SENSITIVITY OF ARTHROPOD AND MICROBIAL COMMUNITIES ASSOCIATED WITH VERTEBRATE CARRION IN RESPONSE TO DELAYED BLOW FLY ACCESS: IMPLICATION FOR CARRION ECOLOGY AND FORENSIC ENTOMOLOGY

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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August 2016

Major Subject: Entomology

ABSTRACT

The objectives of this study were to determine the sensitivity of microbial metabolic community profiles, terrestrial and soil arthropod community structures and function, and soil chemistry dynamics associated with carrion experiencing delayed Diptera colonization.

Bacterial metabolism profiles indicate a significant difference between carrion with immediate insect access (Control) and carrion with delayed insect colonization for seven days and 14 days (Treatments). In contrast, soil samples demonstrated no significant change in soil microbial metabolic profiles in 2013, but exhibited significant difference in 2014 trial. These results suggest high sensitivity of microbial community function on pig carrion, but a stochastic response in the soil microbial ecosystem. This phenomenon may be due to the significant abiotic change in the temperatures as well as the differences in the amount of precipitation between trials.

Soil chemistry profiles were significantly different between Control and Treatment carcasses. Furthermore, significant differences were found between days of decomposition (temporal sensitive) and soil regions (spatial sensitive). Soil nutrients, such as ammonium, phosphate, non-purgeable organic carbon and total nitrogen were sensitive to treatment effects, but nitrate was not.

The treatment effects, community divergence, convergence and resilience for aboveground and belowground arthropods depended on trial, sampling methods (sticky traps, pitfall traps, and sweep nets), taxonomic resolutions (Order, Family, and Genus) and ecological indices (richness, Simpson's diversity, Shannon-Wiener's diversity, evenness, and effective number of species) tested. In general, soil arthropod (including acari) community structures were sensitive to treatment effects only at the Family level. The total abundance of acari was not significantly different across treatments in all-sampling days. For aboveground arthropod community structure and function trapped by sticky traps, significant differences in treatments were detected at the Order and Genus levels for both pitfall traps and sweep nets.

The present study demonstrated that insect succession on carrion by family level is predictable. However, insect succession by genus level demonstrated stochasticity when dealing with disturbances. Hence, both Clementsian and Gleasonian models explained insect succession and scale matters with regards to these ecological phenomena. These data are valuable for a host of applications, such as forensic sciences, disease ecology, and conservation biology.

DEDICATION

To Henry Allan Gleason (1882-1975) who proposed the individualistic ecological succession concept that was largely ignored during the Clementsian era.

ACKNOWLEDGEMENTS

I would like to thank my chair, Dr. Jeffery Tomberlin and committee members Dr. Pete Teel, Dr. Aaron Tarone, Dr. Jacqueline Peterson (Soil and Crop Sciences, TAMU) and Dr. Tawni Crippen (USDA-ARS) for their guidance, advice, and support throughout my study. My committee has helped to shape me into a better ecologist, a better entomologist and at the same time, a better person to be. A special thanks to Dr. Micky Eubanks who was generous to lend me the equipment to conduct the field work successfully. I would like to acknowledge Mr. Edward Riley from the insect systematic laboratory for his assistance in insect identification, Dr. Gregory Dahlem (Northern Kentucky University) for sarcophagids identification, and Dr. Barry O'Connor (Museum of Zoology, University of Michigan, Ann Arbor) for his time and help in the identification of acari. Special thanks to Dr. Roy Norton (retired professor), Dr. Brian Brown (Natural History Museum, Los Angeles County) and Dr. Andrzej Grzywacz (Nicholaus Copernicus University, Poland) for their kind assistance in arthropod identification. Also, I sincerely thank Dr. Masami Fujiwara (Department of Wildlife and Fisheries, TAMU) for providing valuable comments and guidance on theoretical ecology.

This incredible work could not be done without the support of many great people. First, I would like to thank the present and past laboratory members of Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S) Facility: Dr. Micah Flores, Charity Owings, Dr. Meaghan Pimsler, Dr. Baneshwar Singh, Dr. Jonathan Cammack, Dr. Jonathan Parrot, Brandon Lyons, Le Zheng, Ashleigh Faris, Lue Cuttiford, Chelsea Holcomb, and many other members for their support both in the field and in the laboratory. I feel privileged to share the amazing four years (Aug 2012 - Aug 2016) with you all and I am proud to call you colleagues.

Thanks also go to my undergraduate student helpers who assisted me in the field work and laboratory work: Stephanie Thornton (Microbiology), Michael Banfield (Forensic), Jocelyne Soh (Bioenvironmental Science), Adrianna Tirloni (Forensic),

Loren Williams (Forensic, Penn State University), Katherina Kang (Forensic), Aaron Guererro (Forensic), and Caitlin Evers (Forensic). I am grateful to each of you for volunteering your time and effort, which made this research a success. A special thanks to the Texas A&M Field Lab at Snook, Texas where I continued to use the land for pig decomposition studies for two consecutive summers. Not forgetting, Ms. Nina Stanley, who was helpful in soil processing and soil chemistry analyzing work. Sincere thanks to Mr. Nelson (person in-charge of the field site), Mr. Ricky Schraeder (pig supplier), Mr. Peter Krauter (logistic of Berlese funnels), Ms. Mary Frances (statistical analysis), Dr. Inoka Karunaratne (data entry), Dr. Loriann Garcia (logistic of insect-exclusion cages), Dr. Rodrigo Correa (beetle identification), and Mr. Rodrigo Carmo (statistical analysis). This research is also dedicated to the 18 pigs whose sacrifice was useful for the advancement of Science.

I also want to extend my gratitude to the Ministry of Higher Education, Government of Malaysia and Universiti Teknologi MARA, who provided me the full scholarship to study in Texas A&M University throughout the four years period.

Finally, thanks to my parents, brothers and sisters for their continuous support and encouragement throughout this entire process, and to my wife, for her patience, understanding and love.

NOMENCLATURE

mPMI	Minimum post-mortem interval
PMI	Post-mortem interval
Pre-CI	Pre-colonization interval
Post-CI	Post-colonization interval
ANOVA	Analysis of variance
PERMANOVA	Permutational analysis of variance
NMDS	Nonmetric multidimensional scaling
MRPP	Multiple-response permutation procedure
ISA	Indicator species analysis
MMCPs	Microbial metabolic community profiles
ADH	Accumulated degree hour
ADD	Accumulated degree day
CDI	Cadaver decomposition island
PBI	Post-burial interval
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
NPOC	Non-purgeable organic carbon
TN	Total nitrogen
VOC	Volatile organic compounds
MVOC	Microbial volatile organic compounds
MMEs	Mass mortality events
ENS	Effective number of species
EC	Electrical conductivity
OD	Optical density
°C	Degree Celsius
sp.	Species

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Ecosystem and decomposition

The word "ecosystem" was first introduced by the British ecologist Arthur Roy Clapham, which then appeared in a publication of Arthur Tansley (Willis, 1997). Contemporarily, a British-born American limnologist, G. Evelyn Hutchinson and his colleagues suggested that the flow of energy through a lake was the primary driver of the ecosystem. This work was published in his 1961 paper "*The paradox of the plankton*". Hutchinson's student, Eugene P. Odum and his brother further developed a systematic approach to study the ecosystem in detail. Since then, the integrated study of ecosystem ecology has been the framework to examine how nature works through its physical and biological structures and how these properties interact with each other.

Ecosystem, *sensu lato*, is the planet's life support systems for the human species (*Homo sapiens*) and all other forms of life. An ecosystem can be recognized as a unit of biological organization (Swift et al. 1979) defined as the interactions between biotic and abiotic factors in a given geographical area (Campbell et al. 1999). The functioning ecosystem can be divided into three distinct subsystems, which are plants, herbivores and decomposers with the decomposer subsystem being the fundamental determinants of ecosystem structure and dynamics (Swift et al. 1979). An ecosystem can be established by having four basic functions: primary production through autotroph, consumption, decomposition and abiotic storage (Seastedt & Crossley, 1984). In addition, de Groot et al. (2000) suggested that ecosystem functions can be divided into four primary categories namely regulation functions, habitat functions, production functions, and information functions.

A large number of mammals can die from causes other than predation and leave their cadavers to be recycled, for instance, during a natural disaster or mass culling

during an outbreak of zoonotic disease (Fey et al. 2015). Vertebrate carrion represents an ecological unit within a larger ecosystem (Odum, 1969) and is an ephemeral resource that has high nutritive value (Hanski, 1987b). Carrion is also a great example of a resource pulse with their occurrence being brief, infrequent, and containing high-valued resources (Yang, 2004). For example, the caloric value and mass loss estimated from dead brown laboratory mice ranged from 3,146 to 6,064 calories g^{-1} (640-858 mg organic matter) over a temporal gradient (Putman, 1978a). In addition, carrion decomposition introduces nutrients such as nitrogen, potassium, calcium and magnesium back into the ecosystem (Carter et al. 2007). Nitrogen concentrations in soil collected from beneath a decomposing bison (*Bos bison* L. (Artiodactyla: Bovinae)) carcass one year after its placement in the field were approximately 600 μg g^{-1} more than control soil samples (Carter et al. 2007). Rat (*Ratus rattus* L. (Rodentia: Muridae)) carcasses placed in a temperate ecosystem during summer and winter seasons introduced approximately 1.25 to 2.50 mg C g^{-1} (dry weight) into the soil (Carter et al. 2007). Parmenter & Lamarra (1991) measured the decomposition rates and nutrient losses associated with rainbow trout (*Onchorhychus mykiss* (Walbaum)) and pinktail duck (*Anas acuta* L.) carcasses in a marsh in Wyoming, USA. The sequence of total elemental loss rates from the carcasses was K > Na > N > S > P > Ca~Mg. They concluded carrion decomposition can contribute significant amounts of important nutrients that ultimately influence the structure and functioning of an aquatic ecosystem**.**

Recent hypotheses have suggested that many communities may be strongly influenced by transient dynamics after ecological perturbations (Hasting, 2001). The definition of ecological disturbance, both as a system and a reference state, must be defined (Rykiel, 1985). Perturbation is an effect; the response of an ecological component to disturbance or other ecological process (Odum et al. 1979), while a disturbance is defined as a cause or a physical force that causing perturbation (Bazzaz, 1983). The introduction of vertebrate carrion into an ecosystem facilitates a localized succession of invertebrate colonizers (Horn, 1974). In the United States, Motter (1898) conducted a study of the fauna associated with the grave where he examined 150

exhumed bodies. A list of arthropods recovered from these bodies was documented including a variety of gastropods, spiders, mites, beetles, silverfishes, flies (Diptera) and wasps (Hymenoptera). In Tennessee, USA, Reed (1958) used dog (Canidae: *Canis lupus familiaris* L.) carcasses to examine insect communities around decomposing remains and produced a comprehensive checklist of arthropods. One of the earliest carrion studies in the United States was conducted with fetal pigs (Suidae: *Sus scrofa* L.) and the subsequent arthropods succession patterns were described in association with stages of decomposition (Payne, 1965). A total of 522 species representing 3 phyla, 9 classes, 31 orders, 151 families and 359 genera were collected from the remains, with four orders of arthropods (Coleoptera, Diptera, Hymenoptera and Araneae) accounted for 78% of the carrion fauna (Payne, 1965). However, a majority of this early succession research was descriptive rather than hypothesis driven. Carrion has well-documented effects on aboveground arthropods and vertebrate scavengers as well as provides important resource input to underground system (Parmenter & MacMahon, 2009; Ostfeld & Keesing, 2000b). Only a small proportion of necromass is consumed by aboveground arthropods and animal scavengers and this allows large resource input for the belowground organisms. Many soil arthropods evolved to consume these resources effectively (Moore et al. 1988). For example, boreal forest produce huge amount of spruce seeds that are rapidly decomposed and contribute to the increase of soil nitrogen (Zackrisson et al. 1999). Other than plant seeds and litters, animal carcasses can also affect soil (Carter et al. 2007), microbes (Yang, 2004) and plants (Towne, 2000). Hence, carrion contributes direct and indirect effects on dynamics of species diversity and nutrient recycling in the ecosystem (Beasley et al. 2012).

Recyclers of carrion

Organisms associated with vertebrate carrion decomposition have been divided into three trophic levels namely vertebrate scavengers (DeVault et al. 2004), arthropods (Putman, 1978; Norris, 1965) and microbes (Burkepile et al. 2006). Decomposition without the access of scavengers and arthropods will be continued with microbial

decomposition either from endogenous or exogenous sources (Jojola-Elverum et al. 2001; Carter & Tibbett, 2006). Vertebrate scavengers play a role in the decomposition of carrion. For instance, vertebrates were found to scavenge 35% of rodent carcasses made available annually in the Savannah River Site, South Carolina, USA (DeVault et al. 2003). Vertebrates scavenged year around albeit decomposition rates increased during the summer months when warmer resulting in increased competition with invertebrates who also consuming carrion (DeVault et al. 2004). Coyotes (*Canis latrans* Say) were the initial animals to scavenge pig carrion followed by opossums (*Didelphis virginiana* (Kerr)), while turkey vultures (*Cathartes aura* (L.)) skeletonized a pig carcass within one day (Jones, 2011). A decomposition study of deer carcasses recorded 14 species of scavenging mammals (6 visiting species) and 14 species of scavenging birds (8 visiting species). The prominent scavengers included the American crow (*Corvus brachyrhynchos* Brehm), raccoon (*Procyon lotor* (L.)) and Virginia opossum (*D. virginiana*) (Jennelle et al. 2009).

Blow flies (Diptera: Calliphoridae) are widely distributed throughout North America (Whitworth, 2006) and are the primary invertebrate decomposers of carrion. Their arrival patterns vary depending on the species. In the southern United States, *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) initially colonizes vertebrate carrion followed by *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae), which is thought to use carrion in more advance stages of decomposition (Wells & Greenberg, 1994). *Chrysomya rufifacies* is also an introduced species whose larvae predate on the larvae of the native blow fly *C. macellaria* (Wells & Greenberg, 1992). *Chrysomya rufifacies* larvae often eliminate the other species of fly larvae present on vertebrate carrion eventually dominating the carcass and becoming the most successful species in terms of completing survivorship to the adult stage (Wells & Greenberg, 1992; Baumgartner, 1993).

The interactions occurring between arthropods on vertebrate carrion have been examined previously. Some studies have examined blow fly species composition (Wells & Greenberg, 1992; Faria et al. 1999), blow fly niche relationship and exploitation

strategies (Denno & Cothran, 1975; Denno & Cothran, 1976) while others examined the impact of density and species composition on survivorship (Goodbrod & Goff, 1990). For instance, rearing of *Chrysomya megacephala* (Fabricus) (Diptera: Calliphoridae) and *C. rufifacies* in pure cultures at seven different population densities demonstrated an inverse relationship between density and the duration of the larval stage (Goodbrod & Goff, 1990).

Arthropods are known as regulators of the decomposition process in soil systems. From a nutrient point of view, soil arthropods are able to accelerate nutrient release from decomposing organic matter. They do this directly by feeding upon organic matter and associated microflora or indirectly by channeling and mixing of the soil, improving quality of substrate for microflora, and inoculating organic debris with microbes (Crossley, 1977).

The community of soil arthropods is very diverse with feeding habits being used as characters for creating functional groups within an ecosystem (Swift et al. 1979). Grazers (e.g., Collembola) are animals feeding mainly on microorganisms and algae and comminutors (e.g., Isopoda) mainly consume cellulose (Wallwork, 1976). This structural division also suggests a different functional role; grazers affect the microorganisms serving as decomposers in a more direct way by eating them while comminutors affect microorganisms more indirectly by changing the substrate quality (Anderson et al. 1981).

Mites (Acari) and collembolans usually account for about 95% of total soil arthropods diversity (Harding & Stuttard, 1974). Oribatid mites (Oribatida) are the most numerical abundant group in most forested, grassland and desert ecosystem (Santos et al. 1978). Prostigmatid mites (Prostigmata) are occasionally most abundant (Crossley et al. 1992), as are collembolans (Willard, 1974). Astigamatid mites (Astigmata) are rarely encountered, whereas mesostigmatid mites (Mesostigmata) usually dominate only in situations where nematodes are particularly abundant (Elkins & Whitford, 1982). Phoretic mites are known to be associated with carrion beetles (Coleoptera: Silphidae) and necrophagous Diptera (Takaku et al. 1994; Perotti & Braig, 2009). There is a direct

contribution made by soil arthropods to nutrient dynamics in soil. Teuben & Verhoef (1992) compared nutrient concentrations (K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-}, N, C) in the most abundant species and groups of arthropods in two pine forests (*Pinus nigra* Arnold). The result showed those collembolans (Insecta), oribatids (Acari), isopods (Malacostraca), and millipedes (Diplopoda) (in rank order) can increase the Ca^{2+} and Mg^{2+} concentrations, while decreasing N and C concentrations.

Microbes, such as fungi and bacteria, have been documented to initiate the process of decomposition of vertebrate carrion (Jirón & Cartín, 1981). Microbe diversity and function on carrion have been recently described on vertebrate carrion (Pechal et al. 2013; 2014a). They conducted a field study in Ohio, USA examining the impact of primary arthropod colonizers (e.g., blow flies) on microbial community structure and function on vertebrate carrion. The result showed when primary colonizers were excluded from the carrion source, *Proteus* was the dominant (72%) bacterial genus while *Psychrobacillus* (58%) and *Ignatzschineria* (18%) were dominant bacterial genera on insect-accessed carcasses. They also found significant microbial community metabolic profile changes and the mean carcass microbial community metabolic functions with insect access decreased at a greater rate than those where insect colonization was prevented.

History of succession

There are a number of remarkable ecological theories in existence today. Some of the examples of these great hypotheses are the theory of evolution by natural selection (Darwin, 1859; Darwin & Wallace, 1858), the equilibrium theory of island biogeography (MacArthur & Wilson, 1963), ecological succession (Clements, 1916; Gleason, 1917). According to Cherrett (1989), ecological succession is considered one of the oldest and most enduring concepts in ecology. In carrion ecology, the French army veterinarian and entomologist Pierre Mégnin, who working with other medical examiners had documented the succession of insects on exhumed and exposed human corpses (Mégnin 1887; 1894 as in Michaud et al. 2015). Undoubtedly, this documentation gave rise to the

birth of carrion ecology and advances its application in forensic entomology (Byrd & Castner, 2009; Michaud et al. 2015).

According to Clements (1916), De Luc (1806; as cited in Rennie, 1810) was the first to coin the term "succession". Before this term was used scientifically, many pundits believed that plant seeds spontaneously generate or lay dormant for extended periods of time. An American naturalist, Henry David Thoreau, published his observation in 1860 where he described when a pine tree was cut down, an oak tree sprung up, and vice versa, and he further explained that when pine trees were cut down, conditions became favorable for the oaks and the latter grew larger. After a few years, the area became unfavorable for the oaks and in turn, pines were again allowed to grow. This phenomenon is called "forest succession" (Thoreau (1860), as cited in Clements (1916).

Darwin (1859) also observed that a cleared forest eventually supported the same species. Despite these early records, Cowles (1899) from North America described the vegetation succession at the coastal region of Lake Michigan where he used the term "climax" to describe the last and most mature stage of vegetation at that particular region. Cowles (1901) further described that vegetation succession is driven mostly by climate. He assumed that changes in space mirrored those in time, and it is well known today as space-for-time substitution (Pickett, 1989).

Clements (1916, 1936) developed his monoclimax theory of succession based on Cowles' concept, where the main concept was that succession behaved like an organism, developing from different unstable stages called seres, and eventually to a stable stage called the climax, and all of these were under control by the regional climate. Through Clements' experiments, he emphasized that succession as close-ended, sequential, directional, predictable and identified facilitation as its chief mechanism, where pioneer colonizers modify their environment, making it unsuitable for themselves but suitable for others. Clementsian succession has become dogma and is in many ecological textbooks such as in Allee et al. (1949) and Odum (1953).

Soon after publication of succession by Clements, Gleason (1917) and Ramensky (1924) synthesized a competing concept of succession. They emphasized on individualistic concepts that the community's dependence on environmental gradients, life histories of individual species, stochastic events, and variants in arrival times of colonizers. This is in contrast with Clements (1916, 1936), where he had emphasized the directional, sequential replacement of herbaceous and envisioned a successional climax.

To date, most plant ecologists take an intermediate position between the Clements-Gleason dichotomy (Gurevitch et al. 2006) and acknowledge that the method used in vegetation sampling could affect the result of plant succession. To date, the current vegetation dynamics framework recognizes the role of disturbance, site history, species survival and dispersal, and many other causes in determining how species establish, grow, reproduce and communicate with other organisms and environment (Pickett et al. 2009), and these processes involve many other developing ecological disciplines such as soil microbial ecology, community assembly, invasion species ecology, landscape and metapopulation ecology, life history, disturbance and resilience (Pickett et al. 2009).

It is undoubtedly that Clements' approach has been questioned by contemporary ecologists. First, Clements' Aristotelian mode of explanation (Johnson, 1979) seems teleological and vitalistic to modern ecologists, and it is an obsolete view from the cause-effect mechanism used in science today (Pickett et al. 2007). Second, Clements erected complicated jargons and lexicon on his successional model, and had received frustration among ecologists to comprehend the terminological jungle Clements created to explain the many kinds of successional change (Gleason, 1917).

Mathematical models were then proposed to test plant succession hypotheses. Connell & Slatyer (1977) defined three mechanisms with regards to how succession operates. In the facilitation model, only pioneer species are able to colonize, eventually making the habitat more suitable for the establishment of later-succession species and less suitable for the early colonist. As such, pioneer species will be eliminated. In the tolerance model, any species has equal chance to colonize, and those species will modify

the environment, and eventually make it less suitable for early colonists; however, the ability of later succession species to colonize is not affected. Over time, the pioneer species will be eliminated. In the inhibition model, any species has the potential to colonize; those species will modify the environment and making the habitat less suitable for the early colonists, but also inhibit the ability of later-succession species to colonize. Although the work by Connell & Slatyer (1977) underwent rapid scrutiny and refinement, these developments shifted the paradigm in plant succession (Maggi et al. 2011).

Succession of arthropods on carrion

Insects, and their relatives, are known consumers of vertebrate carrion. Their presence can be temporally dependent as related to the decomposition of the carrion source. Some insects are attracted to vertebrate remains shortly after the animal dies (e.g., blow flies), while others are attracted during more advanced periods of decay, and still others being attracted to the dry skin and bones (e.g., skin beetles (Coleoptera: Dermestidae)). When the sequence of insects colonizing carrion is known for a given area and set of circumstances, an analysis of the arthropod fauna on a carcass can be used to determine the time of colonization which can potentially be used to estimate a minimum time of death given certain assumptions (e.g., colonization in fact occurred after death of the individual) (Anderson, 2001).

Payne (1965), from the United States, developed the concept of stages of decomposition and highlighted the rate of tissue removal by insects on two types of pig carcasses (open to insects and insect free). He determined arthropod succession occurred on carrion. Each stage of decay was characterized by a particular group of arthropods, each of which occupied a particular niche. His efforts were an attempt to add quantifiable information to each stage of decomposition and avoid confusion to potential discrepancies with qualitative descriptors being used. As previously stated, a total of 522 species were collected from decomposing pigs. Four orders of arthropods namely Coleoptera, Diptera, Hymenoptera, and Araneida accounted for 78% of the carrion

fauna. Families that were considered significant in the carrion summer study include Calliphoridae, Sarcophagidae, Muscidae, Staphylinidae and Histeridae. Payne & King (1972) further studied the arthropod succession and decomposition of pig carcasses in water and they found a definite arthropod succession occurred among the 102 insect species inhabiting water carrion with each stage of decay had its particular group of scavengers.

Arthropod succession can be influenced by a number of parameters. These include physical properties of the carrion, rate of decomposition, time of day, weather, season, geographical region, exposure, and habitat (Payne, 1965; Anderson, 2001). Arthropod succession patterns on shaded and sunlit carrions in Canada in three different seasons were drastically different where sun-exposed carrion had greater diversity in fauna than shaded carrion (Sharanowski et al. 2008). Factors affecting decomposition (e.g., temperatures, burial depth, and access to insects) and arthropod colonization on corpses have been reviewed (see Campobasso et al. 2001). Benbow et al. (2013) demonstrated there is substantial variability in necrophagous communities and assembly on carrion over decomposition and among seasons in Ohio, USA in which taxon richness were varied evidently among seasons but was generally lower during early decomposition and increased through mid-phase of decomposition process. Furthermore, autumn and winter showed the highest richness during late decomposition.

Little is known about the impact of delayed colonization on the successional trajectories of arthropods colonization and consuming vertebrate carrion. For instance, buried carrion, where decomposition process is affected by burial environment, climatic, edaphic, oxygen content, pH and biological factors, and so were the arthropod community structure and function (Gaudry, 2010). Different researchers have highlighted the shift of insect populations between exposed and buried remains (Mégnin, 1894; Leclercq, 1978; Smith, 1986). The insect structure associated with buried remains might be divided into five main categories of depth namely very shallow, shallow (10-30 cm deep), deep (40-60 cm deep), very deep (from 90 cm) and coffin (2 meter) (Gaudry, 2010). In a shallow grave, the diversity of Arthropoda is considered greater, with the

presence of various families of Diptera (e.g., Calliphoridae, Muscidae, Phoridae), Coleoptera, Hymenoptera, Acari and Collembola. In deep and very deep graves, insect activities are poor and are understudied in such condition (Gaudry, 2010).

Arthropod access to carrion can be delayed due to man-made barriers such as enclosed container or confinement. As previously mentioned, Pechal (2012) examined the arrival of insects on carcasses with delayed insect access in Dayton, Ohio, USA and she found that oviposition occur within the first 24 hours after exclusion net removal on day five. *Phormia regina* (Meigen, 1826) (Diptera: Calliphoridae) was the dominant taxon arriving to carcasses throughout initial active decomposition and black scavenger flies (Diptera: Sepsidae) were dominant during the dry stage. Pechal et al. (2014b) demonstrated there are differences between insect communities attracted to insect access and delayed insect access carcasses. The possibly underlying causes for such observation could be attributed to differences in resource size (Braack, 1987) or priority effects of initial colonizers altering subsequent community structure (Hanski, 1983; Slatkin, 1974).

Carcass mass and size does not influence insect arrival pattern (Hewadikaram & Goff, 1991) as well as microbial community structure assemblage of gravesoil (Weiss et al. 2015). Perez et al. (2014) evaluated the application of insect community structure on swine remains for calculating a post-mortem interval estimate. They found five species of insects that are exceptionally useful in forensic scenario. These hexapods are the larval forms of *Necrophila americana* Linnaeus (Coleoptera: Silphidae), *Fannia scalaris* (Fabricius) (Diptera: Fanniidae), *Co. macellaria*, *P. regina*, and *Lucilia illustris* Meigen (Diptera: Calliphoridae).

Soil ecology

Soil is fundamental to human life; so much so that it has been reflected in our language. The word “human” itself from the Latin *humus*, which means the organic matter in the soil (Hillel, 1991). Many elements are found within the earth’s crust, and a majority of them are found in soil as well. However, hydrogen, carbon, oxygen, nitrogen, phosphorus, sulfur, aluminum, silicon, and alkali and alkaline earth metals are

dominant. Various trace elements can also be found such as iron, cobalt, nickel, copper, magnesium, manganese, molybdenum, and zinc. Soil can be functionally categorized into sand-silt-clay matrix, which contains biomass and "necromass" (Coleman et al. 2004). A heavy clay soil required more power to till than a lighter sandy loam soil (Russell, 1973). Quantifiable approaches can be used to categorize soil in terms of sand, silt, and clay present, which are ranged on a scale of light-intermediate-heavy or sandy-silt-clay (Coleman et al. 2004).

Climate, organisms present, parent materials, and topography all act together over time to form soil (Jenny, 1941; 1980). These factors also affect major ecosystem processes (e.g., primary production, consumption, decomposition and abiotic storage). The top 10-15 cm of the organic, litter, fermentation, and humification zones of forested soils contain the majority of plant roots, microbes, and fauna. Hence, a majority of biological and chemical activities occur in this layer (Coleman et al. 1983; Paul & Clark, 1996). In fact, a majority of microbial and algal-feeding fauna, such as protozoa (Elliott & Coleman, 1977), and rotifers and tardigrades (Leetham et al. 1982), are within 1-2 cm of the surface. Microarthropods and mesoarthropods (e.g., nematodes) are most abundant in the top 5 cm of forest soils (Schenker, 1984) or grassland soil (Seastedt, 1984). Significant numbers of nematodes may be found at several meters' depth in xeric sites such as deserts (Freckman & Virginia, 1989).

Clay soil is an important specific physical property to soils, microbial life, and to plant activity via nutrient availability. Clays are weathered forms of primary minerals, and hence they are referred to as secondary minerals. Coarse clay particles (0.5 μm) often derived from quartz and mica; finer clays (0.1 μm) are clay minerals or weathered products of hydrated ferric, aluminum, titanium, and manganese oxide (Coleman et al. 2004).

Other than soil particle size, another important factor in soil structure is pore spaces within the structure. Soil porosity has strong implications for ecosystem management, especially agroecosystem (Elliott & Coleman, 1988). Input of organic matter to soil is one of the major agents of soil structure.

Organic matter comes from both living and dead organisms. All heterotrophs ingest organic carbon and associated nutrients and assimilate them into carbohydrates, lipids, and proteins (Coleman et al. 2004). The principal decomposers in soil are microbes (i.e., the bacteria, fungi and viruses). Bacteria encompass more than 35 phyla and are probably the most diverse array of organisms on earth (Tiedje et al. 2001). Furthermore, bacteria are undoubtedly the most numerous organisms and have been estimated at 4-6 x 10^{30} cells on Earth. More than 90% of bacteria are living in the subsurface, which is the top 4 km of the Earth's mantle (Whitman et al. 1998). The number of bacteria present in all types of ecosystems (biomes) is estimated to be 2.5 x 10^{29} cells, where the top one meter of soil constituted 2 x 10^{9} cell g^{-1} and 1 x 10^{8} cell g^{-1} in the top one to eight meters of soil (Whitman et al. 1998).

In bulk soil (absence of rhizosphere), the environment for bacteria is usually stressful; however, bacteria still persist in this low-nutrient condition (Morita, 1997). In anoxic or low-redox microsites within soil, decomposition via microbial fermentation or anaerobic respiration with nitrate or other electron acceptors can occur (Coleman et al. 2004). Many genera of prokaryotes, including both bacteria and archaea, have evolved the highly important biochemical traits of fixing nitrogen whereby they rupture the triple covalent bond of dinitrogen (N_2) and produce ammonium (NH_4) which is then taken up by plants or other microbes (Postgate, 1987).

The distribution and abundance of microorganisms in the soil is usually patchy. There can be aggregations of microbes around roots (rizhosphere) (Lynch, 1990), fecal pellets, and other patches of organic matter (Foster, 1994). Interestingly, microorganisms concentrate in the mucus secretions that line the burrows of earthworms (drilosphere) (Bouché, 1975; Lee, 1985). Approximately 35% of the total amount of microbial biomass was found in soil below a depth pf 25 cm. Gram-positive bacteria and Actinomycetes tended to increase in proportional abundance with depth, whereas Gram-negative bacteria, fungi, and protozoa were highest at the soil surface (Coleman et al. 2004).

Animal members of the soil biota are numerous and diverse. The soil fauna can be categorized by their degree of presence in the soil or microhabitat utilization by different life forms. There are four groups of soil fauna namely transient, temporary, periodic and permanent have been identified. Transient species are the organisms that hibernate in the soil but when active are primarily arboreal or live in the plant stratum. An example of this group is the ladybird beetle (Coleoptera: Coccinellidae). Diptera (e.g., crane fly, gnat) also represent the temporary residents of soil as the adults live aboveground while the eggs are laid in the soil and resulting larvae dwell underground where they act as scavengers. Periodic habitants spend their lives belowground, with adults emerging from soil to reproduce. Examples of periodic species include cicada (Insecta: Cicadidae), velvet mites (Acari: Prostigmata) and earwig (Insecta: Dermaptera). As for permanent soil resident, they spend their entire life in the soil, although different stages may live at different depth of soil. These species include Protura, Diplura, Collembola, and some beetles. The morphology of collembolans reveals their adaptations for life in different soil strata. For those species that dwell on the soil surface, the body is usually pigmented, large in size, and equipped with long antenna and a well-developed jumping apparatus called furcular. Within the mineral soil, collembolans tend to be smaller, unpigmented, elongated bodies with reduced furculae (Coleman et al. 2004).

Soil fauna can be divided based on body size. There are currently three recognized categories, microfauna, mesofauna, and macrofauna. The microfauna include free-living protozoa in litter and soils, which belong to two Phyla: Sarcomastigophora (amoebae and flagellates) and Ciliophora (ciliates) (Levine et al. 1980). The mesofauna include Rotifer, Nematoda, and Tardigrada. Microarthropods can be found in most type of soil, and they are known to have a significant impact on the decomposition process in forest habitats. Examples of microfauna are mites and collembolans. Microarthropods density varied during seasons within and between ecosystems. Generally speaking, temperate forest floors with large amount of organic matter support huge number of microarthropods, while tropical forests contain fewer mites and collembolans (Seastedt,

1984). Disturbance and perturbation of soils usually depresses microarthropods numbers. Examples of disturbance, such as forest fire, tillage, and pesticide application typically reduce a population. But they tend to recovery rapidly demonstrating high resilience within the ecosystem (Coleman et al. 2004).

Collembolans are generally considered to be fungivores; however, some species are predacious (Gilmore & Potter, 1993), while others are associated with decomposing plant or animal residue or fecal materials (Coleman et al. 2004). Experiments have shown important impacts of Collembola on nitrogen mineralization, soil respiration, leaching of dissolved organic carbon and plant growth (Filser, 2002). Grazing upon fungal hyphae seems to be the significant contribution by Collembola in the decomposition process. Grazing on fungal hyphae may be selective, thus influencing the fungal community, and subsequently affect the nutrient cycling indirectly (Moore et al. 1987).

Soil mites (Astigmata: Oribatida) usually outnumber the collembolans. Among the mites, soil mites usually dominate the soil, but Prostigmata may develop large populations in cultivated soils with algae on the surface. Immediately following cultivation, Astigmata mite numbers have been observed to increase dramatically (Perdue & Crossley, 1989). Acari, or mites, are chelicerate arthropods related to spiders and scorpions and are the most abundant microarthropods in many types of soils. In rich forest soil, a 100 g sample extracted through a Berlese funnel may contain as many as 500 mites representing almost 100 genera (Coleman et al. 2004). Mites can be grouped into two Superorders namely Parasitiformes and Acariformes. Under the Superorder Parasitiformes, the Order Mesostigmata is one of the important members in soil. The Superorder Acariformes can be divided into two Orders, namely Order Trombidiformes and Order Sarcoptiformes. In Order Trombidiformes, Suborder Prostigama is one of the important suborders. In Order Sarcoptiformes, Suborder Oribatida (Cohort Astigmata) is the major taxon in the soil (Krantz & Walter, 2009). The oribatids are typical soil mites and are usually fungivorous, detritivorous, or both. Mesostigmatid mites are nearly all predators on other smaller arthropods. Acarid mites are found associated with rich,

decomposing nitrogen sources and are seldom abundant except in agricultural soils or stored products.

Other than collembolans and mites, soil also contains microarthropods. Examples include Protura, Diplura, Microcoryphia, Pseudoscorpionida, Symphyla, Pauropoda, and Enchytraeidae (potworms).

The larger insects are termed as macroarthropods. These are arthropods with body length more than 10 mm and up to 15 cm (Shelley, 2002). Pitfall traps have been widely used to sample litter-dwelling macroarthropods, including diplopods, ants (Hymenoptera) and other epigaeic invertebrates (Banerjee, 1970; Majer, 1978).

Macroarthropods may have direct effects on soil structure. Termites (Isoptera) and ants (Hypmenoptera: Formicidae) are important mover of soils. Other macroathropods, such as emerging nymphal stages of cicadas (Hemitpera: Cicadidae), may impact the soil structures. Larval stages of Scarabaeidae beetles sometimes churn the soil in grasslands (Coleman et al. 2004). All these activities rendered the name "soil ecosystem engineers" to soil macroarthropods. Examples of soil macroarthropods include Isopoda, Diplopoda, Chilopoda, Scorpionida, Araneae, Opiliones, Solifugae, Uropygi, Coleoptera, Hymenoptera, Diptera, Isoptera, Gastropoda, and earthworms (Coleman et al. 2004).

In summary, the combination of microbes, nematodes, microarthropods and macroarthropods provides complex food webs in the soil ecosystem. The entire soil fauna are involved in the maintenance of soil health and are all interacted in creating soil quality (Coleman et al. 2004).

Plant little decomposition

Over the past 30 years, more than one thousand publications concerning litter decomposition have appeared in the literature (Berg & McClaugherty, 2003). Litter decomposition involves a complex set of processes including chemical, physical, and biological agents. The bulk of plant litter consists of varying amounts of several major classes of organic compounds depending on plant part (e.g., leaves, stems, roots, bark)

and species. Under aerobic condition, microbial decomposition results in a release of CO_2; however, under more anaerobic condition, anaerobic decomposers may produce organic acid (e.g., acetic acid) instead of CO_2. Generally, leaf litter breakdown follows a sequential pattern with different classes of organic compounds dominating the decay process as it proceeds. Degradation of soluble and low molecular weight compounds dominates the first stage of litter decay. Afterwards, hemicelluloses and then cellulose compounds are degraded. Then, lignin degradation will occur finally (Berg & McClaugherty, 2003). Once litter fall has occurred, fungi will be the first to colonize the material. They penetrate the leaf through openings and invade the fresh substrate. There is also a succession of fungal species, which colonize the litter depending on litter decomposition stage and substrate quality (Berg & McClaugherty, 2003). In the early stage of litter decomposition, climate plays a critical role in the process, while in the later stage, its role is diminished. Finally, in a humus-near stage, the litter decomposition reaches a limited value (threshold) for a total mass loss. At such a stage, the litter would be more stabilized soil organic matter, indicating extremely slow decomposition of the litter mass (Berg, 2000).

Plant decomposition is the result of three combined phenomena: leaching, fragmentation and catabolism. Leaching is the removal of soluble compounds from detritus by water and it is of significant in the early stage of decomposition when nutrients and soluble carbohydrates are still abundant in the litter. The fragmentation of litter into smaller pieces is caused by physical action of soil fauna while catabolism of organic matter is mainly operated by fungi and bacteria (Cotrufo et al. 2000). The rate of decomposition is affected by three main variables and their interactions: the physiochemical environment (e.g., pH), the resource quality (i.e., relative decomposability of litters) and the decomposer organisms (Swift et al. 1979). When resource quality is low and climatic constraints are present, fungi tend to play a more important role in decomposition process than bacteria (Dighton, 1995)

During the decomposition process, elements are converted from organic to inorganic forms (mineralization), which are available for plant uptake. For example,

microbes decompose organic N from organic matter to ammonium (NH_4). Plant will then use ammonium or nitrates for growth by synthesizing amino acids and proteins from it. However, immobilization can occur during decomposition, which is the reverse of mineralization. Immobilization refers to the process in which nitrate and ammonium are taken by soil organisms and therefore become unavailable to plants (Jansson & Persson, 1982).

Biochemical modifications of C, N, and P during plant litter decomposition

Three major patterns of nutrient concentration have been observed in litter during decaying process. Some nutrients are slowly released from the litter, resulting in a linear concentration increase with cumulative mass loss. Other nutrients may be leached from the litter and disappear faster than the litter mass as whole, resulting in linear or curved negative relationship to mass loss. Finally, some nutrients are strongly retained in the litter-microbe complex, resulting in an exponential increase in concentration over mass loss (Berg & McClaugherty, 2003).

Carbon

Cellulose and hemicellulose account for more than 50% of carbon in plant debris. Cellulose is an important source of energy as it serves as fuel for microbial processes such as transformation of nitrogen and sulfur. Over time, the carbon-nitrogen ratio and carbon-sulfur ratio in decomposing materials are reduced. In general, carbon is progressively lost throughout microbial respiration, as cellulose and other labile organic compounds are hydrolyzed and utilized in their growth and maintenance (Berg & McClaugherty, 2003).

Nitrogen

The concentration of N in litter increases during decomposition. This increase may be described in relations to time or as a function of litter mass loss. For boreal Scots pine (*Pinus sylvestris* L.) needle litter, the N concentration may increase about three-fold

during decomposition (starting with ca. 4 mg g^{-1} and increasing up to ca. 12 mg g^{-1}). According to Berg & Staaf (1981), the N content increases during the initial stages of decomposition and then declines. Nitrogen is mineralized during decomposition and is simultaneously immobilized by microbes, resulting in an increase in the concentration of N in the litter, and in the absolute amount of nitrogen if it is transported into the litter from soil or by atmospheric nitrogen-fixing. As decomposition proceeds, the carbon-nitrogen ratio (C:N) declines until the substrate becomes more suitable for microbial action. In some forests, the duration of nitrogen increase may extend for two years or more (Blair & Crossley, 1988).

Phosphorus

The concentration of P in litter increases during decomposition, in a manner very similar to that of N. This relationship can be described as a positive linear function of litter mass loss. Similarly, an increase of four-fold has been documented (from ca. 0.2 to 0.8 mg g^{-1}) (Staaf & Berg, 1982). However, in woody litter decomposition, it will accumulate Ca and P as a result of fungal invasion and translocation of soil. In another study, P concentration decreased initially in all three litter types (e.g., flowering dogwood, red maple, chestnut oak), probably due to leaching of soluble P-containing compounds. This initial leaching loss was followed by a general increase in P concentrations in litter of all three species throughout the study. Phosphorus concentrations in flowering dogwood (*Cornus florida* L.), red maple (*Acer rubrum* L.) and chestnut oak (*Quercus prinus* L.) litter increased by 72, 181 and 76% by the end of the study (Blair, 1988).

Nutrient ratios

Ratios have been used to determine nutrient release patterns (Mafongoya et al. 1997). The C:element ratios indicate the relationship of element release patterns to dry weight losses. If the ratio increases, the element is being released through leaching, or mineralization processes; if the ratio decreases, the element is being accumulated either

biologically or physiochemically; and if the ratio remains constant, the release of the element is related to dry loss. For instance, low C:N ratio (e.g., high N concentrations) decomposers meet their N requirement directly from the litter. At higher initial C:N, net immobilization typically occurs as microbes access N exogenous to the litter and converts it to microbial biomass or exoenzymes (Parton et al. 2007; Frey et al. 2000). While an element:element ratios (e.g., N:P) indicate loss pattern relationships between elements (Lousier & Parkinson, 1978). One of the factors affecting rate of decomposition is resource quality (Damann & Carter, 2013). Resource quality is inversely proportional to the C:N ratio, where the amount of carbon is expressed per single nitrogen atom; a high ratio indicates a poor quality resource and vice versa. A high quality resource means increased availability of carbon and nitrogen mobilization in the ecosystem (Swift et al. 1979). For instance, plant material contains a C:N ratio of 100:1, whereas cow manure contains a ratio of 18:1 (Carter et al. 2007). In this case, cow manure is the higher-quality resource. As for human cadavers, the C:N ratio is approximately 5.5:1 (Carter et al. 2007), indicating that the rate of decomposition is faster for a human cadaver than plant material in the same environment.

Soil chemistry changes during carrion decomposition.

Vertebrate carrion constitute only 0.06% of total above ground biomass compared with 99.94% plant biomass in the semi-arid ecosystem (Parmenter & MacMahon, 2009). This lack of input indicates that carrion contributes potentially only a trivial amount to the ecosystem nutrient budget. However, this estimate does not include the invertebrates, such as the total biomass of ants, termites and earthworms, which are widely distributed and generally present in huge numbers (Lee & Foster, 1991). Hence, the total carrion biomass may be higher than expected and consequently have a greater impact on the surrounding ecosystem.

A dead animal, including human remains, is a high quality resource (narrow C:N ratio, high water content) that releases an intense, localized resource pulse of carbon and nutrients into soil upon decomposition (Carter et al. 2007). Cadaveric materials are

rapidly introduced to belowground floral and faunal communities, which results in the formation of a highly concentrated island of fertility or known as cadaver decomposition island (CDI) (Carter et al. 2007). This CDI is associated with increased soil microbial biomass and microbial activity. In particular, the degradation of proteins, lipids, and carbohydrates will yield N-based, P-based and C-based products, which may be retained in the immediate soil horizon (Benninger et al. 2008). From the ecosystem perspective, excess N inputs can lead to increases in plant growth and eventually leading to increased losses of N via solution leaching and trace gas emission. Consequently, excess soil nutrients may change species composition and cause ecosystem decline (Matson et al. 2002). Other than terrestrial plants, it has been found that freshwater and marine plants are equally responsive to nutrient inputs. This nutrient enrichment, or eutrophication, can lead to highly undesirable changes in ecosystem structure and function (Smith et al. 1999).

Insect, vertebrate scavengers and microbes compete for cadaveric resources. Insects, such as blow flies, are typically the first to detect the presence of carrion (Byrd & Castner, 2009). However, microorganisms present on the carrion can release repellent toxins, such as botulin toxin (Janzen, 1977), to make carrion resources unsuitable to other consumers so that microbes can outcompete other higher organisms. When insects and microbes are less active (e.g., during winter), scavenging becomes dominant (Putman, 1983b).

Larger cadavers tend to be consumed *in situ*, hence allowing cadaveric materials to enter the soil (Towne, 2000) and subsequently form the CDI. Several carrion decomposition studies indicate cadaver breakdown follows a sigmodal pattern, which differs from the breakdown of plant and fecal matters (Coleman et al. 2004). The differences between the patterns of cadaver and plant decomposition are probably due to the complexity of the substrates and presence of skin, which will retain cadaveric moistures (Putman, 1977).

Cadavers might not persist in terrestrial ecosystems as long as woody plant materials (Schoenly & Reid, 1987). The progress of a cadaver through the sigmoidal

decomposition pattern is often associated with stages as previously mentioned (Payne, 1965). These decomposition stages are a convenient means to summarize physiochemical changes, although there is controversy differentiating between decay stages (Moreau et al. 2015). Five stages of decomposition are widely recognized in carrion decomposition studies: fresh, bloated, active decay, advanced-decay and dry and remains stage as proposed by Payne (1965).

Changes in soil nutrients follow a predictable temporal pattern that closely matches the nutrient and mass loss of the aboveground portion of the carrion. For example, a study examining the role of carrion in nutrient cycling in a North American shrub-steppe ecosystem revealed that the sequence of peak nutrient transfer to the soil was first K and Na, followed by N and S, then P and Mg, and finally Ca (Parmenter & MacMahon, 2009). A study conducted at the University of Tennessee Anthropology Research Facility (ARF) in Knoxville, TN, USA examined the potential of carcass enrichment in the soil. This study determined that six of the eight variables tested were significantly different, These include soil moisture content, soil organic content, soil pH, total nitrogen percentage, C:N, and lipid-bound P, suggesting an influx of high quality nutrients into the ARF soil. Furthermore, elevated pH readings, presumably resulting from ammonification of the soil, were observed in areas of high decomposition (area with more than six decomposing bodies) (Damann et al. 2012).

Changes of carcass C, N, and P during carrion decomposition

According to Parmenter & MacMahon (2009), nutrient content within a rat carcass (*Rattus norvegicus* Berkenhaut) changes as a function of percentage mass remaining in the carcass and time. Carcass concentrations of N, K, Na and S, as well as energy content, all declined linearly or logarithmically as the remaining carcass mass decreased; whereas, concentration of P, Mg and Ca increased linearly or exponentially. The energy content (kJ/g) was measured based on the dry mass of the rat carcasses. Dry mass is mainly carbohydrates, proteins and lipids, which are made up from organic carbon and thus they are considered as a carbon source in this context. Water-soluble

salts (K and Na) were lost initially and most rapidly, most likely through leaching of body fluids into the soil and consumption of soft tissues by insect scavengers. Losses of energy (i.e., C), N, and S were closely related with overall soft tissue consumption by scavengers and detritivores as these nutrients are most commonly associated with muscles and body organs. Phosphorus is a component of both bones and soft tissues. Consequently, it was lost from carcasses at slower rates than N and S. Finally, Ca and Mg were the slowest nutrient to leave the carcass. Similar patterns have been documented in the decomposition of rodents (Parmenter, 2005) and fish and ducks (Parmenter & Lamarra, 1991). The nutrient quality of carrion during decomposition exhibit considerable change as microbes and arthropods selectively remove the soft-tissue labile fraction of the animal carcass. As these tissues are removed, N, S, and energy values declined as a function of carcass mass loss, as well as time. Buried carcasses lose these components more rapidly than above ground (surface) carcasses. As predicted, recalcitrant skeletal constituents (e.g., P, Mg, Ca) exhibit increases in concentration with both carcass condition and time. This result is not caused by the enrichment from allochthonous sources, but rather a function of subtraction of other carcass tissues.

Changes of soil C, N, and P during aboveground carrion decomposition

The fresh stage of decomposition starts immediately after death of an animal. A lack of oxygen inhibit aerobic metabolism, which causes cell autolysis (self-digestion) (Vass, 2001). Concomitantly, blow flies and flesh flies (Diptera: Sarcophagidae) colonize the cadaver by ovipositing or larvipositing their immatures (e.g., eggs or larvae) on the carrion. The feeding activity by the fly larvae is a vital step in the breakdown of a cadaver as they can remove the soft tissues efficiently in the absence of scavengers. In addition, soil microbes positively respond to cadaver introduction within 24 hours (Putman, 1978b). The depletion of internal oxygen also create an ideal environment for anaerobic bacteria (e.g., *Clostridium, Bacterioides*) to transform carbohydrates, lipid and protein into organic acid (e.g., lactic acid) and gases (e.g., ammonia, hydrogen sulphide).

However, nutrient flow into soil environment during this stage is unknown. The bloated stage starts when the internal pressure from gas accumulation forces purge fluids to discharge from orifices (e.g., mouth, nose, anus) and flow into soil. The soil enrichment by purge fluid causes an increase in microbial biomass, shift in soil faunal community, C mineralization and increase in soil nutrient status. This effect is similar to the formation of "island of fertility" observed in plant (Zaady et al. 1996) and fecal resources (Willott et al. 2000). The continuous larval feeding activity on the remains results in skin rupture, which allows oxygen back into the cadaver and exposes more surface area for fly larvae and aerobic microbial activity (Putman, 1978b). The active decay stage is characterized by rapid mass loss resulting from peak insect activity. There is substantial release of cadaveric fluids into soil and leading to the formation of CDI. Yet, the status of soil nutrients during active decay stage is unclear.

The lateral extent of a CDI during advanced decay is determined by the size of the cadaver, larval mass and migration, and soil texture. Coe (1978) examined the CDI in sandy loam soil associated with elephant (~1,629 kg) decomposition and determined it extended < 40 cm below the cadaver, 35 cm at 1 m from the cadaver, and 8 cm at 2 m from the cadaver. No penetration into soil was observed at 2.2 m from the cadaver. The CDI during advanced decay represents an area of increased soil carbon, nutrients and pH (Putman, 1978b; Vass et al. 1992). Advanced decay stage is also associated with a significant increase in soil N concentration. The decomposition of a 68 kg human (*Homo sapiens* L.) cadaver resulted in an increase in approximately 525 µg ammonium g^{-1} soil (Vass et al. 1992) by 20 days postmortem. During litter decomposition, the introduction of any organic resource with a C:N greater than 30:1 (e.g., straw, woody materials) will usually result in an initial decrease in the concentration of soil inorganic nitrogen due to immobilization (Green et al. 1995). Therefore, the C:N will narrow during decomposition and inorganic N will be released into the soil upon reaching 20:1 (Swift et al. 1979). However, no study on carrion decomposition has addressed this issue yet. Other nutrients such as P, K, Ca and Mg will enter soil upon carrion decomposition. Advanced decay stage is also typically associated with the death of underlying and

nearby vegetation. The cause of plant death might be due to N toxicity (Thomas et al. 1999). The intense pulse of N associated with cadaver decomposition might also result in a loss of N from the ecosystem through denitrification, volatilization and leaching.

During the dry and remains stages (e.g., after 100 days of death), the cadaver mass loss becomes slow, probably due to the depletion of readily available nutrients and moisture. However, this retardation in nutrient loss does not mean that the concentration of all nutrients in grave soil have returned to basal or initial levels (i.e., resilience) (Benninger et al. 2008). The concentration of P (Towne, 2000), NH_4, K, SO_4, Ca, Cl and Na (Vass et al. 1992) in soil associated with decomposition of a 68 kg human cadaver can remain as high as 50-150 µg g^{-1}. Furthermore, Towne (2000) observed a concentration of inorganic N approximately 600 µg g^{-1} in soil above basal level after one year of bison (*Bos bison* L.) decomposition. Dry and remain stage can be associated with the formation of fruiting structure of the fungi (Sagara, 1995; Hitosugi et al. 2006). "Early phase" fungi fruit in response to high concentrations of ammonia (Yamanaka, 1995) while "late phase" fungi fruit in response to organic N and high concentration of ammonium and nitrate (Yamanaka, 1995).

Benninger et al. (2008) investigated the dynamics of C, N, and P-based compounds in soil beneath the pig cadavers that were placed on the soil surface over a period of 100 days. They found that the cadaver decomposition did not result in a significant difference in soil C and moisture content. However, significant increases were observed in pH as well as the concentration of total N, soil-extractable P, and lipid-P. A significant increase in decomposition fluid conductivity had been observed in simulated clandestine graves in a semi-rural environment, which provide indication of the post-burial interval (PBI) and potential postmortem interval (Pringle et al. 2010).

Studies conducted in prairie (Towne, 2000) and tundra (Brathen et al. 2002) environments have demonstrated that the effect of large herbivore carcasses on the surrounding soil and vegetation can be dramatic and still detectable several years after introduction. A significant increase in inorganic N concentrations was detected in both soil and vegetation surrounding the carcasses. Fertile areas around the carrion favored

different species of vegetation, stimulated biomass production and increased species richness and spatial heterogeneity (Towne, 2000). Carrion decomposition study in a Polish temperate forest also demonstrated significant increases in nutrient concentrations (Melis et al. 2007). Calcium content and pH were found to be higher directly underneath the carcass with a gradient decrease towards the periphery of the decomposition site. This effect was detectable for up to seven years after the death of the animal. Besides, concentrations of nitrate (NO_3) in the soil also differed suggesting a fast turnover of nitrate in the forest ecosystem (Melis et al. 2007).

Belowground carrion decomposition

Carcass' placement either on above, or below, ground can affect the nutrient dynamics associated with grave soil. The aforementioned descriptions are associated with carrion that decomposes above ground. As for the carcasses buried, such decomposition processes have received little attention. Payne & King (1968) proposed a different set of terminology to describe the stages of decomposition belowground (i.e., fresh, inflated, deflation and decomposition, disintegration, skeletonization). This is predominantly due to the absence of primary necrophagous insects (e.g., flies) and vertebrate scavengers. Hence, belowground decomposition is mainly mediated by microorganisms and proceeds less rapidly than above ground decomposition. Buried remains are thought to decompose eight times slower than remains aboveground (Rodriguez, 1997). Soil arthropods and microbial activity have been documented associated with burial carcasses; however, very few soil nutrient studies have been conducted to address the dynamics of C, N, and P associated with cadavers belowground. In a burial environment, the conditions are relatively anaerobic, anaerobic bacteria present in both the soil and body are able to cleave the ammonia from amino acids, thus producing large quantities of ammonia in anaerobic environment, accumulation of ammonia in burial environment can occur (Carter & Tibbett, 2003; Forbes, 2008). Besides, during the skeletonization stage, NH_4, amino acid N, CO_2, total C, total N, and soil pH are elevated (Hopkins et al. 2000).

Comparison of decomposition between animal and human tissues

Stokes et al. (2013) conducted a comparative study on human and animal tissue decomposition. Skeletal muscle tissues from human, pork, beef (*Bos Taurus* L.) and lamb (*Ovis aries* L.) were buried and then the surrounding soil was sampled and analyzed. Results indicated the overall patterns of nutrient fluxes and chemical changes (e.g., K, PO_4, NH_4, and NO_3) across these resources are quite similar; with the ovine tissue are the most similar to human tissue in many of the measured parameters. From the perspective of decomposition chemistry, this study demonstrated soil nutrients (e.g., NO_3, NH_4 and P) increased during the initial stage of decomposition.

The lateral movement of soil chemistry of human cadaver

Aitkenhead-Peterson et al. (2012) examined the extent of lateral movement of nutrients during decomposition of human remains. The results determined the spatial distribution of dissolved organic C (DOC) and organic N (DON) were consistent across replicates. Both compounds were also significantly different compared to the control soils from the upslope, along with other parameters such as orthophosphate-P, ammonium-N, potassium, pH and conductivity. Besides, pH was lower and electrical conductivity was higher in the soil beneath the decomposing human corpses. The soil nutrients examined at the downslope of the human remains were significantly higher compared to the control soil at the upslope, indicating downward movement of decomposition products and this could be an important factor to consider when searching clandestine graves.

Human decomposition products in Cadaver Decomposition Island (CDI) with known postmortem interval

Aitkenhead-Peterson et al. (2015) examined cold water extractable soil C, N, and P in the CDIs below 14 human cadavers at two sites in Texas. Soil samples were collected from beneath the torso of cadavers at various stage of decomposition. The results indicate the concentration of soil nitrate-N remained below ambient soil

concentration for approximately a year. Nitrate-N is reduced because under an anaerobic condition beneath the decomposing human corpse, anaerobic bacteria use nitrate as an electron acceptor or an oxygen source. This reduces the shift of NO_3^- to NO or N_2, which are then released as gases. The increase in NO_3-N concentration in CDIs one year after placement of the remains was likely due to burrowing or drilling activities imposed by soil microarthropod communities and plant root growth that causes aeration and oxygenation. Likewise, ammonium-N cannot be nitrified in soil containing purge fluid as the reaction of nitrification requires oxygen (an aerobic process). Hence, ammonium-N is accumulated and remained high in CDIs. However, ammonium-N can be utilized by fungi and soil bacteria (Sagara, 1976), as such, ammonium-N can be expected to decrease due to microbial uptake. As for the DON, it tends to increase in the CDIs and then decrease after 196 days PMI. This is because only certain bacteria can mineralize DON to ammonium-N under anaerobic environment in the CDI. The data indicate both mineralization (conversion of DON to ammonium) and immobilization (conversion of ammonium into DON) may occur after 176 days of decomposition of the remains. The decline in ammonium-N and DON with a subsequent increase in nitrate-N after one year indicates that normal aerobic soil conditions are returned. For DOC, it tends to remain high for about a year. DOC is a substrate for soil microorganisms along with ammonium-N. DOC is expected that it would be eventually mineralized to CO_2 but not until aerobic conditions are restored in the CDIs. The slow breakdown of DOC and potential loss as methane gas under anaerobic condition makes it a good option for predicting PMI.

Comparison between plant litter and carrion decomposition

The fundamental differences in tissue characteristics and nutritional composition between plant litter and vertebrate carrion lead to substantial divergent decomposition patterns (Table 1.1). The obvious differences can be noted in the rate of decomposition, N concentration, K, and Ca concentrations, where plant litter and animal carrion response differently to these parameters. As for other variables (e.g., C and P

concentration), plant litter and vertebrate carrion both respond similarly during decomposition.

Table 1.1. Comparison between leaf litter and animal carrion decomposition.

Characteristics	Plant litter	Vertebrate carrion	References
Decomposition rate	Slower (decomposition rate constant ranged between 0.01 to 1.06)	Faster (rate of active decay is between mean range of 0.69 to 3.08)	Harmon et al. 2001; Matuszewski et al. (2010)
Decomposition rate depends on C:N ratio	Yes	Not observed	Swift et al. 1979; Parmenter & MacMahon (2009)
Nitrogen concentration	Increase	Decrease	Berg & Laskowski, 2006; Aitkenhead-Peterson et al. (2012).
Carbon concentration	Decrease	Decrease	Berg & McClaugherty (2003)
Phosphorus concentration	Increase	Increase	Berg & McClaugherty, (2003); Parmenter & MacMahon (2009); Aitkenhead-Peterson et al. (2012); Blair, 1988
Sulphur concentration	Increase	Decrease	Berg & McClaugherty (2003); Parmenter &

Table 1.1 (Continued)

Characteristics	Plant litter	Vertebrate carrion	References
			MacMahon, (2009); Blair, 1988
Potassium concentration	Decrease due to leaching, but then steadily increases in concentration	Decrease quickly	Berg & McClaugherty, (2003); Parmenter & MacMahon (2009); Laskowski et al. (1995)
Calcium concentration	Increases initially, and then decreases during decomposition	Continue to increase, particularly during the latter skeletal remains stage of carrion decomposition	Berg & McClaugherty, (2003); Parmenter & MacMahon (2009)
Microbial activity	Occur immediately after litter introduction to soil	Majority of nutrients enter the soil after maggot migration (active decay stage)	Putman (1983)

As previously mentioned, decomposition and soil chemistry profiling of human remains was studied by Aitkenhead-Peterson et al. (2012). They determined the DOC was large, low sulfate (due to anaerobic respiration of microbes), low pH and high conductivity. They observed a significant downslope movement of gravesoil chemistry particularly orthophosphate-P, potassium, dissolved organic carbon and dissolved

organic nitrogen. The authors concluded that cadaver decomposition can have a significant and persistent effect on grave soil chemistry.

A decomposition study with kangaroo (*Macropus giganteus* (Shaw)) carcasses was conducted by Macdonald et al. (2014). They quantified soil nutrient changes in a box gum grassy woodland ecosystem. The results showed a significant redistribution of N within the ecosystem where there was a significant and lasting input of proteins (40 mg/kg) and amino acid (25 mg/kg) into the soil. Based on the findings, they argued for a reconsideration of the models used in ecosystem management to predict the removal of carcasses as an ecosystem management tool, as they provide large and lasting resource islands which influence soil N cycling.

Flies as invasive species

The impacts of invasive species on ecosystem services have attracted worldwide attention (Charles & Dukes, 2007). Various attempts have been made to address the ecosystem processes that are affected by invasive species (Levine et al. 2003; Dukes and Mooney, 2004), but the links between these mechanisms and ecosystem services are largely lacking in the literature.

The impacts of invasive species are often classified as economic, environmental, or social in nature. Economic impacts are those of direct consequence to humans, typically leading to monetary losses (Pimentel et al. 2005). Environmental impacts are those that affect ecosystem structure and function, often referring to loss of biodiversity or unique habitats (Pimentel, 2011). Social impacts focus predominantly on human health and safety (Charles and Dukes, 2007).

The concept of ecosystem health integrates ecology, economics and human health to prevent further degradation of ecosystem stability by human dominant activities (Rapport et al. 1998). One of the devastating effects to ecosystem services was the introduction and establishment of an invasive species (Pejchar & Mooney, 2009). Invasive effects on native biodiversity and community structure are well known, but few studies have examined the mechanisms that lead to these effects (Levine et al. 2003).

Invasive species may alter community structure through exploitation competition (e.g., indirect interactions such as resource use), or interference competition (e.g., direct interactions such as allelopathy in plants) (Callaway & Ridenour, 2004). Invasive species impacts on other species interactions, including predation, herbivory, parasitism and mutualisms, can change the abundance of species with certain key traits that influence ecosystem processes (Chapin et al. 2000). To date, no studies have addressed the invasive impact of *C. rufifacies* to the structure and function of arthropods in North America, and its significant effects on carrion decomposition process is still remain unknown to the ecologists.

Flies as vectors of pathogens

Blow flies, and other carrion-breeders, are known to serve as vectors for a number of pathogens. Infectious diseases have long been known to cause devastating illnesses in humans, crops and livestock (Service, 2012). The field of disease ecology, defined as the ecological study of host-pathogen interaction within the context of their environment and evolution (Kilpatrick & Altizer, 2012). Vertebrate carcasses are consumed by a wide variety of animals (Hanski, 1987a), including larvae of carrion flies in three dipteran families (Calliphoridae, Sarcophagidae and Muscidae) that often dominate in terms of numbers and resource use. A considerable number of these flies have developed various relationships with higher vertebrates, including man and associated domestic animals. The larval stages may feed on animal feces, or in cadavers, or in wounds and sores, or they may pierce skin and breed in flesh (i.e., myiasis). The adults may take advantage of human shelters and feed on man's foodstuffs, or feed on body exudates (e.g., sweat, conjunctival fluids) or on blood. Many of them are also responsible in the transmission of various pathogens, either in mechanical or biological ways (Greenberg, 1971).

Over 100 different pathogens have been recorded from house flies (*Musca domestica* L.), at least 65 of which are known to be transmitted (Service, 2012). For instance, house flies can transmit the viruses responsible for polio, trachoma, Coxsackie

virus and infectious hepatitis, as well as rickettsiae such as Q fever (*Coxiella burnetti*) and numerous bacterial diseases, but mainly enteric ones, such as bacillary dysentery (*Shigella*), cholera, enterotoxic *Escherichia coli*, *Campylobacter*, *Salmonella*, and variety of streptococci and staphylococci. In addition, they can carry eggs and cysts of a variety of helminthes such as *Taenia, Hymenolepis, Dypylidium, Diphyllobothrium, Necator, Ancylostoma, Thelazia, Enterobius, Trichuris* and *Ascaris* (Service, 2012).

Flies as agents of myiasis

For Calliphoridae and Sarcophagidae, these families can cause obligatory or facultative myiasis, which is the colonization of living tissue, to human and animal, depending on species (Zumpt, 1965). Sherman (2000) reported 42 cases of wound myiasis in urban and the suburban United States. The most common species was *Lucila sericata* (Meigen) (Diptera: Calliphoridae), followed by flesh flies (Sarcophagidae) and humpbacked flies (Phoridae). Larvae of both *Lucilia* and *Calliphora* have been found in many parts of the world developing in foul-smelling wounds and ulcerations, especially those producing pus. Occasionally intestinal myiasis is reported (Zumpt, 1965). This is usually caused by eating uncooked foods contaminated with larvae of *Lucilia* and *Calliphora* (Service, 2012).

Insect succession

Data collected from arthropod succession studies could also be used to estimate a minimum post-colonization interval, which could be used to infer a minimum postmortem interval (mPMI) (Tomberlin et al. 2011a, Tomberlin et al. 2011b). The odor released from a corpse changes as the body decomposes. Such shifts result in the remains becoming more attractive to certain species and less attractive to others. Although blow flies arrive very soon after death, they are no longer attracted when remains have passed a particular stage of decomposition, or become mummified or dry (Nuorteva, 1977). For example, *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) preferred decomposed remains to fresh when given a choice

(Erzinclioglu, 1996). In England, *C. vicina* and *Lucilia caesar* (L.) (Diptera: Calliphoridae) appeared on rodent remains within minutes or hours of death. *Lucilia illustris* (Meigen) (Diptera: Calliphoridae) was not attracted to corpses in woodland until 76 hours after death, and to corpse in open grassland until 48 hours after death (Lane, 1975). Real cases in estimating time of death was demonstrated by Goff et al. (1988) where they estimated PMI by arthropod succession in three cases from Hawaiian Islands and the estimated mPMI fitted well with time intervals established by other means with only 0.8-3.0% margin of error. Anderson (2004) was able to determine time of death using blow fly eggs in the early PMI by using available weather records, developmental data and degree day accumulation (ADD) even though the case was over 20 years old.

The recent progress in what factors are governing the process of insect succession has been under investigations. Ma et al. (2012) obtained *Proteus mirabilis* from the salivary glands of the blow fly *L. sericata* and found that this strain of bacteria produced a strong odor that attract blow flies. Ma et al. (2012) demonstrated that the mechanism used by *L. sericata* for detecting a resource can be associated with bacterial quorum sensing. Tomberlin et al. (2012) determined that the physiological state (age) of the insect influences its response. Due to recent evidence, a biochemical interaction between microorganisms and insects by means of microbial volatile organic compound (MVOC) production has been proposed (Davis et al. 2013).

Quorum sensing

Quorum sensing is a term used to describe intercellular signaling in bacteria. It is the regulation of gene expression in response to fluctuations in cell-population density. Bacteria use quorum sensing molecules as a means for intercellular communication (Waters & Bassler, 2005). These signaling molecules are called autoinducers. The bacteria have a receptor that can specifically detect the signaling molecule (Bassler, 2002). When the autoinducers bind to the receptor, it activates transcription of certain genes for functions such as facilitating the bacterial virulence factor, or directing biofilm formation (Williams, 2007). When a bacteria population is low in the environment,

diffusion reduces the concentration of the autoinducers in the surrounding medium to almost non-detectable. However, when the bacterial population grows, the concentration of autoinducers reaches a critical threshold, resulting in a population level gene upregulation (Nadell et al. 2008). In this way, individual cells can sense the local density of bacteria, and through the concentration of autoinducers, the whole population can make a collective decision (Miller & Bassler, 2001).

Quorum sensing bacteria produce and release chemical signal molecules (e.g., autoinducers) that increase in concentration along with bacteria density (Miller & Bassler, 2001). Although several quorum sensing systems are known, the two most well-studied are the acyl-homoserine lactone (AHL) systems employed by many Gram-negative species and the peptide-based signaling systems by Gram-positive species (Whitehead et al. 2001; Kleerebezem et al. 1997). Several cooperative behaviors exhibited by bacteria have been demonstrated to be regulated by quorum sensing. As such, quorum sensing is an indication that bacteria are social (Parsek & Greenberg, 2005). By possessing the ability to communicate, although via chemical signals, microbes behave like eukaryotes. As social organisms, microbes have been examined under the pretext of many social concepts. These social phenomena include cooperation (Griffin et al. 2004), kin selection (Mehdiabadi et al. 2006), altruism (Strassmann et al. 2000), kin discrimination (Strassmann et al. 2011), cheaters and punishment (Wang et al. 2015; Kiers et al. 2003). In the past decade, sociomicrobiology was introduced to address issues especially with regards to biofilm formation and the quorum sensing system (Parsek & Greenberg, 2005). Biofilms are any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substances (ESP).

Quorum sensing is essential for biofilm development for several bacterial species. For example, AHL-based quorum sensing has been shown to influence biofilm maturation for Gram-negative bacterium *Serratia liquefaciens*. Quorum sensing regulates swarming motility in *S. liquefaciens*. In Gram-positive bacteria, the LuxS-type

quorum sensing system in *Streptococcus mutans* is also involved in biofilm development (Ng & Bassler, 2009).

Other than biofilm formation, another type of cooperation includes mutualism. Mutually beneficial social interactions provide a direct fitness benefit to the individuals that perform the behavior, or other recipients, which outweighs the cost of performing the behavior. In microbial systems, this interaction is considered public goods (Diggle et al. 2007). Many bacteria produce numerous factors that are released into environment. One popular example is the production of siderophores (an iron scavenging enzyme). Iron is a major limiting nutrient for bacterial growth because most iron in the environment is in the insoluble form. In order for bacteria to access this limiting factor, they will manufacture these enzymes, and then secrete them into the extracellular space. Once released, the siderophore will sequester the iron, allowing the iron to be metabolically available for the bacteria (Griffin et al. 2004).

Another example of cooperation is altruism, which can be defined as a behavior exhibited being costly to the donor but beneficial to the recipient (Diggle et al. 2007). The possible benefit from altruism is that they provide indirectly towards other individuals who carry the cooperative gene. By helping a close relative reproduce, an individual is still passing its own genes to the next generation indirectly. This interaction is defined as kin selection according to Hamilton (1964). One of the popular examples of altruism is the slime mold, *Dictyostelium discoideum* (a type of soil-dwelling amoeba) (Strassmann et al. 2000). When starving, the amoebae aggregate and form a multicellular slug that can contain 10^4-10^6 cells. This slug migrates to the soil surface, where it transforms into a fruiting body composed of a spherical tip of spores and a stalk consisting of nonviable stalk cells that hold the spores on top. Approximately 20% of the cells "sacrificed" and developed into non-reproductive stalk, elevating the spores and aiding their dispersal (Strassmann et al. 2000).

The cost that bacterial cells pay for quorum sensing is high (Diggle et al. 2007). However, with the sufficient number of collaborators, the benefits outweigh the costs. For example, bioluminescence produced by *Vibrio fischeri*. Individual production of the

enzyme, Luciferase, can be costly and will not be visible if produced solely by a single cell. However, by having the whole population produce luciferase (via quorum sensing induction), *V. fischeri* cells are able to avoid wasting energy on the production of an useless product. Each eukaryotic host uses the light provided by the bacteria for a specific function. For instance, in the squid, *Euprymna scolopes* Berry, bioluminescence is used as an antipredation strategy in which it counter-illuminates itself using the light from *V. fischeri*. Counter-illumination allows the squid to prevent casting a shadow beneath it on bright clear nights when the light from the astronomical objects (e.g., moon and stars) penetrates the seawater (Ruby & McFall-Ngai, 1992; Visick & McFall-Ngai, 2000).

Cheaters exist within the microbial environment. Cheaters are individual that take advantage of the benefits resulting from cooperation (e.g., quorum sensing responses) without contributing. Cheaters have been found in many microbial systems, for example, *Pseudomonas aeruginosa* and *Myxococcus xanthus*. In the production of siderophore, the mutant *P. aeruginosa* which do not contribute, can gain benefits from the wild type *P. aeruginosa* producing it and outcompete the wild type *P. aeruginosa* (Harrison et al. 2008). The bacterium *M. xanthus* exhibits several social behaviors, including formation of spore-forming fruiting bodies during starvation (Vos & Velicer, 2009). However, a mutant *M. xanthus* (does not secrete signaling peptides) exhibited cheating during development, being overrepresented among resulting spores relative to their initial frequency in the mixture (Velicer et al. 2000). Those cheaters may be common in nature and have created the tragedy of the commons among social bacteria.

There are many methods to "punish" the cheaters. For example, interactions between leguminous plants and rhizobial bacteria that fix N within the root nodule of the host plants) (Kiers et al. 2003). In this mutualisms relationship, instances where the rhizobia in a nodule will not provide nitrogen for their plant host due to high metabolism cost, but the plant responds by decreasing the oxygen supply to that nodule, which reduces the growth rate of the bacteria (Denison, 2000).

Interkingdom communication between bacteria and insects

Bacteria consumed by immature blow flies (larvae) feeding on decomposing organic resources survive transtadially (through larval molting and pupation) and are present in emergent adults (Ahmad et al. 2006). *Proteus mirabilis*, a Gram-negative bacterium, which causes 90% of all *Proteus*-induced infections in humans (Liu, 2009) is commonly found associated with dogs, cattle, and birds, and can cause nosocomial infections when colonizing human feces in hospital settings. Bacteria associated with carrion release volatile organic compounds (VOCs) that facilitate attraction and oviposition by blow flies (LeBlanc & Logan, 2010). Similarly, myiasis-producing flies attracted to wounds are attributed to certain bacteria activities (Khoga et al. 2002). *Proteus mirabilis* has been found to elicit oviposition response in *Co. macellaria* (Chaudhury et al. 2010).

Bacteria and fruit flies (Diptera: Drosophilidae) share a common cell-cell communication system and have a common evolutionary origin (Waters & Bassler, 2005). This phenomenon is demonstrated through the examination of *aarA* gene expression. The inner membrane protein AarA of *Providencia stuartii* is required for the release of an extracellular QS signals. The homolog of AarA in the fruit fly *Drosophila melanogaster* Meigen, is a rhomboid protein RHO that control fly wing vein development and eye organization. Expression of *P. stuatii* aarA in a *D. melanogaster rho* mutant rescued wing vein development, while expression of *rho* in *P. stuartii* aarA mutant rendered the QS signal released from the membrane (Waters & Bassler, 2005).

Ma et al. (2012) hypothesized that bacteria quorum sensing could play a role in regulating fly behavior. As previously mentioned, they obtained *P. mirabilis* from the salivary glands of the blow fly *L. sericata*. This bacterium is known to swarm (quorum sensing response) and attract blow flies. The authors discovered six novel genes for swarming. They also determined that if these genes were removed, swarming would cease. However, swarming could be rescued with the application of the fly attractants: lactic acid, phenol, NaOH, KOH and ammonia. Furthermore, they observed that fewer blow flies were attracted to the bacteria incapable of swarming. This study is of

ecological significant, as it represents a new facet of trophic interactions between resources and those entities competing, or collaborating, to consume them. In a bigger picture, swarming molecules could be one of the players in regulating the entire ecosystem processes and interkingdom communication.

Using the same wild-type *P. mirabilis*, Tomberlin et al. (2012) conducted a study to access *L. sericata* over the wild-type *P. mirabilis* (which is able to swarm) and the swarming-mutant *P. mirabilis* strain. The results demonstrated that sex of the blow fly did not significantly influence response, but age and diet did. Seven-day-old flies had a significant greater probability of responding to the wild type than to the mutant, regardless of diet, but the percentage of milk-fed flies that responded was significantly lower than the percentage of blood-fed flies that responded. The blood-fed flies oviposited whilst the milk-fed did not. The 14-day-old flies oviposited predominately on the mutant. These results indicate that a mechanism used by *L. sericata* for detecting, and responding to, a resource can be associated with bacterial quorum sensing, and that the physiological state of the insect influences its responses.

These results depict a possible driving force of carrion decomposition process. Microbial communities associated with decomposition remains produce quorum sensing signals, which would be eavesdropped by arthropods in order to colonize the resource. This interaction provides insight how flies detect, locate and utilize resources. This discovery opens the door for explaining variation in arthropod community structure on carrion, as well as arthropod succession on decomposing carrion, and its potential application in forensic entomology in the determination of pre-colonization interval (Tomberlin et al. 2011a). Pre-colonization interval has been defined as the interval between deaths and extends to colonization by arthropods (begins with exposure phase, acceptance phase and stops at acceptance phase). This length of this interval varied depending on multiple factors in biotic and abiotic condition. The exposure phase begins at death and continues until the remains have been detected by arthropods either visually or through olfactory. The detection phase entails two stages namely (i) activation, which

involves detection by the target arthropod; and (ii) searching, which is the arthropod's behavioral response to the stimulus originating from remains (Tomberlin et al. 2011b).

VOC released by bacteria are the primary mechanism governing blow fly attraction, acceptance and colonization of such resource (Chaudhury et al. 2010). Results from the study showed not all species of bacteria produced the same effective attractants at the same rate. For example, *P. mirabilis, Proteus vulgaris, Providencia rettgeri, Providentia stuartii*,and *Klebsiella oxytoca* produced relatively more attractive factors than *Enterobacter* spp. and *Serratia liquefaciens*. Also, the relative volatility of the effective chemicals may have impacts on fly behavior (Chaudhury et al. 2010).

Genetic variation plays an important role in behavioral ecology (Miller et al. 2011). Using the *Drosophila* model, Miller et al. (2011) demonstrated some strains prefer to lay eggs only on food with yeast present, whereas others oviposit in yeast-free substrates. Furthermore, some strains prefer oviposition inoculated with specific yeast strains (Anagnostou et al. 2010). This phenomenon established a clear interkingdom communication between microbes and *Drosophila*, while demonstrating the variation in signals related to microbes that regulate insect behavior. Yeasts are not the only microbes that impact *Drosophila* behavior, bacteria such as *Wolbachia* infection in fruit flies can modify their attraction to a resource (Panteleev et al. 2007). For instance, Panteleev et al. (2007) showed females of *D. melanogaster* infected with *Wolbachia* exhibited changes in oviposition substrate preference (preferred both wheat and oat diets instead of wheat diet alone) and the infected female fruit flies are more resistant to the entomopathogenic fungus, *Blauveria bassiana* than uninfected females. Furthermore, males infected with the bacterium are more competitive than uninfected males. In another study, *Wolbachia* and *Lactobacillus* species have also been shown to affect mating preferences in *Drosophila* (Sharon et al. 2010). The experiment used two different populations of *D. melanogaster* (one reared from molasses and the other on starch medium). Initial results showed "molasses flies" preferred to mate with other molasses flies while "starch flies" preferred to mate with other starch flies. However, results showed antibiotic treatment abolished mating preference, suggesting that the fly

microbiota was responsible for this phenomenon. Further investigations revealed that symbiotic bacteria changed the level of cuticular hydrocarbons sex pheromones (Sharon et al. 2010). The study further discussed the possibility of bacterially induced mating preference contribute to speciation and evolution in nature.

Davis et al. (2013) provide a review of interactions between microorganisms and insects by the means of MVOC production. Davis et al. (2013) hypothesized that insect olfactory responses to emissions from fungi and bacteria inhabiting their environment are much more common than previously thought, and that these signals represent evolutionary reliable infochemicals. In general, there are numerous instances of MVOCs being closely related with insect-feeding behaviors (Wertheim et al. 2005), but some MVOCs are also powerful repellents (Engelberth et al. 2004).

Emissions from microorganisms *in situ* may signal potential oviposition site or habitat suitability. For example, MVOC produced by the bacteria *Enterobacter agglomerans* increase oviposition rates of the apple maggots fly, *Rhagoletis pomonella* Walsh (Diptera: Tephritidae) on fruit (Lauzon et al. 1998). Similarly, Chaudhury et al. (2010) observed that blood inoculated with bacteria isolated from the primary screwworm, *Cochliomyia hominivorax* Coquerel (Diptera: Calliphoridae) collected from infested animal wounds was an attractive oviposition site for adult flies. Likewise, tsetse, *Glossina* spp. (Diptera: Glossinidae) are attracted to typical fungal odors such as 1-octen-3-ol that are also associated with many mammals (Steiner et al. 2007). In Australia, Emmens & Murray (1983) conducted a study on bacterial odor as oviposition stimulants for the Australian sheep blow fly, *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). They used extracts from unsterile sheep fleeces (wool) seeded with *P. aeruginosa, P. mirabilis, Enterobacter cloacae* or *Bacillus subtilis*. Results showed all bacteria exhibited an equal oviposition stimulus to *L. cuprina*. However, with increasing length of incubation, significant differences were noted where the cultures of *P. mirabilis, E. cloacae,* and *B. subtilis* becoming contaminated with increasing number of *P. aerugionosa,* and the response of the flies to the culture extracts becoming greater. The authors explained although contamination with *P. aeruginosa* appeared to be the cause

of the increased oviposition; pure *P. aeruginosa* cultures did not elicit high responses. The response to cultures of *P. aeruginosa* was obviously enhanced by interactions with other bacteria. A volatile study on stable fly, *Stomoxys calcitrans* L. (Diptera: Muscidae) suggested that a strain of *Citrobacter freundii* highly stimulated the oviposition activities by adults (Romero et al. 2006). Other than cyclorrhaphans, MVOCs are also important stimulants for many mosquito (Diptera: Culicidae) species (Lindh et al. 2008). Gravid *Aedes aegypti* L. (Diptera: Culicidae) mosquitoes use volatiles in the form of carboxylic acids and methyl esters emitted from alpha and gamma Proteobacteria to direct egg laying in desirable habitats (Ponnusamy et al. 2008). Furthermore, skin normal floral such as bacteria on mammals and birds are known to affect host preference and settling behavior of the malaria vector, *Anopheles gambiae* Giles (Diptera: Culicidae) (Verhulst et al. 2009). In another study, Trexler et al. (2003) found that gravid female *Aedes albopictus* Skuse (Diptera: Culicidae) oviposited more frequently in water that has been inoculated with bacteria such as *Psychrobacter immobilis, Sphingobacterium multivorum* and a *Bacillus* species. On the other side, MVOCs also can deter mosquito oviposition. A mixture of bacteria originating from a natural larval habitat containing *Pseudomonas, Stenotrophomonas, Enterobacters, Pantoea, Klebsiella, Acinetobacter, Aeromonas,* and *Bacillus* reduced oviposition by gravid *A. gambiae* (Huang et al. 2006). The study suggested that communities, rather than individual bacteria species, are important in releasing the complexes of MVOCs which needed to elicit or repel oviposition.

In some ecosystems, bacterial or fungal volatiles can facilitate insect aggregations (Tillman et al. 1999; Wertheim et al. 2005). Bentz & Six (2006) demonstrated that the mutualistic fungus *Grossmania clavigera* produces sterols that are required for the biosynthesis of aggregation pheromones by *Dencroctonus ponderosae* Hopkins (Coleoptera: Scolytinae). In addition, Dillon et al. (2000) showed that a component of the locust pheromone, guaiacol, derived from locust fecal pellets triggers locust mating aggregations.

Necrophagous flies are important colonizers of decaying organic resources and are highly responsive to MVOCs from decaying carcasses. Carrion flies use sulphide compounds emitted by bacteria to identify decaying carcasses for oviposition (Stensmyr et al. 2002). Frederickx et al. (2012) studied the VOC associated with cadaveric materials (i.e., putrescene, cadaverine, butan-1-ol, butanoic acid, indole, dimethyl disulfide, and phenol) using electroantennography and olfactory behavioral assays. They found that *L. sericata* responded to di-methyl disulphide, putrescine, butan-1-ol. And, in general, females were more sensitive to these compounds than males. A recent study showed that oviposition site-seeking females blow flies (*L. sericata* and *P. regina*) do not respond to an oviposition pheromone. Instead, they appear to co-opt semiochemicals associated with feeding flies as resource indicators (Brodie et al. 2014). Skin beetles, *Dermestes maculatus* De Geer (Coleoptera: Dermestidae), are attracted to MVOCs emitted from pig carcasses; however, the attraction was varied with the stages of decomposition. The beetles were most attracted to carrion during post-bloated stage corresponding with a significant production of benzyl butyrate that could be produced by the bacteria *Clostridium butyricum*. This bacterium performs anaerobic fermentation of glucose into acetic acid, hydrogen, carbon dioxide, and butyric acid in association with the decaying pig carcasses (Von Hoermann et al. 2011; Zhang et al. 2009; Popoff, 1984). Similarly, burying beetles, *Nicrophorus vespillo* L. and *Nicrophorus vespilloides* Herbst (Coleoptera: Silphidae) both respond to sulfur-containing MVOCs commonly emitted by fresh carrion (Kalinova et al. 2009).

Several dipteran predators use MVOCs to locate prey insect species. *Medetera* sp. (Diptera: Dolichopodidae), a bark beetle predator, is highly attracted to wood material inoculated with the fungus *Ophiostoma ips* or a bacteria strain *Burkholderia* sp., which indicates the presence of the prey insects (Boone et al. 2008). Furthermore, many hymenopteran parasitoids use MVOCs associated with living prey to locate food source for their offspring. For example, wood wasps employ MVOCs from the bacterial and fungal symbionts of wood-boring insects to locate hosts (Madden, 1968).

MVOCs emitted from pathogen can also deter insects. Recent work demonstrated that female house fly, *M. domestica,* detect the presence of harmful entomopathogenic fungi in the odor profile of animal feces and that females accordingly avoid ovipositing in these resources (Lam et al. 2010). The study showed that five fungus-derived volatile compounds (dimethyl trisulfate, an unknown, 2-phenylethanol, citronellal, and norphytone) elicited responses from house fly antenna. In behavioral assay, dimethyl trisulfide and 2-phenylethanol significantly reduced oviposition by house flies.

Additionally, herbivorous lepidopterans may use information from MVOCs to select host plants (Tasin et al. 2011). In their experiment, the host plants, *Vitis vinifera* (grapevine) were infected with a variety of microorganisms, and fitness of the larvae feeding on the plants was correlated to the infected state of the host plant. The results showed an oviposition preference for volatiles that is significantly correlated with the fitness of the substrate. Both the volatile signal and the quality of the plant as larval food were found to be influenced by the introduction of microorganisms. This study demonstrates the effects and the interactions between plant-microorganism on insect population dynamics (Tasin et al. 2011).

The metabolism of sulfur-containing amino acids can occur in several bacteria inhabiting the carcasses such as *Brevibacterium, Corynebacterium, Micrococcus, Staphylococcus, Arthrobacter* and lactic acid bacteria (Schulz & Dickschat, 2007). It seems like the arthropods appear to recognize unique time-based MVOC profiles and use them to determine if a resource is appropriate for their needs (Chaudhury et al. 2010; Tomberlin et al. 2012). If MVOCs produced by bacterial in the necrobiome are being used by insects to evaluate resource quality, then this temporal aspect of microbial utilization needs to be considered when accessing insect species composition in forensic studies (Ma et al. 2012; Tomberlin et al. 2012).

The production of secondary metabolites has a temporal aspect. For example, when a bacterium first colonizes a freshly deceased animal, the bacteria first find itself in a nutrient-rich environment. In this situation, most bacteria employ primary metabolism that enhance growth and facilitates reproduction as their highly priority functions (Görke

& Stülke, 2008; Davis et al. 2013). However, when the resource and nutrient depleted over time, the bacterium utilizes other strategies and begin activating lower priority pathways that indirectly promote survival or are useful to metabolize alternative substances as energy source and produce unique secondary metabolites (Davis et al. 2013). In other words, in a nutrient-rich environment, the microbes produce a particular VOC profile that corresponds to stress-free growth, but under stress condition (limitation in nutrients), a different package of MVOCs may be produced as the metabolic pathways shift to enable adaptation to changing resource availabilities (Davis et al. 2013). Consequently, as decomposition progresses, some microbes may not possess the metabolic pathway necessary to utilize the changing resource and are replaced by species who can metabolize the changing resource, thus resulting in a fluctuating microbial community structure, and with it, a shifting volatile profiles (Davis et al. 2013).

Application of ecology in forensic entomology

The fields of ecology and evolution have always been closely linked together; at least can be seen from the classical examples of Darwin's finches (Grant, 1999). To understand the evolution of an organism, it is vitally important to understand its ecology. Fundamentally, ecological forces are the sources of selection and drift that ultimately impact the evolution of genes and species (this could be the reason for speciation). As such, studying the ecology of the organisms without considering the evolutionary pressure that shape its biology is not scientifically sound (Benbow et al. 2015b). As carrion decomposes in an ecosystem, it is expected that ecosystem exerts evolutionary pressures on all the organisms (e.g., prokaryotes, archaea, eukaryotes, fungi, arthropods, and vertebrates) that utilize carrion within the ecosystem (e.g., through competition, predation, parasitism etc.). Similarly, the evolution of decomposers (and coevolution of their competitors or predators) in their local environments will impact the ecological processes (e.g., lipolysis, putrefaction, microbial community structure, insect succession etc.) associated with carrion (Benbow et al. 2015a).

Through continuous events of evolution and co-evolution over time, this raises another fundamental question: can carrion serve as a means for speciation? It is known that organism change/evolve to adapt to the new and changing environment over time (Levins, 1968). When that organism dies, what is left is considered the history-based resource (i.e., what the organism fed during lifetime determines its carrion quality when it dies as determined by its C:N or C:P ratio. In other words, how and what it was fed actually shaped its body nutritional composition). As such, the feeding history of a particular organism could result in the variations of carrion quality (Sterner et al. 1993). It is expected that the decomposers and scavengers are then evolved to detect and compete for the best carrion (in terms of nutritional quality) by evolving better adaptations and/or developing new strategies in winning the competition or escaping from predation, and ultimately evolved into a new species of decomposer/scavenger. If the above statement is true, then carrion (with its pre-determined resource quality) is able to shape the necrophagous community structure and function in an ecosystem. Furthermore, new species may evolve from carrion through competition and adaptation. Note that this hypothesis is derived from the concept of resource-driven sympatric speciation (Dieckmann & Doebeli, 1999). Although carrion-speciation hypothesis has not been proven, yet it is an interesting direction of research for the future.

Obviously, the link between ecology and evolution is also applicable in the carrion system. Two major publications aiming to link basic sciences such as ecology to forensic entomology have been published by Tomberlin et al. (2011a; 2011b), in addition, two textbooks related to these two disciplines are also available (Tomberlin et al. 2015; Benbow et al. 2015b). All of these publications intended to emphasize the importance of returning forensic entomology to its ecological roots by providing a science based conceptual framework.

Tomberlin et al. (2011b) proposed a conceptual framework for forensic entomology. The idea was that forensic entomology should be framed in terms of multidisciplinary ecological concepts to advance understanding of the carrion decomposition process and to explain observed error and variation. As carrion insects

exhibited wide array of behaviors before, during and after utilization of carrion resource, Tomberlin et al. (2011b) categorized at least four important elements composing the behavioral ecology of arthropods that use carrion: (i) evolutionary underpinnings of effective foraging, (ii) carrion signaling characteristics, (iii) control modes of arthropod behavioral cascades, and (iv) mechanisms of host location and selection.

Evolutionarily, an organism will employ the best strategy to increase its fitness maximally by capitalizing on necessary resources (e.g., food, mate) while minimizing energy expenditure. This theory is called optimal foraging theory (OPT) (MacArthur & Pianka, 1966). This arthropod foraging event involved a series of decision steps with important neural processes (Vinson, 1976). Therefore, it is likely that arthropods make decision when locating carrion. In the framework proposed by Tomberlin et al. (2011b), different neurobiological events and the ensuing arthropod choices divide the PMI continuum into five phases namely exposure, detection, acceptance, consumption, and dispersal.

At the broadest level, the PMI is divided into precolonization interval (pre-CI) (which consists of exposure, detection, acceptance phases) and the postcolonization interval (post-CI) (which includes the consumption and dispersal phases) (Tomberlin et al. 2011b). The idea behind this proposed framework is to provide a flexible list of terms to describe ecologically relevant phases of decomposition, allowing researchers to describe and communicate the temporal and each biological aspect of studies. Universal application of this framework in future research would allow for a more systematic and solid understanding of ecology and evolution within the practice of forensic entomology (Tomberlin et al. 2011a). This framework also clearly demonstrated the gap of knowledge between ecology and applied sciences such as forensic entomology and identifies the research areas need to be done to complete the whole picture of carrion decomposition.

Tinbergen's four questions (i.e., causation, ontogeny, phylogeny and adaptation) should always be asked in all carrion insect behavioral studies whenever carrion is exposed to the environment. Questions such as "how does this behavior occur?" and

"how does it change over time" are significantly important from a forensic perspective. Questions such as "How did it develop?" and "how does it affect reproductive fitness" are important to understand the natural variation during decomposition process (Tomberlin et al. 2011b).

Understanding the timing of arthropod colonization of a body is useful in estimating a post-CI. However, many abiotic factors can affect entomological-derived post-CI (Catts, 1992). Substantial variation in the arrival and succession of arthropods on remains can reduce the reliability and accuracy of using entomological succession data in criminal cases (Tomberlin et al. 2011b). One of the major assumptions in forensic entomology is that arthropods are predictable, and are following a set of "golden rules" made from past observations, such as the descriptions of insect succession or community assembly (Tomberlin et al. 2011b). However, this idea has not been tested vigorously in replicated field or laboratory studies, and even some of them were having pseudoreplication that went unnoticed by researchers (Michaud et al. 2012).

Tomberlin et al. (2011b) discussed the use of quantitative genetics in decreasing error in forensic entomology. Molecular research is well established in species identification of carrion insect, but an understanding of the role of genetics in development and behavior of necrophilous arthropods will help decrease error in forensic entomology. The fundamental principle of quantitative genetic research is that phenotypes can be affected by genetic differences among individual, environment, and/or by interactions between the two (equation: P = G + E + GE) where P is the phenotype, G is the genotype, E is the environment, and GE is the interaction between genotype and environment. The concept of plasticity (an environmental response) in blow fly developmental phenotype is more appreciated in forensic entomology (studies such as species-specific developmental times under laboratory-controlled treatments- see Byrd & Butler (1996)). It is also important to determine population-specific development, as different populations render different observations in developmental rates (Gallagher et al. 2010). It is clear that, even within a relatively small geographical area, there are differences between populations of forensically important flies. Hence, it

is not only important to identify the blow fly species correctly, but also important to know the originating source populations (Tomberlin et al. 2011b).

Ecological genetics is devoted to understand the inheritance of ecologically relevant phenotypes (Conner & Harlt, 2004). Natural variation in developmental rate, life-history traits, phenology, photoperiod sensitivity, stress tolerance, foraging strategy, oviposition preference, mating behaviors, disease resistance, predator avoidance behaviors and many other biological properties affect the survival and /or fitness of an organism (Tomberlin et al. 2011b). Moreover, this variation affects the duration of each phases in the framework as proposed by Tomberlin et al. (2011b). Thus, understanding the underlying causes of natural variation would lead to a greater appreciation of the variation associated with both pre-CI and post-CI. It is possible to incorporate all these variations in a mathematical model when estimating mPMI, when all ecological aspects have been well understood and appreciated through rigorous empirical experimentations.

Forensic entomology

Forensic entomology is a rapidly growing forensic science discipline with significant volume of new literatures has been published in the last decades from North America, Europe and Asia. It has a long documented history first noted in China (1235 AD) by the criminal investigator, Sung Tźu (宋慈) in his medico-legal text book entitled "洗冤集录" published in 1247 and being translated into English as "*The Washing Away of Wrongs*" (McKnight, 1981). Sung Tźu described a murder case by stabbing near a rice field. He then examined the wounds (more than ten injuries) on the deceased and noticed it was caused by a sickle. He also noticed that the victim still had his clothes on and presence of personal belonging, indicated that the case was not a robbery. It must be a murder associated with anger reason. Sung Tźu was then asked the deceased's wife whether her husband had serious conflict recently with any third party. The wife told Sung Tźu that her husband was not in serious conflict with anyone, but there was a man who requested a loan recently but was rejected by her husband. The day after the murder, he called all workers who live nearby to bring their own sickles and he said

whoever failed to bring sickle will be deemed culprit. A total of 70-80 sickles were laid on the ground during a hot afternoon. One of the sickles was attended by blow flies which were attracted to the invisible traces of blood. By further questioning, the owner of the sickle was then confessed to his crime by knocking his head on the ground. Documentation of Sung Tźu represented the first use of insect biology and behavior in solving criminal case. Not only did he include accounts of cases that he involved, but he also described fly behavior on decomposing remains, patterns of invasion at different natural body openings, and different insects' attraction to wounds (Walker, 2014).

According to Benecke (2001), Leclercq and Lambert confirmed that the preference of blow flies to blood and was laying eggs into blood of the deceased in 1976. In 15th century, artwork such as "Dances of the Death" (*Danse Macabre* in French) represented an artistic genre of late-medieval allegory. These paintings were produced to remind people of the fragility of their lives and the emptiness of earthly life. In 16th century, an ivory carving of "Skeleton in the Tumba" was produced. This sculpture depicts a corpse lying inside a coffin with some of its internal organ exposed, and maggots were almost reduced the corpse to skeleton. In this era, decomposing corpses were the common subject among artists during the premodern Europe where the dead bodies were easily obtained for observation. Around 1600, the English poet, William Shakespeare wrote that human "fat themselves for maggots" provided a glimpse into the public understanding of decay in the 17th century England. In fact, popular belief that rotting meat actually produced maggots was prevailed. People at that time made no connection between flies and maggots. This phenomenon was called spontaneous generation (also known as abiogenensis). The disproof of this hypothesis was the foundation in the science of biology, and marked the advent of scientific experiment on forensic entomology. An Italian physician named Francesco Redi in 1668, conducted experiment to collect empirical evidence to discover how and why maggots appeared on rotting meat. Through his experiment, Redi demonstrated that maggots appeared on the objects (a dead fish and a row chunk of veal) in the open jars, on which flies had been able to land, but not in the gauze-covered jars. In other experiment, Redi captured dead

flies and dead maggots and put in sealed jars with dead animals, and no maggot appeared, but when the same thing were repeated for living flies, live maggots were seen. Redi then published his results as *Esperienze Intorno alla Generazione degl'Insetti* and he was careful to express his new views so that it would not become the target by the theological tradition of church. Redi's famous adage was *omne vivum ex vivo*, which means "all life comes from life" (Gottdenker, 1979; Amici, 2001). In 1767, the botanist Carl von Linné made the famous statement that three flies would destroy a horse as fast as a lion would (Müller, 1774), which acknowledged that the flies are indeed great decomposers by taking into account its prodigious reproductive potential of flies. M. Orfila and C. Lesueur published two handbooks on exhumation, where they compiled a list of necrophagous insects presence on the exhumed cadavers (Orfila & Lesueur (1831), as cited in Benecke (2001)). Their work established a relationship between specific insects and stages of decomposition. The first application of forensic entomology in the determination of PMI was given by Dr. Bergeret d'Arbois in France who solved a case of a mummified child behind the mantelpiece at that time. Skeletonized remains of a child were found behind a chimney during building reconstruction. He pointed out that insect evidence was accepted as proof that the current occupants of the building were not the murderers, but the previous tenants (Bergeret, (1855), as cited in Perotti et al. (2009)). Although his results were clearly questionable as he was assuming the development of the adult flies took about a year. Mégnin (1894) were the first to attempt to evaluate the insect succession on corpses and establishing a method to estimate postmortem interval from successional sequences. One of his notable books, *La Faune des Cadavres: Application de Entomologie a la Medicin Legale*, served in large part to make the medical and legal profession aware that entomological evidence could be useful in death investigation. In this book, he expanded his former theory of four insect waves to eight successional waves and for buried corpses, he reported two waves (Perotti et al. 2009).

In the United States, insect association with human remains was studied by Fowler (1888). After that, Motter (1898) listed the insect fauna from 150 grave

disinterments from the City of Washington. He reported pseudoscorpions, Thysanura, Coleoptera, Diptera, Homoptera, earthworm, Acari, Araneidae, Myriapoda, Hymenoptera, Gastropoda, Crustacea, Psocoptera, and Isoptera. Since then, prominent publications related to the taxonomy of forensic importance dipterans were published mainly in the North America. Aldrich (1916) published a monograph on the Sarcophagidae and uses the distinctive male genitalia as identification tool. Knipling (1936) who provided descriptions and keys to the common maggots of flesh flies. Hall's 1948 monograph which is entitled *The Blowflies of North America*, made possible the accurate identification of adults and immature of most species in the family Calliphoridae. Presently, the field is recognized as having three sub-divisions namely urban entomology, stored products entomology and medicolegal entomology. The latter field relates primarily to the determination of the time of death (minimum post mortem interval or m-PMI) which inextricably linked with the broader scientific discipline of medical entomology, taxonomy and forensic pathology (Catts & Haskell, 1990).

The forensically important flies and related arthropods in North America are fairly well understood by entomologists. However, recent introduction of invasive species may affect the ecology of native blow flies and is speculated to affect medicocriminal cases (Baumgartner, 1986). Currently, four species of calliphorids from the Old World have been found in America continent (Baumgartner & Greenberg, 1984; Gagné, 1981; Greenberg, 1988) namely *C. rufifacies*, *Chrysomya albiceps* (Wiedemann), *C. megacephala* and *Chrysomya putoria* (Wiedemann). The effect of these invasive species on the succession of indigenous North American blow flies on carrion is not known and will require detailed study (Wells & Greenberg, 1992). To date, the maggots of these invasive species have been collected from forensic cases in the southern U.S. (Hall & Haskell, 1995). One of the interesting ecological questions is how these invasive species can establish themselves in a new environment, how do they diminish interspecies competition with the natives, and what factors allow them to co-exist in a similar guild.

Another sub-discipline called veterinary forensic entomology which examined the time and cause of death of wildlife has also developed after the utilization of forensic entomology on human corpses (Anderson, 1999; Watson & Carlton, 2003; Anderson & Huitson, 2004). Tomberlin & Sanford (2012) contributed a comprehensive book chapter on the use of forensic entomology in animal abuse and wildlife death investigations. Sanford (2015) presented several cases where pets can be trapped and die inside dwelling with their decomposing owners, and they both were colonized by similar insect species. The results highlighted that there was potential contamination of insect specimens between human corpse and the pet. Hence, a reliable molecular technique should be developed to differentiate the origin of insect specimen in such cases.

Entomotoxicology is a branch of forensic entomology, mainly deals with the cause of death and the effects of drugs on the rate of development on developing fly larvae either increase or decrease the development time (Pounder, 1991; Goff & Lord, 1994; Introna et al. 2001, Rashid et al. 2008; Gosselin et al. 2011). Apart from that, many case reports pertaining human decomposition and determination of mPMI using developing larvae have been published in the literatures from many countries including the United States (Goff, 1992; Catts & Goff, 1992; Nolte et al. 1992). Since methamphetamine (MA) is becoming a common illegal recreational drug, a GC-MS method for the detection of MA in *Calliphora vomitoria* L. was developed and validated. Results showed that MA produced a significant increase in the developmental time from egg to adult, increase mortality rate of pupa and the average length of larvae and pupae were significantly larger than control (Magni et al. 2014). Despite of MA, the effect of methylphenidate hydrochloride, phenobarbital, and methylphenidate hydrochloride associated with phenobarbital have been evaluated on three species of *Chrysomya* in Brazil. The emergence interval was similar among all experimental groups, but larval and pupal viabilities were affected in different ways (Rezende et al. 2014).

Household products were recently found to have in vitro effects on Calliphoridae larvae development. Aubernon et al. (2015) examined several common household products such as bleach, perfume, hydrochloric acid, caustic soda, insecticide, mosquito

repellent, and gasoline. The results showed hydrochloric acid, insecticide, and gasoline killed all larvae. On the other side, bleach and perfume did not affect the survival rate and developmental time of *L. sericata*.

Cuticular hydrocarbons analyses have demonstrated its potential uses in the determination of larval age and hence the postmortem interval for a cadaver. Moore et al. (2013) found there were distinguish features within the hydrocarbon profile over the period of the larvae life cycle, with significant chemical changes occurring from younger larvae to postfeeding larvae. Pechal et al. (2014c) identified unique hydrocarbon profiles for two adult Calliphoridae species viz. *Co. macellaria* and *C. rufifacies*. The results showed that hydrocarbon profiles shifted as adults aged for both species, hence, adult flies found at death scenes could be used to improve mPMI estimate.

GC-MS analysis of cuticular lipids in new and old insect puparia has been demonstrated as a potential approach to estimate postmortem interval. The change in puparia lipid composition over time could potentially provide new indices for estimating time of death (Frere et al. 2014).

Recently, with the publication of "*Forensic Entomology: International Dimensions and Frontiers*" by Tomberlin & Benbow (2015), the field of forensic entomology has advanced into more accurate and precise experimental designs, employing computational modeling with powerful statistical tools, sophisticated molecular techniques (i.e., Next Generation Sequencing), application of microbiology, soil chemistry, engineering behavioral and community ecology in the practice and research of forensic entomology. Most importantly, the emphasis of the fundamental concepts of ecology in the application of insects in forensics has been highlighted and will be influential in determining the future direction of this discipline.

Forensic acarology

In 1878, Brouarde described a case of a mummified newborn child that was inhabited by several arthropods, including a butterfly larvae and acari. Monsier Perier then identified the butterfly larvae as *Aglossa* (Lepidoptera: Pyralidae). Mégnin reported

that the whole body of the mummified child was covered with a brownish layer composed of mite skins and mite feces. Inside the cranium, he found large numbers of a single mite species. Mégnin calculated that on the whole body, 2.4 million acari were counted either dead or alive. He then conducted further calculation and estimated after 15 days, the first generation with 10 females and five males had developed; after 30 days, 100 females and 50 males; after 45 days, 1000 females and 500 males. Eventually, after 90 days, the population of mites achieved 1 million females and 500,000 males were present. Based on his calculation, Mégnin made a conservative guess and reported that the corpse must have been abandoned for at least 5 months but most likely 7-8 months. Mégnin stated this case as his "*premiere étude medico-légale*" or his first medico-legal study (Brouardel (1879) as cited in Benecke (2001)).

More than a century ago, Antonio Berlese (1863-1927), an Italian entomologist, invented a device to isolate microfauna from soil and leaf litter, which laid the foundation for the comprehensive recognition of the coprophilous and necrophilous mite fauna. He described most Mesostigmata species associated with ephemeral habitats and has emphasized on members of the Macrochelidae family (Perotti & Braig, 2009). Some of the dung-visiting insects, especially flies, also visit carcasses, and due to this reason, some phoretic mites have been reported from dung and carcasses (Berlese (1918), as cited in Perotti & Braig (2009)).

Phoresy, phoresis or phoresia, are terms describing a phenomenon where one animal uses another animal as transportation. The interaction between the carrier and the phoront should be temporary and it normally ends when the phoront lands in a new habitat by detaching itself from the carrier (Perotti & Braig, 2009).

During carrion decomposition, dipterans may be the first invertebrate scavengers to arrive on the carcass and lay their eggs thereafter. The mites then detach from their carrier flies and subsequently feed on the fly eggs, other arthropods as well as microbes present on the decaying body. Since the mites breed faster than their fly carriers, they can provide vital information about the time of colonization of the carcass. Hence,

identification of specific phoront can confirm the presence of its carrier (Perotti & Braig, 2009).

One of the uprising fields worth to mention in the context of forensic entomology is the advent of forensic acarology, where mites could be useful in forensic investigations. By identifying species of mites associated with decomposing remains and understanding its biology and ecology, it is possible to determine the m-PMI and the primary location of the corpse. Rasmy (2007) indicated forensic acarology could be potentially a new area in criminal investigation. The first case of forensic acarology was mentioned by Mégnin in 1878 (Perotti, 2009). Then, Goff (1991) used the mites associated with human remains to establish PMIs in homicide cases on the island of Oahu, Hawaii. Since then, species of mites have been identified that have potential forensic uses (Desch, 2009; Perotti et al. 2009; Turner, 2009; Solarz, 2009; OConnor, 2009a; Perotti et al. 2010; Silahuddin et al. 2015). Recently, *Proctolaelaps euserratus* (Mesostigmata: Melicharidae) has been found associated with animal and human decomposition, and considered as a new potential marker for later stage of decomposition, namely butyric fermentation and dry decomposition (Mašán et al. 2013). Mites from the suborders Mesostigmata, Prostigmata, Astigmata and Oribatida were recovered from decomposing vertebrate remains and could be useful to provide indicators for forensic scientists in determining mPMI or the primary location of death (Silahuddin et al. 2015). The presence of larval and nymphal stages of ticks (Ixodida) on insects of forensic importance have been recently documented by Saloña-Bordas et al. (2015) where the authors found *Ixodes ricinus* (Acari: Ixodidae) on three beetle species belonging to families Silphidae and Geotrupidae collected from Spain and England. This record represents the first time that phoresy of ticks on forensic beetles was recorded. In Malaysia, *Macrocheles scutatiformis* (Mesostigmata: Macrochelidae) has been recently recorded from monkey (*Macaca fasicularis*Raffles) and rabbit (*Oryctolagus cuniculus* (L.)) carcasses in a secondary forest (Hanifah et al. 2015).

OConnor et al. (2015) collected mite specimens from a human corpse from Texas and identified *Myianoetus muscarum* (L.) (Astigmatina: Histiostomatidae). This genus

are biologically associated with dipterans and are known to inhabit decaying organic materials such as manure (Scheucher, 1957), carrion (Russell et al. 2004), guano (Willmann, 1937), halophilic vegetation (Fain, 1976) and the nest of vertebrates and insects (Scheucher, 1957). Because these mites can be associated with human remains, and thus is of forensic importance. Accurate identification of these mites could be useful to provide information in the future (OConnor et al. 2015). Several genera in the family Histiostomatidae are specialist in vertebrate carrion and are known to occur on human remains (OConnor, 2009a). The genera *Spinanoetus* Scheucher, *Pelzneria* Scheucher, and *Peripatetes* Mahunka, are associated with beetles of the family Silphidae and Staphylinidae (Scheucher, 1957; Mahunka, 1976; OConnor, 2009a), while *Myianoetus* is associated with flies of numerous families. One species of *Myianoetus*, identified as *Myianoetus diadematus* Willman, was found in large number on an old remain of a child in the basement of a home in Germany (Russell et al. 2004). Similarly, Pimsler et al. (2016) reported *Myianoeus muscarum* (L.) (Acariformes: Histiostomatidae) was associated with the muscid fly, *Synthesiomyia nudiseta* (van de Wulp) (Diptera: Muscidae) during three indoor medicolegal forensic entomology cases in Texas, USA.

Forensic microbiology

Bacteria fingerprinting has been used in forensic investigations as there are many crimes have occurred through biological warfare (bioweapons) such as the use of plant toxin (ricin), bacteria (anthrax and bulbonic plague) and viruses (small pox, measles). There are approximately 85-90% of the bacteria genome is composed of non-repetitive DNA, and mostly is protein-coding DNA. Therefore, different bacterial genomic region from highly conserved regions, such as the small subunit ribosomal RNA genes (16S rRNA), offer potential sites for accurate species analysis. The 16S rRNA is present in all bacteria and contains conserved region that allow identification to the bacterial phylum by using a universal primer during PCR. Although this region can offer species identification based on the available database, however, not all existing bacteria are

represented. Hence, some comparisons only infer phylogenetic relationship but not the actual identification (Crippen & Singh, 2015).

Recent developments in molecular technologies have allowed greater chances in identifying microbial species and increase the efficacy of forensic investigations. For example, the anthrax causative agents, *Bacillus anthracis*, were mailed to the victims and had caused several fatalities to whoever contacted the mails. Forensic microbiologists used DNA sequencing to determine the strain of the bacteria and eventually pin-point the personnel who had access to the source of that particular strain of pathogen. The genetic evidence obtained from this bacterium helped the investigators to narrow down the list of suspects to the individual who had actually committed the crimes (Crippen & Singh, 2015).

During human corpse or carrion decomposition, bacteria or microbes on the body produce microbial volatile organic compounds (MVOCs) through their normal metabolisms (Tomberlin et al. 2012). On the other hand, arthropods are known to respond to MVOCs, either by attraction or repellence (arthropods use MVOCs to access resource quality) (Ma et al. 2012; Davis et al. 2013). Due to the facts that arthropod colonizing a corpse/carrion is an important factor in determining the mPMI (Tomberlin et al. 2012), thus the identification of the MVOCs and its successional sequence released during a carrion decomposition can serve as an indicator for mPMI. This MVOCs succession can be a novel method in forensic investigation when the insect data are not available.

Besides, forensic microbiologists are exploring the possibility to link an individual to any object after it is been touched (Fierer et al. 2005). A study was done to link residual skin bacterial communities collected from computer keyboards to the individuals who touched the keys by comparing the bacterial communities from pyrosequencing analyses of the bacteria swabbed from the keys and the fingertips (Fierer et al. 2010). The results showed promising results as a potential tool in forensic investigations where the individual's fingertips and the keys they touched shared similar

bacterial communities and have distinctive bacterial profiles compared to other individuals.

Microbial geo-signatures have been explored to determine the geographic origin of the host by looking at the specific marker of microbial DNA (Crippen & Singh, 2015). A study compared the gut microbiota from human subjects from Venezuela, Malawi and the United States demonstrated differences in the gut microbial ecology due to age and geography/cultural tradition of the hosts (Yatsunenko et al. 2012).

Another useful application of forensic microbiology is source tracking. For example, *Escherichia coli*, was used to track the source of environmental pollution of waterways (Stoeckel et al. 2004). Outbreak of parasitic diseases such as cryptosporidiosis and giardiasis have been identified using molecular biology to pinpoint the genotype, subtype and also the source of infection, the food handlers (Quiroz et al. 2000; Cacciò et al. 2005).

Furthermore, DNA barcoding have been used to establish the herbivorous insect diet and interactions with plant (Jurado-Rivera et al. 2009). In forensic entomology investigation, blood pool isolated from blood-sucking lice can be analyzed for human DNA and link the relationship of two human individuals (Lord et al. 1998). Similarly, human flesh consumed by the maggots can be analyzed genetically for possible human identification (Wells et al. 2001; Campobasso et al. 2005; Wells & Skaro, 2014). Mosquito blood meals can be used to determine on which host that these mosquitoes fed, as these studies are epidemiologically important in the surveillance of vector-borne diseases on humans or animals (Mukabana et al. 2002; Ngo & Kramer, 2003). Similarly, blood-fed Chagas disease vectors (Triatoma bugs; Hemiptera: Reduviidae) have been studied for the blood source (Pizarro & Stevens, 2008) and host DNA have been isolated from ticks (Kirstein & Gray, 1996).

In term of biological conservation and biodiversity, leeches have recently been promoted as an indirect source of DNA from terrestrial mammal species (Schnell et al. 2012). Additionally, carrion flies-derived DNA analysis may also serve as a novel tool for mammalian diversity survey. DNA extracted from 201 carrion flies collected in

tropical habitats of Cote d'Ivoire and Madagascar for mammal DNA using multiple PCR retrieved 26 species of mammals inhabiting the distinct forest strata in both countries and displaying a broad range of body sizes (Calvignac-Spencer et al. 2013).

Very little is known about the microbiology of grave. However, microorganisms are known to play a vital role in the decomposition of organic matter. Janaway (1996) described the succession from predominant aerobic to anaerobic bacterial community along the cadaver decomposition. Since then, not much literature has been published on microbial activity during carrion decomposition. Pechal et al. (2013) studied the structure and functional activity of epinecrotic microbial communities associated with carrion. The result showed the microbial functional activity increased through decomposition in spring, summer and winter while it decreased in autumn. Furthermore, four major phyla associated with the carcasses throughout decomposition have been identified through 454-pyrosequencing which include Proteobacteria, Firmicutes, Actinobacteria and Bacteriodetes.

Pechal et al. (2014a) proposed the potential use of bacterial community succession by high-throughput metagenomics sequencing in estimation of minimum postmortem interval. The results showed different phyla and families of bacterial communities dominated the pig carcasses at different day of decomposition. For instance, Moraxellaceae was dominant on Day 0, it was succeeded by Enterobacteriaceae on Day 1, and Aerococcaceae on Day 2 and Planococcaceae on Day 3.Generally, there was negative relationship between phylum / family richness and time of decomposition. Besides, Pechal et al. (2014a) suggested a working framework for utilizing bacterial communities to estimate physiological time (h°C). Decomposition also changed soil bacterial profiles as indicated in Olakanye et al. (2014). The results demonstrated that bacterial diversity and richness changed during pig decomposition. There was temporal and spatial community shift relative to buried material as well as taphonomic and environment parameters. In addition to that, the authors also suggest that denaturing gradient gel electrophoresis (DGGE) can potentially become a useful forensic tool for clandestine grave location.

Recently, Carter et al. (2015) demonstrated that there was seasonal variation of postmortem microbial communities associated with swine carcasses where soil microbial was different in summer and winter. As such, observations in winter might not be applicable in summer. Finley et al. (2015a) provide a comprehensive review on the potential applications of soil microbial ecology and next-generation sequencing in criminal investigations. Framework for the classification of necrobiomic microorganisms to estimate postmortem microbial clocks was proposed and several metagenomics molecular approaches have been highlighted to be employed in the study of forensic soil microbiology.

Damann et al. (2015) studied the bacterial community succession in decaying human bone for estimating postmortem interval and the results indicated partially skeletonized remains maintained a presence of bacteria associated with human gut, whereas bacterial composition of dry skeletal remains maintained a community profile similar to soil communities. The authors suggested community membership (unweighted) may be better for estimating PMI from skeletonized remains than community structure (weighted).

Recently, carrion microbiome studies utilizing next-generation sequencing (NGS) techniques have been applied to characterize complex microbial communities by providing microbial taxonomy via phylogenetic placement and at the same time, estimating taxon's relative abundance in the community. Metcalf et al. (2013) employed both 16S and 18S rRNA amplicon deep sequencing to characterize the postmortem microbial community changes in the abdominal cavity, soil, and on the skin in a mouse model system, The authors discovered drastic, quantifiable, and repeated changes in both bacterial and microbial eukaryotic communities at each site during decomposition. Similar to Pechal et al. (2014a), Metcalf et al. (2013) demonstrated microbial succession on ephemeral resources. Also, Metcalf et al. (2013) provided the first NGS-based characterization of microbial eukaryote community change during decomposition. Similar to bacterial communities, the microbial eukaryote community changed significantly during decomposition and consistently over time. The Rhabditidae

nematode, *Oscheius tipulae* Lam & Webster, dominated the community during the advanced decay stages of decomposition. The nematode population increase was likely a response to the proliferation of bacteria communities associated with the carrion (Benninger et al. 2008; Carter et al. 2008). These findings indicated that the trophic interactions should be taken into account when studying the ecology of decomposition.

Researchers started to question whether carcass mass is a variable in affecting microbial succession in soil during decomposition. Weiss et al. (2015) investigated the effect of carcass mass (i.e., 1, 20, 40 and 50 kg) on the soil microbial communities by utilizing bacterial (16S) and eukaryotic (18S) rRNA genes in soil samples. The results showed that time of decomposition was a significant factor on the microbial community, but carcass mass was not. There was significant increase in alpha diversity for carcasses of differing mass in pre-carcass rupture (Day 0-6 postmortem) versus post-carcass rupture (Day 9-15 postmortem) microbial communities.

Application of insect succession in determining the mPMI

The odor emitting from corpse changes in intensity and composition as the body decomposes, becoming more attractive to certain species and less attractive to others as time progresses. Although blow flies arrive very soon after death, they are no longer attracted when remains have passed a particular stage of decomposition, or become mummified or dry (Nuorteva, 1977). For example, *C. vicina* preferred decomposed remains to fresh when given a choice (Erzinclioglu, 1996). In England, *C. vicina* and *Lucilia caesar* (L.) appeared on rodent remains within hours or minutes of death. *Lucilia illustris* (Meigen) was not attracted to corpses in woodland until 76 hours after death, and to corpse in open grassland until 48 hours after death (Lane, 1975).

Phormia regina is often reported to arrive later on remains than other blow flies, being attracted a day or two after death (Denno and Cothran, 1976). In Missouri, experiments showed that only a few of *P. regina* were collected in the first day but many more were collected on carcasses between 24 to 28 hours old, and significantly increased in collection from 48 to 72 hours old (Hall & Doisy, 1993). In contrast, *P. regina* adults

were collected from the remains immediately after death, albeit no eggs were collected until two days later (Anderson & VanLaerhoven, 1996). This phenomenon is explained by Erzinclioglu (1996) that in some cases, adults are attracted to remains immediately perhaps to obtain the protein meal required for ovary and testes development. *Cochliomyia macellaria* is also reported as a late comer and has been attracted to remains aged 18-48 hours after death (Hall & Doisy, 1993). However, in South Carolina and Georgia, USA, *C. macellaria* is the primary colonizer of carrion during the summer (Tomberlin et al. 1998; Tomberlin et al. 2005).

The species involved in the sequential colonization of the remains and their times of arrival will vary from region to region. In tropical region such as Hawaii, the first colonizer were the calliphorids *L. cuprina, C. megacephala*, and *C. rufifacies*, then followed by sarcophagids such as *Sarcophaga haemorrhoidalis, Parasarcophaga ruficornis, Sarcophaga occidua* and *Helicoba morionella,* although individual species varied with region (Early & Goff, 1986). In contrast, the first colonizer in Tennessee were *Lucilia coeruleiviridis* (Macquart) and *P. regina* (Reed, 1958), while in South Carolina, the first colonizer was *C. macellaria* (Payne, 1965). Time of colonization of insect species and group also vary significantly with geographical region. In many areas, dermestid beetles are considered to be late colonizer, frequently arriving when only skin and bone remains, sometimes months after death (Smith, 1986). In Hawaii, some adult dermestids were collected as early as 3 to 10 days after death (Early & Goff, 1986). In Canada, dermestid larvae were first collected from pig carrion in exposed pasture after 21 days after death.

There are two basic ways to estimate PMI using entomological data. The first method is based on the time period needed for each represented species to develop to the growth form collected at the death scene. Most of the specimens are maggots, primarily consist of blow flies and flesh flies. Those specimens showing the oldest stage on corpse are assumed to manifest the PMI provided the corpse was exposed and conditions were suitable for insect activity following death. The second basic approach to determine PMI

is based on the composition of the arthropod community as it relates to expected successional patterns (Catts & Goff, 1992).

Mass mortality events (MMEs)

Death is a ubiquitous demographic process and the final biological destiny for all living organisms. Many factors can lead to mortality on daily, seasonal and annual basis and these factors include resource limitation, stochastic events, exceeding physiological threshold, senescence, and interactions with predators, pathogens, and parasitoids (Fey et al. 2015). Although all organisms eventually die, the timing and magnitude of death within a population varies greatly. Mass mortality events (MMEs) represent demographic catastrophes that can instantaneously affect all life stages (Lande, 1993) and can rapidly eliminate a substantial proportion of a population over a short duration (Reed et al. 2003).

MME of varying spatial and temporal scales influence biological communities in the present day and are often a natural phenomenon (Stokstad, 2014; La & Cooke, 2011). For instance, background mortality levels of sea stars are occasionally punctuated with MMEs driven by outbreaks of wasting syndrome (Stokstad, 2014), which have led to rapid population losses in species such as purple sea stars, *Pisaster ochraceus* (Brandt), along both coasts of North America. In another example, during fall and spring migrations, the majority of the North American populations of Eared Grebes (*Podiceps nigricollis* Brehm) pass through the Salton Sea, California, USA *en route* to or from wintering areas in the Gulf of California. Tens or hundreds of thousands also winter at the Salton Sea (Jehl, 1988), where maximum daily counts have exceeded one million individuals. The most persistence causes of mortality are adverse weather during migration and disease (Jehl, 1996). MMEs such as these may trigger local extinction by reducing population levels at which loss of genetic diversity (bottleneck effect), demographic stochasticity, or Allee effects (decline in individual fitness at low population density) can drive population to extinction (Lande, 1993). Population loss through MMEs can alter the structure of food webs by abruptly generating resource

pulses, removing predators or competitors (Ostfeld & Keesing, 2000b), or disturbing mutualist interactions (Thébault & Fontaine, 2010). Furthermore, MMEs can cause severe economic costs as well as disrupt ecosystem services such as pollination (Potts et al. 2010).

Fey et al. (2015) examined 727 published MMEs from across the globe, affecting 2407 animal populations. The results showed that the magnitude of MMEs has been intensifying for birds, fishes, and marine invertebrates; invariant for mammals; and decreasing for reptiles and amphibians. They found that the increase in MMEs appears to be associated with a rise in disease emergence, biotoxicity, starvation, and events produced by multiple interacting stressors.

From the perspective of carrion ecology, what are the impacts of MMEs to ecosystem? First, the sudden influx of overwhelmingly ephemeral resources could induce environmental toxicity and pollution (DeVault et al. 2003), which will then kill off the adjacent inhabitants (e.g., plant and soil arthropod communities) at the affected location of MMEs. Also, the potential leaching of harmful N and S compounds from animal carcasses to ground water is another environmental hazards (Kalbasi et al. 2005). Second, there is possibility that the death site serve as the source of a disease outbreak (either epidemic or epizootic) as pathogens proliferation on carrion masses are greater than usual (Kalbasi et al. 2005). Since there is a carrying capacity (*k*) or density-dependent factors that limits the population and distribution of necrophagous communities at that particular location of MMEs, it is hypothesized that the rate of carrion decomposition could be delayed due to the insufficient quantity of decomposers and scavengers *in-situ* to provide their eco-services to the affected area.

When the carrion decomposition process is delayed, what are the consequences that might occur to the ecosystem? I believe that delayed carrion decomposition is an ecological perturbation unique to certain spatial and temporal scales. In the present study, I aim to examine several variables (i.e., microbes, arthropods, soil chemistry) associated with delayed carrion decomposition for an extended period similar to the event of mass mortality. To date, little research has been done on the context of delayed

carrion decomposition (Pechal et al. 2012; 2013, 2014a, 2014b) and we have no complete understanding about the ecosystem sensitivity and resilience following MMEs in natural system. The present work could provide the foundation in understanding the impacts of MMEs to the environment (i.e., delayed of carrion decomposition process for an extended period), and the responses of ecosystem (resistance and resilience) towards this perturbation.

Carrion ecology

Detritus is defined as any source of nonliving organic matter (Swift et al. 1979). It is an important component of recycling energy and nutrients in ecosystem (Swift et al. 1979; Barton et al. 2013a; 2013b). Thus far, most of the detrital pool has been derived from phototrophic organic sources such as leave litter, grass, or decaying algae. Many studies continue to provide evidences that litter decomposition is fundamentally important in regulating ecosystem processes such as nutrient and energy cycling, community interactions, and food web network stability and resilience (Hawlena et al. 2012). However, there has been very little research focused on heterotrophically derived component of the necromass compared to phototrophically origin detritus (Benbow et al. 2015b).

Carrion (Anglo-French *carogne*;Vulgar Latin *caronia*) has historically been defined as dead and decaying flesh or as a carcass of an animal and has been widely considered as vertebrate animals (Benbow et al. 2015b); although, carrion can also be carcasses of microscopic eukaryotes (e.g., rotifer, cladocerans, nematodes), and macroinvertebrates (e.g., insects, crabs, and cephalopods) (Beaver, 1973, 1977; Hawlena et al. 2012; Yang et al. 2008). As previously mentioned, the importance of carrion to the detrital energy and nutrient foundation in ecosystem has long been regarded as insignificant compared to biomass generated from plant litter; however, recent research has demonstrated that carrion can be significantly important to ecosystem processes often through indirect interactions among several trophic groups (Towne, 2000; Yang et al. 2008; Parmenter & MacMahon, 2009, Barton et al. 2013a).

MMEs do impact ecosystems by giving a huge amount of nutrient influx into the surrounding soil or marine ecosystem, either locally or globally. Examples of mass mortality events such as the effects of mass cicada emergence (Yang, 2004), mass salmon die-offs (Tiegs et al. 2009) or whale falls (Smith & Baco, 2003). Other than this direct effect, Hawlena et al. (2012) discovered a pathway how terrestrial predators regulate ecosystem processes via indirect control over soil community function. Grasshopper (Orthoptera) herbivores stressed by spider predators have a higher body C:N (non-consumptive effects) than do grasshoppers raised without spiders. This change in elemental content does not slow down grasshopper decomposition, but perturb, belowground community function by decelerating the subsequent decomposition of plant litter. This legacy effect of predation on soil community function appears to be regulated by the amount of herbivores protein entering the soil.

Benbow et al. (2015b) compiled excellent chapters on carrion ecology. Their efforts also represent the first book of its kind on this subject. The topics ranged from ecological mechanisms of carrion decomposition, evolutionary ecology to application of carrion ecology. Recent technological advances such as high-throughput metagenomic sequencing, unpiloted drones to high-resolution satellite remote imaging have opened many new opportunities to study the ecology and evolution of organisms, including interkingdom communication via semiochemicals. Carrion ecology is getting more attention among ecologists, entomologists and biologists, and there is increasing quantity in literatures related to carrion decomposition studies globally (Tomberlin & Benbow, 2015). The future of carrion ecology is promising. With the advent of the latest technological advancements, it is now possible to explore new and historically understudied natural systems, in such a way that will most surely uncover new genomes, species and ways how organisms interact (Benbow et al. 2015b).

Current concepts in carrion ecology

Carrion is a valuable resource to an ecosystem (Yang, 2004). The carrion provides ephemeral or transient microhabitats and food source for various organisms

ranging from microorganism to immature stages of arthropods. The purging fluid from carrion that seeped into the soil will also provide amino acid rich nutrient and subsequently fertilize the soil through a series of nutrient recycling process (Carter et al. 2007).

Ecosystems are dynamics and can be quite stochastic. Plants grow and die, animal feed on plants and on one another, and decomposers recycle the chemical elements that make up the biotic portion of any ecosystem. Abiotic factors (temperature, rainfall, sunlight, seasonality) also have a major influence on the kind of community that will establish. Over an extended period, it is possible to see trends in the way of a community changes and to recognize that climate greatly influences the kind community that becomes established in an area. The concept that communities proceed through a series of recognizable, predictable changes in structure over time is called succession. The relatively stable, long-lasting community that is the result of succession is called a climax community (Enger & Smith, 2015). In order to understand the concept and scope in this study, several ecological terms need to be defined precisely:

Perturbation, disturbance, resistance, and persistence

Perturbation is defined as the response of an ecosystem to a disturbance. It can be further characterized by direction, magnitude and persistence (Odum et al, 1979), while "disturbance" is a cause for the perturbation (which includes stress). It can be further categorized as destruction, discomposition, interference and suppression (Bazzaz, 1983).

There are many perturbations to natural systems (Pimm, 1984). Some perturbations may involve changes in species abundances (e.g., winter which temporarily depressed bird populations) (Strong et al. 1984); others may involve the removal of some or all species (e.g., secondary plant succession) and eventually lead to longer recovery duration. Obviously, the definition of resilience, persistence, and resistance give rise to problems of scale when we try to measure them in the field (Connell & Sousa, 1983). It is apparent that large perturbations will disappear slower than the small ones. The recovery time following perturbation may be relatively

unrelated to the size of perturbation, but also other factors such as resistance (Pimm, 1984). Persistence, which is harder to deal with, will not only depend on the system, but also on the properties of the disturbances, or vice versa. It is observable that long-lived organisms (e.g., trees) will be less resilient and more persistence in numbers than short-lived annual plants on a time scale measured in years (Connell & Sousa, 1983). Both resilience and resistance maybe the answers to why some systems are dominated by organisms with short or long life histories (Pimm, 1984; Burke & Laurenroth, 2011).

Perturbations also need to be defined spatially. Disturbing 1 m^2 is not merely a smaller perturbation than disturbing 1 km^2. In the latter, the boundary is smaller compared to the total area. Perhaps in this situation, immigration and emigration are most likely to be less significant than birth and death process (Pimm, 1984).

Persistence, as defined by Pimm (1984), is the time a variable lasts before it is changed to a new value. Turnover is the reciprocal of persistence. Resistance is defined as the degree to which a variable is changed, following perturbation (Pimm, 1984).

Stability and resilience

Stability refers to a steady state or a stable point and they are the subjects of disturbance and can potentially be permanently altered or destroyed by a disturbance (Rykiel, 1985). A system is deemed stable if and only if variables measured return to the initial equilibrium following perturbation. A system is locally stable if this return is known to apply only for small perturbation and globally stable if the system returns from all possible perturbations (Pimm, 1984).

Resilience is defined as the capacity of an ecosystem to absorb disturbance without shifting to an alternative state and losing function and services (Holling, 1973). The process therefore encompasses two separate processes: “resistance”, which is the magnitude of disturbance that causes a change in structure and “recovery”, the speed of return to the original structure (Holling, 1996a; Tilman & Downing, 1994).

Resilience can also be defined as the speed with which a system returns to equilibrium state following a perturbation (DeAngelis, 1980). A number of different

meanings have been attached to the term "stability". The most common interpretation is that a system is stable when it tends to return to an equilibrium point from which it has been displaced. Another closely related concept, relative stability, is a measure of both the resistance of the system to perturbations and the speed with which it returns to an equilibrium point following a perturbation. This property has been referred to as system "resilience" (Webster et al. 1975). The faster the disturbed system returns from its initial displacement back to the equilibrium point, the shorter its recovery time, T_R, and the greater its resilience is. Therefore, $1/T_R$ is taken as a measure of system resilience (DeAngelis, 1980). O'Neill (1976) noted that T_R decreased (i.e., shorter recovery time) as energy input (rate of flow of energy into system via the autotroph compartment) per unit standing crop in the steady state increased. This observation might be expected, for instance, the tundra model had the longest recovery time and hence the lowest resilience. The pond ecosystem, with a relatively low standing crop and high rate of biomass turnover, had the shortest recovery time and hence the highest resilience (DeAngelis, 1980). Odum & Pinkerton (1955) defined "power capacity" for ecological systems as the quantity of energy processed per unit living tissue, and hypothesized that greater power capacity would result in greater capability to counteract change, or greater resilience.

Pimm & Lawton (1977) examined the observation that a food chain is seldom deeper than four or five trophic level. They employed a set of Lotka-Volterra equations to describe the flow of biomass through a variety of species. They found as the number of trophic levels in a chain of species increases, the average recovery time in a chain of species increases. In other words, the resilience decreases, making the system remain away from equilibrium longer following perturbations. Similar result was obtained by DeAngelis et al. (1978) and they pointed out that decreases in recovery time can result when the energy flux through the system is increased.

In nutrient recycling, chemical energy passes through successive trophic levels. It is degraded towards low-quality thermal energy, which is unable to perform useful work. Some nutrients may held very tightly by the system and recycled many times before they are lost as output from the system (DeAngelis, 1980). Pomeroy (1970) highlighted that

coral reefs and rain forests are examples of systems with tight nutrient cycles. When there is disturbance occurs in this system, recovery may be very slow because there is little exchange of nutrients coming from the outside the systems. The factor determining the resilience of food web or trophic model (with no feedback) is the energy or biomass flux per unit standing crop (DeAngelis, 1980).

Neubert & Caswell (1997) published a mathematical model to quantify resilience. According to them, resilience can be assessed as the rate at which perturbations to a stable ecological system decay. Ecological responses to perturbation are characterized quantitatively by stability (does the system return to its original state after perturbation, or it does not?), and quantitatively by resilience, or its reciprocal return time, which measure how rapidly a stable system returns to its original states after a perturbation (Webster et al. 1975; Beddington et al. 1976; Harrison, 1979, DeAngelis 1980, 1992; Pimm, 1982, 1984, 1991). Many ecologists, both theoretical and experimental, have studied what kind of ecosystem characteristics affecting resilience. These include energy flow (O'Neill, 1976), nutrient loads and nutrient cycling (DeAngelis, 1980), environmental stochasticity (Ives, 1995), life history strategies (Lepš et al. 1982), food chain length (Pimm & Lawton, 1977), food web connectance (Pimm, 1979) and connectivity (Armstrong, 1982), herbivory (Lee & Inman, 1975) and omnivory (Pimm & Lawton, 1978, Pimm, 1979). Pimm & Lawton (1979) predicted that resilience should decrease as food chains get longer while DeAngelis et al. (1989) predicted that resilience should increase as the turnover rate of a limiting nutrient increases. These hypotheses were tested by Carpenter et al. (1992), who measured the flow of phosphorus through a lake ecosystem before and after food web manipulation. In 1984, Tuesday Lake in Wisconsin was dominated by planktivorous minnows. Carpenter and his team in 1985 added another trophic level to Tuesday Lake by introducing piscivorous largemouth bass, while removing enough minnows to maintain total fish biomass (Carpenter et al. 1987). As a result, the lake in 1984 was planktivorous-dominated system; while in 1986, the lake had shifted to piscivore-dominated system. The resilience calculated was 0.035 for 1984 and 0.005 for 1986 (seven times larger

resilience in planktivorous system than piscivorous system). The findings in Carpenter et al. (1992) supported the food chain length and nutrient turnover rate hypotheses and concluded that the piscivore-dominated lake is less stable in the long run.

Mechanism of succession and ecosystem stability

Braack (1987) defined succession on carrion as the addition, not replacement, of species to the community present on the carcass. The addition of species arises when new resource becoming available and these resources most often arise as a consequence of the action of one or more of the species members in the community. For instance, the emergence of blow fly larvae at the carcass stimulates the arrival of large numbers of predatory histerid beetles; the feeding activities of the maggots leave a liquid deposit on the carcass which attract moisture-seeking piophilid and other dipterans; the continued feeding efforts of the fly larvae eventually exposed the stomach-content in initially undamaged carcasses which is then utilized by scarabaeidae; the departure of maggots create "empty space" or unoccupied niche for more individuals of certain species such as clerids (Coleopterea: Cleridae) (Braack, 1987).

Physically speaking, the remaining parts of carcasses after the dry stage of decomposition are skin, bones, hooves and horn-sheaths, which will be utilized by dermestid, Trogidae (Coleoptera) and Tineidae (Lepidoptera). The substrate is non-replenishing and is depleted over time in direct proportion to the population of arthropods it supports. According to Braack (1987), carrion therefore represents an ephemeral resource with no steady progression to a stable climax community having a reasonable prospect of long term existence.

Disturbances to ecosystems, due to human activity, date back to preneolithic cultures. In Britain, prehistoric human populations set fires to clear land for agricultural purposes (Smith, 1970). Other disturbances not associated with man are natural fires (e.g., due to dry and hot summer or lighting strike), landslides, severe storms, and other biological activities such as intense grazing (e.g., the bison on North American plains). Within the past several thousand years, much of the forest of North America has been

severely destroyed by fire at least once every few hundred years, within the life span of the dominant conifers (Heinselman & Wright, 1973). These major sources of perturbation are so widespread even before the man intervention became common. After a severe disturbance, there is usually a burst of regeneration that, once established, suppresses later regeneration. Henry & Swan (1974) noted that the white pine trees that got established after catastrophes in the late 17th century dominated the forest for 200 to 250 years thereafter, suppressing all later tree invasion. The existence of dominant, widely spaced age-classes species resulting from such regeneration processes after perturbations is an indication that succession has not yet stopped in an equilibrium assemblage (Connell & Slatyer, 1977).

A system is stable if it persists despite perturbations. In real communities, disturbances are being continually offered to the ecosystem and challenged its stability in the form of variations in physical condition, invasions of competing species, or natural enemies. Margalef (1969) pointed out that ecosystems persist either by: (i) giving way to the perturbation and subsequently recovering to the original state, or by (ii) not giving way at all. He then proposed that these could be called "adjustment or lability" for the first situation and "conservation, endurance, or persistence" for the second scenario. In Margalef (1969) definition on succession, the process of succession represents "adjustment" stability, if all succession on a site led to a similar species composition at equilibrium, as proposed by Clemens (1916), this would be global stability. If different species composition were reached, then the system would have multiple stable points. If a community resists perturbation, there will be no succession since there is no change (Connell & Slatyer, 1977).

Considering the maintenance of species structure is vital during ecosystem recovery from a perturbation. However, discussion on stability of any community should be considered on three scales: the time, the space, and the intensity of perturbation. In other words, to judge stability, we need to decide how long and over what space the present species structure must persist in the face of a given intensity of perturbation (Connell & Slatyer, 1977). Therefore, to be able to judge the degree of stability of the

species composition of a community, two characteristics must be fulfilled: (i) an area large enough to ensure an early succession species persist in the system, (ii) an observation period at least as long as the longest generation time of any of the species and also long enough so that the whole range of kinds and intensities of perturbations will have had a chance to occur.

The importance of scale in ecology

Over the past years, researchers have come to realize that ecology itself is scale-dependent (Levin, 1992; Schneider, 2001). Gotelli et al. (2010) emphasize the importance of this scale-dependency. They gave an example that a process that occurs at small spatial scales, namely competition between individuals, plays a crucial role even at the large scale of an entire country. It is thought that competition is played out at small scale through interactions between individual organisms (e.g., birds in this case). It is difficult to imagine how the interaction between two birds can be influential at large scale. In fact, there is evidence that the role of competition diminished at biome level (Russell et al. 2006). Gotelli et al. (2010) then assembled a dataset on the distribution of birds at the scale of a country (Denmark). They expected that competition would no longer be influential at this scale, and that habitat would be most important in controlling where bird species live (as different types of vegetation defined habitat types). Surprisingly, analyses of data showed that habitat appeared unimportant, but that competition was significant in determining which bird species lived where. Again, this finding approved the conclusion in Diamond (1975) who assumed competition as the primary determinant in the assembly of species communities although his theory has been challenged by many ecologists of his time, for example, by Connor & Simberloff (1979). McGill (2010) pointed out that ecologists need to ask which force(s) is most important at a given scale. He suggests the first step towards identifying scale dependencies of this kind is to collect more data on what controls species distribution and other variables (e.g., richness, abundance) across scales. However, this will lead to many distinct scale diagrams. He pointed several questions about how to rescale

depending on the organism, given that scale of 1 m are unlikely to be the same for bacteria and elephants.

Because there is no single scale at which ecosystems should be described, there is no single scale at which models should be constructed (Levin, 1992). We must find ways to quantify patterns of variability in space and time, to understand how patterns change with scale, and to understand the cause and consequence pattern (Levin, 1989). Cross-scale studies are critical to complement more traditional studies carried out on narrow single scales of space, time, and organizational complexity (Holling, 1996a), just as γ-diversity (species diversity regionally) are needed to complement β-diversity (species diversity among-localities), and β-diversity are needed to complement within-community measures of α-diversity (species diversity locally) (Whittaker, 1960; Whittaker, 1975).

For the present study, it is considered a plot-based ecological study where the study site (Snook, Texas) used in this study is of microscale in spatial (within 1 m^2 to 100 ha) and microscale in temporal (within 1-500 years) according to the standards used in Delcourt & Delcourt (1992).

Species structure in a steady-state equilibrium

Connell & Slatyer (1977) examined this question on two different spatial scales (i.e., smaller and larger spatial scale). For smaller scale, the changes in species composition will depend on whether individuals are more likely to be replaced by a member of their own or another species. The species of replacing individual will depend upon how the condition at the spot had become modified during the previous occupation (whether the condition is favorable for the offspring of the initial species, or become unfavorable for the initial species but favorable for the successive species, or the spot remains neutral for initial species and successive species, with no obvious advantage given any species). For larger spatial scale, it depends on which condition has occurred in smaller spatial scale. As in Horn (1974) stated that, if stability is defined as the absence of species turnovers and population fluctuations, then stability increases

tautologically with succession. According to his definition, succession will stop when the composition of the community is not changing. However, Connell & Slatyer (1977) have found no evidence of a community of sexually reproducing individuals in which it has been demonstrated that the average species composition has reached a steady-state equilibrium. They concluded that in general, succession never stops.

Challenges in carrion ecology

My research represents a developing subfield in ecology, known specifically as carrion ecology. However, there are several concerns that need to be clarified. First, carrion is an ephemeral nutritional resource with no new energy input (therefore not a primary producer or subsequent consumer in trophic cascade). Hence, carrion itself poses a question whether it represents an "ecosystem" by strict definition, as carrion does not fit the description of an ecosystem (i.e., ecosystem shall meet the four primary functions: primary production, consumption, decomposition and abiotic storage) (Lindeman, 1942). Second, although carrion serves as food source for wide variety of consumers including vertebrate scavengers and invertebrate detritivores, can it be placed at the basal location of food chain? A food chain (or web) by definition should be originated from a primary producer (i.e., photosynthetic plants or even chemolithotrophs) (Pimm et al. 1991). Therefore, putting carrion (as a non-primary producer) as the base of a food web could be controversy among food web researcher. Similarly, can scavengers and detritivores represent a trophic level by itself? To answer these questions, Moore et al. (2004) develop an integrative framework for understanding the impact of detritus that emphasize the ontogeny and heterogeneity of detritus and the various ways that explicit inclusion of detrital dynamics alters generalization about the structure and functioning of food webs. They determined detritus increases system stability and persistence, and having substantial effects on trophic structure and biodiversity. Wilson et al. (2011) argued that carrion consumption, or scavenging, is a type of detrital feeding that should have widespread consequences for the structure and stability of food webs. In his review, facultative scavenging is a ubiquitous and

phylogenetically common strategy, and In fact, more energy is transferred per link via scavenging than predation. Third, is carrion a stable ecosystem? If yes, by definition, carrion should be qualified to own properties such as succession, climax, resilience and resistance (Pimm, 1984). But most ecologists struggle with explaining such ecological phenomena (e.g., resilience, or climax community etc.) on carrion (Braack, 1987). According to Pimm (1984), resilience is not defined for an unstable system. If carrion is an unstable system, perhaps the species richness, abundance, connectivity and other observable ecological phenomena (e.g., insect successional sequence) reported from many carrion and forensic entomological studies are merely observations of stochastic events (He & Mladenoff, 1999; Kreyling et al. 2011). If it is a pure stochastic and random event, then did insect succession really occur on carrion? Will Gleason (1917) (who proposed vegetation succession phenomena depend upon the phenomena of the individual plant and is determined by environmental selection) sufficiently explain insect successional phenomena on carrion? Gleason (1917) summarized that the effective changes in the environment may lead to significant changes in the vegetation of an area. If these changes involve the establishment of a new association, the phenomenon is known as succession. If we think from a microscale perspective, carrion is indeed a "changing environment" (i.e., different stages of decomposition) which results in shifts of the insect community structure through ecological separation (Peschke et al. 1987), and this indicates Gleasonian successional model is applicable in explaining insect succession phenomena on carrion although it is not a stable ecosystem, *per se*. Fourth, ecologists use "perturbation" to describe disturbances in ecosystems, such as fire, storm or biological activities (introduction of an invasive species) (Pimm, 1984); and, as a functional ecosystem, there should be responses following perturbation (either recovery or collapse of ecosystem). However, can carrion (as a non-living object) respond to a perturbation? Or shall we emphasize that it is the response strictly by the necrobiome (*sensu* Benbow et al. 2013)? The above questions stated are thus far the main concerns needed to be addressed immediately in order to put carrion, as an chemical energy entity,

in an appropriate context in Ecology so that it can be discussed effectively among the scientific community.

The introduction of carrion into an ecosystem can be considered as a resource pulse (Yang et al., 2008), which is the event of increased resource availability over time combine low frequency, short duration and large magnitude. In my study, introduction of carrions (resource pulses) could serve as a "disturbance" to a stable ecosystem. The carrion will then be consumed by variety of necrophagous guilds and transformed into chemical energy through the trophic cascade. During this process, dynamics of arboreal and underground arthropods, microbes on carcasses and in the soil, soil chemistry response can be quantified by using different levels (e.g., species, population, or community) and indices (e.g., abundances, richness, microbial metabolism function or nutrient concentration) over time of decomposition (day) or Accumulated Degree Hour (ADH). In the experiment design, the control group (pig carrions that allow insect succession) represented the current state of succession, with the time noted when is the community of necrophagous insect reaches maturity (climax community) In this context, we defined the species of arthropod achieve climax community by observing its highest abundance along the decomposition process. As for the treatment groups, insect accession will be inhibited on pig carrions for 7 days and 14 days and then exposed them to the insects accordingly. These treatments are termed interference disturbance, where there is inhibition of energy or matter exchange process (that is delayed insect colonization in this case). It is possible to quantify the perturbation of the ecosystem to these disturbances by looking at the dynamical changes in insect succession, arthropod abundance, microbial function and soil nutrients. The change of climax community (change or loss of stability) will be termed as the shift of climax, which is represented by different hypothetical curves at Figure 1.1. The residual between the control curve and the hypothetical curve will be identified as variation, which indicates the variability of responses in ecosystem towards the stress caused by the disturbance. The variation in perturbation of an ecosystem is critical from the forensic perspective. As forensic entomologist used to estimate mPMI based on the arrival of insect on the corpse. The

variations caused by interference disturbance (e.g., delayed insect colonization on corpse) could result in bias when estimating mPMI based on insect succession. Therefore, it is essential to determine how well the ecosystem response to the stress and how soon the ecosystem can recover from it (resilience). From the application side, these important ecological aspects should be addressed and given recognition in the practice of forensic entomology.

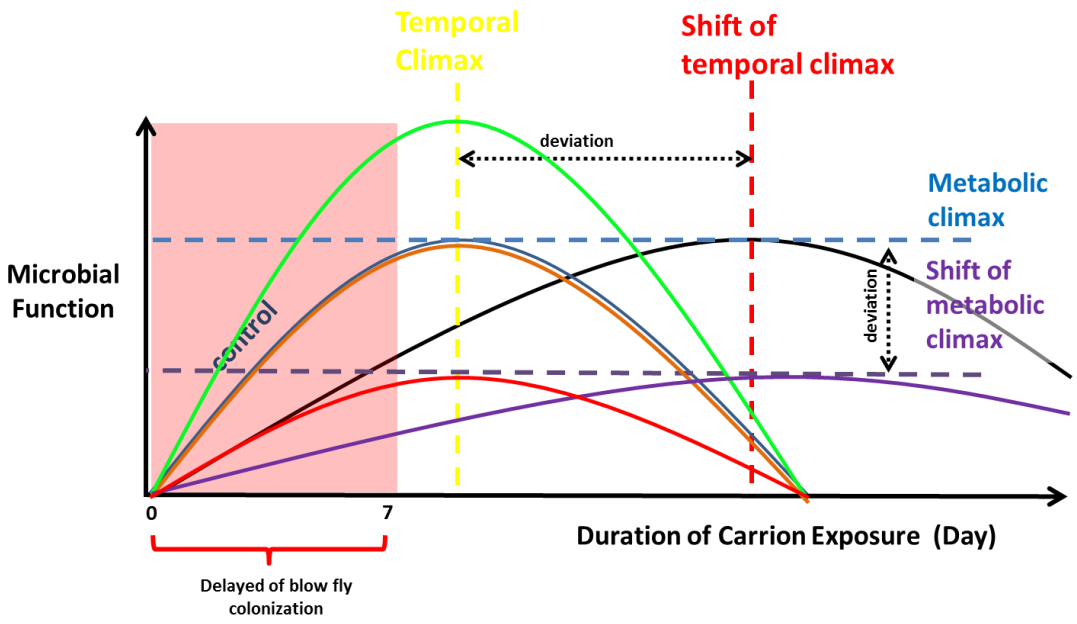

Figure 1.1. Hypothetical perturbation and resilient of insect succession on pig carrion following interference disturbance in an ecosystem.

OBJECTIVES

A review of the literature indicates that a variety of factors play essential roles in the decomposition of vertebrate carrion. In fact, the items discussed most likely do not represent all factors that are important in this process. In fact, the impact of delayed colonization of vertebrate remains by the primary invertebrate consumers on the small

(the resource itself) and large scales (the host environment) has not been studied in detail.

The objectives of this research were to link together the various process (e.g., soil and terrestrial arthropods with soil chemistry and microbiology) to better define the mechanisms impacting the decomposition process of vertebrate carrion. I specifically examined the following factors in response to delayed primary arthropod (i.e., dipteran) colonization of vertebrate remains, with hypotheses stated as below:

(i) Microbial metabolic community profiling of delayed vertebrate decomposition.

Ho: There is no shift in microbial metabolic community profiling in response to delayed vertebrate decomposition

Ha: There is a shift in microbial metabolic community profiling in response to delayed vertebrate decomposition

(ii) Arthropod community structure and function associated with delayed vertebrate decomposition.

Ho: There is no shift in arthropod community structure and function in response to delayed vertebrate decomposition

Ha: There is a shift in arthropod community structure and function in response to delayed vertebrate decomposition

(iii) Soil chemistry dynamics of delayed vertebrate decomposition.

Ho: There is no significant difference in soil chemistry dynamics in response to delayed vertebrate decomposition

Ha: There is significant change in soil chemistry dynamics in response to delayed vertebrate decomposition

(iv) Soil arthropod community structure and function in delayed vertebrate decomposition.

Ho: There is no shift in soil arthropod community structure and function in response to delayed vertebrate decomposition

Ha: There is a shift in soil arthropod community structure and function in response to delayed vertebrate decomposition

A decomposition study with delayed blow fly colonization is vitally important to carrion ecology as insect colonization can be deterred and delayed due to abiotic factors such as extreme weather or storage condition. This research has practical applications in forensic entomology as it may provide additional information to predict minimum time of death. Future directions from this study include modeling of ecological variables (arthropod richness, abundance, microbial function, soil chemistry etc.) in determining ecosystem health from the perspective resistance and resilience in various disturbed ecosystems such as mass mortality events or environmental pollutions.

CHAPTER II

MICROBIAL METABOLIC COMMUNITY PROFILING OF DELAYED VERTEBRATE DECOMPOSITION

INTRODUCTION

Decomposition of organic matter is central to the cycling of energy and matter in all ecosystem (Swift et al. 1979). Carrion, leaf litter and dung are rich organic matter. They have high nutritive value and are often competed for by various scavengers such as insects (Hanski, 1987b). Physiochemical and biotic condition in these microhabitats change rapidly and select for fast exploitation. Dung beetles (Coleoptera: Scarabaeidea) move parts of the dung pat to their underground nest, where larval development is safe from fierce competition with other coleopteran or dipteran larvae, or predation and physical hazards on the soil surface (Halffter & Edmonds, 1982).

Up to 90% of organic matter generated by plants enters the detritus pool (Swift et al. 1979). The green world hypothesis states that terrestrial herbivores consume relatively little plant biomass because the population of herbivores is balanced by many ecological interactions such as predation, parasitism, intra- and intercompetition, as well as restriction of plant nutrients, plant defense and other abiotic factors (Hairston et al. 1960). The 10% consumed by other animals is utilized for growth. And, of that 10%, what is not used is eventually excreted. Regardless, those nutrients bound within the body of the consumer will eventually be returned to the ecosystem when the animal dies, which represents the carrion aspect of nutrient cycling (Schoenly & Reid, 1989). Carrion represents only a small part of the total detritus pool in most ecosystems (Swift et al. 1979).

Carrion is considered a "Cadaver Decomposition Island" which has created a spatially distinct hotspot of biological and chemical activity that affects associated organisms (*sensu* Carter et al. 2007). Environmental variables such as temperatures and humidity strongly influence rates of carrion decay (Carter et al. 2010). Lauber et al.

(2014) determined soil microbial communities have a significant impact on the rate of carrion decomposition. In addition, changes in species diversity within and across trophic levels in association with the carrion source can significantly alter its decomposition (Gessner et al. 2010). Field experiments that varied in the composition of plant litters revealed that lower biodiversity slows the rate of litter decomposition (McLaren, 2014). The dispersal of nutrients away from carrion is largely driven by the activity of arthropod and vertebrate detritivores and scavengers, and their predators (Payne et al. 1968; DeVault et al. 2003). Carrion nutrients are then flowed through belowground pathways by bacteria and fungi, which subsequently break down the large and complex large organic molecules into a simple form which can be reuse by plant, and through the consumption of plants, animal uses these nutrients to make various body tissues, including the predators of herbivores, which nutrients were transferred between trophic cascades (Bornemissza, 1957; Carter et al. 2008). Some loss of energy occurs through the release of carbon dioxide gases from decomposing carrion (Putman, 1978b). The mineralization of key nutrients by microbes, such as nitrogen and phosphorus, make them available for plant uptake (Towne, 2000). Ultimately, all living organisms will turn into carrion pool in all types of ecosystems upon their death. Thus, carrion ecology is the central of a sustainable and functional ecosystem, without an efficient nutrient recycling process; the ecosystem function might be interrupted and possibly collapsed due to insufficient continuous supply of nutrients to the generations of organisms (Barton et al. 2012).

Recycling of carrion nutrients and energy is often facilitated by insect communities (Putnam, 1978, Parmenter & Lamarra, 1991, Carter et al. 2007), and vertebrate scavengers (Parmenter & MacMohan, 2009). In addition, the nutrients introduced by the cadavers is usually associated with the increased of soil microbial biomass, microbial activity (C mineralization) and nematodes abundance (Carter et al. 2007). Furthermore, the fruiting structure of certain fungi, the ammonia and the postputrefaction fungi have been found repeatedly in association with decomposed mammalian cadavers (Carter & Tibbett, 2003). Carter & Tibbett (2006) conducted

microbial decomposition studies on goat's skeletal tissues under different temperatures and the results showed skeletal muscle tissue can be immediately used as a source of nutrients by the soil microbial biomass and this utilization can be greatly influenced by temperature.

Documentation on microbial decomposition on carcasses has been reported on marine mammals. Smith et al. (1989) reported large communities of bacteria, vesicomyid clams, mytilids mussels and gastropods supported by an oil-rich whale skeleton at 1240 m off California, in Santa Catalina Basin. Microbial decomposition on land mammals have been conducted by Pechal et al. (2013) where pig carcasses had been used to demonstrate that microbial functional activity throughout decomposition in different seasons. Pyrosequencing had been employed to identify the bacteria taxa which were potentially useful for estimating the minimum post-mortem interval (Pechal et al. 2014a). Metcalf et al. (2013) provided a detail understanding of bacteria and eukaryotic ecology within a decomposing corpse system and suggest that microbial community data can be developed as a forensic tool for estimating PMI. A detail review on postmortem microbiology and human decomposition is provided by Damann & Carter (2013) and Crippen & Singh (2015).

Soil microbial ecology has been incorporated into forensic application as a tool in estimating PMI. The soil epinecrotic microbial communities, the microorgamisms on and in decomposing heterotrophic biomass, have recently gaining attention. These microbial communities are comprised of bacteria, fungi, protists (Benbow et al. 2013). Soil are extremely heterogeneous terrestrial ecosystems that contain multiple layers of both organic and inorganic compounds, which are made up from both living and the remnants of decomposing animals, plants, bacteria, fungi and other microorganisms (Turbé et al. 2010). Edaphic microorganisms such as algae, bacteria and fungi form the majority of the soil biomass and are ubiquitous in the soils. These microorganisms represent the large portion of the Earth biomass, with approximately 10^6 to 10^7 grams of microbial biomass per square meter of surface soil (Baldrian et al. 2012). Approximately 80% of edaphic bacteria are found in the pores between soil particles, free or attached to

particle surfaces such as the ultrathin water films surrounding soil particles (Stotzky, 1997; Ranjard & Richaume, 2001).

There is a shift in microbial community activity in vertebrates during decomposition, notably the shift from aerobic bacteria, namely *Staphylococcus* and *Enterobacteriacae*, to the anaerobic bacteria, *Clostridia* and *Bacteroides* (Carter et al. 2008; Howard et al. 2010; Pechal et al. 2014). The endogenous enteric-associated bacteria dominate the cadaver at the beginning of decomposition (Carter et al. 2008; Tuomisto et al. 2013), and then the decomposition is proceeded by endogenous bacteria from gastrointestinal tract and other microorganisms that spread to other parts of body (Can et al. 2014). The aerobic bacteria dominated the body and depleting oxygen from the cadaver, which encourage the growth of anaerobic bacteria and facilitate the microbial succession (Hyde et al. 2013; Metcalf et al. 2013). Upon the depletion of oxygen, endogenous anaerobics, Firmicutes in the Lactobacillaceae family and Bacteroidetes in the Bacteroidaceae family increase in the abdominal cavity of the carcasses (Metcalf et al. 2013). These bacteria produce gaseous hydrocarbons and ammonia compounds that bloat the cadaver and eventually rupture the decaying skin (Fiedler & Graw, 2003).

Lauber et al. (2014) employed next-generation sequencing methods and demonstrated that the rate in which carrion decomposes increases in the presence of a diverse set of microorganisms in soil. The study showed mice placed on soil containing intact, endogenous microbial communities decayed at rate 2-3 times faster than that of mice placed on soil that was sterilized. In another study, meta-analysis demonstrated that reduction in detrivores diversity result in significant reduction in the rate of decomposition (Srivastava et al. 2009).

Pechal et al. (2013) demonstrated for the first time the use of metabolic profiling to access carrion decomposition, and the potential to use this technique for carrion decomposition research is evidenced. The results showed that the microbial metabolic profiles described significant functional changes in the community during decomposition both within and among seasons. The results were in-lined with studies in aquatic habitats

(Burkepile et al. 2006; Dickson et al. 2011). However, a more detailed research using microbial functional data in conjunction with entomological data is needed to better understand the complexity and their relationship during carrion decomposition, as Pechal et al. (2013) demonstrated that insects may have moderating effects on decomposing by mediating microbial structure and function. Despite of biotic factors, barrier can also has effect on arthropod and microbial assembly on carrion. Pechal et al. (2014b) examined the effects of delayed insect access on carrion decomposition and found alteration s in insect community assembly.

Scientists used to assumed that similar environment and similar microbes should have similar function, However, Stickland et al. (2009) suggested that the implicit assumption in ecosystem models (i.e., microbial communities in the same environment are functioning equally) is wrong, indicating the importance in community composition and adaptation of microbial communities to past resource environment. Under similar environment, animal carcasses may have different necrophagous arthropods and microbial assembly, which could result in different rate of decomposition or other changes in biochemical activities. Furthermore, barrier such as cages that delayed arthropod colonization on carcasses may have impacts on arthropods and microbial diversity and function.

The objective of this study is to investigate microbial metabolic community profiling of delayed vertebrate decomposition. The differences of microbial metabolic community profiling on pig carcasses and in the soil were compared. Furthermore, microbial metabolic community profiling between years (summers 2013 and 2014) were also compared.

METHODS

Site description and experiment design

Swine carcass (*S. scrofa* L.) decomposition study was conducted at a field site associated with the Field Laboratory, Texas A&M University, College Station, Texas, USA (30°33’ 18.54’’ N 96°25’38.71’’ W, 68 m a.s.l.). The perimeter of the study area

was approximately 371 m and the area was about 7,943 m^2 (Figure 2.1 and 2.2). The study site was subtriangular in shaped, with an oil drill and several oil tanks located at the right, approximately 180 m and 100 m to the study site, respectively. There was sandy soil at the oil pumping site while the study site was consisted of clay soil. There was a small stream located at the north of the study site. The east and the south edges of the study site were steep cliffs that 6 m lower from the level of study site (cliffs approximately 62 m a.s.l.) The vegetation at the study site is considered blackland prairie ecoregion (http://www.texasalmanac.com). Common vegetation found at the study site included Johnsongrass (*Sorgum halepense* L.) (dominant cover plant, covered approximately 75% at the study site), oak (*Quercus* spp.), annual sunflower (*Helianthus annuus* L.), thistles (*Cirsium* spp. Mill.), Western horse nettle (*Solanum dimidiatum* Raf.), Camphorweed (*Heterotheca subaxillaris* (Lam.)), muskmelon (*Cucumis melo* L.), jujube (*Zizyphus jujube* Miller),wild purple morning glory (*Ipomoea cordatotriloba* Dennst. tievine), pink evening primrose (*Oenothera speciose* Nutt.), poison ivy (*Toxicodendron radicans* (L.) Kuntze) and arrow-wood (*Viburnum dentatum* L.) (See appendix A).

Studies were conducted during the summers of 2013 (16 June - 26 July) and 2014 (15 June - 25 July). A total of nine pig carrions euthanized by blunt force trauma were used each year. The sex and weight of each pig carcasses was determined prior to placement in the field. The Texas A&M University Institutional Animal Care and Use Committee required no animal use protocol, as the swine were deceased at the time of acquisition. The carcasses were double bagged and placed in the field within 1 hour after death at approximately 1700 hours. Carcasses were randomly placed along three transects minimally 20 m apart based on a Latin Square design (Figures 2.3 and 2.4). All carcasses were oriented with their heads to cardinal north and dorsal side towards the east. No site was used more than once over the course of two years.

Figure 2.1. Map showing the study site near to Snook, Texas (30°55’ N 96°42’ W) (red arrow showing the location of carrion placement) (Google Map 2013).

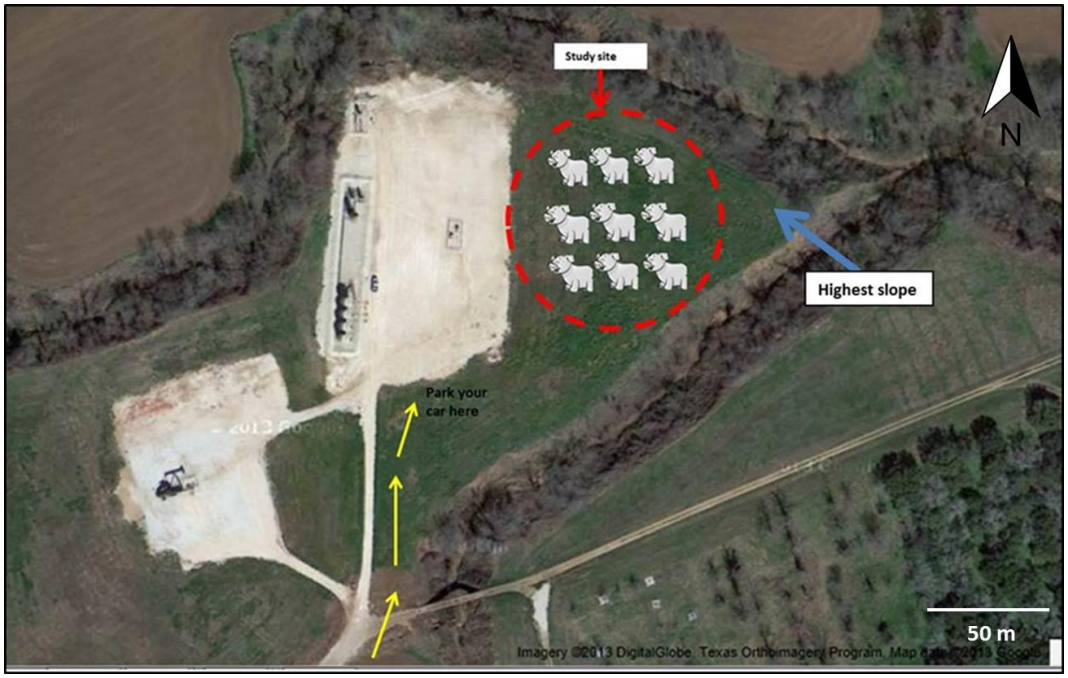

Figure 2.2. Map showing the location of carrion placement (red circle). On the left is a sandy area with an oil drill and machineries. Yellow arrows indicate the vehicle trail getting to the site. Blue arrow shows the highest slope at the study site (Google Map 2013)

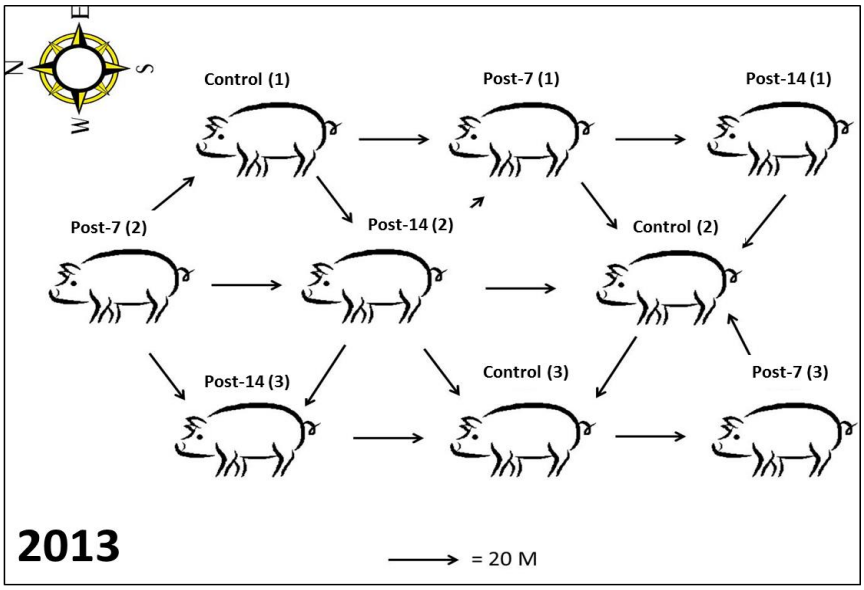

Figure 2.3. Latin square design for positioning the pig, *Sus scrofa* L., carcasses in the field located in Snook, Texas during summer 2013 (trial 1). Post-7 and Post-14 were the treatments while the number in brackets referred to the number of replicate.

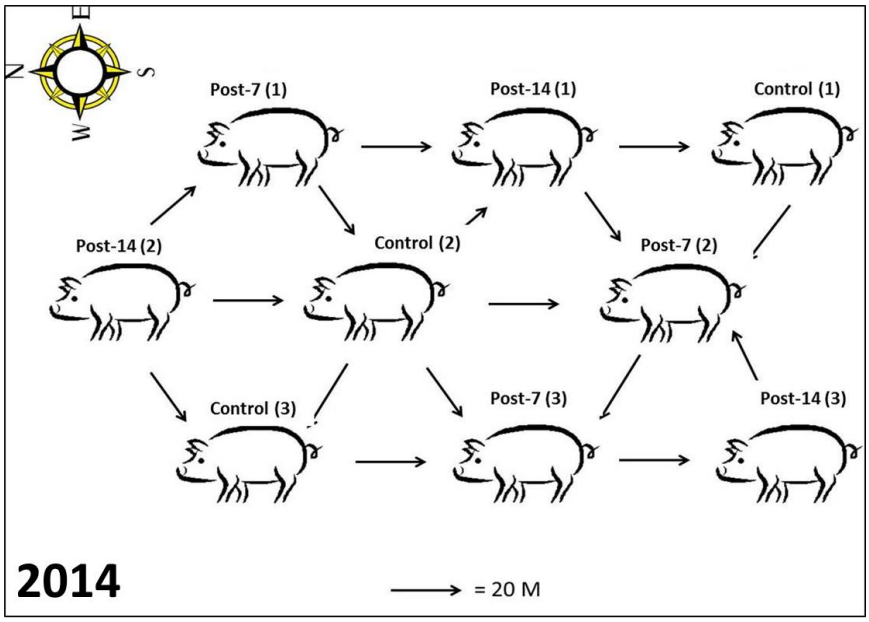

Figure 2.4. Latin square design for positioning the pig, *Sus scrofa* L., carcasses in the field located in Snook, Texas during summer 2014 (trial 2). Post-7 and Post-14 were the treatments while the number in brackets referred to the number of replicate.

All carcasses were placed on an autoclaved chicken wire measuring 0.61 m x 0.91 m gurney and then covered with a galvanized steel cage (0.61 m height x 0.91 m width x 1.22 m length), which was wrapped in chicken wire to prevent vertebrate scavenging. The nine carcasses were then randomly assigned to one of three treatments. Six carcasses were enclosed in an individual 1.8 m x 1.8 m x 1.8 m Lumite® screen (18 x 14 mesh size) portable field cages (BioQuip Products, Rancho Dominquez, CA, USA) (Figure 2.5). Cages were removed from three carcasses 7 day post-enclosure, while the cages covering the remaining three were removed 14 day post-enclosure. The remaining three that were not enclosed in the lumite cages served as the controls (Figure 2.6). This approached inhibited access of the primary arthropod colonizers to the remains in the lumite cages.

Stage of decomposition as defined by Payne (1965) for each carcass was recorded (see Appendix E) three to four times per day for the first two weeks, and two observations per day for the remainder of the experiment. Carcasses were considered in the fresh stage from initial death until bloating. The bloat stage was defined as when the abdomen was expanded due to gas accumulation. The active decay stage started when the abdomen ruptured and deflated. The carcasses were in advanced decay stage when bones were visible, and larval dispersion from the carrion for pupation was evident. The dry and remains stage occurred when the whole carcass was skeletonized, with little tissues left and skin beetles (Coleoptera: Dermestidae) observed feeding on the remains. All insect activities observed from day 0 until Day 40 were recorded and representative specimens either immature or adult stages were collected as voucher specimens and some of these insect specimens were deposited in the Texas A&M University Insect Collection (TAMUIC voucher #722). See Chapter 4 and 5 for detailed description of associated belowground and aboveground arthropods, respectively.

Figure 2.5. Random carcass was enclosed with insect exclusion cage for either 7 days or 14 days. In the picture above, it was designated as Post-14 ("R" indicated Post-14 group and "3" indicated the replicate number).

Figure 2.6. Anti-scavenging cage and stone weight placed over each swine, *Sus scrofa* L., carcass placed in the field located in Snook, Texas during summers 2013 and 2014 to prevent vertebrate scavenging.

Climatological data such as temperatures and precipitation were recorded. Three NexSens DS1923 micro-T temperatures loggers (Fondriest Environmental, Inc., Alpha, OH, USA) (Figure 2.7) were placed at the study site. Each data logger was attached to the top edge (0.3 m) of the galvanized steel cage covering the carcasses of Control, Post-7 and Post-14. Ambient temperature was recorded every 60 min for 40 days. Temperature data were converted into accumulated degree hours (ADH) based on the following formula:

$$\mathrm{ADH} = \sum_{i=1}^{n} (\emptyset - \emptyset_0)$$

where $\emptyset$ is the ambient temperature (in degree Celsius), while the minimum threshold temperature is $\emptyset_0$ (Higley & Haskell, 2009). The minimum development temperature threshold was set as 0°C for this chapter.

A rain gauge placed at the study site was used to record precipitation daily during the study period. The rain gauge was placed on the top of a wooden stake at 1.3 m above the ground, and approximately 1 m north from the carcass labelled as "Control 2" (Figure 2.8).Weight of each pig carcass was measured during each sampling period by attaching a hanging scale (minimum sensitivity = 500 g) to the gurney previously described and lifting the remains off the ground (Figure 2.9).

Microbial communities of all pig carcasses and soil were sampled on day 0, 7, 14, 21, and 40 posmortem. Sterile cotton applicators (Fisher Healthcare, USA) were used to collect microorganisms from bucca (under the tongue and inner cheek), skin area (left side of the abdomen), and anal orifice (swabbed for approximately 60 s each site) (Figure 2.10). In every sampling day, systematic swabbing were employed for not to repeat sampling areas (e.g., skin) throughout the decomposition process (Figure 2.11).

Figure 2.7. Three NexSens DS1923 Micro-T temperatures loggers randomly placed on the anti-scavenging cages (approximately 0.3 m above the ground) in the field located at Snook, Texas during summers 2013 and 2014. (Image downloaded from http://www.fondriest.com/reviews/humidity-sensors) (Accessed on 22 March 2015).

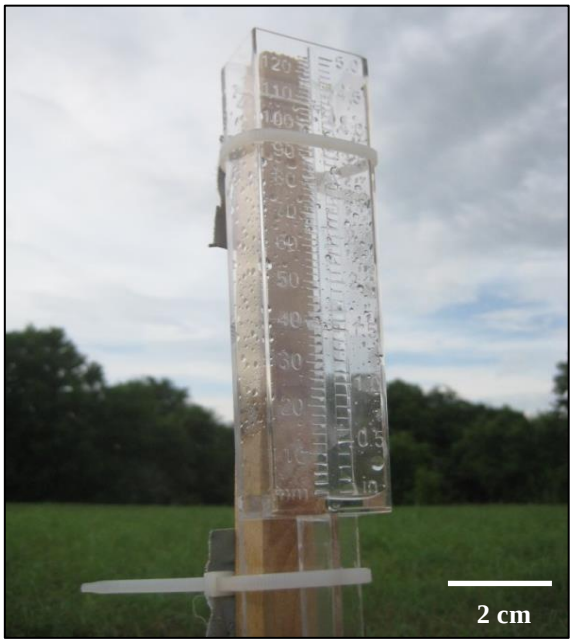

Figure 2.8. A rain gauge was placed on a wooden stake about 1.2 m height from the ground and approximately 1 m north from a swine, *Sus scrofa* L., carcass in the field at Snook, Texas during summers 2013 and 2014.

Figure 2.9. Weighing of pig, *Sus scrofa* L., carcass biomass in the field using a hanging scale during summers 2013 and 2014 at Snook, Texas. A sterilized platform made of aluminium mesh (0.9 m x 1.2 m) was placed at the bottom of each swine carcass for lifting purposes.

Figure 2.10. Sterile cotton applicators were employed to collect microbial samples from (A) oral (B) skin (C) anal region of the swine, *Sus scrofa* L., carcasses during summers 2013 and 2014 at Snook, Texas.

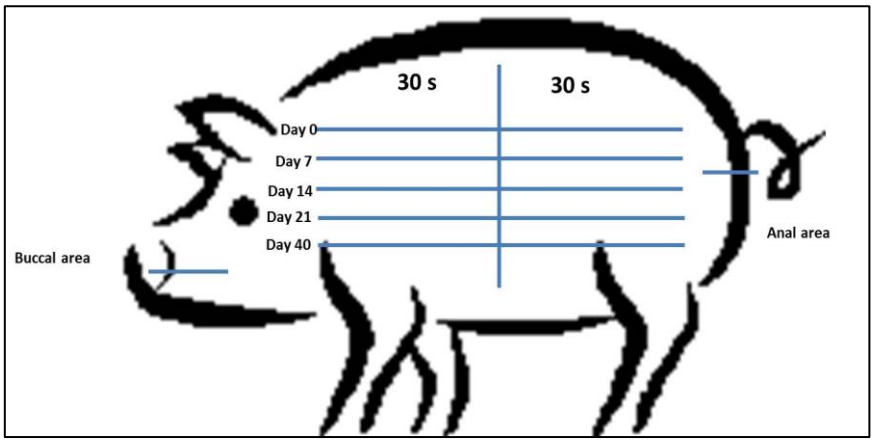

Figure 2.11. Imaginal lines for systematic skin swabbing on pig, *Sus scrofa* L., carcass. These lines were intended to prevent repetitive swabbing on the same skin area during each sampling day (image not to scale).

Soil samples were collected from beneath and at the side of carcasses (designated as soil lateral), along with a control sample, which was taken from a site five meters north of the carcass (Figure 2.12). Approximately 400 g soil sample were collected using a plastic trowel (at three different non-repetitive sampling sites randomly selected from one location) and measured using a steel tin can (170 g capacity) (Figure 2.13). Equipment was disinfected using Lysol in every single use. Soil samples together with swab samples were kept in cooler box (L: 70 cm; W: 40 cm; H: 45 cm) filled with ice (~4°C) to avoid DNA disintegration and to reduce microbe's metabolism activity temporarily. Samples were processed accordingly and inoculated into Biolog EcoPlate™ (BIOLOG Inc., Hayward, CA, USA) (Figure 2.14) for functional analyses within five hours of collection and kept in a cooler box (~4°C) until processing. All samples were processed for microbial function according to methods in Pechal et al. (2013) and Webber & Legge (2010).

Briefly, samples were added individually to 50 ml Falcon tubes (VWR™ International, Randor, PA, USA) containing 40 ml of sterilized 25% Ringer solution (contained NaCl, CaCl and KCl) and 15 sterilized 3 mm glass beads (Fisher Scientific, Fair Lawn, NJ, USA). Samples were then homogenized using a vortex for 2 min. Samples were centrifuged at 800 g for 2 min and the supernatant was retained. The Biolog Ecoplate™ were inoculated with 100 µl supernatant aliquots per well. Plates were then incubated at 25°C in darkness. Absorbance, or overall plate metabolic activity, were measured at 590 nm every 12 h up to 108 h or until the average plate absorbance reached 0.7 OD using Tecan Sunrise™ (Tecan Group Ltd., Männedorf, Switzerland) (Figure 2.15) with Magellan™ software version 7.0 (Tecan Group Ltd., Männedorf, Switzerland). A summary of Biolog protocol was shown in Figure 2.16 (see Appendix B for details). The mechanisms on how the Biolog works is exhibited in Figure 2.17. The name of the carbon sources impregnated in the Biolog Ecoplate is attached at Appendix C.

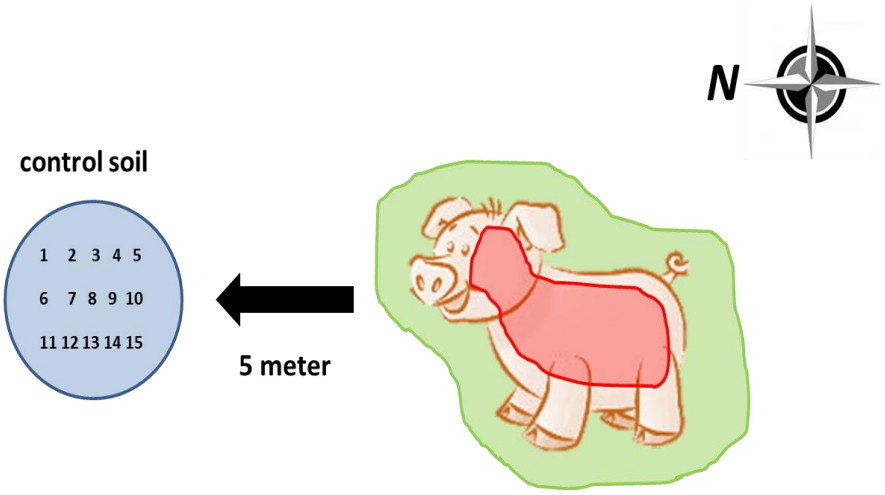

Figure 2.12. Locations of soil sample collection for microbial community functional analysis throughout the carrion decomposition process in summers 2013 and 2014 in the field site located at Snook, Texas. Red = soil beneath; Green = soil lateral; Blue = soil 5 meter. The numbers (1 - 15) in the control soil circle indicates 15 different sampling spots and these numbers were also applied to both soil beneath and soil lateral of the carrion. Three random non-repetitive numbers were chosen as the unique soil collection spots in each sampling day to prevent repetitive sampling (image not to scale).

Figure 2.13. Collection of soil beneath the pig, *Sus scrofa* L., carcass using a sanitized plastic trowel. The soil was then transferred into a sterilized tin can (170 g capacity), and poured into a Ziploc bag, and mixed. The soil samples were then kept in a cooler at 4°C and transported to lab.

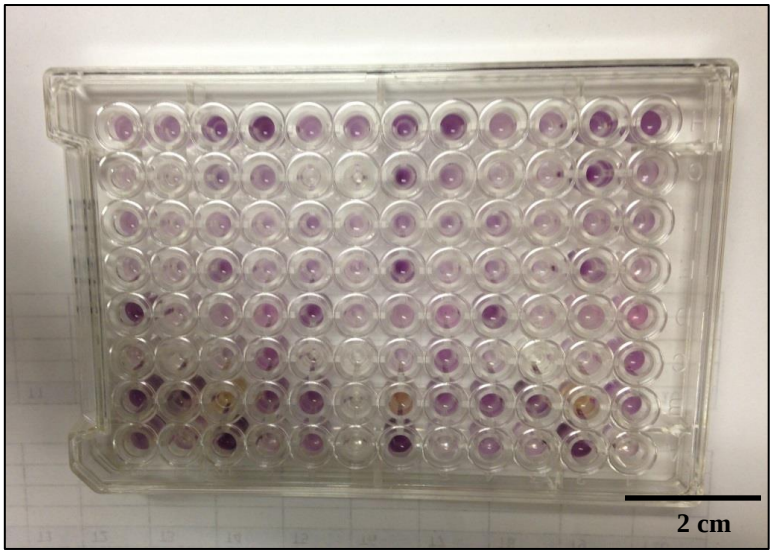

Figure 2.14. A Biolog Ecoplate™ has been inoculated with microbiological sample collected from the field. Purplish colored-well indicated certain degrees of bacterial metabolism (Image courtesy of Stephanie Thornton (2013)).

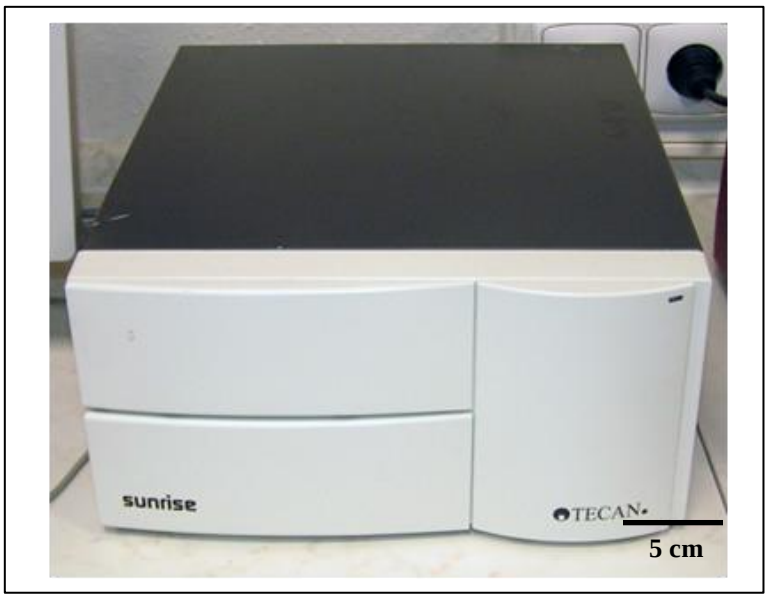

Figure 2.15. Plate reader Tecan Sunrise™ used in this study to measure average OD of Biolog EcoPlate™ for both 2013 and 2014 trials (Image downloaded from http://www.biotrans.cas.cz).

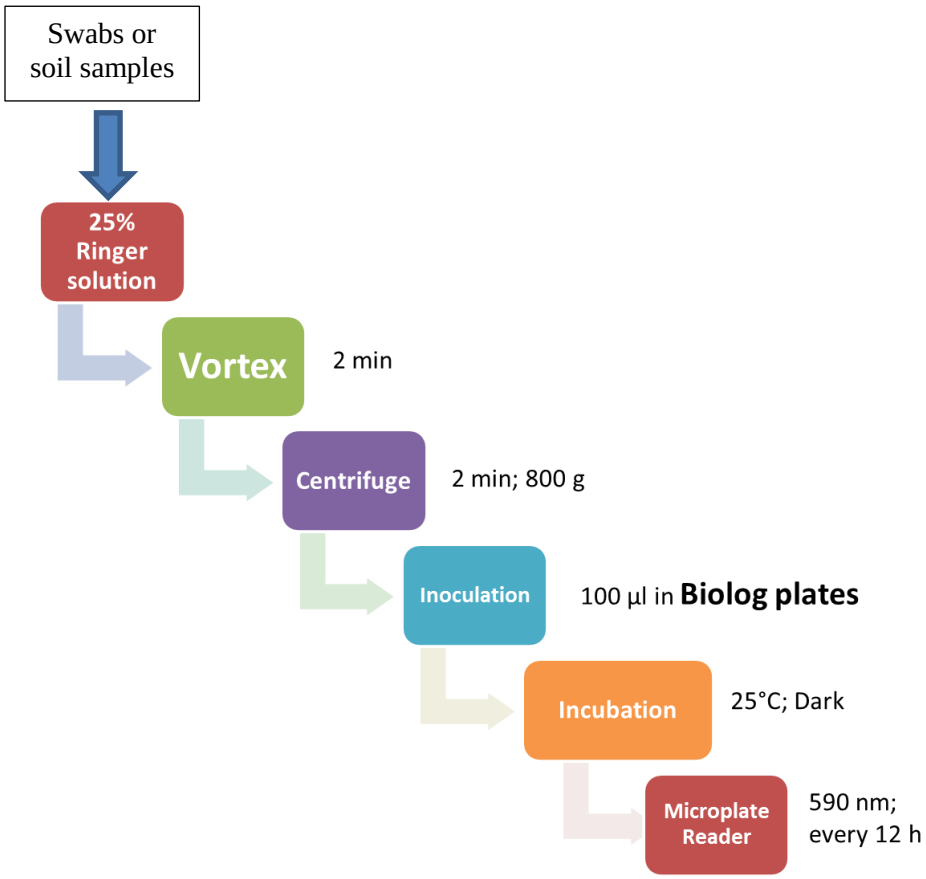

Figure 2.16. Flow chart showed the simplified laboratory protocols for swab or soil sample preparation for MMCPs reading. For more details, see Appendix B.

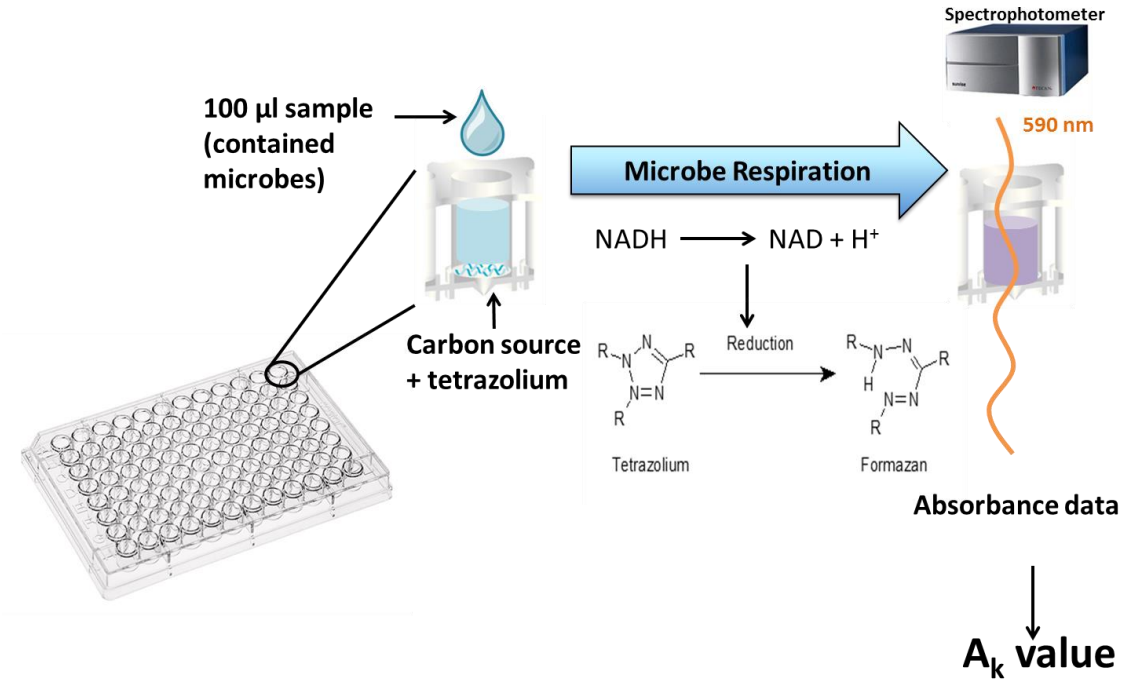

Figure 2.17. Biochemical mechanisms of Biolog EcoPlate™ for the detection of bacterial metabolism. When a 100 µl sample from carcass or soil is inoculated into a 96-well Biolog EcoPlate (which contained 31 carbon sources with triplicates), bacteria metabolism will then reduce tetrazolium salts into formazan (an artificial chromogenic product of tetrazolium by dehydrogenases) which give rise to purple color. A spectrophotometer or a plate reader (i.e., Tecan Sunrise™) is then used to detect the optical density (OD) of each well in Biolog EcoPlate. The absorbance data (e.g., average OD of each well) obtained will then convert into A_k value using formula to obtain the normalized value of each well (by omitting the water value) (see Appendix D for detail).

Statistical analyses

Microbial community metabolic activity was determined using the following formula. Initially, data for each carbon were normalized by subtracting the mean absorbance (A_i) determined for the water wells (A_o) and then dividing it by the sum of the corrected plate absorbance as described by Weber & Legge (2010). Doing so accounted for possible density difference among samples. The A_k was the normalized well (individual carbon substrate) metabolic activity.

$$A_k = \frac{A_i - A_0}{\frac{1}{31}\sum_{i=1}^{31}(A_i - A_0)}$$

Negative well responses were coded as zeros for further data analysis. Microbial community function (using A_k value) from each sample was tested statistically for effects of sampling days, treatments, regions (oral, skin, anal, soil beneath, soil lateral, soil 5 meter), and their interactions using permutation analysis of variance (PERMANOVA) followed by multiple comparisons tested with Bonferroni corrections using the R statistical package (R Core Team, 2013). Bonferroni corrections were used to test for significance of pair-wise comparisons without an increased probability of rejecting the null when it was actually true (Type I error) (Cabin & Mitchell, 2000). Non-metric multidimensional scaling (NMDS) was used to evaluate microbial metabolic community profiling (MMCP) between treatments over days using the package Vegan function Adonis in R (Oksanen et al. 2013). Multi-response permutation procedures (MRPP) was then used for testing statistical differences between overlay groups of MMCPs within the ordination using methods described elsewhere (Biodini et al. 1985). Indicator species analysis (ISA) completed MRPP by assigning significant indicator values to carbon substrates that were indicative of community functional separation among treatments and over time (McCune & Grace, 2002). The indicator value described which carbon substrate best explained microbial community function in each treatments (Control, Post-7 and Post-14) as related to decomposition days and sampling regions.

Statistical program JMP® Pro version 11.0.0 (SAS Institute Inc., NC, USA) was employed in this study. Functions such as student-T test, analysis of variance (ANOVA) and Tukey-Kramer HSD post-hoc test were performed on the weather data, carrion biomass data, as well as optical density (OD) readings obtained from swab and soil samples through spectrophotometry methods as described previosuly.

RESULTS

Weather data in summer 2013

The mean temperature was 30.59 ± 7.81°C, with maximum 47.67 ± 4.48°C and minimum 15.5 ± 0.00 °C. Total accumulated degree hour (ADH) for 2013 trial was 29219.70 (base temperature 0 °C). According to the nearest National Weather Station (KCLL) at Easterwood Field Airport, College Station, Texas (data downloaded from www.wunderground.com). There were nine rain events and five thunderstorms recorded during the study period. Total precipitation during the study period was 39.12 mm as recorded from rain gauge.

Weather data in summer 2014

The mean temperature was 29.27 ± 6.49 °C, with maximum 43.00 ± 1.80°C and minimum 19.00 ± 0.00 °C. Total accumulated degree hour (ADH) for 2014 trial was 28090.70 (base temperature 0 °C). There were 13 rain events, 11 thunderstorms and two fog events recorded during the study period. Total precipitation during the study period was 171.45 mm as recorded from rain gauge.

Weather comparison between summers 2013 and 2014

Generally, combined data showed mean temperature in summer 2013 is higher than mean temperature in summer 2014 (Figure 2.18). Two-tailed T test was employed to compare two years temperature data and the results showed a significant difference ($p = 0.0004$) (Table 2.1). Regarding precipitation, although summer 2014 showed higher amount of precipitation compared to summer 2013 (Figure 2.19), however, two-tailed T-test showed not significance between these two years ($p = 0.2725$). Table 2.2 showed the comparison of precipitation of both years. Accumulated Degree Hours for summer 2013 and 2014 was demonstrated in Figure 2.20, where the ADH in summer 2013 was higher than summer 2014. As expected, a significant difference in ADH between the two trials ($t (1964.141) = -2. 1944$, $p = 0.0142$).

Table 2.1. T-test on ambient temperature between summers 2013 and 2014 at Snook, Texas.

Group	n	T ratio	df	P value
2013	985	-3.55963	1938.492	0.0004*
2014	985			

Table 2.2. T-test on the amount of precipitation between summers 2013 and 2014 at Snook, Texas.

Group	n	T ratio	df	P value
2013	41	1.111543	43.0544	0.2725
2014	41			

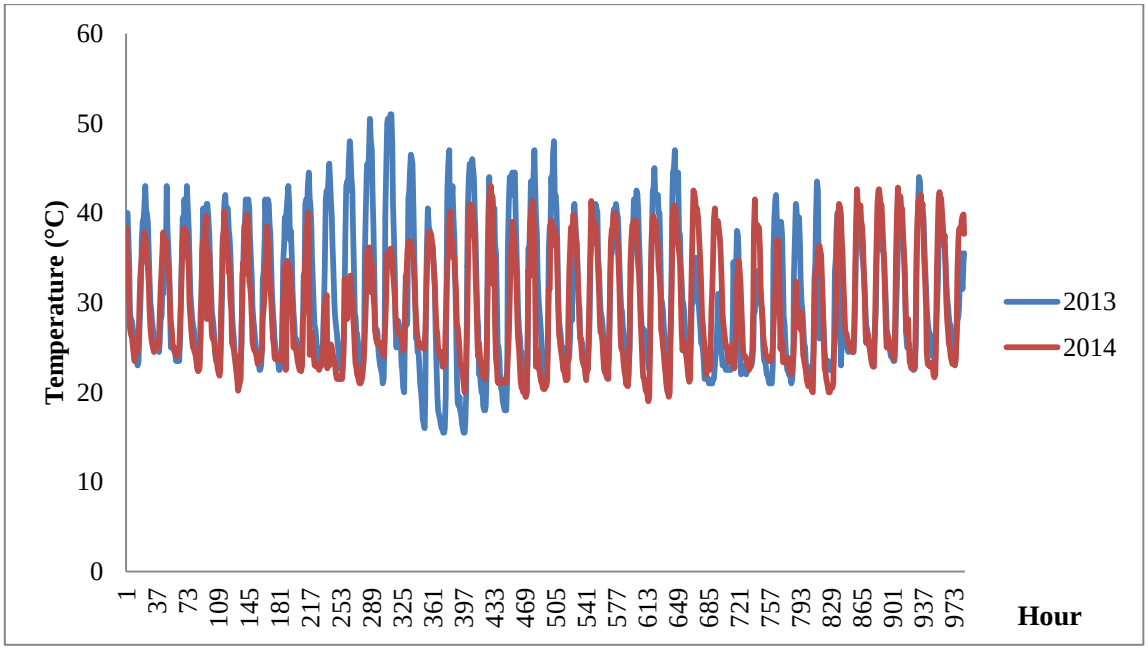

Figure 2.18. Comparison of average ambient temperatures recorded from micro-T temperature loggers (0.3 m above ground) over time (hour) between summers 2013 and 2014 at the study site at Snook, Texas.

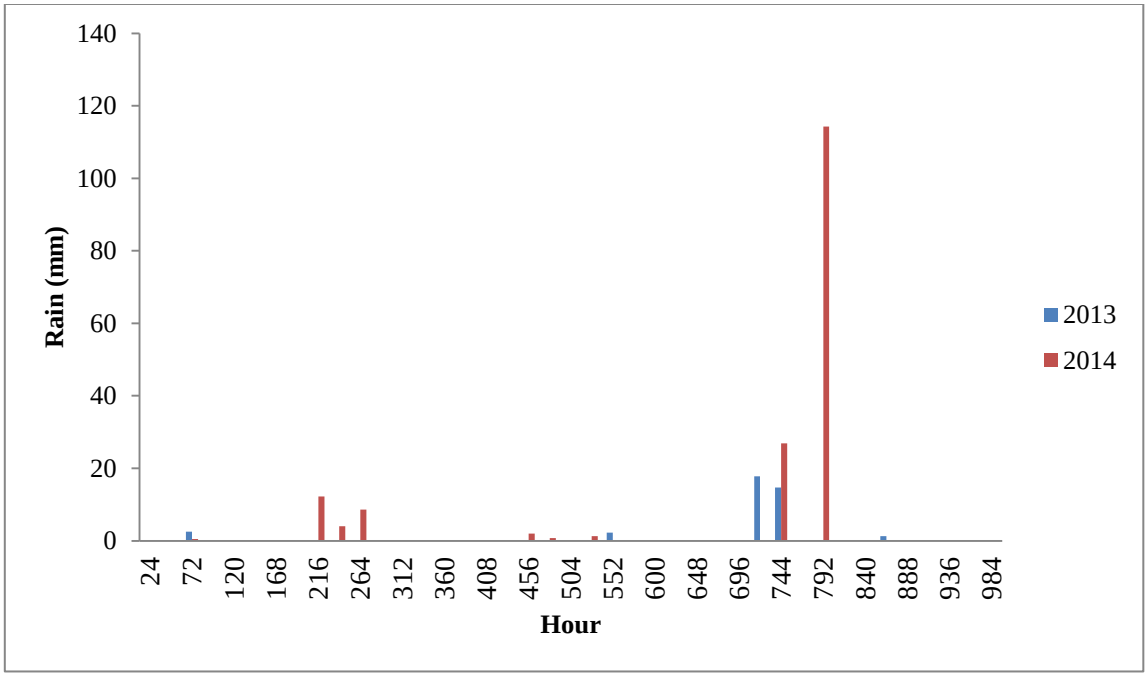

Figure 2.19. Comparison of the amount of precipitation (mm) between summers 2013 and 2014 at the study site at Snook, Texas.

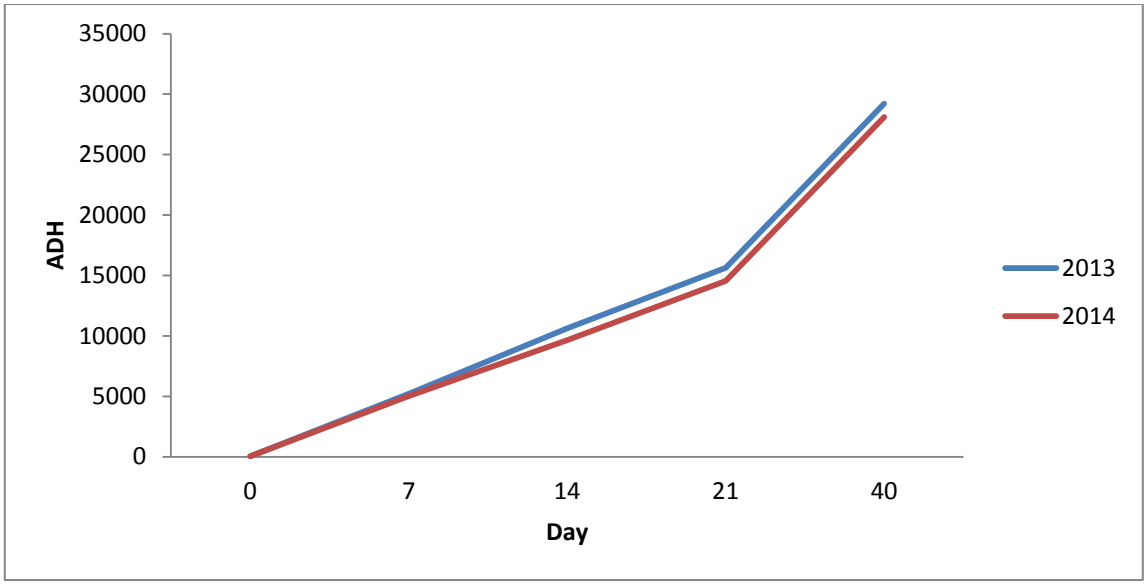

Figure 2.20. Accumulated Degree Hour (ADH) for summers 2013 and 2014 at the study site at Snook, Texas (base temperature 0 °C).

Sex, weight, and time of death of the pig carcasses in 2013 and 2014

A total of nine pig carcasses were used in each field trial. In summer 2013, five females and four males were placed in the field, while in summer 2014, six females and three males were deployed. The mean weight of pig used in both 2013 and 2014 trials were 26.33 ± 4.29 kg and 23.55 ± 4.68 kg, respectively (Table 2.3).

Table 2.3. Sex, weight, and time of death of pig carrion used in this study during summers 2013 and 2014 at the field site located at Snook, Texas.

Group	2013 (16 June 2013)				2014 (15 June 2014)			
	Sex	Weight (kg)	Time of death	Time of placement in field	Sex	Weight (kg)	Time of death	Time of placement in field
Control (1)	F	22.5	17:29	20:37	M	25	16:26	17:35
Control (2)	M	22.5	17:28	20:49	F	27	16:21	17:38
Control (3)	F	30	17:22	20:58	M	24	16:19	17:42
Post-7 (1)	M	28	17:27	19:32	M	25	16:08	18:09
Post-7 (2)	F	30	17:42	18:50	F	27.5	16:13	18:08
Post-7 (3)	F	28	17:26	20:08	F	29.5	16:17	18:12
Post-14 (1)	M	18	17:38	19:53	F	17.5	16:24	18:14
Post-14 (2)	M	30	17:27	20:23	F	15.5	16:10	17:51
Post-14 (3)	F	28	17:24	19:12	F	21	16:15	18:05
Average		26.33 ± 4.29 kg				23.55 ± 4.68 kg		

M = Male; F = Female.

Biomass loss of pig carcasses

Summer 2013

The biomass loss of pig carcasses showed similar trend between treatments as well as between years. 80% of the biomass losses for Control carcasses and 50% of the

biomass losses for Post-7 and Post-14 carcasses occurred within the first week of each trial (Figure 2.21). By Day 40, most carcasses lost approximately 80-100% of their original weight. Treatment significantly ($p < 0.05$) impacted weight loss over time (Table 2.4). Day 0 was the first day of experiment. On Day 7, Control and Post-7 showed a significant difference in weight loss. On Day 14, Day 21 and Day 40, Control and Post-14 showed a significant difference between each other.

Summer 2014

Treatment significantly ($p < 0.05$) impacted weight loss over time. The biomass loss pattern was similar with that in summer 2013, where the biomass of control was declined sharply within the first week (95.43%), whilst the Post-7 and Post-14 were lost almost 50% of their original weight. On Day 14, the Control group's biomass loss achieved 97.40%. In Post-7 group, because the insect exclusion cages were removed on Day 7, the biomass loss increased up to 50% within a week. As for Post-14 group, the carcasses were still enclosed in insect exclusion cages, and the biomass loss was steadily declined to approximately 70%. On Day 21 and Day 40, all carcasses were exposed to insect colonization and the biomass loss for Control and Post-7 groups were almost identical, and Post-14 group was increasing steadily and achieved 93.21% on Day 40 (Figure 2.22).

The ANOVA results showed that there were significant differences between groups in every sampling day. For Day 7, biomass lost was significant difference between Control x Post-7 ($p = 0.0001$), and Control x Post-14 ($p = 0.0001$). For Day 14 and Day 21, two comparison groups namely Control x Post-14 and Post-7 x Post-14 were significant difference. For Day 40, Post-7 vs Post-14 was significant difference, however, Control vs Post-14 was marginally significant difference ($p = 0.0547$). Table 2.5 demonstrated the results of ANOVA and Tukey HSD tests between groups for each sampling day. Table 2.6 showed the comparison of significant biomass loss between summers 2013 and 2014.

There was no significant of percentage biomass loss between years for each treatment group ($p > 0.05$) (Table 2.7). Comparison of rate of decomposition between treatment groups for both 2013 and 2014 trials was demonstrated in Figure 2.23 - 2.25.

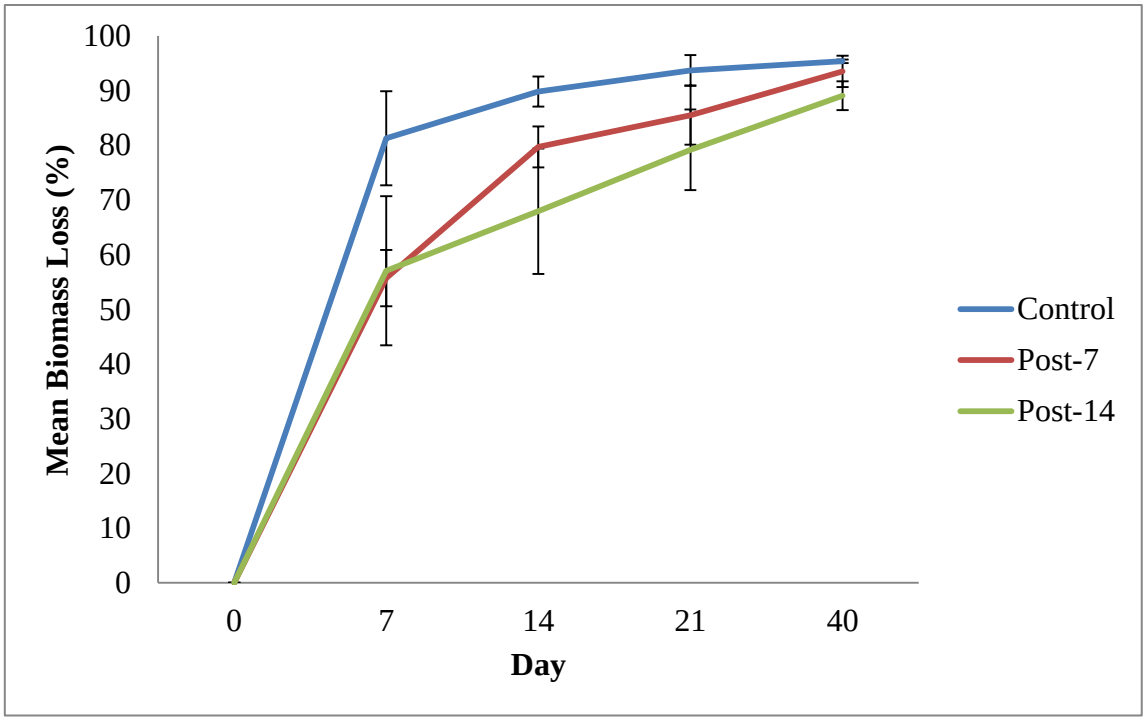

Figure 2.21. Mean percentage of biomass loss of pig carcasses over time (days) between treatments in summer 2013 at the field site located at Snook, Texas.

Table 2.4. ANOVA and Tukey-Kramer HSD on percentage of pig carrion biomass loss between treatments according to carrion decomposition days in summer 2013 at Snook, Texas.

Day 7 (ANOVA)

Source	Df	SS	MS	F ratio	Prob > F
Treatment	2	1243.7365	621.868	6.5191	0.0313*
Error	6	572.3501	95.392		
C. Total	8	1816.0866			

Tukey HSD

Level	Differences	Std Err Dif	Lower CL	Upper CL	P value
C vs Post-7	25.5833	7.9756	1.1160	50.0506	0.0421*
C vs Post-14	24.23667	7.9756	-0.2306	48.70397	0.0518•
Post-14 vs Post-7	1.34667	7.9756	-23.1206	25.81397	0.9844

• Marginal significant difference.

Day 14 (ANOVA)

Source	Df	SS	MS	F ratio	Prob > F
Treatment	2	719.3923	359.696	7.0324	0.0267*
Error	6	306.8887	51.148		
C. Total	8	1026.2810			

Tukey HSD

Level	Differences	Std Err Dif	Lower CL	Upper CL	P value
C vs Post-14	21.8800	5.8394	3.9638	39.7961	0.0223*
Post-7 vs Post-14	11.7433	5.8394	-6.1728	29.6595	0.1903
C vs Post-7	10.1366	5.8394	-7.7795	28.0528	0.2681

"C" represents Control carcasses.

Table 2.4 (Continued).

Day 21 (ANOVA)					
Source	Df	SS	MS	F ratio	Prob > F
Treatment	2	318.40496	159.202	5.2452	0.0482*
Error	6	182.1111	30.352		
C. Total	8	500.5160			

Tukey HSD				
Level	Differences	Std Err Dif	Lower CL	Upper CL	P value
C vs Post-14	14.5266	4.4982	0.7252	28.3280	0.0411*
C vs Post-7	8.2300	4.4982	-5.5714	22.0314	0.2389
Post-7 vs Post-14	6.2966	4.4982	-7.5047	20.0980	0.3987

Day 40 (ANOVA)					
Source	Df	SS	MS	F ratio	Prob > F
Treatment	2	63.2369	31.6185	6.2800	0.0338*
Error	6	30.2088	5.0348		
C. Total	8	93.4458			

Tukey HSD				
Level	Differences	Std Err Dif	Lower CL	Upper CL	P value
C vs Post-14	6.3133	1.8320	0.6922	11.9344	0.0317*
Post-7 vs Post-14	4.4700	1.8320	-1.1511	10.0911	0.1102
C vs Post-7	1.8433	1.8320	-3.7777	7.4644	0.6000

“C” represents Control carcasses.

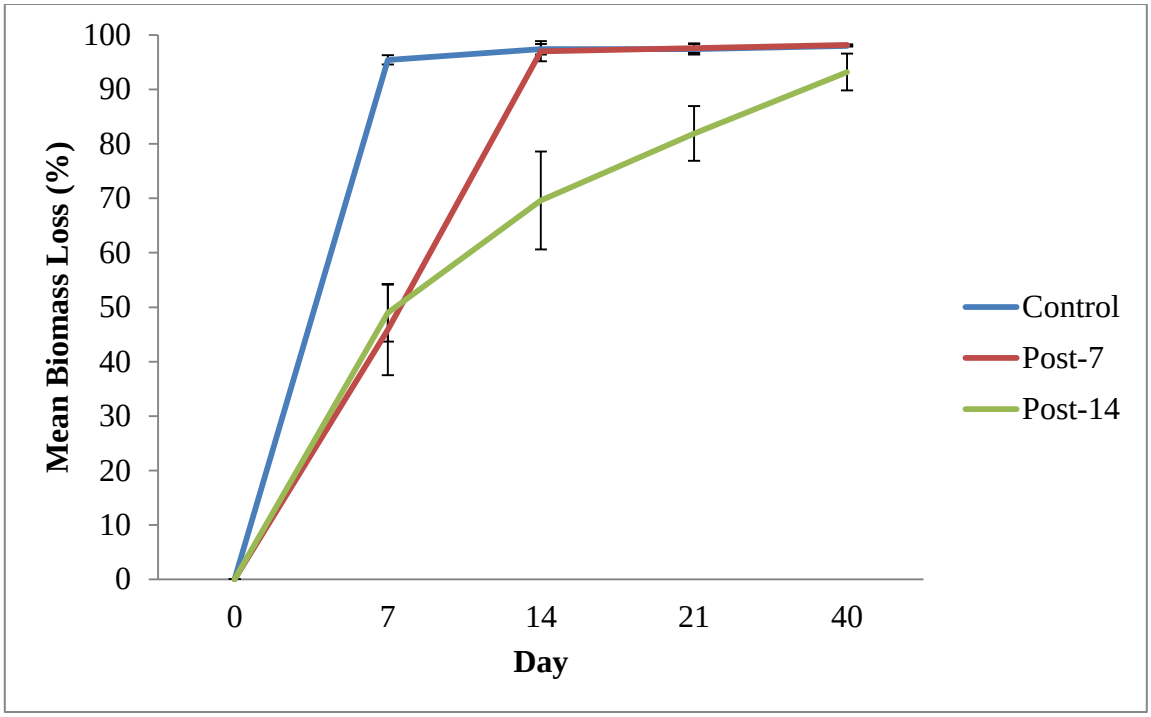

Figure 2.22. Mean percentage of biomass loss of pig carcasses between treatments according to carrion decomposition days in summer 2014 at Snook, Texas.

Table 2.5. ANOVA and Tukey-Kramer HSD on percentage of pig carrion biomass loss between treatments in summer 2014 at Snook, Texas.

Day 7 (ANOVA)

Source	Df	SS	MS	F ratio	Prob > F
Treatment	2	4628.5539	2314.28	70.7857	<0.0001*
Error	6	196.1649	32.69		
C. Total	8	4824.7187			

Tukey HSD

Level	Differences	Std Err Dif	Lower CL	Upper CL	P value
C vs Post-7	49.5872	4.6686	35.2631	63.9112	0.0001*
C vs Post-14	46.4756	4.6686	32.1515	60.7996	0.0001*
Post-7 vs Post-14	3.1116	4.6686	-11.2125	17.4356	0.7904

Table 2.5 (Continued).

Day 14 (ANOVA)					
Source	Df	SS	MS	F ratio	Prob > F
Treatment	2	1516.1195	758.06	26.6793	0.0010*
Error	6	170.4828	28.414		
C. Total	8	1686.6022			

Tukey HSD					
Level	Differences	Std Err Dif	Lower CL	Upper CL	P value
C vs Post-14	27.6329	4.3523	14.2794	40.9864	0.0017*
Post-7 vs Post-14	27.4317	4.3523	14.0782	40.7852	0.0018*
C vs Post-7	0.2012	4.3523	-13.1523	13.5547	0.9988

Day 21 (ANOVA)					
Source	Df	SS	MS	F ratio	Prob > F
Treatment	2	485.2565	242.628	26.7833	0.0010*
Error	6	54.3535	9.059		
C. Total	8	539.6101			

Tukey HSD					
Level	Differences	Std Err Dif	Lower CL	Upper CL	P value
Post-7 vs Post-14	15.6721	2.4574	8.1321	23.2121	0.0017*
C vs Post-14	15.4791	2.4574	7.9391	23.0191	0.0018*
C vs Post-7	0.1930	2.4574	-7.3469	7.7396	0.9966

“C” represents Control carcasses.

Table 2.5 (Continued).

Day 40 (ANOVA)					
Source	Df	SS	MS	F ratio	Prob > F
Treatment	2	47.6881	23.8441	6.1650	0.0351*
Error	6	23.2060	3.8677		
C. Total	8	70.8941			

Tukey HSD					
Level	Differences	Std Err Dif	Lower CL	Upper CL	P value
Post-7 vs Post-14	4.9518	1.6057	0.0251	9.8785	0.0491*
C vs Post-14	4.8111	1.6057	-0.1155	9.7378	0.0547•
Post-7 vs Post-14	0.1407	1.6057	-4.7859	5.0673	0.9958

"C" represents Control carcasses; •Marginal significant difference.

Table 2.6. Comparison of statistically significant pig carrion biomass loss between treatments according to carrion decomposition days during summers 2013 and 2014 at Snook, Texas.

Day	2013	2014
0	Nil	Nil
7	C vs Post-7	C x Post-7
	C vs Post-14•	C x Post-14
14	C vs Post-14	C x Post-14
		Post-7 vs Post-14
21	C vs Post-14	C x Post-14
		Post-7 vs Post-14
40	C vs Post-14	C vs Post-14•
		Post-7 vs Post-14

"C" represents Control carcasses; •Marginal significant difference.

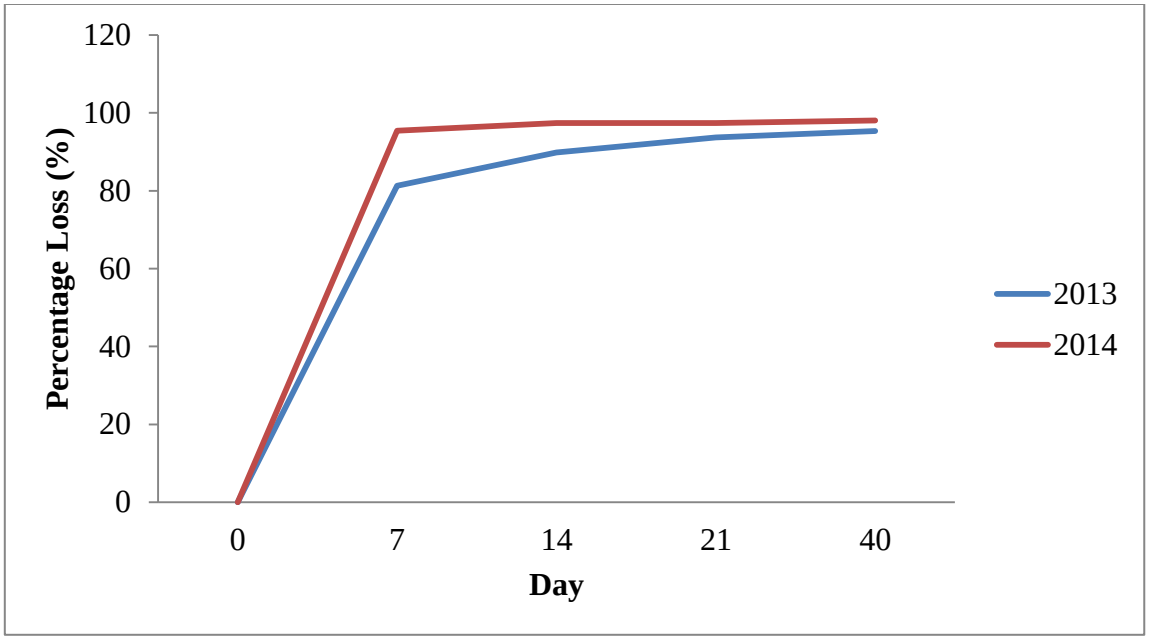

Figure 2.23. Rate of decomposition (percentage of biomass loss) of Control carcasses between years in the field located at Snook, Texas.

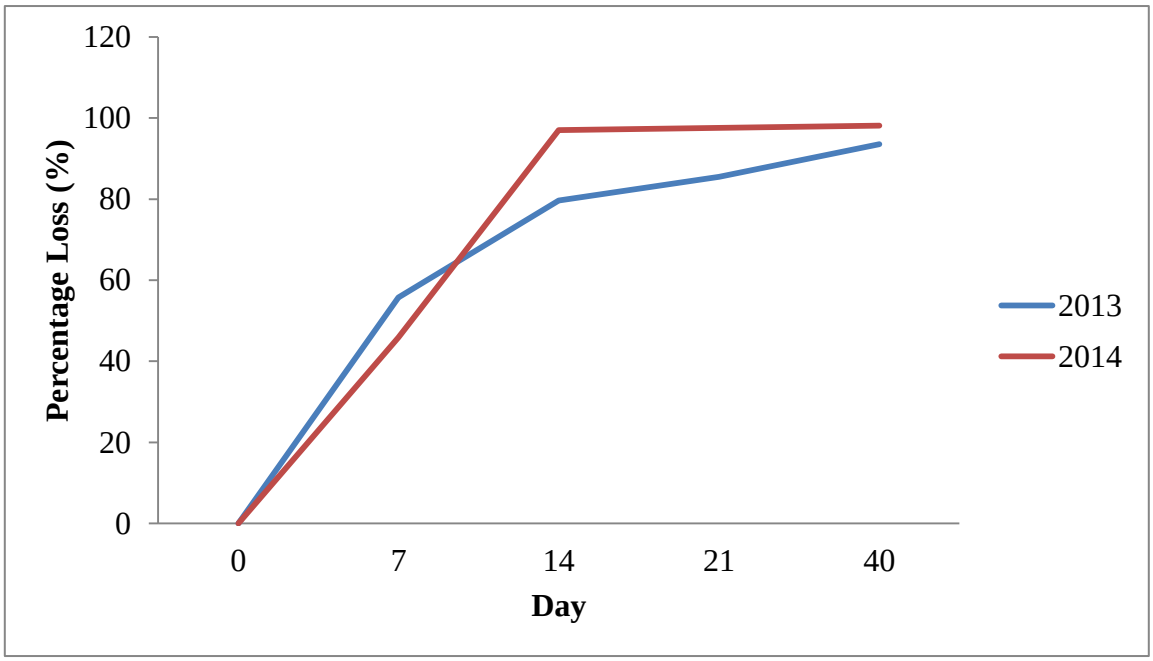

Figure 2.24. Rate of decomposition (percentage of biomass loss) of Post-7 carcasses between years in the field located at Snook, Texas.

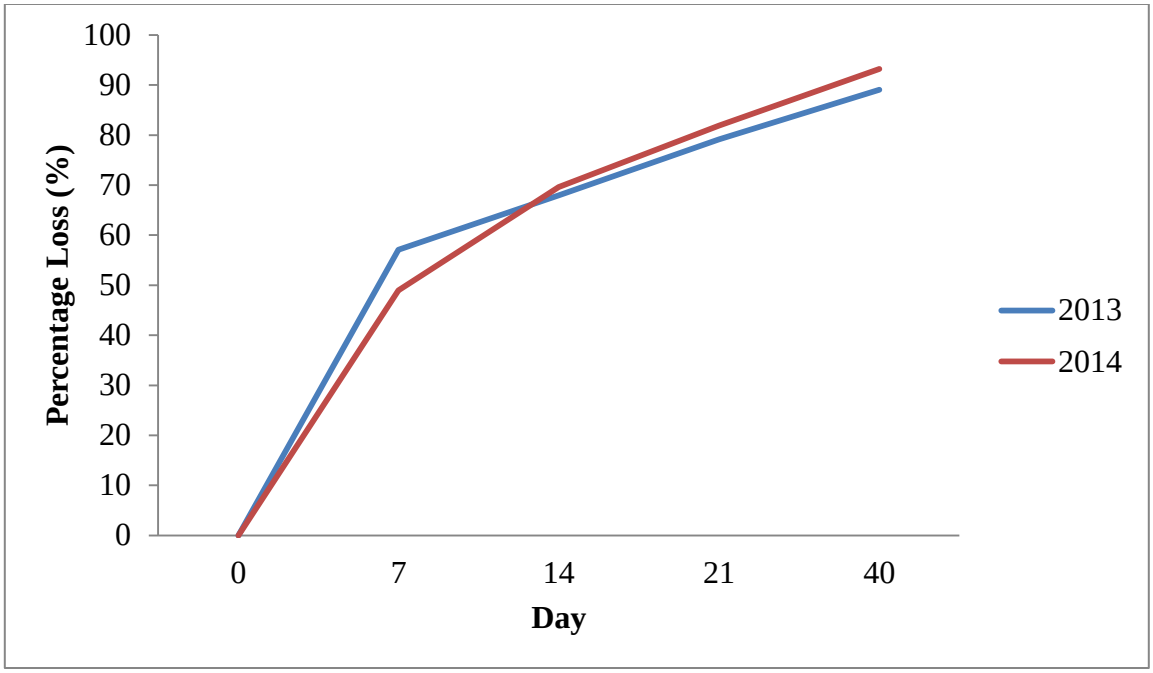

Figure 2.25. Rate of decomposition (percentage of biomass loss) of Post-14 carcasses between years in the field located at Snook, Texas.

Table 2.7. T-test on percentage of pig carrion biomass loss between years for each treatment group at Snook, Texas.

Control

Variable	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%	T ratio	df	p
2013	72.0387	37.7919	9.758	51.11	92.967	0.3939	27.89	0.6966
2014	77.6513	40.2032	10.38	55.388	99.915			

Post-7

Variable	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%	T ratio	df	p
2013	62.8800	35.2216	9.094	43.375	82.385	0.3478	27.40	0.7306
2014	67.7253	40.8632	10.551	45.096	90.355			

Post-14

Variable	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%	T ratio	df	p
2013	58.6473	33.1464	8.5584	40.291	77.003	0.0070	27.96	0.9944
2014	58.7347	34.2900	8.8536	39.745	77.724			

Microbial metabolic community profiles (MMCPs)

Summer 2013

A total of 540 samples were collected in summer 2013 for MMCPs analysis (i.e., 270 swabs from pig carrion and 270 soil samples). Stress test (0.1936, $r^2 = 0.8463$) indicated reducing dimensions was appropriate. Interactions between day and treatment as well as day and region were determined (Table 2.8). No significant difference due to carrion biomass was determined (df = 6; $r^2 = 0.0171$; p = 0.835). A significant difference in MMCPs between pig and soil samples was determined (df = 1, $r^2 = 0.0839$; p = 0.001).

Table 2.8. Analysis of the overall microbial metabolic community profiles for pig and soil samples in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F model	P value
Day	1	4.4051	0.003*
Treatment	2	2.6373	0.002*
Region	5	8.0248	0.001*
Day x Treatment	2	0.8996	0.585
Day x Region	5	1.4838	0.026*
Treatment x Region	10	1.3412	0.014*
Day x Treatment x Region	10	0.7148	0.989

Pig samples 2013

Stress test (0.2035, $r^2 = 0.7768$) indicated reducing dimensions was appropriate. Figure 2.26 showed the NMDS plot of stress for all pig samples. PERMANOVA was employed to test the difference. The results demonstrated Treatment ($p = 0.016$) and Day x Region ($p = 0.027$) were significantly difference. Multi Response Permutation Procedure (MRPP) was then employed to test whether there is a significant difference between two or more groups of sampling units. The results showed there was significant difference between Treatment (A value: 0.01556; Significant of Delta: 0.001 based on 999 permutations). Table 2.9 showed the statistical results on pig samples in summer 2013. Figure 2.27 demonstrated the NMDS ordination by Treatments for pig samples. A pattern was observed on Control group (red dots) throughout the ordination, where some parts of the Control group were clustered separately from the Post-7 (designated as M in the legend) and Post-14 groups (designated as R in the legend) whereas both treatments groups (Post-7 and Post-14) were mixed throughout the ordination and there was no clear separation among them.

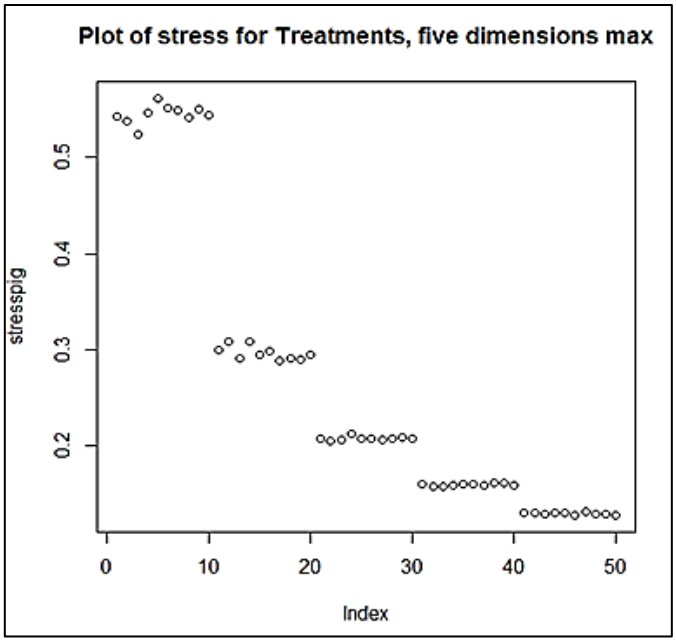

Figure 2.26. NMDS plot of stress for pig samples collected in summer 2013 at Snook, Texas (Stress test 0.2035, $r^2 = 0.7768$).

Table 2.9. Microbial metabolic community profiles for pig samples in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F model	P value
Treatment	2	2.9609	0.001*
Day	1	3.6251	0.001*
Region	2	2.4657	0.001*
Treatment x Day	2	0.8852	0.619
Treatment x Region	4	0.9866	0.507
Day x Region	2	0.9253	0.559
Treatment x Day x Region	4	0.6979	0.945

We are interested to know which Treatment group was significantly different from each other. Hence pairwise comparisons using PERMANOVA with Bonferroni's correction was performed to test the MMCPs between treatments on pig samples. The

results exhibited that Control x Post-7 and Post-7 x Post-14 were significantly different, with p value 0.005 and 0.001, respectively. There was no significant difference in Post-7 x Post-14 (p = 0.119). Table 2.10 showed the statistical results for the comparison between Treatments. Mean microbial function activity (average Optical Density, OD) on pig samples was plotted according to Treatments (Figure 2.28). Figure 2.29 exhibited the microbial function on pig samples by regions.

Table 2.10. Pairwise comparisons of MMCPs between Treatments on pig samples (summer 2013) at Snook, Texas after Bonferroni's correction.

Factor		df	SS	MS	F Model	R^2	P value
Control x Post-7	Treatment	1	0.6488	0.6488	2.6441	0.0292	0.005*
	Residual	88	21.5926	0.2454		0.9708	
	Total	89	22.2413			1.0000	
Control x Post-14	Treatment	1	0.9887	0.9887	4.3588	0.04719	0.001*
	Residual	88	19.9599	0.2268		0.95281	
	Total	89				1.0000	
Post-7 x Post-14	Treatment	1	0.289	0.2890	1.4616	0.0163	0.119
	Residual	88	17.401	0.1977		0.9837	
	Total	89	17.690			1.0000	

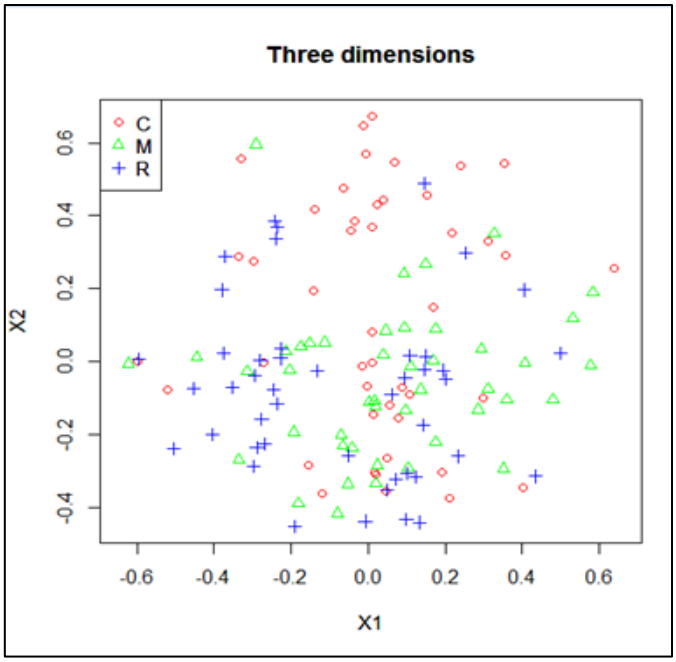

Figure 2.27. NMDS ordinations of normalized pig carcass microbial community activity according to treatments in summer 2013 at Snook, Texas (C = Control; M = Post-7; R = Post-14).

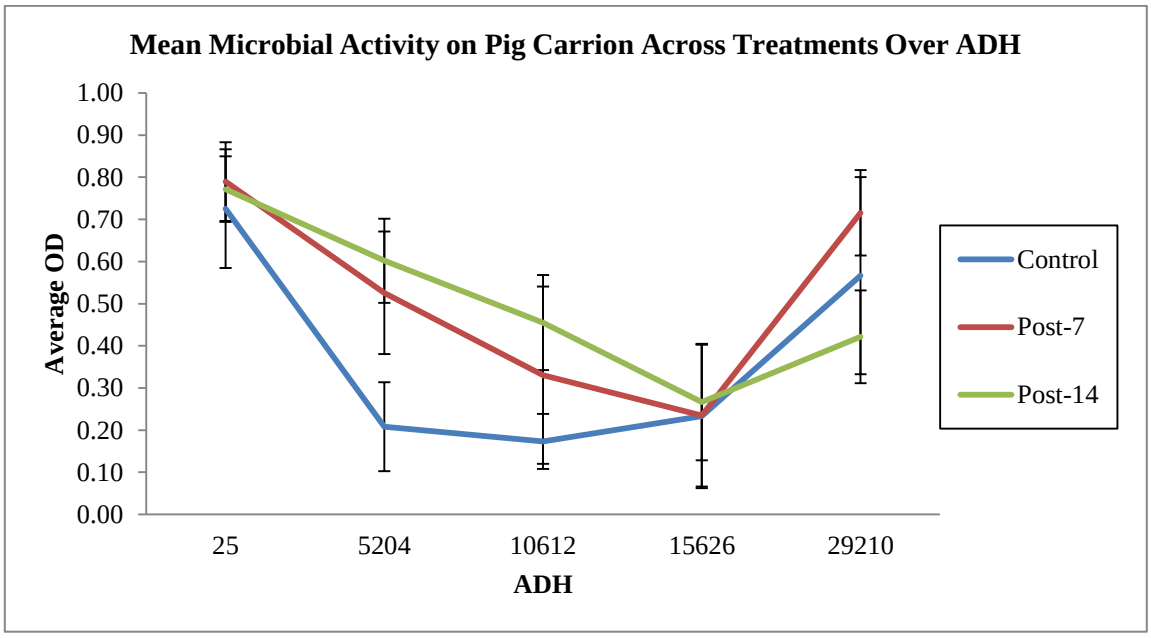

Figure 2.28. Mean microbial function (average OD) on pig samples (oral, skin, and anal) over Accumulated Degree Hour (ADH) during summer 2013 at Snook, Texas.

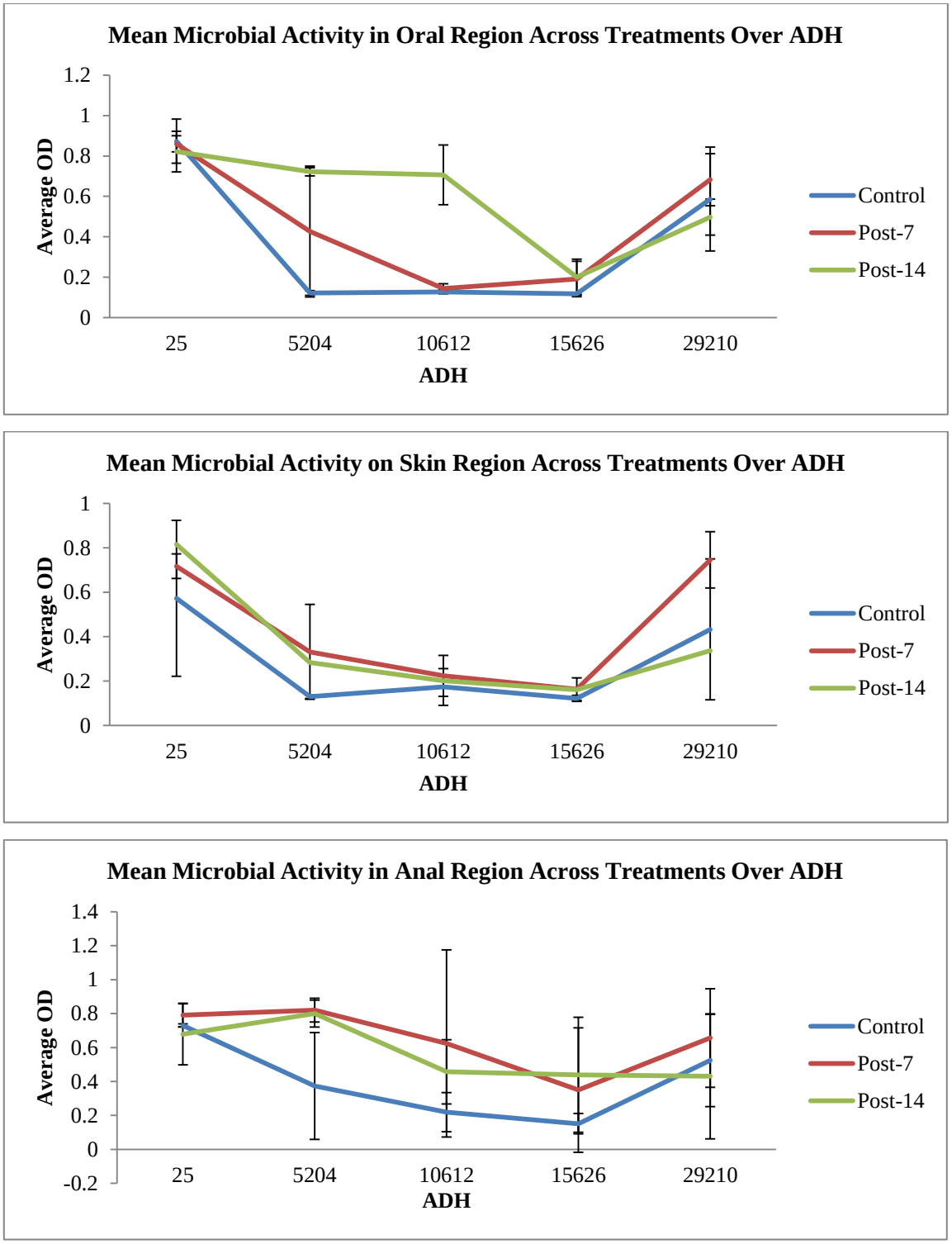

Figure 2.29. Mean microbial function on pig samples by regions in summer 2013 at Snook, Texas. Above. Oral region. Center. Skin area. Bottom. Anal region.

MMCP associated with regions (oral, skin, and anal samples) was significantly ($p < 0.05$) different. MRPP analysis was conducted on regions on pig samples and A value showed 0.0138 with Significant of Delta of 0.001. Pairwise comparisons using PERMANOVA was performed to test the MMCPs between regions on pig samples. The results demonstrated that Oral x Skin was not significant difference from each other ($p = 0.313$) while Oral x Anal and Skin x Anal were significant difference ($p = 0.008$ and 0.001, respectively). Table 2.11 showed the statistical results for the comparison between Regions. Mean microbial function (average Optical Density, OD) on pig samples was plotted according to Regions (Figure 2.30). Anal region (red dots) demonstrated certain degree of isolation from skin and oral region, while the skin and oral regions were mixed randomly without any distinct pattern.

Table 2.11. Pairwise comparisons of MMCPs between Regions on pig samples (summer 2013) at Snook, Texas after Bonferroni's correction.

Factor		df	SS	MS	F Model	R^2	P value
Oral x Skin	Region	1	0.2596	0.2596	1.1078	0.0124	0.313
	Residual	88	20.6249	0.2343		0.9876	
	Total	89	20.8846			1.0000	
Oral x Anal	Region	1	0.5822	0.5822	2.6879	0.0296	0.008*
	Residual	88	19.0614	0.2166		0.9704	
	Total	89	19.6437			1.0000	
Skin x Anal	Region	1	0.7624	0.7623	3.406	0.0373	0.001*
	Residual	88	19.6970	0.2238		0.9627	
	Total	89	20.4594			1.0000	

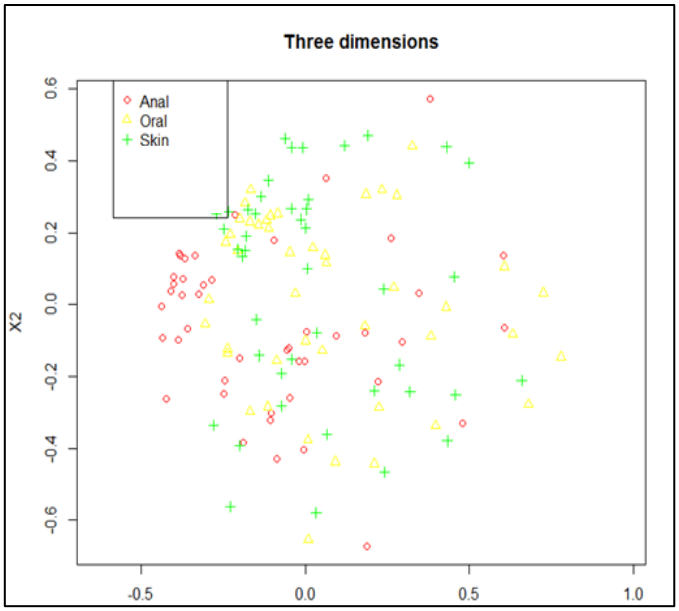

Figure 2.30. NMDS ordinations of normalized pig carcass microbial community activity according to pig regions (oral, skin and anal) in summer 2013 at Snook, Texas.

MMCPs based on Day was significant difference ($p = 0.001$) (Table 2.12, Figure 2.31). This observation was strengthened by the analysis by MRPP which showed A value 0.06735 with Significant of Delta 0.001. Pairwise comparisons by PERMANOVA was conducted and the results showed all pairs were significant difference ($p < 0.05$), except the pair of Day 14 x Day 21 ($p = 0.705$).

Table 2.12. Pairwise comparisons of microbial function between Days on pig samples in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40
0	-	0.001*	0.001*	0.001*	0.001*
7	0.001*	-	0.024*	0.005*	0.008*
14	0.001*	0.024*	-	0.729	0.003*
21	0.001*	0.005*	0.729	-	0.002*
40	0.001*	0.008*	0.003*	0.002*	-

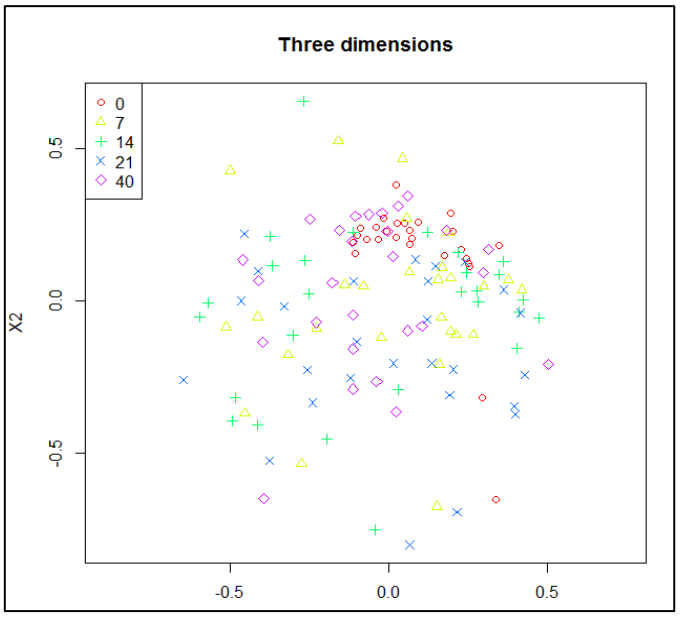

Figure 2.31. NMDS ordinations of normalized pig carcass microbial community activity according to carrion decomposition days (Day 0, 7, 14, 21, and 40) in summer 2013 at Snook, Texas.

Indicator carbon source on pig samples 2013

When all samples were pooled together, there was four indicator carbon sources associated with microbial metabolic community. There were Tween 40, Itaconic acid, D,L-α-glycerol phosphate, and N-Acetyl-D-glucosamine. By separating the soil samples from the pooled data, the indicator carbon source on pig samples demonstrated the same results, which consisted of four carbon substrates as above. Among the treatments, Control x Post-7 and Control x Post-14 was significantly difference by microbial function. Hence, carbon indicator analysis was performed on these groups and the results showed three carbons that were indicative namely D,L-α-glycerol phosphate, D-xylose, and D-malic acid for Control x Post-7 group. There was four indicator carbons demonstrated from Control x Post-14 group, there were Tween 40, itaconic acid, N-Acetyl-D-glucosamine, and D-xylose. By comparison, D-xylose was the only common carbon source shared between these groups. Note that when each treatment group was analyzed for treatment-specific indicator carbon source, there was no indicator carbon demonstrated. As for detail comparison between Treatments, between Days, and between Days x Regions, statistical results were shown at Table 2.13, 2.14 and 2.15, respectively.

Table 2.13. Indicator carbon analysis based on MMCPs for pig samples in summer 2013 at Snook, Texas.

Factor	Carbon source	Indicator value	p value
All samples (pig + soil samples)	Tween 40	0.5335	0.009*
	Itaconic acid	0.3993	0.004*
	D,L-α-Glycerol phosphate	0.4141	0.037*
	N-Acetyl-D-Glucosamine	0.3630	0.010*
Pig samples (include oral, skin and anal)	Tween 40	0.5330	0.010*
	Itaconic acid	0.3627	0.021*
	D,L-α-Glycerol phosphate	0.4919	0.017*
	N-Acetyl-D-Glucosamine	0.3856	0.012*
Oral samples	Tween 40	0.5330	0.006*
	Itaconic acid	0.3627	0.028*
	D,L-α-Glycerol phosphate	0.4919	0.017*
	D-Malic acid	0.4034	0.043*
	N-Acetyl-D-Glucosamine	0.3856	0.029*
Skin samples	Tween 40	0.5330	0.013*
	Itaconic acid	0.3627	0.025*
	D,L-α-Glycerol phosphate	0.4919	0.014*
	N-Acetyl-D-Glucosamine	0.3856	0.024*
Anal samples	Tween 40	0.5330	0.009*
	Itaconic acid	0.3627	0.027*
	D,L-α-Glycerol phosphate	0.4919	0.015*
	N-Acetyl-D-Glucosamine	0.3856	0.014*

Table 2.13 (Continued).

Factor	Carbon source	Indicator value	p value
Control	Nil	Nil	Nil
Post-7	Nil	Nil	Nil
Post-14	Nil	Nil	Nil
Control x Post-7	D,L-α-Glycerol phosphate	0.6469	0.008*
	D-Xylose	0.5820	0.020*
	D-Malic acid	0.5746	0.008*
Control x Post-14	Tween 40	0.7154	0.002*
	Itaconic acid	0.5260	0.003*
	D,L-α-Glycerol phosphate	0.5983	0.002*
	D-Xylose	0.5683	0.028*
Post-7 x Post-14	Nil	Nil	Nil

Nil = No carbon source indicator.

Table 2.14. Carbon source indicators between days on pig samples in summer 2013 at Snook, Texas.

Day	Carbon source	Indicator value	p value
0	Nil	Nil	Nil
7	Tween 40	0.7675	0.025*
	D-Malic acid	0.6466	0.004*
	N-Acetyl-D-Glucosamine	0.5328	0.029*
14	D,L-α-Glycerol phosphate	0.6963	0.037*
	N-Acetyl-D-Glucosamine	0.5881	0.009*
21	Nil	Nil	Nil
40	α-Ketobutyric acid	0.7373	0.002*
	γ-Hydroxybutyric acid	0.6726	0.016*

Nil = No carbon source indicator.

Table 2.15. Carbon source indicators between Regions and Days on pig samples in summer 2013 at Snook, Texas.

Day	0	7	14	21	40
Oral	Glucose-1-phosphate Phenylethylamine L-Phenylalanine L-Serine	Nil	4-Hydroxy benzoic acid L-Threonine L-Serine α-Cyclodextrin D-Mannitol	Nil	α-cyclodextrin D-mannitol
Skin	D-Glucosaminic acid	D-Galacturonic acid	Pyruvic acid methyl ester N-Acetyl-D-Glucosamine α-Ketobutyric acid	D-Galacturonic acid L-Arginine	α-ketobutyric acid
Anal	Nil	Nil	D,L-α-Glycerol phosphate α-Ketobutyric acid D-Mannitol	Glycyl-L-Glutamic acid	L-Asparagine L-Threonine γ-Hydroxybutyric acid α-Ketobutyric acid i-Erythritol

Nil = No carbon source indicator.

Soil samples 2013

When only soil samples were analyzed using NMDS, the minimum stress result rendered was 0.1886, which indicated a great representation in reduced dimensions, with r^2 = 0.8455. Figure 2.32 showed the NMDS plot of stress for soil samples. PERMANOVA was then performed on soil microbial function data and the results showed Day, Region, and Day x Region were significantly different (p = 0.004, 0.001, and 0.001, respectively). Table 2.16 showed the statistical results using PERMANOVA with 999 permutations on soil samples. The results were then analyzed by using MRPP and similar result was obtained where Region was significantly different (A = 0.04343 and Significant of Delta = 0.001). Pairwise comparisons were then performed to test which region of soil was significantly different (Table 2.17). The results demonstrated soil beneath x soil lateral, soil beneath x soil 5 meter, and soil lateral x soil 5 meter, were significant difference ($p < 0.05$). Figure 2.33 exhibited the NMDS ordination of soil samples by regions. Mean microbial function (average OD) in soil samples was plotted according to Treatments (Figure 2.34). Figure 2.35 showed the mean microbial function across treatments over ADH in soil samples according to regions.

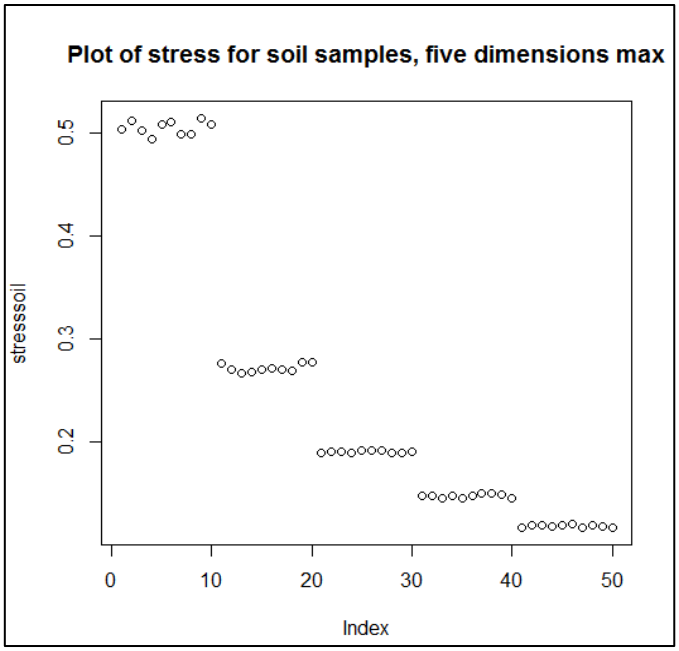

Figure 2.32. NMDS plot of stress for soil samples collected in summer 2013 at Snook, Texas (Stress test 0.1886 with $r^2 = 0.8455$).

Table 2.16. Microbial metabolic community profiles for soil samples in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F model	P value
Day	1	2.7671	0.004*
Treatment	2	1.2751	0.152
Region	2	6.7900	0.001*
Day x Treatment	2	0.8308	0.687
Day x Region	2	2.3092	0.001*
Treatment x Region	4	1.2551	0.105
Day x Treatment x Region	4	0.6284	0.975

Table 2.17. Pairwise comparisons of MMCPs between Regions of soil samples in summer 2013 at Snook, Texas after Bonferroni's correction.

Factor		df	SS	MS	F Model	R^2	P value
Beneath x	Region	1	0.3329	0.3329	5.3615	0.0574	0.001*
Lateral	Residual	88	5.4645	0.0621		0.9425	
	Total	89	5.7975			1.0000	
Beneath x 5	Region	1	0.6957	0.6957	9.1886	0.0945	0.001*
meter	Residual	88	6.6631	0.0757		0.9054	
	Total	89	7.3588			1.0000	
Lateral x 5	Region	1	0.2796	0.2796	4.5918	0.0495	0.001*
meter	Residual	88	5.3586	0.0609		0.9504	
	Total	89	5.6382			1.0000	

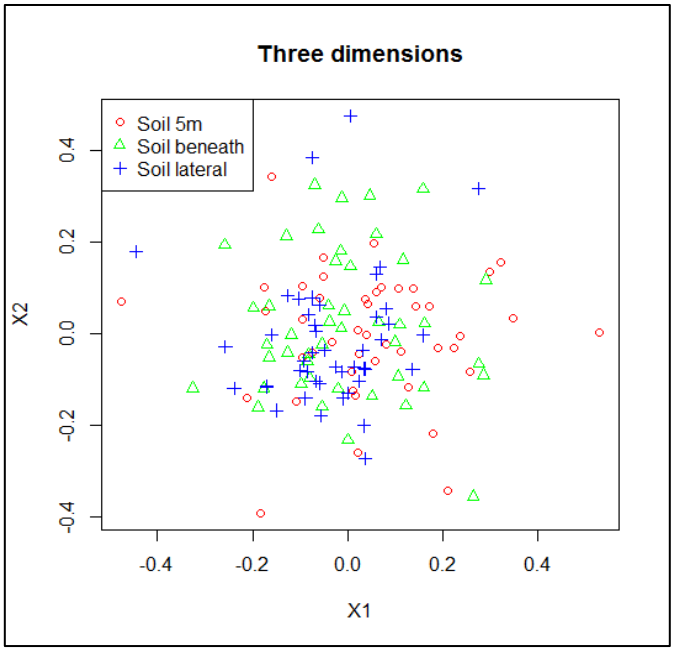

Figure 2.33. NMDS ordinations of normalized soil microbial community activity by soil regions in summer 2013 at Snook, Texas.

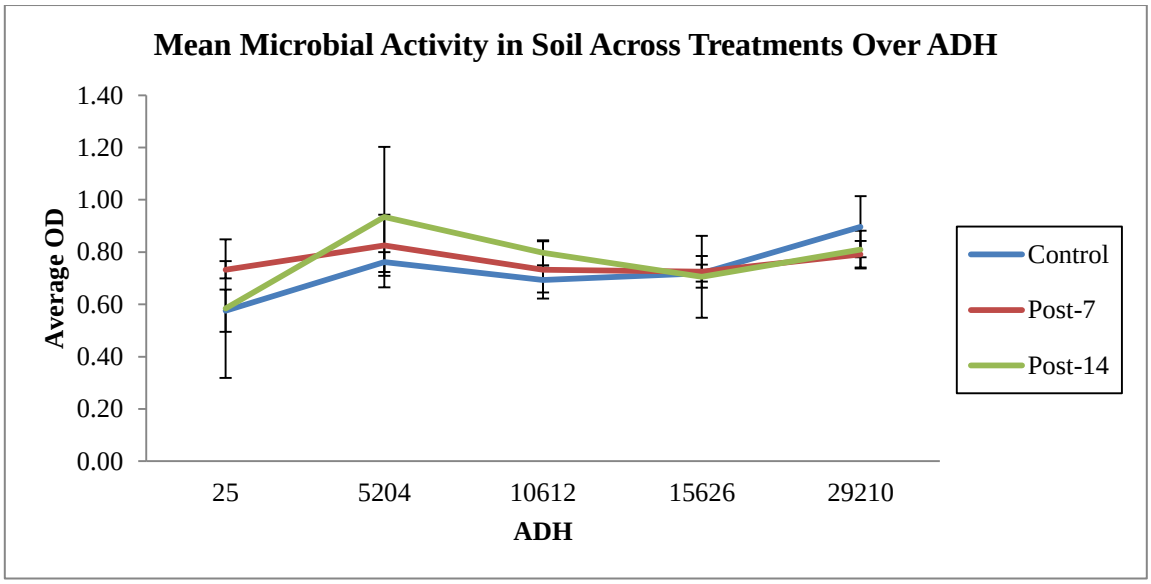

Figure 2.34. Mean microbial function (average OD) in soil samples (soil beneath and soil lateral) over Accumulated Degree Hour (ADH) during summer 2013 at Snook, Texas.

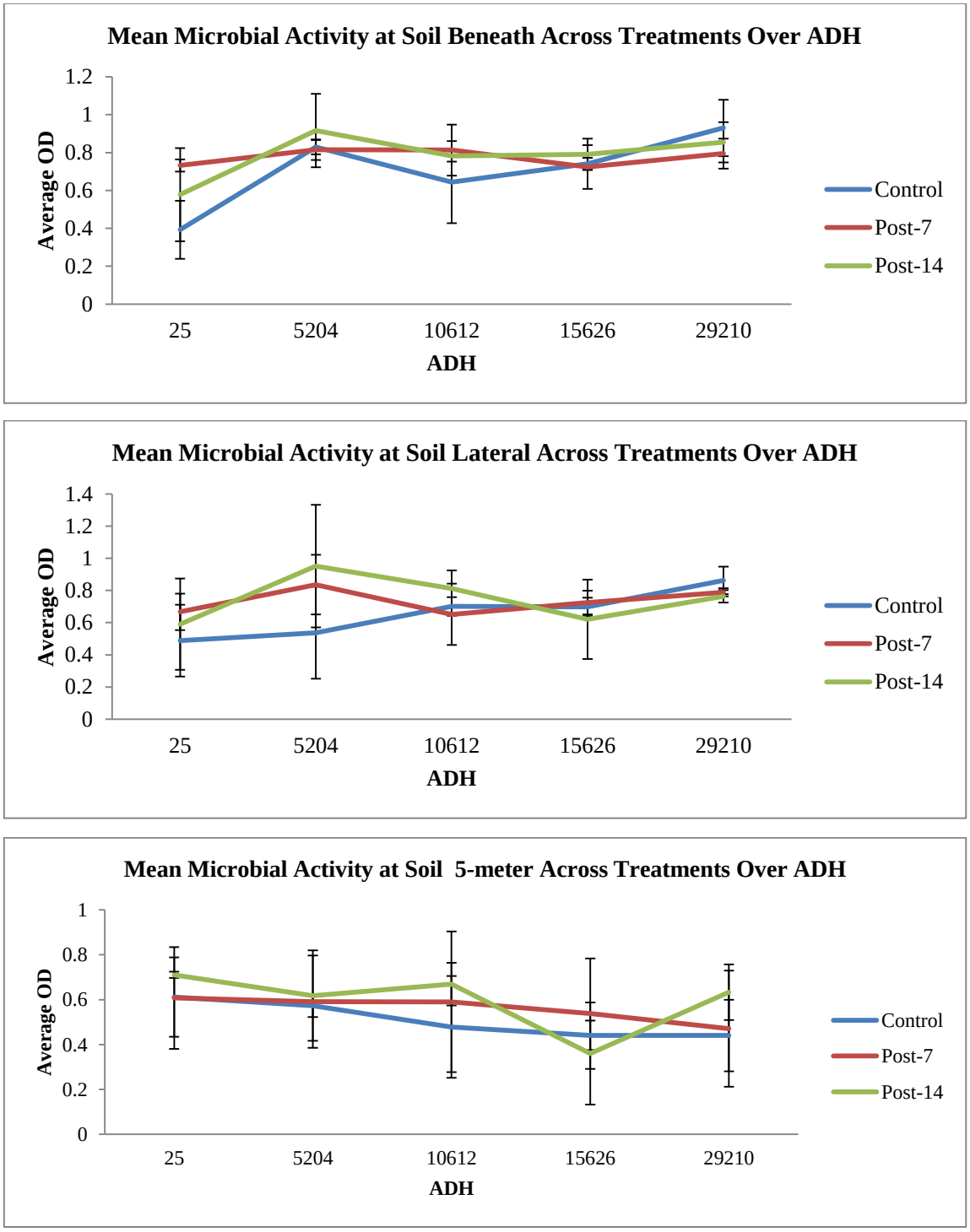

Figure 2.35. Mean microbial function across treatments over ADH in soil samples according to soil regions in summer 2013 at Snook, Texas. Above. Soil beneath. Center. Soil lateral. Bottom. Soil 5 meter.

Microbial function was significantly different between days in soil samples. MRPP analysis rendered A value of 0.04343 and Significant of Delta 0.001 based on 999 permutations. PERMANOVA was performed and results were showed in Table 2.18 Figure 2.36 showed the NMDS plot of microbial function in soil samples by Days. Minimum stress value was 0.1886 and r^2 for minimum stress configuration was 0.8453.

Table 2.18. Pairwise comparisons of microbial function between Days on soil samples in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40
0	-	0.01*	0.024*	0.002*	0.002*
7	0.01*	-	0.258	0.023*	0.171
14	0.024*	0.258	-	0.380	0.459
21	0.002*	0.023*	0.380	-	0.315
40	0.002*	0.171	0.459	0.315	-

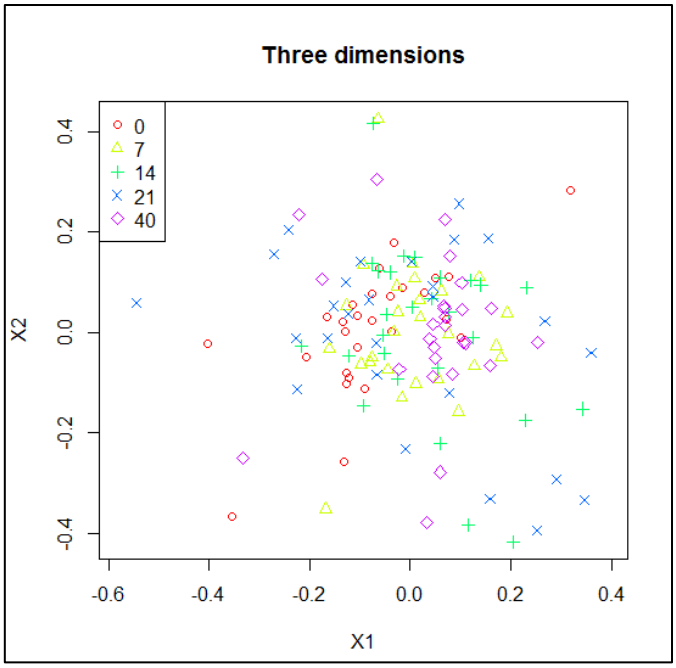

Figure 2.36. NMDS ordinations of normalized microbial function in soil samples by carrion decomposition days in summer 2013 at Snook, Texas.

Indicator carbon source in soil samples 2013

When all soil samples were pooled together, ISA results showed only two carbons that were indicative namely Itaconic acid and Glucose-1-phosphate. The regions of soil and days were significant difference when compared with microbial metabolic community profiles. Hence, indicator carbon source was compared between regions of soil and days. For soil regions, there was two carbon sources, Glucose-1-phosphate and Itaconic acid, were indicative for soil beneath, soil lateral and soil 5-meter. A summary of carbon indicators associated with soil regions and days is shown at Table 2.19. ISA was conducted on specific soil region with specific day, and the result summary is exhibited at Table 2.20.

Table 2.19. Indicator carbon analysis based on MMCPs for soil samples in summer 2013 at Snook, Texas.

Factor	Carbon source	Indicator value	p value
All soil samples	Itaconic acid	0.4197	0.009*
	Glucose-1-phosphate	0.3942	0.015*
Soil Beneath	Itaconic acid	0.4197	0.011*
	Glucose-1-phosphate	0.3942	0.019*
Soil Lateral	Itaconic acid	0.4197	0.013*
	Glucose-1-phosphate	0.3942	0.017*
Soil 5 meter	Itaconic acid	0.4197	0.010*
	Glucose-1-phosphate	0.3942	0.011*
Day 0	Tween 80	0.4207	0.029*
Day 7	D-galacturonic acid	0.3959	0.039*
	N-acetyl-D-glucosamine	0.3758	0.045*
	α-Cyclodextrin	0.5057	0.050*
Day 14	α-Cyclodextrin	0.5728	0.023*
	Tween-40	0.4476	0.050*
Day 21	Tween 40	0.4374	0.029*
Day 40	Nil	Nil	Nil

Nil = No carbon source indicator.

Table 2.20. Indicator carbon analysis based on MMCPs for soil Regions according to Days in summer 2013 at Snook, Texas.

Region by Day	Soil Beneath	Soil Lateral	Soil 5 meter
Day 0	Putrescine Tween 40 Tween 80	Itaconic acid D-galactonic acid γ-lactone	2-hydroxy benzoic acid Glucose-1-phosphate D-cellobiose
Day 7	Putrescine Tween 40 Tween 80	D-galactonic acid γ-lactone	2-hydroxy benzoic acid Glucose-1-phosphate D-cellobiose
Day 14	Putrescine Tween 40	Itaconic acid D-galactonic acid γ-lactone	2-hydroxy benzoic acid Glucose-1-phosphate D-cellobiose
Day 21	Putrescine Tween 40 Glycyl-L-glutamate acid	Itaconic acid D-galactonic acid γ-lactone	2-hydroxy benzoic acid Glucose-1-phosphate D-cellobiose
Day 40	Putrescine Tween 40 Glycyl-L-glutamate acid	Itaconic acid D-galactonic acid γ-lactone	2-hydroxy benzoic acid Glucose-1-phosphate D-cellobiose

Summer 2014

A total of 540 samples were collected in summer 2014 for MMCP analysis (i.e., 270 swabs from pig carrion and 270 soil samples). Stress test (0.1669, r^2 = 0.9106) indicated a great representation in reduced dimensions. When all data (pig and soil samples) was pooled together, the results indicated Day, Treatment, and Region were significantly difference ($P < 0.05$). Table 2.21 showed the statistical results for the overall microbial metabolic activity in summer 2014. Another two variables were added in the analysis namely Biomass and Type of Sample (pig vs soil). Statistical results showed MMCPs had no significant difference with biomass of pig carrion (df = 6; r^2 = 0.0213; p = 0.429). However, there was significant difference in MMCPs between pig and soil samples (df = 1, r^2 = 0.0915; p = 0.001).

Table 2.21. Analysis of the overall microbial metabolic community profiles for pig and soil samples in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F model	P value
Day	1	5.0880	0.001*
Treatment	2	2.4754	0.002*
Region	5	7.7599	0.001*
Day x Treatment	2	1.3519	0.134
Day x Region	5	1.2512	0.128
Treatment x Region	10	1.0938	0.268
Day x Treatment x Region	10	0.6849	0.988

Pig samples 2014

Microbial samples collected from oral, skin and anus of pig samples were processed for MMCPs analysis. Stress test (0.1946, r^2 = 0.8179) indicated a good representation in reduced dimension. Figure 2.37 showed the NMDS plot of stress for all

pig samples in summer 2014. PERMANOVA was employed to test the difference. The results demonstrated Treatment ($p = 0.001$), Day ($p = 0.003$) and Region ($p = 0.006$) were significantly difference. Multi Response Permutation Procedure (MRPP) was then employed to test whether there is a significant difference between two or more groups of sampling units. The results showed there was significant difference between Treatment ($A = 0.0105$; Significant of Delta = 0.003 based on 999 permutations); Day ($A = 0.0673$; Significant of Delta = 0.001) and Region ($A = 0.0123$; Significant of Delta = 0.003). Table 2.22 showed the statistical results on pig samples in summer 2014. Figure 2.38 demonstrated the NMDS ordination by Treatments for pig samples. However, no distinctive pattern was observed through this ordination, although Control and Post-14 group was significant difference.

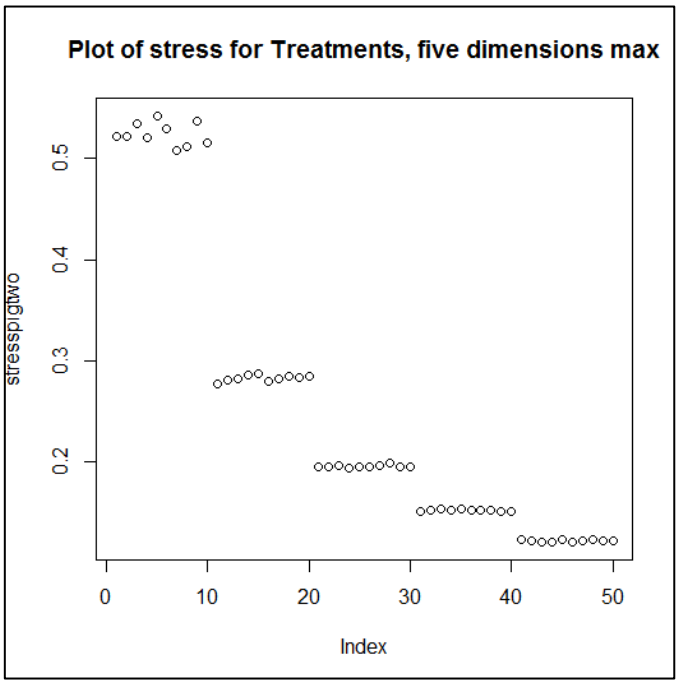

Figure 2.37. NMDS plot of stress for pig samples collected in summer 2014 at Snook, Texas (Stress test 0.1669, $r^2 = 0.9106$).

Table 2.22. Microbial metabolic community profiles for pig samples in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F model	P value
Treatment	2	2.2801	0.001*
Day	1	2.9223	0.003*
Region	2	2.1124	0.006*
Treatment x Day	2	1.2285	0.212
Treatment x Region	4	0.7982	0.845
Day x Region	2	1.0749	0.347
Treatment x Day x Region	4	0.5229	0.997

Pairwise comparisons with Bonferroni correction using PERMANOVA were performed to test the MMCPs between treatments on pig samples collected in summer 2014. The results demonstrated that Control x Post-14 was significantly difference with $p = 0.001$. However, there were no significant difference in Control x Post-7 and Post-7 x Post-14 ($p = 0.09$ and 0.171, respectively). Table 2.23 showed the statistical results for the comparison between Treatments. Mean microbial function on pig carcasses over ADH was plotted in Figure 2.39, as well as mean microbial function by different regions of pig carrion (Figure 2.40)

Table 2.23. Pairwise comparisons of MMCPs between Treatments on pig samples in summer 2014 after Bonferroni correction.

Factor			df	SS	MS	F Model	R^2	P value
Control	x	Treatment	1	0.3031	0.3031	1.6183	0.0181	0.09•
Post-7		Residual	88	16.4850	0.1873		0.9819	
		Total	89	16.7882			1.0000	
Control	x	Treatment	1	0.6888	0.6888	3.8091	0.0415	0.001*
Post-14		Residual	88	15.9133	0.1808		0.9585	
		Total	89	16.6022			1.0000	
Post-7	x	Treatment	1	0.2632	0.2632	1.3792	0.0154	0.171
Post-14		Residual	88	16.7933	0.1908		0.9846	
		Total	89	17.0565			1.0000	

• Marginal significant difference.

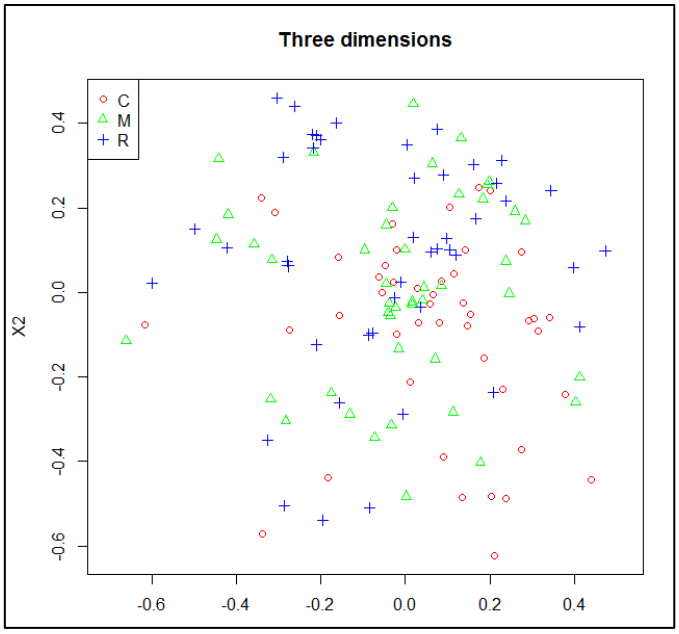

Figure 2.38. NMDS ordinations of normalized pig carcass microbial community activity by Treatments in summer 2014 at Snook, Texas (C = Control; M = Post-7; R = Post-14).

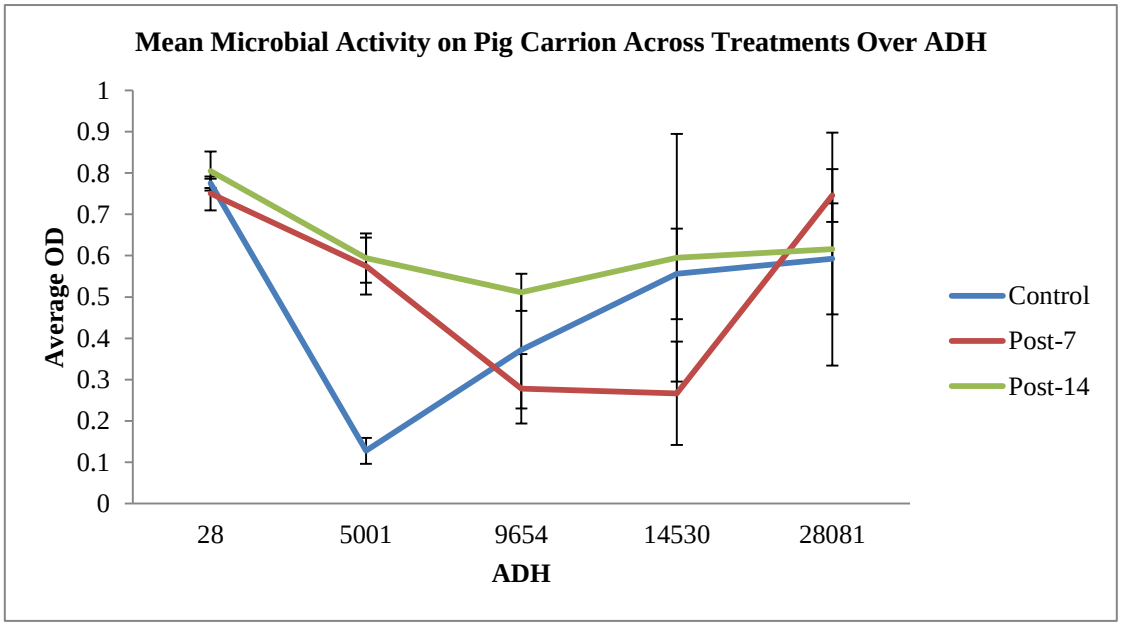

Figure 2.39. Mean microbial function (average OD) on pig carcasses (oral, skin, and anal samples) across treatments over ADH in summer 2014 at Snook, Texas.

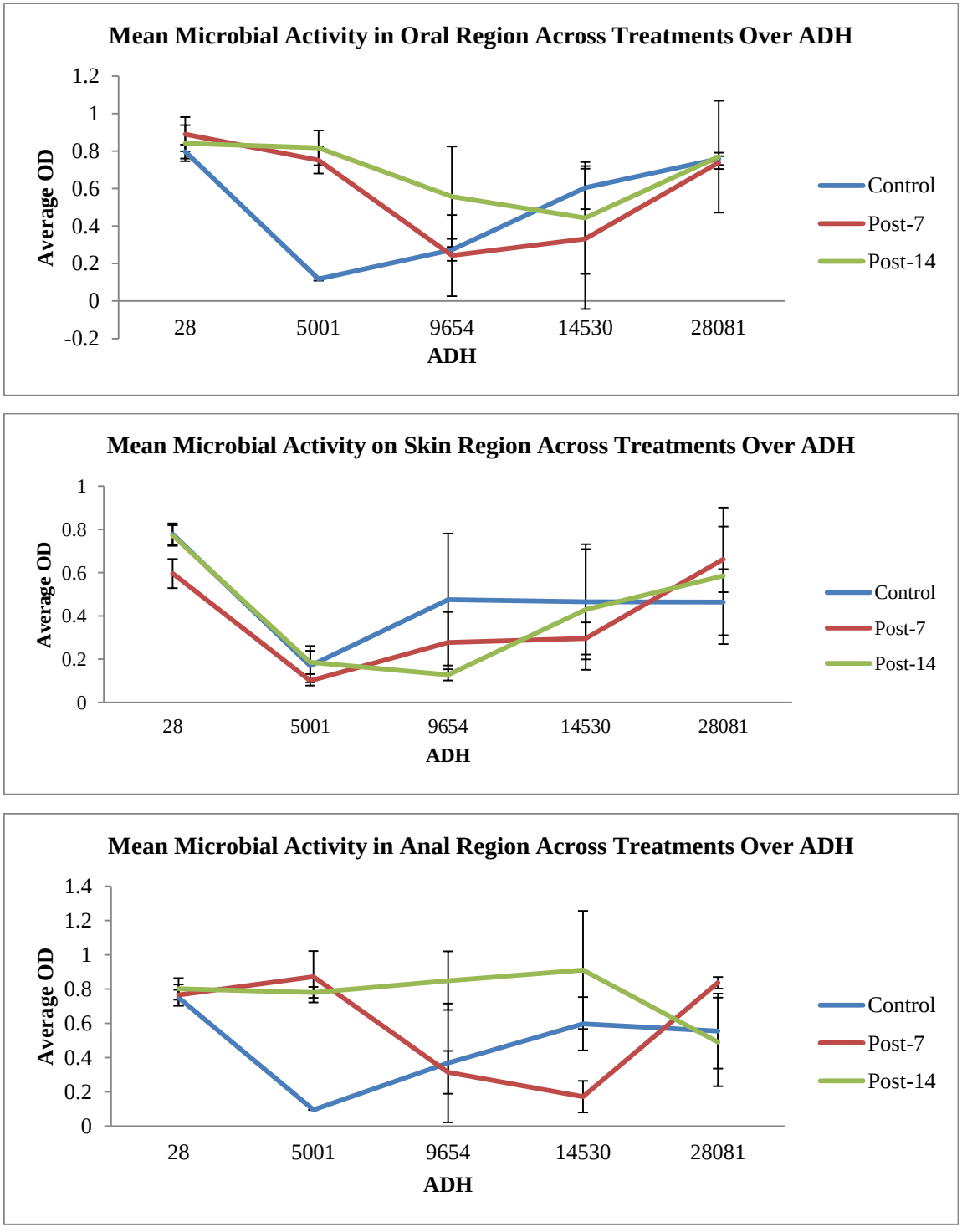

Figure 2.40. Mean microbial function (average OD) on pig carcasses by regions across treatments over ADH in summer 2014 at Snook, Texas. Above. Oral region. Center. Skin region. Bottom. Anal region.

The MMCPs on pig samples by Day was significant difference ($p = 0.003$), this indicated that microbial function was differed over time. This observation was strengthened by the analysis by MRPP which showed A value 0.06735 with Significant of Delta 0.001. Pairwise comparisons with Bonferroni correction using PERMANOVA were performed to test the MMCPs between Days on pig samples collected in summer 2014. The results demonstrated that Day 0 x Day 7, Day 0 x Day 14, Day 0 x Day 21, Day 0 x Day 40, Day 7 x Day 40, Day 14 x Day 40, and Day 21 x Day 40, were significantly difference ($p < 0.05$). However, there was no significant difference in Day 7 x Day 14, Day 7 x Day 21, and Day 14 x Day 21. Table 2.24 showed the statistical results for the comparison between Treatments. Table 2.25 showed the summary of pairwise comparisons of microbial function between Days on pig samples after Bonferroni's correction. Figure 2.41 showed the NMDS ordination by Day for pig samples. There were separations among the data points, especially Day 0 with Day 7, 14, 21 and 40, where all these data points were significant difference ($p < 0.05$) from each other.

Table 2.24. Pairwise comparisons of MMCPs between Days on pig samples in summer 2014 at Snook, Texas after Bonferroni correction.

Factor		df	SS	MS	F Model	R^2	P value
Day 0 x Day 7	Day	1	1.2384	1.2383	8.0157	0.1336	0.001*
	Residual	52	8.0337	0.1544		0.8664	
	Total	53	9.2721			1.0000	
Day 0 x Day 14	Day	1	1.0574	1.0574	7.8811	0.1316	0.001*
	Residual	52	6.9768	0.1341		0.8684	
	Total	53	8.0342			1.0000	
Day 0 x Day 21	Day	1	0.7949	0.7949	6.2006	0.1065	0.001*
	Residual	52	6.6663	0.1282		0.8935	
	Total	53	7.4612			1.0000	
Day 0 x Day 40	Day	1	0.5273	0.5272	6.04	0.1041	0.001*
	Residual	52	4.5394	0.0873		0.8959	
	Total	53	5.0666			1.0000	
Day 7 x Day 14	Day	1	0.296	0.2960	1.2132	0.0228	0.245
	Residual	52	12.689	0.2440		0.9772	
	Total	53	12.985			1.0000	
Day 7 x Day 21	Day	1	0.4113	0.4112	1.7276	0.0322	0.071•
	Residual	52	12.3785	0.2380		0.9678	
	Total	53	12.7898			1.0000	
Day 7 x Day 40	Day	1	0.9914	0.9913	5.0285	0.0882	0.001*
	Residual	52	10.2516	0.1971		0.9118	
	Total	53	11.2430			1.0000	

Table 2.24 (Continued).

Factor		df	SS	MS	F Model	R2	P value
Day 14 x Day 21	Day	1	0.3351	0.3350	1.5389	0.0287	0.113
	Residual	52	11.3216	0.2177		0.9713	
	Total	53	11.6566			1.0000	
Day 14 x Day 40	Day	1	0.6355	0.6355	3.5942	0.0646	0.001*
	Residual	52	9.1947	0.1768		0.9354	
	Total	53	9.8302			1.0000	
Day 21 x Day 40	Day	1	0.4595	0.4595	2.6896	0.0492	0.006*
	Residual	52	8.8842	0.1708		0.9508	
	Total	53	9.3437			1.0000	

• Marginal significant difference.

Table 2.25. Summary of pairwise comparisons of microbial function between Days on pig samples in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40
0	-	0.001*	0.001*	0.001*	0.001*
7	0.001*	-	0.245	0.071	0.001*
14	0.001*	0.245	-	0.113	0.001*
21	0.001*	0.071	0.113	-	0.006*
40	0.001*	0.001*	0.001*	0.006*	-

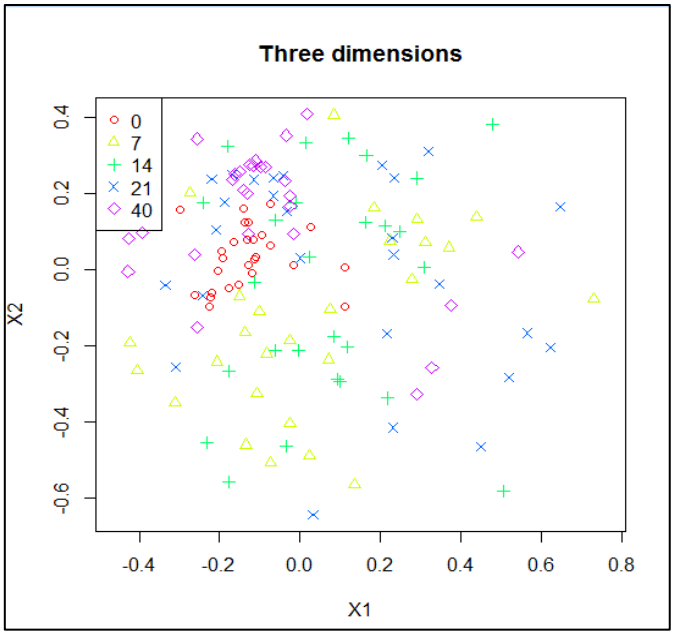

Figure 2.41. NMDS ordinations of normalized pig carcass microbial community activity by carrion decomposition days in summer 2014 at Snook, Texas.

Other than Treatment effect, Regions (oral, skin, and anal samples) were shown significantly different ($p < 0.05$). MRPP analysis was conducted on regions on pig samples and A value showed 0.0123 with Significant of Delta of 0.003. Pairwise comparisons using PERMANOVA were performed to test the MMCPs between regions on pig samples. The results demonstrated that Oral x Anal was not significant difference from each other ($p = 0.217$) while Oral x Skin and Skin x Anal were significant difference ($p = 0.009$ and 0.004, respectively). Table 2.26 showed the statistical results for the comparison between Regions. Mean microbial function activity (average Optical Density, OD) on pig samples was plotted according to Regions (Figure 2.42).

Table 2.26. Pairwise comparisons of MMCPs between Regions on pig samples in summer 2014 at Snook, Texas after Bonferroni's correction.

Factor		df	SS	MS	F Model	R^2	P value
Oral x Skin	Region	1	0.4643	0.4643	2.4463	0.0270	0.009*
	Residual	88	16.7043	0.1898		0.9730	
	Total	89	17.1686			1.0000	
Oral x Anal	Region	1	0.2206	0.2206	1.268	0.0142	0.217
	Residual	88	15.3105	0.1739		0.9858	
	Total	89	15.5312			1.0000	
Skin x Anal	Region	1	0.4779	0.4779	2.431	0.0269	0.004*
	Residual	88	17.2999	0.1965		0.9731	
	Total	89	17.7778			1.0000	

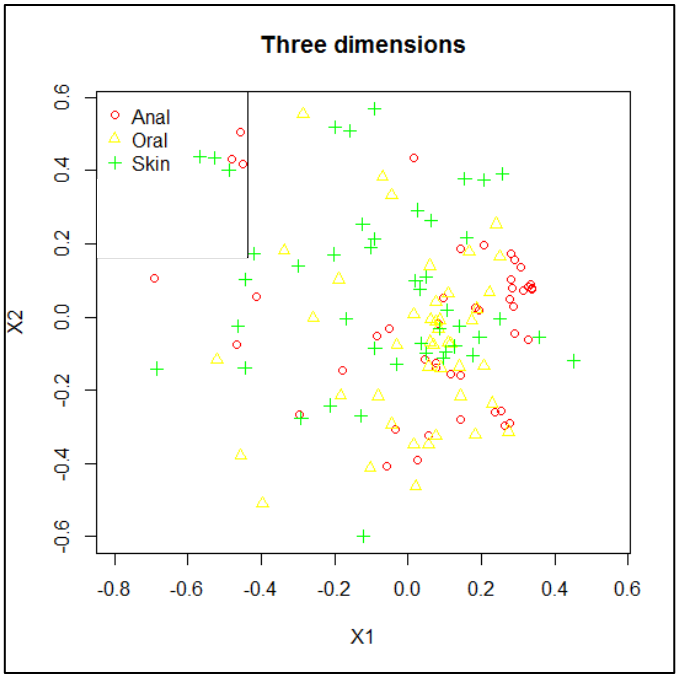

Figure 2.42. NMDS ordination of normalized pig carcass microbial community activity according to pig regions (oral, skin and anal) in summer 2014 at Snook, Texas.

Indicator carbon source on pig samples 2014

In summer 2014, two carbon substrates were indicative when all data (pig and soil samples) were pooled together namely Glucose-1-phosphate and putrescine. As for pig carcasses, there was no indicator carbon present at oral, skin and anus region. Among the treatments, Control x Post-7 and Control x Post-14 were significantly difference by microbial function, hence, carbon indicator analysis was performed on these groups. The results showed two carbon subtracts that were important for Control x Post-7 namely Glucose-1-phosphate and Putrescine. For Control x Post-14 group, seven carbon substrates were the significant indicators (Table 2.27). Comparison of carbon indicators between Days is shown in Table 2.28.

Table 2.27. Indicator carbon analysis based on MMCPs for pig samples in summer 2014 at Snook, Texas.

Factor	Carbon source	Indicator value	P value
All samples (pig + soil samples)	Glucose-1-phosphate	0.4312	0.009*
	Putrescine	0.3831	0.005*
Pig samples (oral + skin + anal)	Nil	Nil	Nil
Oral samples	Nil	Nil	Nil
Skin samples	Nil	Nil	Nil
Anal samples	Nil	Nil	Nil
Control x Post-7 (Pig samples)	Putrescine	0.5613	0.020*
	D-Galactonic acid γ-lactone	0.5406	0.049*
Control x Post-14 (Pig samples)	Tween 40	0.7334	0.002*
	Tween 80	0.6725	0.001*
	D-Galactonic acid γ-lactone	0.5585	0.003*
	Putrescine	0.5419	0.034*
	Itaconic acod	0.5228	0.036*
	α-Cyclodextrine	0.6582	0.026*
	α-D-Lactose	0.5925	0.043*
Post-7 x Post-14 (Pig samples)	Tween 40	0.6527	0.027*
	Tween 80	0.6244	0.014*
	D-Galacturonic acid	0.5793	0.001*

Table 2.27 (Continued).

Factor	Carbon source	Indicator value	P value
Control x Post-7 x Post-14	Glucose-1-phosphate	0.4483	0.034*
	Putrescine	0.4149	0.010*
	D-Galacturonic acid	0.3517	0.050*

Table 2.28. Indicator carbon analysis based on MMCPs for pig samples according to carrion decomposition day in summer 2014 at Snook, Texas.

Factor	Carbon source	Indicator value	P value
Day 0	Putrescine	0.4869	0.019*
	L-Threonine	0.7120	0.005*
Day 7	Nil	Nil	Nil
Day 14	4-Hydroxy benzoic acid	0.5194	0.025*
	D-Galacturonic acid	0.5888	0.036*
	L-Serine	0.5491	0.029*
Day 21	D-Galacturonic acid	0.7362	0.001*
	Tween 80	0.5983	0.007*
	D-Glucosaminic acid	0.5707	0.007*
	L-Asparagine	0.5236	0.032*
	α-D-Lactose	0.7479	0.001*
Day 40	D-Galactonic acid γ-lactone	0.5484	0.013*
	Glycogen	0.5105	0.018*
	D-Cellobiose	0.6352	0.011*
	γ-Hydroxybutyric acid	0.9025	0.006*
	α-Clycodextrine	0.8269	0.019*

Soil samples 2014

Soil samples in summer 2014 were analyzed using NMDS and the minimum stress result rendered was 0.1763 with r^2 = 0.8669, which indicated a great representation in reduced dimensions. Figure 2.43 showed the NMDS plot of stress for soil samples for summer 2014. PERMANOVA was then performed on soil microbial function data and the results showed Day, Treatment, and Region was significant difference ($p < 0.05$). Table 2.29 showed the statistical results using PERMANOVA with 999 permutations on soil samples. The results were then analyzed by using MRPP and similar result was obtained where Day was significant difference (A= 0.0468; Significant of Delta = 0.001), Region was significantly difference with A= 0.0388 and Significant of Delta= 0.001) as well as Treatment (A= 0.0055; Significant of Delta= 0.027). Pairwise comparisons using PERMANOVA with Bonferroni's correction were then performed on Day, Treatment, and Region. For Day, all pairs of comparison were significant difference ($p < 0.05$) except Day 7 x Day 14 and Day 21 x Day 40 (Table 2.30) Table 2.31 provided a summary of day by day comparison for soil microbial function. For Treatment, Control x Post-7 and Post-7 x Post-14 were significant difference ($p = 0.028$ and 0.036, respectively) while Control x Post-14 was not significant difference ($p = 0.08$), although it is marginally significant different (Table 2.32). For Region, the results demonstrated soil beneath x soil lateral, soil beneath x soil 5 meter, and soil lateral x soil 5 meter, were significant difference ($p < 0.05$) (Table 2.33). Figure 2.44 exhibited the NMDS ordinations of soil samples by regions. Mean microbial function in soil samples (beneath and lateral only) over ADH in summer 2014 was demonstrated in Figure 2.45. Moreover, mean microbial functions according to soil regions (beneath, lateral and 5 meter) were provided in Figure 2.46. Figure 2.47 showed NMDS ordinations of normalized soil microbial community activity by Days and Figure 2.48 exhibited the NMDS ordinations of soil microbial community function by Treatments.

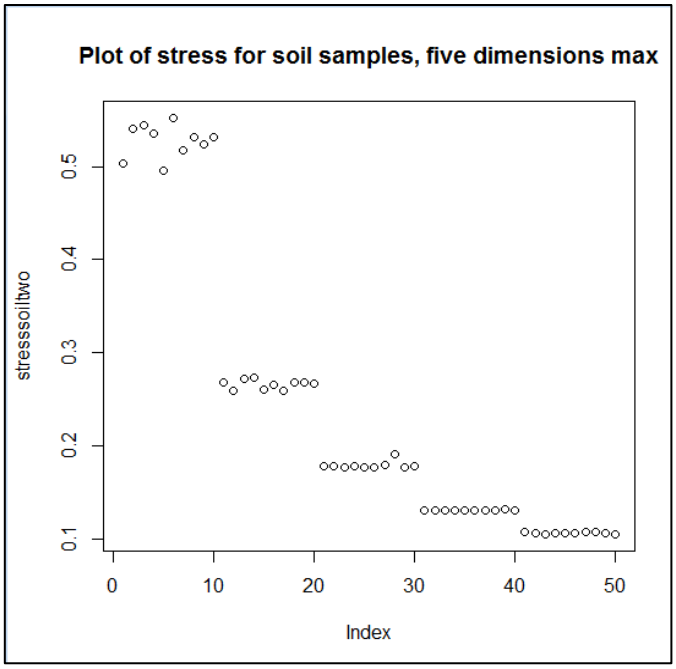

Figure 2.43. NMDS plot of stress for soil samples collected in summer 2014 at Snook, Texas (Stress test 0.1763 with $r^2 = 0.8669$).

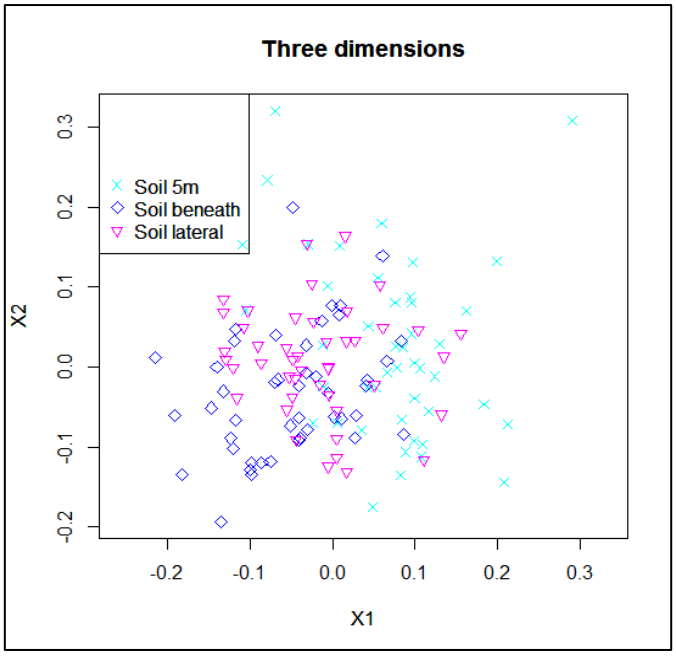

Figure 2.44. NMDS ordinations of normalized soil microbial community activity by soil regions in summer 2014 at Snook, Texas.

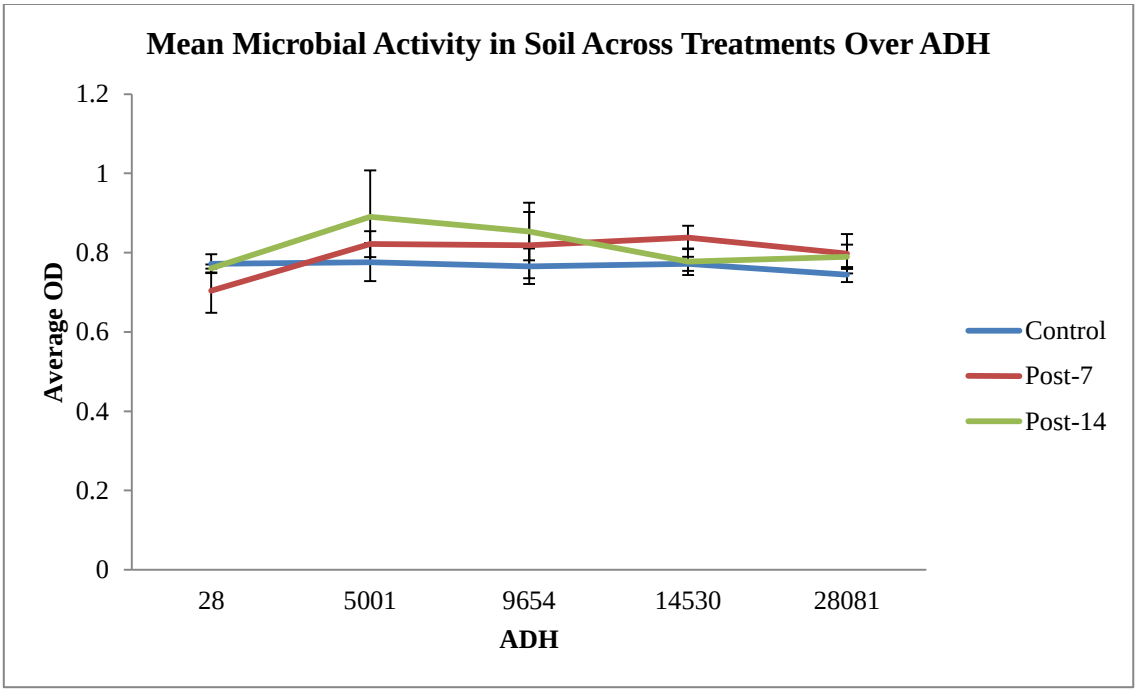

Figure 2.45. Mean microbial function (average OD) in soil samples (soil beneath and lateral) across treatments over ADH in summer 2014 at Snook, Texas.

Table 2.29. Microbial metabolic community profiles for soil samples in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F model	P value
Day	1	8.3016	0.001*
Treatment	2	2.1860	0.002*
Region	2	6.9527	0.001*
Day x Treatment	2	1.4915	0.083•
Day x Region	2	1.2426	0.235
Treatment x Region	4	1.3009	0.084•
Day x Treatment x Region	4	0.9332	0.568

• Marginal significant difference.

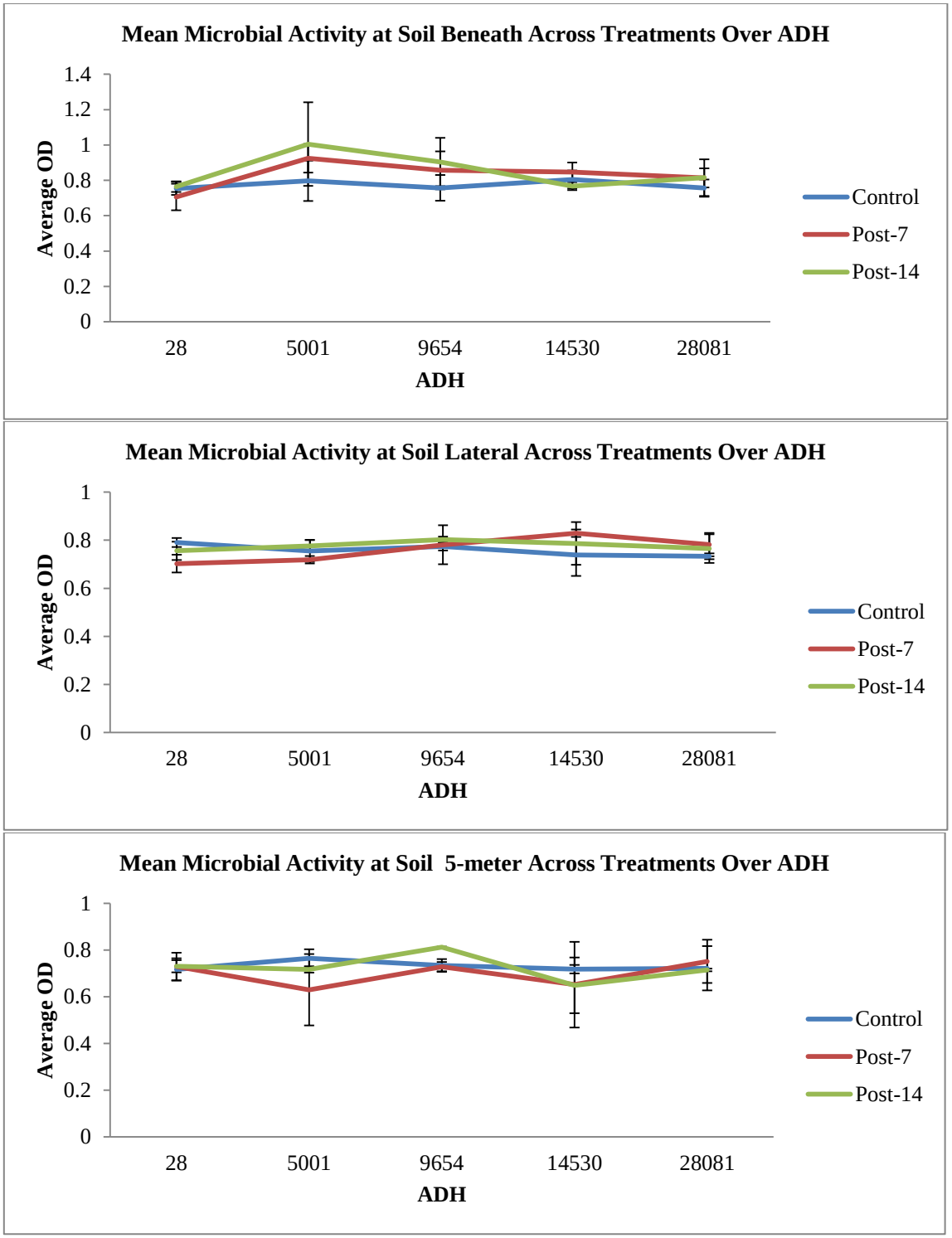

Figure 2.46. Mean microbial function (average OD) in soil sample by regions across treatments over ADH in summer 2014 at Snook, Texas. Above. Soil beneath, Center. Soil lateral. Bottom. Soil 5 meter.

Table 2.30. Pairwise comparisons of MMCPs between carrion decomposition days for soil samples in summer 2014 at Snook, Texas after Bonferroni correction.

Factor		df	SS	MS	F Model	R^2	P value
Day 0 x Day 7	Day	1	0.0992	0.0992	4.8464	0.0853	0.001*
	Residual	52	1.0645	0.0204		0.9147	
	Total	53	1.1637			1.0000	
Day 0 x Day 14	Day	1	0.1131	0.1131	6.0953	0.1049	0.001*
	Residual	52	0.9649	0.0185		0.8951	
	Total	53	1.0780			1.0000	
Day 0 x Day 21	Day	1	0.1250	0.1250	5.566	0.0967	0.001*
	Residual	52	1.1683	0.0224		0.9033	
	Total	53	1.2934			1.0000	
Day 0 x Day 40	Day	1	0.1560	0.1560	7.7354	0.1295	0.001*
	Residual	52	1.0487	0.0201		0.8705	
	Total	53	1.2047			1.0000	
Day 7 x Day 14	Day	1	0.0199	0.0199	0.8420	0.0159	0.583
	Residual	52	1.2300	0.0236		0.9841	
	Total	53	1.2499			1.0000	
Day 7 x Day 21	Day	1	0.0681	0.0681	2.4723	0.0454	0.003*
	Residual	52	1.4334	0.0275		0.9546	
	Total	53	1.5016			1.0000	

Table 2.30. (Continued).

Factor		df	SS	MS	F Model	R2	P value
Day 7 x Day 40	Day	1	0.1295	0.1295	5.1275	0.0897	0.001*
	Residual	52	1.3138	0.0252		0.9102	
	Total	53	1.4433			1.0000	
Day 14 x Day 21	Day	1	0.0521	0.0512	2.032	0.0376	0.013*
	Residual	52	1.3338	0.0256		0.9624	
	Total	53	1.3859			1.0000	
Day 14 x Day 40	Day	1	0.0921	0.0921	3.9447	0.0705	0.001*
	Residual	52	1.2141	0.0233		0.9294	
	Total	53	1.3062			1.0000	
Day 21 x Day 40	Day	1	0.0277	0.0276	1.0159	0.0192	0.432
	Residual	52	1.4176	0.0272		0.9808	
	Total	53	1.4453			1.0000	

Table 2.31. Summary of pairwise comparisons of microbial function between carrion decomposition days for soil samples in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40
0	-		0.001*	0.001*	0.001*
7	0.001*	-	0.583	0.003*	0.001*
14	0.001*	0.583	-	0.013*	0.001*
21	0.001*	0.003*	0.013*	-	0.432
40	0.001*	0.001*	0.001*	0.432	-

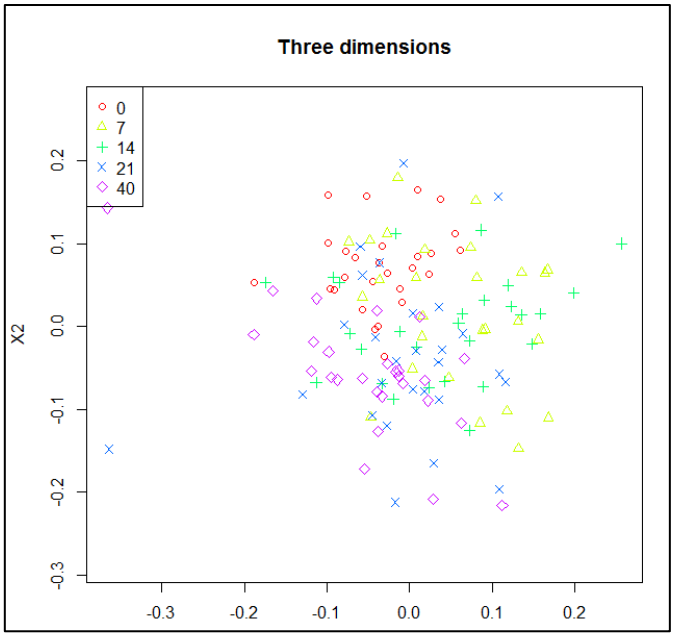

Figure 2.47. NMDS ordinations of normalized soil microbial community activity by carrion decomposition days in summer 2014 at Snook, Texas.

Table 2.32. Pairwise comparisons of MMCPs between Treatments on soil samples in summer 2014 at Snook, Texas after Bonferroni correction.

Factor			df	SS	MS	F Model	R^2	P value
Control	x	Treatment	1	0.0510	0.0510	2.0118	0.0224	0.028*
Post-7		Residual	88	2.2323	0.0253		0.9776	
		Total	89	2.2834			1.0000	
Control	x	Treatment	1	0.0343	0.0343	1.6123	0.0180	0.080*
Post-14		Residual	88	1.8760	0.0213		0.9820	
		Total	89	1.9104			1.0000	
Post-7	x	Treatment	1	0.0557	0.0557	1.9589	0.0218	0.036*
Post-14		Residual	88	2.5044	0.0284		0.9782	
		Total	89	2.5601			1.0000	

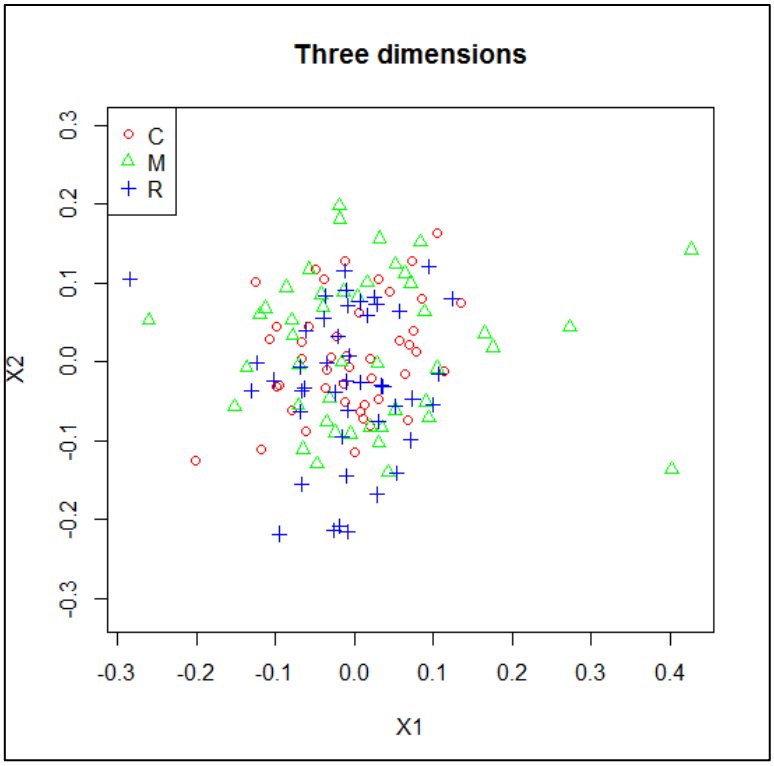

Figure 2.48. NMDS ordinations of normalized soil microbial community activity by Treatments in summer 2014 at Snook, Texas. C denotes Control, M denotes Post-7 and R denotes Post-14 carcasses.

Table 2.33. Pairwise comparisons of MMCPs between Regions of soil samples in summer 2014 at Snook, Texas after Bonferroni correction.

Factor		df	SS	MS	F Model	R^2	P value
Beneath x Later	Treatment	1	0.0762	0.0762	3.8864	0.0423	0.002*
	Residual	88	1.7262	0.0196		0.9577	
	Total	89	1.8025			1.0000	
Beneath x 5 meter	Treatment	1	0.2514	0.2514	9.2972	0.0956	0.001*
	Residual	88	2.3798	0.0270		0.9044	
	Total	89	2.6313			1.0000	
Lateral x 5 meter	Treatment	1	0.1212	0.1212	5.0915	0.0547	0.001*
	Residual	88	2.0962	0.0238		0.9453	
	Total	89	2.2175			1.0000	

Indicator carbon source in soil samples 2014

When all soil samples were pooled together, ISA results showed seven carbons that were indicative namely Glucose-1-phosphate, D-L-Glycerol phosphate, D-Malic acid, D-Xylose, 4-Hydroxy benzoic acid, Phenylethylamine, and 2-Hydroxy benzoic acid. The regions of soil were significant difference ($p = 0.001$) when compared by their microbial metabolic community profiles. Thus, indicator carbon analysis was performed to compare differences within and between regions of soil. For within region, seven carbon species were indicative for soil beneath, eight carbon species for soil lateral and seven carbon species for soil 5 meter, although many carbon species were repetitive. As for the comparison between regions, soil beneath x soil 5 meter had four indicative carbons, namely D-Malic acid, α-Cyclodextrin, Phenylethylamine, and 4-Hydroxy benzoic acid. Four carbon species were indicative for soil beneath x soil lateral and three carbon species for soil lateral x soil 5 meter (Table 2.34). Similarly, indicator carbon sources between Days were provided in Table 2.35 and Table 2.36 demonstrated the indicator carbon sources by Treatment.

Table 2.34. Indicator carbon analysis based on MMCPs for soil samples by Regions in summer 2014 at Snook, Texas.

Factor	Carbon source	Indicator value	P value
All soil samples	Glucose-1-phosphate	0.3767	0.025*
	D-L-Glycerol phosphate	0.3716	0.024*
	D-Malic acid	0.4277	0.006*
	D-Xylose	0.3664	0.041*
	4-Hydroxy benzoic acid	0.3819	0.011*
	Phenylethylamine	0.3718	0.018*
	2-Hydroxy benzoic acid	0.2398	0.023*
Beneath x 5 meter	D-Malic acid	0.4686	0.005*
	α-Cyclodextrin	0.4323	0.036*
	Phenylethylamine	0.3985	0.020*
	4-Hydroxy benzoic acid	0.3928	0.014*
Beneath x Lateral	D-L-Glycerol phosphate	0.3907	0.005*
	β-Methyl-D-Glucoside	0.3638	0.011*
	4-Hydroxy benzoic acid	0.3895	0.009*
	2-Hydroxy benzoic acid	0.3261	0.009*
Lateral x 5 meter	Glucose-1-phosphate	0.3751	0.031*
	D-Malic acid	0.4186	0.024*
	D-Xylose	0.3847	0.024*

Table 2.34 (Continued).

Factor	Carbon source	Indicator value	P value
Beneath samples	Glucose-1-phosphate	0.3767	0.025*
	D-L-Glycerol phosphate	0.3716	0.024*
	D-Malic acid	0.4277	0.006*
	D-Xylose	0.3664	0.041*
	4-Hydroxy benzoic acid	0.3819	0.011*
	Phenylethylamine	0.3718	0.018*
	2-Hydroxy benzoic acid	0.2398	0.023*
Lateral samples	Glucose-1-phosphate	0.3767	0.024*
	D-L-Glycerol phosphate	0.3716	0.026*
	D-Malic acid	0.4277	0.009*
	D-Xylose	0.3664	0.039*
	D-Glucosaminic acid	0.3590	0.046*
	4-Hydroxy benzoic acid	0.3819	0.009*
	Phenylethylamine	0.3718	0.021*
	2-Hydroxy benzoic acid	0.2398	0.025*
5 meter samples	Glucose-1-phosphate	0.3767	0.023*
	D-L-Glycerol phosphate	0.3716	0.021*
	D-Malic acid	0.4277	0.011*
	D-Xylose	0.3590	0.050*
	4-Hydroxy benzoic acid	0.3819	0.006*
	Phenylethylamine	0.3718	0.021*
	2-Hydroxy benzoic acid	0.2398	0.018*

Table 2.35. Indicator carbon analysis based on MMCPs for soil samples by carrion decomposition days in summer 2014 at Snook, Texas.

Factor	Carbon source	Indicator value	P value
Day 0	α-Cyclodextrin	0.4068	0.019*
Day 7	Putrescene	0.4021	0.025*
	D-Malic acid	0.5310	0.011*
	L-Threonine	0.5361	0.037*
	α-Ketobutyric acid	0.5127	0.034*
	D-Mannitol	0.4432	0.029*
Day 14	i-Erythritol	0.5464	0.004*
Day 21	Tween 40	0.3798	0.049*
Day 40	L-Threonine	0.6635	0.009*
	Glycyl-L-Glutamic acid	0.5274	0.048*
	D-Cellobiose	0.4431	0.026*
	N-Acetyle-D-Glucosamine	0.3711	0.027*

Table 2.36. Indicator carbon analysis of carbon sources for soil samples by Treatments in summer 2014 at Snook, Texas.

Factor	Carbon source	Indicator value	P value
Control	Nil	Nil	Nil
Post-7	Nil	Nil	Nil
Post-14	Nil	Nil	Nil
Control x Post-7	Glucose-1-phosphate	0.5510	0.027
	Putrescene	0.5366	0.033
	2-Hydroxy benzoic acid	0.2535	0.038
	Glycogen	0.5662	0.025
Control x Post-14	D,L-α-Glycerol phosphate	0.5516	0.019*
	Glucose-1-phospahte	0.5435	0.047*
	β-Methyl-D-Glucoside	0.5310	0.024*
	4-Hydroxy benzoic acid	0.5612	0.009*
Post-7 x Post-14	D-Malic acid	0.6286	0.005*
	α-Clycodextrin	0.5801	0.050*
	D-Glucosaminic acid	0.5339	0.041*
	Phenylethylamine	0.5649	0.003*
	4-Hydroxy benzoic acid	0.5445	0.044*
	Putrescine	0.5365	0.043*
	2-Hydroxy benzoic acid	0.3466	0.005*

Nil = No carbon source indicator.

Comparison of microbial metabolic community profiles between summers 2013 and 2014

Year effect was incorporated in the overall data (pig and soil samples) and analyzed using NMDS, MRPP and PERMANOVA analysis. The NMDS analysis indicated that the stress value was 0.1910 and r^2 was 0.8582. Figure 2.49 showed the NMDS plot of stress for the overall data. Pooled NMDS ordination plots according to Day, Treatment and Region were demonstrated in Figure 2.50, 2.51 and 2.52, respectively. MRPP results showed A value 0.03115 and Significant of Delta 0.001 based on 999 permutations. The results demonstrated that MMCPs for Year, Day, Treatment, and Region were significantly different. As for interactions, Year x Day, Year x Treatment, Year x Region, Day x Region, Treatment x Region and Year x Treatment x Region were significantly difference (Table 2.37). Furthermore, NMDS ordinations of pig samples between Year was demonstrated in Figure 2.53 (with minimum stress 0.2109 and r^2 0.7720), as well as soil samples between Year in Figure 2.54 (minimum stress 0.1807 and r^2 0.8629). Again, replicate and biomass were not significant difference ($p = 0.498$ and 0.703, respectively). However, type of samples showed a significant difference ($p = 0.001$). Due to the significant difference between years, the data was separated by Year and analyzed individually. Hence, pool data of both years were not analyzed.

Table 2.37. Analysis of the microbial metabolic community profiles for all samples collected in both trials (summers 2013 and 2014) at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F model	P value
Year	1	23.3920	0.001*
Day	1	7.3160	0.001*
Treatment	2	3.0430	0.001*
Region	5	13.4211	0.001*
Year x Day	1	2.0702	0.019*

Table 2.37 (Continued).

Factor	df	F model	P value
Year x Treatment	2	2.0950	0.007*
Day x Treatment	2	1.2144	0.191
Year x Region	5	2.4050	0.001*
Day x Region	5	1.6868	0.005*
Treatment x Region	10	1.2463	0.035*
Year x Day x Treatment	2	0.9664	0.487
Year x Day x Region	5	1.0846	0.304
Year x Treatment x Region	10	1.2274	0.037*
Day x Treatment x Region	10	0.6997	0.997
Year x Day x Treatment x Region	10	0.7047	0.998

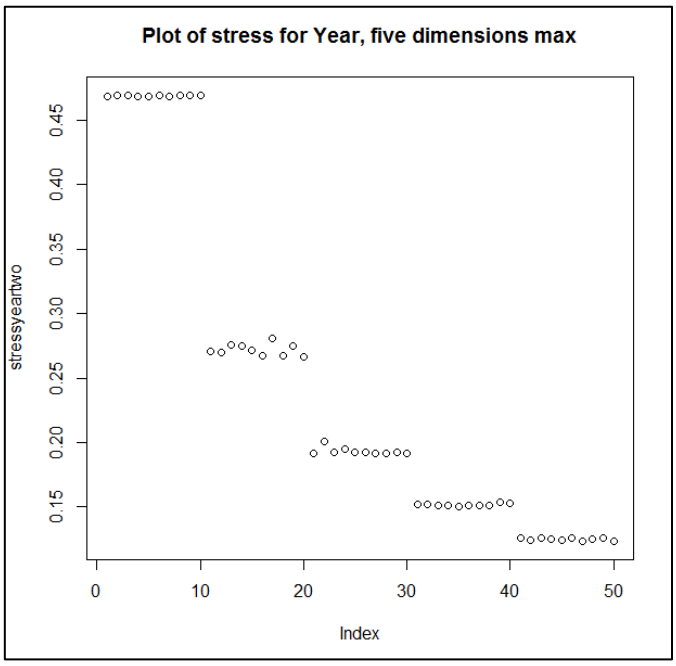

Figure 2.49. NMDS plot of stress for overall data collected in summers 2013 and 2014 at Snook. Texas (Stress test 0.1910; $r^2 = 0.8582$).

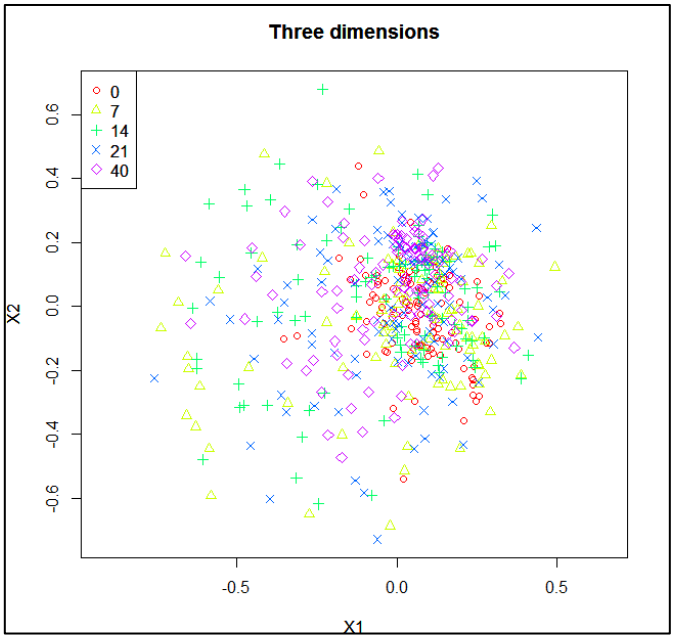

Figure 2.50. NMDS ordinations according to carrion decomposition days pooled from overall data in summers 2013 and 2014 at Snook, Texas.

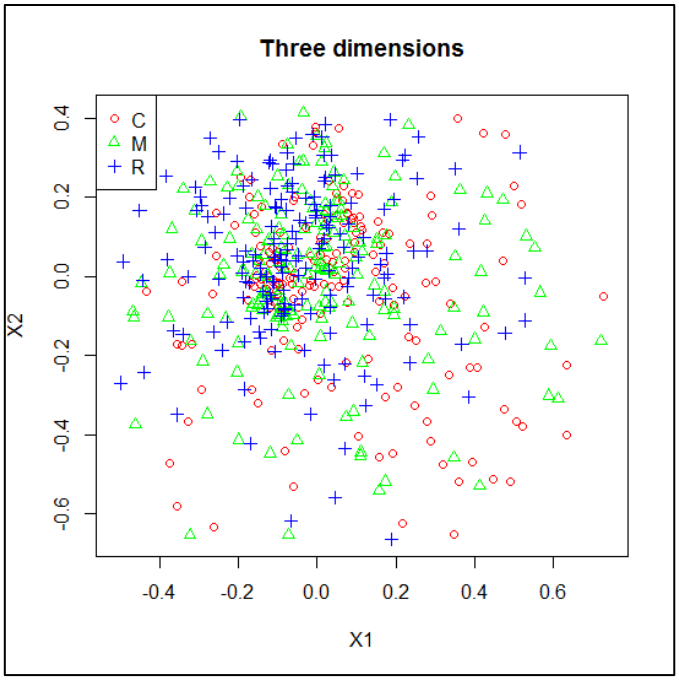

Figure 2.51. NMDS ordinations according to Treatments pooled from overall data in summers 2013 and 2014 at Snook, Texas (C denotes Control; M denotes Post-7; R denotes Post-14).

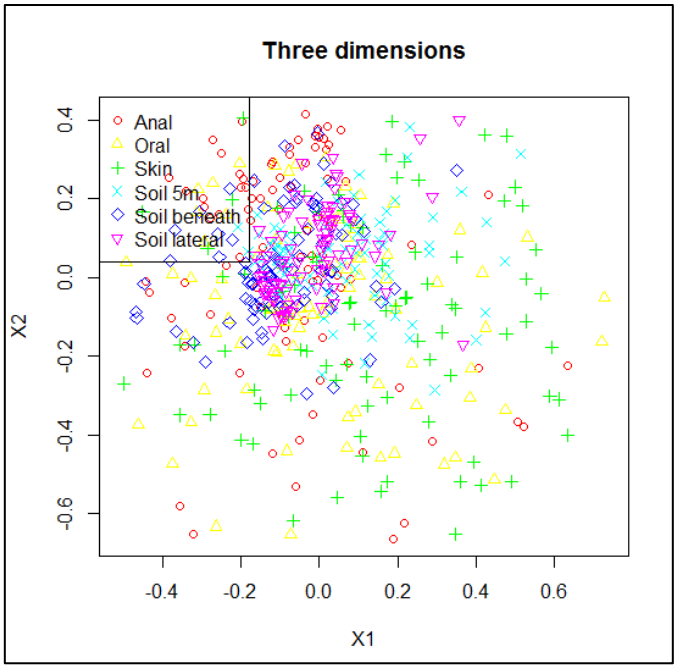

Figure 2.52. NMDS ordinations according to all Regions (included pig and soil regions) pooled from overall data in summers 2013 and 2014 at Snook, Texas.

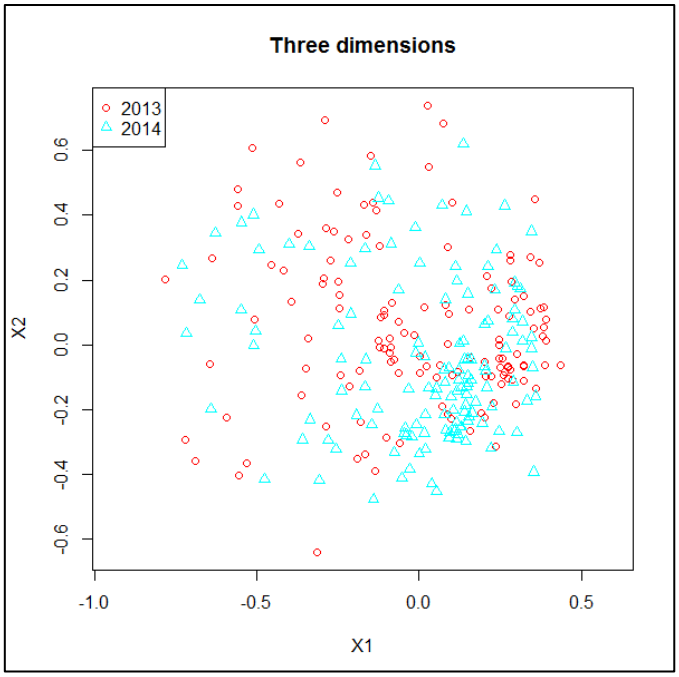

Figure 2.53. NMDS ordinations according to Years pooled from all pig samples in summers 2013 and 2014 at Snook, Texas.

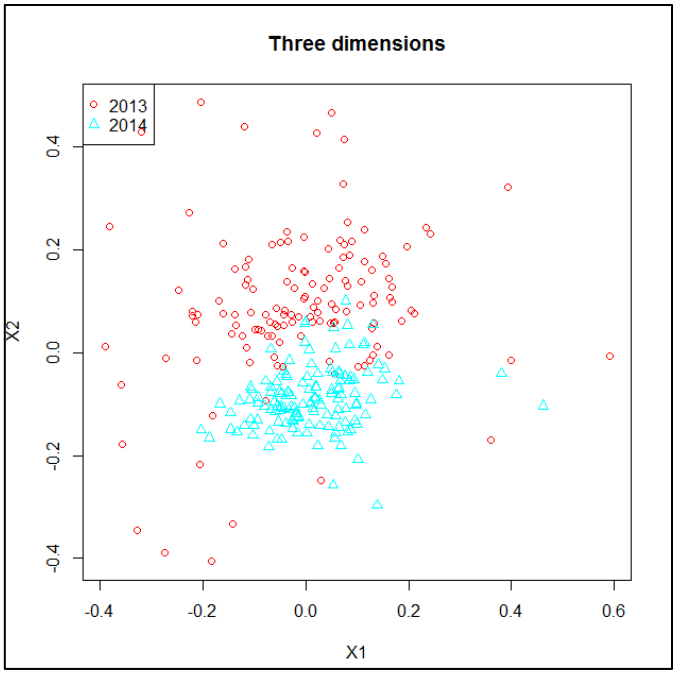

Figure 2.54. NMDS ordinations according to Years pooled from all soil samples in summers 2013 and 2014 at Snook, Texas.

Comparison of MMCPs significant results for both 2013 and 2014 field trials

The MMCP results from 2013 and 2014 trials were compared to determine which factors were consistent in their MMCP over the two consecutive summers. As for the pig carcasses, Treatment was significantly different for both years. This result was consistent and indicated that the loss of microbial function resistance on pig carrion. Among the Treatments, Control x Post-14 was found consistently significant different in both years. For the soil samples, Regions were significant difference in both 2013 and 2014 trials. Treatments were not significant difference in 2013 (which indicates resistance in soil ecosystem), but it was significantly different in 2014 trial. This phenomenon indicates that there was stochastic soil microbial functional response between years, probably contributed by the differences in abiotic factors such as temperatures, ADH or precipitation. As for the indicator carbon substrate, γ-Hydroxybutyric acid was the only consistent indicator carbon substrate for pig samples collected on Day 40 for both trials while Glucose-1-phosphate was the consistent carbon source indicator for soil samples

collected in both years (Table 2.38) (see Appendix N for ISA comparison between years).

Table 2.38. Comparison of significant MMCPs for pig and soil samples collected in summers 2013 and 2014 at Snook, Texas.

Factor	2013	2014
Type of sample (pig vs soil)	Significant difference	Significant difference
Replicate (within and between year)	No significant difference	No significant difference
Pig (All data)	Treatment Day Region	Treatment Day Region
Treatment (Pig)	Control x Post-7 Control x Post-14	Control x Post-14
Day (Pig)	0 x 7 0 x 14 0 x 21 0 x 40 7 x 14 7 x 21 7 x 40 21 x 40	0 x 7 0 x 14 0 x 21 0 x 40 7 x 40 14 x 40 21 x 40

Table 2.38. (Continued).

Factor	2013	2014
Region (Pig)	Oral x Anal	Oral x Skin
	Skin x Anal	Skin x Anal
Soil (All data)	Day	Treatment
	Region	Day
		Region
Day (Soil)	0 x 7	0 x 7
	0 x 14	0 x 14
	0 x 21	0 x 21
	0 x 40	0 x 40
	7 x 21	7 x 21
		7 x 40
		14 x 21
		14 x 40
Region (Soil)	Beneath x Lateral	Beneath x Lateral
	Beneath x 5 meter	Beneath x 5 meter
	Lateral x 5 meter	Lateral x 5 meter
Indicator Carbon (Pig)	Tween 40	Nil
	Itaconic acid	
	D,L-α-Glycerol phosphate	
	N-Acetyl-D-Glucosamine	
Day 0 (Pig)	Nil	Putrescine
		L-Threonine

Table 2.38. (Continued).

Factor	2013	2014
Day 7 (Pig)	Tween 40 D-Malic acid N-Acetyl-D-Glucosamine	Nil
Day 14 (Pig)	D,L-α-Glycerol phosphate N-Acetyl-D-Glucosamine	4-Hydroxy benzoic acid D-Galacturonic acid L-Serine
Day 21 (Pig)	Nil	D-Galacturonic acid Tween 80 D-Glucosaminic acid L-Asparagine α-D-Lactose
Day 40 (Pig)	α-Ketobutyric acid γ-Hydroxybutyric acid	D-Galactonic acid γ-lactone Glycogen D-Cellobiose γ-Hydroxybutyric acid α-Clycodextrine
Oral	Tween 40 Itaconic acid D,L-α-Glycerol phosphate D-Malic acid N-Acetyl-D-Glucosamine	Nil

Table 2.38. (Continued).

Factor	2013	2014
Skin	Tween 40	Nil
	Itaconic acid	
	D,L-α-Glycerol phosphate	
	N-Acetyl-D-Glucosamine	
Anal	Tween 40	Nil
	Itaconic acid	
	D,L-α-Glycerol phosphate	
	N-Acetyl-D-Glucosamine	
Indicator Carbon (Soil)	Itaconic acid	Glucose-1-phosphate
	Glucose-1-phosphate	D-L-Glycerol phosphate
		D-Malic acid
		D-Xylose
		4-Hydroxy benzoic acid
		Phenylethylamine
		2-Hydroxy benzoic acid
Beneath	Itaconic acid	Glucose-1-phosphate
	Glucose-1-phosphate	D-L-Glycerol phosphate
		D-Malic acid
		D-Xylose
		4-Hydroxy benzoic acid
		Phenylethylamine
		2-Hydroxy benzoic acid

Table 2.38. (Continued).

Factor	2013	2014
Lateral	Itaconic acid	Glucose-1-phosphate
	Glucose-1-phosphate	D-L-Glycerol phosphate
		D-Malic acid
		D-Xylose
		D-Glucosaminic acid
		4-Hydroxy benzoic acid
		Phenylethylamine
		2-Hydroxy benzoic acid
5 meter	Itaconic acid	Glucose-1-phosphate
	Glucose-1-phosphate	D-L-Glycerol phosphate
		D-Malic acid
		D-Xylose
		4-Hydroxy benzoic acid
		Phenylethylamine
		2-Hydroxy benzoic acid
Day 0 (Soil)	Tween 80	α-Cyclodextrin
Day 7 (Soil)	D-galacturonic acid	Putrescene
	N-acetyl-D-glucosamine	D-Malic acid
	α-Cyclodextrin	L-Threonine
		α-Ketobutyric acid
		D-Mannitol
Day 14 (Soil)	α-Cyclodextrin	i-Erythritol
	Tween-40	

Table 2.38. (Continued).

Factor	2013	2014
Day 21 (Soil)	Tween 40	Tween 40
Day 40 (Soil)	Nil	L-Threonine Glycyl-L-Glutamic acid D-Cellobiose N-Acetyle-D-Glucosamine

DISCUSSION

In this study, Biolog EcoPlate™ was used extensively to describe microbial community metabolic profiles throughout carrion decomposition as an indicator of overall microbial function and specific carbon source utilized by necrobiome. First of all, statistical results demonstrated that there was no replicate effect ($p > 0.05$) in both pig and soil samples, within and between trials. However, there was significant difference ($p < 0.05$) between trials (summer 2013 vs summer 2014) (see Table 2.37) in terms of MMCPs, both in pig and soil samples. This difference could be due to differences in abiotic conditions (e.g., temperature, ADH, and precipitation) experienced each year.

When measuring microbial metabolic function, it should be bear in mind that microbial community such as fungi and bacteria are stochastic in nature, either spatially or temporally, especially on ephemeral resources such as carrion (Ramette & Tiedje, 2007; Pechal et al. 2013). However, what drives these community shifts among taxa on carrion has yet to be elucidated (Crippen et al. 2015). Many previous researches had arrived at the same conclusion where temperature altered microbial composition and cause functional shift (Zogg et al. 1997). Carbon utilization by microbes was more active in the warmer summer and soil temperature, rather than soil moisture, strongly influenced microbe carbon used, structure as well as functional dynamics (Bell et al.

2009). A study conducted to determine the effects of temperature on metabolic rate and found that the temperature is one of the primary determinants of biological time and ecological roles (Gillooly et al. 2001). Carter et al. (2008) also found that temperature affects microbial decomposition of rat cadavers (*Rattus rattus* (L.)) in soils. Similarly, Pechal et al. (2013) found overall mean functional activities was significantly different between years, with no significant interaction. Although precipitation was not significant between years, however, the difference was 132.33 mm more rain received in summer 2014 than summer 2013. Study found changes in precipitation altered fungal community composition and may cause changes in bacterial and fungal overall abundance, with changes in precipitation being the major factor to have a much greater effect on the community composition (Castro et al. 2010). Carter et al. (2010) concluded that grave soil moisture content can modify the relationship between temperature and cadaver decomposition and that soil microbe can play a vital role in cadaver breakdown. These results suggest that epinecrotic microbial activity on ephemeral resource is sensitive to external environmental factors such as the change of ambient temperatures. Furthermore, it is unknown whether the existence microbiome on each pig was the same before the launching of experiment, although all pigs were supplied from the same farm (which considered sharing a similar environment and similar diet). It is important to note that the assumption of microbial communities in the same environment are functionally equivalent is incorrect (Strickland et al. 2009). A study demonstrated that microbial community structure of intestine can be influenced by unique history of each community and intrinsic temporal dynamics (Dethlefsen et al. 2006). Another reason was concerned regarding the study site. It is unclear whether the repeated use of the same study site for two consecutive summers rendered any effect to the soil condition, although the locations of pig in the second trial were different from the first trial, by shifted 5 meters to the East (higher slope) from their original location in 2013. Evans & Wallenstein (2012) suggest that environmental history can affect contemporary rates of biogeochemical processes both through changes in abiotic drivers and through changes in microbial community structure. Considering a total of nine pig carrion (similar to

mass mortality event in nature) was quite impactful to the local study site which was about 371 m in perimeter and an approximate 7,943 m^2 in area, thus, the excessive carrion-derived nutrients (act as fertilizer) may already change the microbial community structure and function for the 2014 trial. In fact, efforts such as applying fertilizer or additional soil organic matter may affect microbial composition (Steenwerth et al. 2002).

For pig samples, both trials showed consensus that Day, Treatment and Region were significant different ($p < 0.05$) (see Table 2.38). Among the treatment groups, Control x Post-14 was the only group that was consistently significant difference in both trials, although in 2013 trial, Control x Post-7 was significant different in MMCPs. These results were in contrast with Pechal et al. (2013) where they found no significant difference in MMCPs between treatments (i.e., pig carcasses with and without insect access, along with three sampling periods on days 1, 3 and 5), although Pechal et al. (2013) found changes in the relative abundance of four main bacterial phyla (*Proteobacteria, Firmicules, Actinobacteria* and *Bacteroidetes*) over days. The possible explanation for these contrasting results may be due to different duration of insect exclusion on carrion, season and location of study. Carrion with insect excluded (e.g., hidden or burial corpses) performs decomposition in a different pathway compared to carcasses with immediate insect access, as insect-excluded carrion tends to decompose microbiologically rather than entomologically (Dent et al. 2004). After death, the microorganisms present in the intestines and respiratory tracts invade the body tissues. Aerobic organisms consume and deplete the oxygen inside the body and at the same time setting up favorable conditions for anaerobic microorganisms that take the remains through the putrefactive stage. These anaerobic organisms are usually alimentary tract-origin but may also migrate from the soil and air into the remains in the later stages of decomposition (Evans, 1963). Microbial activity based on Day showed comparison pairs of 0 x 7, 0 x 14, 0 x 21, 0 x 40, 7 x 40, and 21 x 40 were consistently significant difference ($p < 0.05$) in both trials, and without interactions occurred for both years (see Table 2.38). This observation was in agreement with Pechal et al. (2013) as they also observed significant different MMCPs between the initial day (Day 0) and each

subsequent day of decomposition (Days 1, 3 and 5). In our study, significant different in MMCPs between days was even detected on Day 40, indicating a substantial change on microbial function from fresh stage to dry remains stage. Bacterial function is closely related to its structure (Fuhrman, 2009). As such, we can hypothesize that the significant change in bacterial function are related to the change in bacterial structure. Hyde et al. (2013) sequenced bacterial samples collected from human cadavers and showed a shift from aerobic bacteria to anaerobic bacteria in all body sites and demonstrated variations in bacterial community structure between bodies. Bacteria associated with Diptera, such as *Ignatzschineria* and *Wohlfahrtimonas* were common during bloated stage. After dehydration, bacteria associated with soil, such as *Acinetobacter*, were common at most body sites (Hyde et al. 2014). Similar observation was also noted in litter decomposition, where there was increasing bacterial diversity as decomposition proceeded and substrate quality decreased (Dilly et al. 2004). As for the pig region, Skin x Anal was the only region consistently demonstrated statistically different from other regions in both trials. These results may suggest different types of body sites harbor different types of microbes. As in agreement with Grice et al. (2009) who analyzed 20 distinct skin sites of humans and revealed that physiologically comparable sites harbor similar bacterial communities. Pechal et al. (2013) found that there was significant difference in microbial functional activity between the buccal (oral) and skin communities. However, no anal sample was obtained in their study. In the present study, microbial function of oral cavity and skin was found significantly different only in 2014 trial. Oral microbiome maybe different from individual to individual, and has been demonstrated to have strong correlation with health and diseased states of the host (Curtis et al. 2011). Therefore, this may explain discrepancies in results between different studies.

Soil samples, in both trials, exhibited a different pattern and trajectory between trials (see Table 2.38). Ecologists are debating the relative role of deterministic and stochastic determinants in shaping soil organism community structure (Caruso et al. 2012). The inconsistency in soil MMCP may indicate stochastic process in soil ecosystem which changes the degree of edaphic microbe sensitivity to disturbance.

Landscape sensitivity is the response of landscape systems to perturbation and an unstable system behave chaotically while stable systems resist change until threshold values of system parameters are exceeded (Thomas, 2001). Although both trials agreed statistically that Day and Region were significantly different in all soil samples, however, treatment was found no significant difference ($p = 0.152$) in summer 2013, but was significantly different ($p = 0.002$) in 2014 trial (pairwise comparisons showed all treatment groups were significantly different from each other) (see Table 2.32). Soil ecosystem is expected to have high resilience when encountered disturbances compared to carrion ecosystem, which can be seen in 2013 trial, however, there are many factors affecting soil resiliency, including soil type, vegetation, climate, land use, scale, and disturbance regime (Seybold et al. 1999). Lal (1993) defined soil stability as the susceptibility of soil to change under natural or anthropogenic perturbations. In comparison, soil resilience refers to soil ability to restore its life support processes after being stressed. Compost amendment has been reported to impact soil microbial activities or community composition. Saison et al. (2005) conducted a study on the resilience of soil microbial community associated with compost amendment and found that no resilience of microbial characteristics was observed 6-12 months after amendment with high amount of compost. These findings suggest that carrion used in this study (total weight of pig carrion introduced were 237 kg and 212 kg, in 2013 and 2014 trials, respectively) could give rise to a large amount of organic matter or high nutrient influx into the study site and probably the cause no resilience in soil microbial community. Comparison of MMCPs by Day demonstrated that 0 x 7, 0 x 14, 0 x 21, 0 x 40, and 7 x 21 were significantly different in both trials. These results suggest that the initial soil condition was affected by decomposition materials over time, at least metabolically by epinecrotic bacterial communities. Similar observation was also noticed on pig samples where MMCPs on initial day of experiment (i.e., Day 0) was significantly different with other sampling periods. Likewise, Pechal et al. (2013) also demonstrated that decomposition day was significantly different in their 2010 field trial in Xenia, Ohio. Howard et al. (2010) characterized the soil microbiota associated with a decomposing

swine carcass. The results showed that microbial structure and functional change during vertebrate decomposition. The lipolytic bacterial counts were initially the lowest on Day 0 (before pig carcass exposure) and increased to the peak between days 9 and 12 (active decomposition).the lipolytic bacteria then decreased and leveled at days 15 through 71. Conversely, the proteolytic bacterial count were the highest at day 0, slowly decreased at days 3 (fresh stage) and 6 (bloated stage) with a rapid decline at day 9 followed by a second major decline at day 28 (advance stage), they then leveled through the remaining time period (Howard et al. 2010). During litter decomposition, microbial succession (change of community structure over time) was observed and this process is strongly influenced by synergic interaction among functional groups and litter chemical composition (Torres et al. 2005). As for the soil regions, all regions including soil beneath, soil at the side of carcass (soil lateral), and even soil collected from 5 meter away from carcass (which should serve as Control to the other two sites) showed a significant difference ($p < 0.05$) with each other for two consecutive summers. These results suggest a slight distant from the pig carcasses (in this case, the soil beneath and soil lateral, which were approximately 30 cm away from each other) may result in a significant change of microbial metabolic function. In other words, soil beneath the pig carrion had a higher microbial function compared to soil lateral, and soil lateral had a slightly higher microbial activity compared to soil at 5 meter away (see Figure 2.35). Benninger et al. (2008) investigated the biochemical alteration of soil beneath a decomposing carcass and found that cadaver decomposition did significantly increases in the concentration of soil pH, total nitrogen, soil-extractable phosphorus, and lipid-phosphorus. These nutrients served as additional resources for soil microbes (Shen et al. 2011) and could eventually change the structure and function of soil microbiome.

The identification of species associated with a group of samples is a common aspect of ecological research. In this study, Indicator Species Analysis (ISA) was employed extensively to permit statistically rigorous assessments of indicator species (Bakker, 2008). The application of indicator species analysis is to clarify whether responses are driven by all or a subset of species in the community (Bakker, 2008). By

definition, the identification of indicator species requires comparison between two or more groups, including experimental treatments within a site, different sites, or measurements of the same site at different times. When multiple groups are samples, the identification of significant indicators will depend on the scale in the typology at which comparisons are conducted (Dufréne & Legendre, 1997; Hess et al. 2006). In this study, ISA was used to determine which carbon substrate in Biolog EcoPlate^{TM} responsible for the observed microbial activities on samples.

For the pig samples, there was no consensus in indicator species in both trials, with no indicator species of carbon substrate obtained in 2014 trial (see Table 2.38). There was four indicator carbons seen in 2013 trial namely Tween 40, Itaconic acid, D, L-α-Glycerol phosphate and N-Acetyl-D-Glucosamine. Similar carbon substrates were also observed in oral, skin and anal samples from the pig, with additional carbon species (D-Malic acid) for the oral samples. Indicator analysis was then applied along the decomposition process over time, and there was only a single carbon substrate that was overlapped in both trials, γ-Hydroxybutyric acid, which occurred on Day 40 in both years. Generally, there was no similarity among indicator carbon species between two trials. In 2013, Tween 40, D-Malic acid and N-Acetyl-D-Glucosamine were associated on Day 7; D,L-α-Glycerol phosphate and N-Acetyl-D-Glucosamine on Day 14; α-Ketobutyric acid and γ-Hydroxybutyric acid on Day 40, while there was no carbon indicator on Day 21. In 2014, Putrescine and L-Threnine were indicative on Day 0; 4-Hydroxy benzoic acid, D-Galacturonic acid, and L-Serine on Day 14; D-Galacturonic acid, Tween 80, D-Glucosaminic acid, L-Asparagine and α-D-Lactose on Day 21; D-Galactonic acid γ-lactone, Glycogen, D-Cellobiose, γ-Hydroxybutyric acid and α-Clycodextrine on Day 40, while no indicative carbon species on Day 7.

For the soil samples, two carbon substrates (Itaconic acid and Glucose-1-phosphate) were indicative in 2013 trial while seven carbon species (Glucose-1-phosphate, D-L-Glycerol phosphate, D-Malic acid, D-Xylose, 4-Hydroxy benzoic acid, Phenylethylamine and 2-Hydroxy benzoic acid) were the significant indicators in 2014 trial (see Table 2.38). Among these carbon species, only one carbon species occurred in

both trials namely Glucose-1-phosphate. When comparing decomposition days, only Tween 40 occurred on Day 21 for both years. In 2013 trial, Tween 80 was indicative on Day 0; D-galacturonic acid, N-acetyl-D-glucosamine and α-Cyclodextrin on Day 7; α-Cyclodextrin and Tween-40 on day 14; Tween 40 on Day 21, and no carbon indicator was detected on Day 40. While for 2014, α-Cyclodextrin was found associated with Day 0; Putrescene, D-Malic acid, L-Threonine, α-Ketobutyric acid and D-Mannitol on day 7; i-Erythritol on day 14; Tween 40 on day 21; L-Threonine, Glycyl-L-Glutamic acid, D-Cellobiose and N-Acetyle-D-Glucosamine on Day 40.

Most environmental microbiological organisms are unculturable and thus researchers are unable to determine an accurate assessment of the diversity of microbial communities. With the advent of Community Level Physiological Profiling (CLPP) techniques, such as Biolog EcoPlate™, scientists are able to describe microbial populations in terms of quantitative data about phenotypes and are used to describe the complexity of a microbial community in terms of its metabolic diversity (Marshall & Sweat, 2008). Research had shown that community function (carbon source utilization) and community stability (resistance to disturbance) are a function of the structural composition of the community (Cook et al. 2006). Although Biolog EcoPlate™ is not designed to identify the true bacterial community structure, however, it is possible that based on the metabolic profiling, certain bacteria taxa can be deduced from its metabolic fingerprints. There was another type of plate which is called Biolog GN MicroPlate system which has been evaluated for the identification of some plant-pathogenic bacteria (Jones et al. 1993). Similarly, Biolog substrate has been developed to be utilized in the identification of *Legionella* spp. (Mauchline & Keevil, 1991). In another report, the Biolog performed well with many genera, but having some problems when encountered with some strains of *Klebsiella*, *Enterobacter*, and *Serratia* (Miller & Rhoden, 1991; Klingler et al. 1992). Although Biolog offers advantages, as it is a relatively simple protocol and ease of use, however, limitations of Biolog must be acknowledged. Some of the critiques for such technique include the bias in the method towards rapidly growing bacteria, the need to ensure similar inoculum sample size in the wells, the need

to reduce time between sampling and inoculation of the microplates and the difficulties with meaningful data analysis and interpretation (Weber & Legge, 2010).

The meaning of differences in MMCPs remains unclear (Garland, 1997). Although researchers tend to use MMCPs as an indicator of *in-situ* carbon source utilization. The interpretation makes two assumptions that have not been proven. The first assumption is that color development is a function of the proportion of organisms in the community that are able to utilize the specific carbon sources within a well. This may not be true due to following reasons (i) differential growth rates among organisms utilizing the same sole carbon source (ii) the lack of direct linkage between growth and tetrazolium dye reduction (Winding & Hendriksen, 1997) (iii) cross-feeding among organisms within a single well. If the first assumption is true, it would only indicate that the relative rates of carbon source utilization reflect the relative distribution of phenotypic potentials in the community. Second assumption is that phenotypic potential is closely related with community function. Again, this may not be correct as microorganisms may possess phenotypic potential that is not directly relevant in their natural community. Hence, changes in phenotypic potential may be the result of selective forces not directly measured in MMCPs, suggesting that a shift in profile may be structurally relevant, but functionally misleading (Garland, 1997).

Despite of all these limitations, it is still possible, with sufficient awareness, to hypothesize which structure of bacterial community could utilize carbon sources for energy supply, although bacteria could also use secondary substrate in the absence of preferred carbon source (Görke & Stülke, 2008).

To link between the utilization of indicative carbon substrate with bacteria community structure, the following examples are provided. Polysorbates, such as Tween 40 (polyoxyethylene sorbitan monopalmitate) and Tween 80 (polyoxyethylene sorbitan monooleate) are a class of emulsifiers used in some pharmaceutical, cosmetic and food preparation industries as vehicles for fat-soluble compounds (López et al. 2000). Tween 40 differs structurally from Tween 80 in that it has palmitic acid as its fatty acid side-chain while Tween 80 has oleic acid (O'Sullivan et al. 2004). Tween have been shown to

enhance the growth of several species of mycobacteria (Cutler et al. 1987) as well as growth factors for lactic acid bacteria such as *Lactobacillus* (Williams et al. 1947; Partanen et al. 2001). Howe & Ward (1976) also reported that *Pseudomonas* is capable in utilizing Tween 80 as carbon source. A new species of bacteria, *Paucibacter toxinivorans,* isolated from lake sediment, was tested for carbon source utilization. Out of 96 carbons tested, Tween 40 was the only one used by all strains (Rapala et al. 2005). Also, *Acinetobacter* and *Serratia* are able to utilize both Tween 40 and 80 (Boothe & Arnold, 2002). Note that Pechal et al. (2013) used only 29 carbon sources (out of 31 carbons) where both tweens were excluded from their analyses as they were considered as positive controls. However, the current study did not remove the tweens as we found tweens are not strictly positive controls as some species of bacteria in the genera *Bacillus, Escherichia, Flavobacterium, Klebsiella, Lactococcus, Pasteurella, Serratia* and *Staphylococcus* were not able to grow on tweens (Boothe & Arnold, 2002).

Itaconic acid (methylenesuccinic acid, $C_5H_6O_4$) is well known as a precursor for polymer synthesis and has been involved in industrial processes for decades (Schaechter, 2009). Itaconic acid can be metabolized by *Pseudomonas* and *Salmonella* species (Martin et al. 1961; Cooper & Kornberg, 1964). However, many aerobic bacteria are known to grow on itaconate as their sole source of carbon (Cooper & Kornberg, 1964). Similarly, the up-take of D,L-α-Glycerol phosphate and Glycerol by *Pseudomonas aeruginosa* was reported (Siegel & Phibbs, 1979). *Bacillus*, *Escherichia, Flavobacterium, Klebsiella, Serratia* and *Staphylococcus* were reported to metabolize D,L-α-Glycerol phosphate (Boothe & Arnold, 2002). N-Acetyl-D-Glucosamine ($C_8H_{15}NO_6$) is a monosaccharide derivative of glucose and it is significant in several biological systems. In the ocean, as on land, N-Acetyl-D-Glucosamine is a major component of structural polymers in bacteria, plants and animals. Chitin, a homopolymer of N-Acetyl-D-Glucosamine, is a structural material in many marine invertebrates, insects, fungi and algae (Riemann & Azam, 2002). The mechanism of N-Acetyl-D-Glucosamine transport and metabolism have been reported in *Escherichia coli* (Plumbridge, 1990), *Escherichia vulneris* (Boothe & Arnold, 2002), *Bacillus subtilis*

(Freese et al. 1970), *Klebsiella pneumoniae*, *Lactococcus lactis*, *Pseudomonas aeruginosa*, *Serratia liquefaciens, Serratia plymuthica* (Boothe & Arnold, 2002), *Staphylococcus aureus* (Imada et al. 1977) and *Vibrio furnissii* (Bassler et al. 1991). Riemann & Azam (2002) demonstrated that Acetyl-D-Glucosamine were widely uptake among pelagic marine bacteria such as Firmicutes, Cytophaga-Flavobacterium-Bacteroides (CFB), α-Proteobacteria, Oceanospirillum, Vibrionaceae and Alteromonadaceae.

D-Malic acid ($C_4H_6O_5$) is an organic compound that is used as a food additive. Malic acid has two stereoisomeric forms (L- and D- enantiomers), however, only the L-isomer exists naturally (Singhal et al. 1997). Knichel & Radler (1982) found 14 Gram-negative bacteria and two yeast strains were using D-Malic acid as sole carbon source. The bacteria were identified as *Pseudomonas putida*, *Pseudomonas fluorescens, P. aeruginosa* and *Klebsiella aerogens*.

Putrescine (or known as tetramethylenediamine) is a foul smelling organic compound (Haglund, 1996). Putrescine, along with cadaverine, is produced by the breakdown of amino acids in living and dead organisms (Raina & Jänne, 1975). Putrescine and cadaverine are compounds usually associated with the decaying process (Gill-King, 1997). However, these two compounds were not detected from decaying corpses in several studies (Dekeirsschieter et al. 2009; Vass et al. 2004). Fredericks et al. (2012) demonstrated that blow flies behavior is influenced by the dose of volatile organic compounds (VOCs). Besides, females and males showed different behavior for VOCs. Female flies only responded to dimethyl disulfide (DMDS) and putrescine. A new species of bacteria, *Anaerovorax odorimutans*, was described as a strictly anaerobic, putrescene-fermenting bacterium (Matthies et al. 2000). On the other hand, *P. aeruginosa, Pseudomonas fragi*, *P. putida, S. plymuthica* were able to metabolize putrescine (Boothe & Arnold, 2002). Furthermore, marine bacteria are known to remove two amines, putrescine and cadaverine (Höfle, 1984). L-Threonine is an α-amino acid that is used in the biosynthesis of proteins. Bell & Turner (1977) studied the route of L-Threonine degradation in bacteria and four strain of *Pseudomonas* were able to catalyze

it. Furthermore, wild strain of *Serratia marcescens* was able to degrade threonine rapidly (Komatsubara et al. 1978). Boothe & Arnold (2002) showed that *P. aeruginosa, P. putida, Escherichia vulneris, Flavobacterium breve and Flavobacterium indologenes* were able to use L-Threonine. Note that Putrescine and L-Threonine were the indicator carbon species on Day 0 (2014 trial) on pig samples. Based on the literature and to infer bacterial community from the metabolic profiling, it is possible that *Pseudomonas* (which has proteolytic enzymes) was the active bacterial genera during fresh stage of decomposition. Although this hypothesis needs to be confirm by sequencing the bacteria of the necrobiome.

4-Hydroxy benzoic acid is a phenolic derivative of benzoic acid. A novel haloarchaeal strain, *Haloarcula* sp. was reported growing aerobically on 4-Hydroxy benzoic acid (Fairley et al. 2002). Moreover, photosynthetic bacteria *Rhodopseudomonas palustris* had been grown anaerobically on 4-hydroxybenzoate (Merkel et al. 1989). Chen et al. (2011) demonstrated that a broad spectrum endophytic fungi, *Phomopsis liquidambari*, was capable to grow on phenolic 4-Hydroxy benzoic acid as the sole carbon and energy source. D-Galacturonic acid is a carboxylic acid. It is the main component of pectin, in which it exists as the polymer polygalacturonic acid (Mohnen, 2008). Bacteria such as *Escherichia, Klebsiella* and *Pseudomonas* were able to metabolize D-Galacturonic acid (Boothe & Arnold, 2002). L-Serine is an amino acid and is one of the naturally occurring proteinogenic amino acids. Only the L-stereoisomer appears naturally in proteins. L-Serine is considered a non-essential amino acid and plays a central role in cellular proliferation (de Koning et al. 2003). *Campylobacter jejuni*, an intestinal bacterium which is of microaerophilic and asaccharolyitc, was reported capable in the catabolism of L-Serine, which is a vital mechanism for the growth of this bacterium in *vivo* (Velayudhan et al. 2004). L-Serine was the indicator carbon species on Day 14, as it is possible that the pig's intestine content had been exposed during this stage. L-Serine can be metabolized by *Escherichia, Flavobacterium, Klebsiella, Pasteurella volantium, Pseudomonas, Serratia, Staphylococcus arlettae* and

Staphylococcus lentus. Furthermore, lactococci (*Lactococcus lactis*) had been shown to catalyze serine and threonine (Konings et al. 1989; Poolman, 1993).

D-Glucosaminic acid (2-amino-2-deoxy-D-gluconic acid) is a group of carboxylic acid and is a component of bacterial lipopolysaccharides and a chiral synthon. It can be prepared by oxidation of D-glucosamine catalyzed by glucose oxidase (Pezzotti et al. 2005). Two new species of *Pseudomonas* isolated in farm soil of South Korea were described to utilize D-Glucosaminic acid (Kwon et al. 2003). Labrenz et al. (1999) isolated eight Gram-negative, aerobic, pointed and budding bacteria from various depth of the hypersaline, heliothermal and meromictic Ekho Lake (Vestfold, East Antartica) and demonstrated through Biolog system that *Roseobacter litoralis* and *Roseobacter denitrificans* utilized D-Glucosaminic acid. Also, soil bacteria such as *Agrobacterium vitis* and *Agrobacterium rubi* were able to oxidize D-Glucosaminic acid as sole carbon source (Bouzar et al. 1993). This may indicate that soil bacteria had become dominant on pig carrion after 21 days of decomposition.

L-Asparagine is a non-essential α-animo acid that is used in the biosynthesis of proteins. Stereospecific aspariginases were detected in the extracts of many gram-negative bacteria including *E. coli* (Willis & Woofolk, 1975), *Erwinia carotovora* (Howard & Carpenter, 1972) and mycobacteria (Lyon et al. 1970). Furthermore, L-Asparagine can be utilized by *Acinetobacter* spp., *Flavobacterium, Klebsiella, Pseudomonas* spp. and *Serratia* spp. (Boothe & Arnold, 2002).

α-D-Lactose ($C_{12}H_{22}O_{11}$) is a dissacharide carbohydrate formed by the condensation of one galactose and one glucose molecule and α-D-Lactose is the primary sugar in the milk. Lactic acid bacteria (LAB) are known to utilize α-D-Lactose, including *Lactococcus*, *Lactobacillus* and *Enterococcus* (Leong-Morgenthaler et al. 1991; Kleerebezemab et al. 2000; Hagedorn et al. 2003). *Escherichia*, *Pasteurella* and *Serratia* are also users of α-D-Lactose (Boothe & Arnold, 2002). This carbon source became indicative on Day 40 on pig samples. It is possible that lactic acid bacteria was one of the dominant taxa at this dry-remains stage, however, it could be due to other bacteria as well. D-Galactonic Acid γ-Lactone ($C_6H_{10}O_6$) is a group member of

carboxylic and acetic acids. Studies showed *K. pneumoniae, E. coli* and *Salmonella* sp. were able to utilize this carbon source (Bochner et al. 2001; Boothe & Arnold, 2002; Kauko et al. 2010)

γ-Hydroxybutyric acid ($C_4H_8O_3$) is a carboxylic acid that present in food such as wine, beef and small citrus fruit (Weil & Rosen, 1983). *Staphylococcus lentus* is reported to use γ-Hydroxybutyric acid as carbon source for energy production (Boothe & Arnold, 2002). Tóth et al. (2001) isolated a new bacteria species, *Schineria larvae* (= *Ignatzchineria larvae*), from the flesh fly larvae *Wohlfahrtia magnifica* (Diptera: Sarcophagidae). The biochemical test using Biolog GN plate demonstrated that *Ignatzschineria larvae* showed a positive reaction to γ-Hydroxybutyric. In this study, necrophagous flies, including sarcophagids, were observed on Day 40, although the population was low. Diptera are known to vector microorganisms (Greenberg, 1973) and it is possible that flies transmit the bacteria to the carrion as previous studies shown that *Ignatzschineria* and *Wohlfahrtiimonas* have been isolated from animal carcasses and human remains (Pechal, 2012; Hyde et al. 2014). Both bacteria had also been isolated from myiasis wounds, septicemia and cellulitis patients (Le Brun et al. 2015; Thaiwong et al. 2014; Rebaudet et al. 2009; de Dios et al. 2015). Interestingly, γ-Hydroxybutyric was the indicator carbon on Day 40 postmortem for both 2013 and 2014 trials. This result could indicate the presence of *Ignatzschineria* and *Wohlfahrtiimonas* on pig carrion, as these bacteria increased in abundance after purge to a maximum relative abundance of nearly 90%, but decreasing again to about 6% relative abundance as wet biomass was lost and tissue began to dry out (Hyde et al. 2015). In contrast, Iancu et al. (2015) found that the final apparent community proliferated in rectum of a swine carcass from week 18 to week 21 consisted of only two Gammaproteobacteria, *I. larvae* and *Wohlfahrtiimonas chitiniclastica.* Furthermore, *I.* larvae, *W. chitiniclastic* and *Arsenophonus nasoniae* (a son-killer bacterium of the wasp *Nasonia vitripennis*) was found in the swine mouth cavity after more than 100 days of experimentation (Iancu et al. 2015).

Glycogen is a readily mobilized storage form of glucose. It is a very large polymer of glucose residues that can be broken down to release glucose molecules when energy is needed (Berg et al. 2002). Glycogen can be metabolized by *Escherichia, Flavobacterium* spp., *Pasteurella* spp., *P. putida, Serratia* spp. and *S. lentus* (Boothe & Arnold, 200). *Fibrobacter succinogenes*, a strictly anaerobic cellulolytic bacterium living in the rumen of cattle, uses cellulose, glucose or cellobiose, as well as glycogen as carbon and energy source (Gaudet et al. 1992). D-Cellobiose is a disaccharide carbohydrate. Several cellobiose-fermenting yeasts have been identified namely *Torulopsis, Brettanomyces* and *Candida* species (Parekh & Wayman, 1986). Bacteria such as *Escherichia, L. lactis, S. plymuthica* and *S. lentus* were reported to metabolize cellobiose.

Cyclodextrines are a family of cyclic oligosaccharides composed of α-(1,4) linked glucopyranose subunits (Del Valle, 2004). α-Cyclodextrine is a soluble fiber derived from corn (Comerford et al. 2011). Boothe & Arnold (2002) found *Flavobacterium* spp., *Lactococcus* and *S. plymuthica* utilized α-Cyclodextrine. Furthermore, *Bacillus subtilis* is able to grow on α-, β- and γ-Cyclodextrine as a carbon source (Kamionka & Dahl, 2001).

Melvin et al. (1984) and Carter et al. (2007) identified and suggested that the decomposition of soft tissue is caused by enteric obligate and facultative bacteria from the genera *Clostridium, Bacteroides, Staphylococcus*, and the Enterobacteriaceae. These genera are therefore proposed by the present study based on the carbon usage indicated by Biolog system. Furthermore, previous decomposition studies have recorded a shift from communities dominated by aerobic bacteria (*Staphylococcus* and Enterobactericeae) to those dominated by anaerobic bacteria (*Clostridia* and *Bacteroides*) (Janaway et al. 2009; Hyde et al. 2013). Pechal et al. (2013) found Proteobacteria was the predominant phyla for carcasses (both insect inclusion and insect exclusion groups) followed by Firmicutes. Proteobacteria remained predominant throughout the five decomposition days for insect exclusion carcasses, while Firmicules decreased as decomposition progressed. However, for the insect accessed carcasses,

Proteobacteria decreased throughout the decomposition process while Firmicutes became the predominant phyla as decomposition progressed. Fusobacteria was a significant indicator phylum of the overall epinecrotic community on the first sampling day while Actinobacteria significantly represented the skin communities (Pechal et al. 2014a). Over the course of decomposition, family Moracellaceae was the most dominant with Pasteurellaceae, Enterobacteriaceae and Aerococcaceae. Planococcaceae was the most dominant family on the fifth day, along with Clostridiaceae (Pechal et al. 2014a). Similarly, Hyde et al. (2015) also found Proteobacteria was dominant on human skins during the first 2 days of decomposition, and then Firmicutes increased in abundance on these sites during later stages of decomposition. Actinobacteria, although at a lower relative abundance, also increased in the later phases of decomposition. For human rectum samples, Hyde et al. (2015) found that Firmicutes and Bacteroides were the most abundant phyla before purge, and that Proteobacteria dominating the most after purge. Other human cadavers were dominated by *Ignatzscheneria* and *Acinetobacter* while others were dominated by *Clostridium* and *Acinetobacter*. Pechal et al. (2014a) and Hyde et al. (2015) both agree that Moraxellaceae (*Acinetobacter* sp.), Xanthomonaadaceae (*Ignatzschineria* sp.) and Clostridiaceae (*Clostridium* sp.) are important groups of decomposers across host type. In contrast, Metcalf et al. (2013) demonstrated that Pseudomonadaceae (a Gammaproteobacteria) as the significant family in the soil and on the skin of mouse carcasses.

Looking into the genus level, one of the human corpses as reported in Hyde et al. (2015), found that *Pseudomonas* dominated the oral community structure before purge. In fecal samples, *Ignatzscheneria* increased in abundance after purge, and decreased as wet biomass was lost. Meanwhile, *Corynebacterium* was the most abundant after most wet biomass was disappeared. For all skin samples, *Pseudomonas* was dominant before purge and *Ignatzscheneria* increased in abundance after purge, decreasing again as wet biomass was lost (Hyde et al. 2015). In another human corpse, fecal samples were comprised mainly of *Bacteroides* and *Porphyromonas*; Enterococcaceae and Planococcaceae in the mouth; and *Acinetobacter* and *Clostridium* in the skin samples.

Again, these results suggest variations in the bacterial community structure before and after purge in each human cadaver. It is known that microbial community changes are sensitive to factors including environmental and edaphic conditions, variations in the human microbiome, and differences among host species (Metcalf et al. 2013). These abiotic and biotic variables remain as the major challenges in the interpretation and applicability of these works in real life.

Hyde et al. (2013) conducted another human decomposition study and found the bacteria from lower gastrointestinal tract and body cavity were *Clostridium, Lactobacillus, Eggerthella* and *Bacteroides* while the mouth samples collected during pre-bloat stage showed *Streptococcus, Prevotella* and *Veillonella*. Their results indicated that *Clostridium* is abundant at the end of stage in most of the gastrointestinal (GI) tract. *Bifidobacterium* was among the top ten genera detected in only the transverse colon end-bloat sample, while *Lactobacillus* was relatively abundant in all GI tract samples from a human corpse. One member of Enterobacteriaceae, *Escherichia*, was detected in the lower GI tract samples for both pre-bloat and end-bloat samples (Hyde et al. 2013).

For the soil samples in this study, a total of two significant carbon species were detected in 2013 trial and seven carbon substrates in 2014 trial. Furthermore, indicator species analysis showed that there were variations in carbon indicators over time (i.e., decomposition day) for both trials. The following are the indicative carbon species with their possible bacteria users as energy sources.

Glucose-1-phosphate ($C_6H_{13}O_9P$) is a glucose molecule with a phosphate group on the 1'-carbon. Bacteria that metabolize Glucose-1-phosphate include *Bacillus, Escherichia, Flavobacterium, Klebsiella* and *Serratia* spp. (Boothe & Arnold, 2002). Lactic acid bacteria such as *Lactobacillus sanfranciscensis* can also metabolize Glucose-1-phosphate as energy source (Lahtinen et al. 2011). *Clostridium* is reported to ferment Glucose-1-phosphate and glucose via Embden-Meyerhof pathway to pyruvate (Ljungdahl & Eriksson, 1985). Intracellular pathogenic bacterium such as *Listeria monocytogenes* is also utilizing Glucose-1-phosphate as growth substrate (Ripio et al. 1997).

D-Xylose (a pentose monosaccharide) is a five-carbon aldose that can be catabolized or metabolized into useful products by a variety of organisms. *Lactococcus lactis* is reported to metabolize D-Xylose through two different pathways (i.e., phosphoketolase pathway and the pentose phosphate (PP)/glycolytic pathway) (Tanaka et al. 2002). Acetic acid bacteria such as *Acetobacter xylinus,* was able to utilize D-Xylose in the production of cellulose (Ishihara et al. 2002). D-Xylose and other pentose are widespread in nature, and *E. coli* is able to grow on it (Gottschalk, 2002; Shimizu, 2013). Other than *E. coli* and *Lactobacillus*, *Bacillus* spp. was found to use xylose isomerase to convert D-Xylose to xylulose, which is then phosphorylated to enter the pentose phosphate pathway (Rygus et al. 1991). Furthermore, genetically modified *Klebsiella planticola* is able to ferment D-Xylose to ethanol (Tolan & Finn, 1987). The freshwater bacterium, *Caulobacter crescentus* (Caulobacteraceae), was found using D-Xylose as a carbon and energy source (Stephens et al. 2007).

Phenylethylamine ($C_8H_{11}N$) is an organic compound and a natural monoamide alkaloid. Phenylethylamine is widely distributed in plants and animals, and can be produced by certain algae, fungi and bacteria (Smith, 1977; Güven et al. 2010; Kim et al. 2012). *Pseudomonas putida* and *S. liquefaciens* are examples of Phenylethylamine users (Boothe & Arnold, 2002). *Eschericia coli* K12 grows on 2- Phenylethylamine as sole carbon and energy source by converting it, via phenylacetaldehyde, to phenyacetic acid (Parrott et al. 1987).

2-Hydroxy benzoic acid (also known as salicylic acid) is a type of phenolic acid with chemical formula $C_7H_6O_3$ that occurs in nature as a free compound, linked by an ether bridge, glycosidically linked, esterified (Raskin, 1992). 2-Hydroxy benzoic acid can be degraded anaerobically by denitrifying pseudomonads (Tschech & Fuchs, 1987). Thomas et al. (2001) investigated the anoxic degradation of phenol and other phenolic compounds by a defined mixed culture of *Alcaligenes feacalis* and *Enterobacter* spcies. The culture was shown capable in degrading high concentrations of phenol under anoxic condition.

D-L-Glycerol phosphate is a mixture of D- and L-glycerophosphate enantiomers. It is produced from glycerol by the enzyme activity of glycerol kinase. D-L-Glycerol phosphate can be metabolized by *Bacillus, Escherichia, Flavobacterium, Klebsiella*, *Serratia* spp. and *Staphylococcus* spp. (Boothe & Arnold, 2002; Lascelles & Burke, 1978). Besides, *Corynebacterium glutamicum* and *B. subtilis* are able to grow on D-L-Glycerol phosphate (Lindner et al. 2012).

D-Mannitol ($C_6H_{14}O_6$) is one of the most abundant energy and carbon storage molecules in nature, produced by many different organisms including bacteria, yeast, fungi, algae, lichen and plants (Song & Vieille, 2009). D-Mannitol is utilized by *E. vulneris, E. coli, Pasteurella volantium, P. aeruginosa, S. plymuthica, S. arlettae* and *S. lentus* (Rosenberg & Hardy, 1984; Boothe & Arnold, 2002). Gram-negative soil bacteria that fix nitrogen such as *Rhizobium* spp. are reported to metabolize D-Mannitol (Kuykendall & Elkan, 1976). Other than bacteria, fungus is reported to use D-Mannitol as well, as reported in *Aspergillus candidus* by Strandberg (1969).

i-Erythritol ($C_4H_{10}O_4$) is a sugar alcohol (polyol) that occurs naturally in some fruits and fermented food (Shindou et al. 1988). i-Erythritol may be used to support the growth of *Gluconobacter* bacteria (an acetic acid bacteria) (Voss et al. 2010). The causative agent of brucellosis, *Brucella* sp., is metabolizing erythritol and the role of erythritol metabolism in virulence is suspected (Sangari et al. 2000). Metabolism of erythritol by *Propionibacterium pentasaceum* (a Gram-positive, rod shape bacterium) has been studied (Wawszkiewicz & Barker, 1968). It is known that following the establishment of an anaerobic environment, carbohydrates, lipids, and proteins are transformed into inorganic acids (e.g., propionic acid, lactic acid). Perhaps the presence of propionic acid favors the establishment of *Propionibacterium* who metabolizes propionic acid as energy source (Himmi et al. 2000). Erythritol uptake and metabolism were also reported in wood-rotting mushroom, *Schizophyllum commune*, as sole carbon sources for growth (Braun & Niederpruem, 1969).

The decomposition of a cadaver results in the release of the chemical entities of the body through autolysis and putrefaction (Dent et al. 2004). During decomposition

process, cadaveric materials will enter the soil (gravesoil) providing a localize pulse of nutrients which results in the formation of a concentrated island of fertility, also known as cadaver decomposition island (CDI) (Carter et al. 2007). This island is characterized as having increased in soil microbial biomass and microbial activity. The degradation of proteins, lipids and carbohydrates will render carbon-based, nitrogen-based and phosphorus-based products which may be retained in the surrounding soil environment (Stokes et al. 2009). Macdonald et al. (2014) further confirmed that carrion decomposition causes large and long lasting effects on soil amino acid and peptide flux which influence soil N cycling. Although concentrations of metal ions in the gravesoil (especially burial environment) can lead to localized condition of toxicity, which can further prevent microbial activity in the soil (Janaway, 1996). However, an extensive collection of metallic artefacts usually contains insufficient concentrations of metal ions to result in significant retardation of decomposition (Carter & Tibbett, 2008). In general, microbial degradation is described as having three phases. The initial lag phase is characterized by microbial or enzymatic enrichment. During the second phase the substrate is rapidly degraded. This is followed by a declining phase that results from a lack of readily available substrate (Ajwa & Tabatabai, 1994). In this study, we observed mortality of the adjacent plants surrounding the swine carcasses along the decomposition process, probably due to nutrient toxicity. However, CDI could also act as fertilizers to the growth of adjacent plant (*Helianthus annus*) with growth rate of 2.5 cm day^{-1} (see Appendix K).

Soil microorganisms are directly responsible for most of the CO_2 returned to the atmosphere from soil organic matter (Hopkins, 2009). Microorgansims in the soil can be divided between two ecological strategies. Those that respond quickly to addition of fresh substrate are referred as zymegenous component of the biomass (*sensu* Winogradsky, 1924) while those who survived on older and more stable organic matter are termed autochthonous component of the biomass (*sensu* Winogradsky, 1924). In fact, the zymegenous and autochthonous categories are considered analogous to *r*-selected (i.e., rapid progeny proliferation following addition of resource pulse) and *K*-

selected organisms (i.e., those maintaining a constant population), respectively. The micro-environmental condition in the gravesoil (e.g., anoxia, high concentrations of metal ions) will select for a particular community of microorganisms who are able to tolerate the condition to the extent that they will become a dominant component of the microbial communities (Hopkins, 2009).

Putman (1978b) highlighted that the total amounts of carbon dioxide evolution from carrion with and without blow fly colonization remains the same. In winter and spring (when no blow fly activities observed), release of carrion materials is exclusively through respiratory activity of microorganisms. Approximately 100 cal g^{-1} dry weight (25-20 mg organic matter) may be released from within the carcass (brown mice weighing between 18-28 g), a further 20-30 cal g^{-1} dry weight (median 4 mg organic matter) leach into the soil and are released through respiration of soil animals. There is about 3% of all available carrion materials are immobilized (i.e., the absorption of decomposed organic matter by microorganisms). In summer and fall, the respiration of blow fly larvae and microorganisms within a carcass responsible for some 120-130 cal g-1 dry weight (30-35 mg organic matter) while respiratory breakdown of leachates accounts for about a further 30 cal g-1 dry weight (6-9 mg organic matter). Again, these results affirmed that a total of 3% of the available carrion is released through respiratory activity (Putman, 1978).

It has been known that increased microbial activity can begin within 24 h after the cadaver or skeletal muscle tissue being buried (Putman, 1978; Tibbett et al. 2004; Carter & Tibbett, 2006). Stokes et al. (2009) used mouse (*Mus musculus*) cadavers buried at a depth of 1.5 cm in three different types of soil (i.e., sand, sandy clay loam, and loamy sand) to study microbial activity. The results showed significant increase in microbial activity in all soil types, with maximum microbial activity was observed within the first 10 days. The variation observed in the peak levels of microbial activity is thought to result from the different communities present within each soil.

Hopkins et al. (2000) observed pig carrion decomposition in shallow graves. Grave soils have been found to have higher levels of total carbon, microbial biomass and

total nitrogen even after 430 days. Increased rates of respiration and N mineralization have also been detected when compared to control soils. Carter & Tibbett (2003) reported the presence of several strains of Ascomycetes, Deuteromycetes, and saprotrophic Basidiomycetes as early stage decomposers recovered from gravesoil.

Howard et al. (2010) conducted a study on soil microbial community associated with the decomposing swine carcass and identified *Arthrobacter, Bacillus, Brevibacterium, Kurthia, Pseudomonas* and *Serratia*, as well as 18 isolates in the genus *Acinetobacter* (a Gram-negative lipolytic bacterium). Howard et al. (2011) then performed BLAST analysis using the 16S rRNA-gene sequence and identified the isolates as the following *Acinetobacter* species: *A. baumannii, A. haemolyticus, A. junii, A. johnsonii* and *A. gerneri*. The results of this study revealed the association of *Acinetobacter* spp. with carrion.

Lewis (2011) found that there was significant difference in microbial community metabolic activity between seasons for both carrion and soil samples, and carrion samples were significantly different from soil samples. Our results were in-lined with Lewis (2011) where microbial function on swine carrion samples were significantly different from soil samples ($p = 0.001$) (see Table 2.18). Although our study conducted on the summer seasons for two consecutive years, significant difference was noted between years for the same season. Lauber et al. (2014) found that mice placed on soil with intact microbial communities reach advanced stages of decomposition 2 to 3 times faster than those placed on sterile soil. Furthermore, microbial communities associated with skin and gravesoils of carrion during active and advanced decay stages were significantly different between sterile and control soils, suggesting microbial communities have a significant impact on the rate of decomposition.

Pechal et al. (2013) demonstrated microbial community functional change during vertebrate carrion decomposition and provided some results on bacterial community (at phyla level) through 454-pyrosequencing. Metcalf et al. (2013) provided the bacterial and microbial eukaryotic high-throughput sequence time-series dataset for the skin, abdominal cavity and gravesoil associated with decomposing animals. Pechal et al.

(2014a) (note that the paper was available online in 2013) performed high throughput metagenomic sequencing on swabs collected from pig carrion to assess bacterial community succession. Both Pechal et al. (2014a) and Metcalf et al. (2013) proposed that the microbial community or necrobiome could be a potential tool as forensic indicator within the criminal justice system, with a new framework and standard operating procedure of using high throughput metagenomic sequencing in future forensic investigations (Pechal et al. 2014a). The results generated from the current study posed some technical questions, could microbial metabolic community profiling (MMCPs) be useful in the application of forensic sciences? Could we use indicator carbon species analysis (ISA) to determine minimum postmortem interval? Ecologically speaking, MMCPs can be affected by the delayed of dipteran colonization (the treatment effects as in this study), days and years of decomposition (temporal effect), and regions of the pig or soil (spatial effect). Thus, validation studies need to be done to address the above ecological issues before implementing microbial functions and carbon indicators into applications such as forensic investigations or any biomonitoring services (e.g., environmental quality assessment). Nevertheless, similar microbiological framework and standard operating procedures proposed by Pechal et al. (2014a) could be applied to MMCPs if the results were validated in the field and proven accurate and reliable. Another important publication by Cobaugh et al. (2015) who studied soil microbial community structure and function below decomposing human cadavers. They have demonstrated that soil communities exhibit postmortem patterns by undergo distinct functional and structural changes. Also, they found evidence that human-associated microbes persist in the soils for surprisingly long period of time, suggesting a possible role in using soil communities as forensic evidence in cases where body remains have been moved from the original location of decomposition. Besides, their low abundance in natural soils and significant increases during decay making them good candidates as biomarkers for long-term postmortem interval estimates. Finley et al. (2015a, 2015b) published excellent reviews on epinecrotic microbial communities associated with human decomposition and gravesoils (i.e., soil beneath decomposing human), they also

provided a framework for using soil microbial communities to estimate postmortem microbial clock and made comparison between sequencing techniques, and discussed their potential use in forensic investigations.

Recently, Weiss et al. (2015) investigated the effects of carcass mass on the soil microbial community. They concluded that time of decomposition was a significant influence on the microbial community structure, but carcass mass was not. Interestingly, these results were in agreement with the current study, although our study only focused on microbial metabolic community function. We did not see carcass biomass have significant difference ($p > 0.05$) in microbial function as well. Furthermore, this can be inferred from the replicate effect being no significant difference from each other ($p > 0.05$). Each pig (replicate) used in our study had different biomass (varied from 15.5 – 30 kg), suggesting that biomass of carcasses did not play a significant role in MMCPs.

With the advent of molecular technologies such as next generation sequencing (NGS), DNA extraction methods from gravesoils are becoming important. Finley et al. (2015c) assessed four different methods of microbial DNA extraction of cadaver soil samples for criminal investigations and the statistical result revealed a significant correlation between the yields and days in the soil using phenol-chloroform method. Although this study did not employ NGS in sequencing bacterial community associated with pig carrion, our attempt by understanding microbial metabolic community function over the 40 days of carrion decomposition provides ecological insights on microbial metabolism on carcasses throughout the whole decomposition process.

To date, terrestrial necrobiome associated with human corpses and gravesoils have been studied in the United States (Howard et al. 2010, 2011; Pechal et al. 2013, 2014a; Metcalf et al. 2013; Lauber et al. 2014; Cobaugh et al. 2015; Weiss et al. 2015; Metcalf et al. 2015), including the first forensic entomological and microbiological survey (with microbial species identified) in Romania (Iancu et al. 2015). As for aquatic necrobiome, Benbow et al. (2015) was the first to describe aquatic bacterial succession using high-throughput metagenomic sequencing on vertebrate remains submerged in a

freshwater habitat, and provide initial evidence for their potential use in forensic investigation.

CONCLUSIONS

Current study demonstrated that there was a shift in microbial metabolic community profiling of between insect accessed carrion (control) and insect delayed vertebrate decomposition (treatments). Hence, the null hypothesis was rejected. There was a significant difference between years. For microbial function on pig carcasses, there was significant difference between regions, as well as decomposition days, suggesting microbial metabolic profiles were different temporally and spatially. As for soil samples, there was variation in microbial metabolic community profiles between years, indicating a stochastic microbial response in soil ecosystem. Soil samples collected from two sites (beneath and lateral of the carrion) in cadaver decomposition islands showed a significant difference among each other, and also significantly different with the control soil as well. Similarly, microbial metabolic community profiles on soil samples changes over time, indicating a successional pattern of microbial function. Indicator species analyses were conducted on pig and soil samples and 19 carbon substrates and 20 carbon substrates, respectively, were identified statistically. Indicative carbon species were consistent by region (either on pig or in the soil), but changing over time, suggesting changing microbial community structure along the course of decomposition while microbial community structure was distinctive between pig region or soil collection site. Although microbial community structure was not determined in this study, and thus could not correlate with microbial function. However, the carbon indicators provide some ecological insights regarding possible bacteria genera that are commonly associated with decomposing carrion. In terms of application, the results generated from in this study could provide baseline data of mean microbial function activity on carrion, with or without delayed insect colonization, to be used in determining the time of death or mPMI, or to determine whether blow fly colonization has been delayed on corpse due to wrapping or being kept in a concealed environment.

Such changes in microbial function could also implicate the application of forensic microbiology as most of the studies conducted on necro-microbiome were from the human or animal carcasses without delay in dipteran colonization. Thus, this study provides the evidence that delay of dipteran colonization could impact microbial function. Besides, similar applications can be employed by using carbon indicators as demonstrated in this study. Moreover, forensic microbiologists could use both parameters (i.e., mean microbial activity and carbon indicators) together to determine mPMI and possible bacterial community on cadaver. Framework and standard operating procedures of using MMCPs and indicative carbon sources could be proposed for the use in forensic microbiology. Most importantly, both mean microbial function and indicator carbon substrates need validation studies in the field using human corpses to correlate with the time of death (mPMI), to determine the potential error rate, reliability (precision), accuracy (validity), and reproducibility in order to apply in forensic cases, complying to the *Daubert's* standard. Last but not least, MMCPs and indicator species analysis could be useful in monitoring environmental health and quality by assessing the ability of recovery (resilience) in the soil microbial functions following natural perturbations (e.g., forest fire, flash flood, mass mortality event) or man-made disturbances (pollution, eutrophication, radiation exposure etc.) to the environment. Microbiological methods such as Biolog is sensitive to detect subtle or mild ecosystem perturbations, hence it is suggested that MMCPs could be developed as a novel tool to evaluate environmental status, provide risk assessment and to monitor ecosystem functions for resource sustainability.

CHAPTER III

SOIL CHEMISTRY DYNAMICS OF DELAYED VERTEBRATE DECOMPOSITION

INTRODUCTION

Soil is an organo-mineral assemblage, and its formation and properties are largely depends on biological, chemical and physical processes (Lavelle & Spain, 2001). According to the definition by the Soil Science Society of America (1997), soil is defined as an unconsolidated, stable, three-dimensional assemblage of organic, mineral and organo-mineral materials with a characteristic biota and located at the earth's surface. Soil have five principal roles in ecosystems namely: (i) Mechanical support, which providing mechanical support for plant life; (ii) Habitat provision for vast varieties of soil organisms essential to its functioning; (iii) Storage of organic matter ranging from freshly-fallen leaf litter, dead plant root, to decomposed animal carcasses. These biological matters are important energy sources for many soil organisms and an essential structure to the organization and stability of the soil matrix; (iv) Element release from anion and cation exchange sites in the soil. Soil contains many elements such as zinc, iron abd calcium that are vitally important for many biological processes. Decomposition of organic materials liberates the contained elements in inorganic forms (mineralization) in a controlled or "slow-release" way for uptake by plant roots and other soil organisms; (v) Water storage. Soil supports the growth of plants and other organisms by acting as a water store. However, the capacity of water storage depends on soil depth, soil particle size and density and location in the landscape (Buol, 1995).

It is estimated that 99% of the organic matter in a terrestrial ecosystem is derived from plant decomposition (e.g., leaf and stem litter, root litter and exudates) (Swift et al. 1979). Therefore, plant decomposition has received much attention over the last several decades (Bjørnlund & Christensen, 2005) compared to the decomposition of dead mammals, which has been largely neglected (Allee et al. 1949). Little is understood about the fate of carcass-derived carbon and nutrients and cadaver components

contributed to the ecosystem and food web (Carter et al. 2007). In fact, studies on nutrients from animal carcasses or human cadavers entering the soil have recently emerged in the literature since 2000, with considerable articles being published from researchers such as Shari L. Forbes, David O. Carter, Mark Tibbett, Jacqui Aitkenhead-Peterson, Laura A. Benninger, and many others.

For example, one study showed the average annual bison (*Bos bison* L.) biomass in 988 ha of North American tallgrass prairie from 1998 to 2004 was 92,432 kg (personal communication with E.G. Towne as cited in Carter et al. 2007). An average mortality rate of 5.6% resulted in an annual bison cadaver input of about 5,000 kg and this represented more than 1 % of the organic matter input in some terrestrial ecosystem (Carter et al. 2007).

Decomposition results in the release of the chemical components of the body through autolysis and putrefaction (Dent et al. 2004). Carcasses that are not consumed by vertebrate scavengers will be utilized by microbes and invertebrate consumers (Putman, 1987). During decomposition, cadaveric materials will enter the soil providing a localized pulse of nutrients which results in the formation of a concentrated island of fertility known as Cadaver Decomposition Island (CDI) (Carter et al. 2007). This sudden nutritious patch area is associated with increased soil microbial biomass and microbial activity, as it is the result of protein, lipid and carbohydrate degradation which yield rich nitrogen-based, phosphorus-based, and carbon-based products into the soil directly beneath the cadavers and possibly to the surrounding areas (Benninger et al. 2008; Aitkenhead-Peterson et al. 2012).

Gravesoil is any soil that is associated with cadaver decomposition, regardless of the species of vertebrate or whether decomposition takes place on or within the soil (Efremov, 1940; Carter et al. 2007). Literature from the early and mid-20th century tried to understand gravesoil through empirical observations (Illingworth, 1926; Mant, 1950) with a predominant amount of the literature focused on associated insect activity (Bornemissza, 1957; Payne et al. 1965). Insects, scavengers and microbes are known to compete for cadaveric resources (Payne et al, 1965; Janzen, 1977; DeVault et al. 2004).

Insects are usually the first to consume a cadaver (Putman, 1978; DeVault et al. 2004). Smaller cadavers (i.e., rodents) tend to be consumed *ex-situ* so that the amount of cadaveric materials entering the soil might be negligible (Putman, 1983). Adult or larger cadavers tend to be consumed *in situ*, which allows cadaveric materials to enter the soil (Coe, 1978; Towne, 2000) or be left on the soil surface as recalcitrant residues such as hair, nails, or desiccated skin (Putman, 1983). Therefore, a significant amount of cadaveric materials may transfer into the soil when arthropods and microbes dominate cadaver decomposition or the cadaver is too large to be carried away by a scavenger (Carter et al. 2007).

The decomposition process is often associated with a number of stages namely fresh, bloated, active decay, advanced-decay and dry-remains stage (Payne, 1965). Although decomposition stages are a convenient method in categorizing the progress of decomposition process, it is more often presented in continuum of change rather than discrete series (Schoenly & Reid, 1987). The progress of cadaver decomposition is largely affected by temperature; hence, Accumulated Degree Day (ADD, average thermal summation of a day) can be used to compensate for differences in temperature (Vass et al. 1992).

The majority of organisms associated with carrion exploit these resources for the energy contained in the chemical bonds of the organic molecules, such as C, N, P and S. The release of inorganic C from the organic matter as CO_2 through the respiration of decomposer organisms is the major return route of C to the atmosphere, thus balancing the flux of CO_2 from atmosphere into biomass through photosynthesis (Hopkins, 2009). Inorganic nutrients such as P, S, Ca and K are rarely limiting to microbial activity in soil. Nitrogen is released in inorganic forms such as NH_4- through mineralization during organic matter decomposition. Conversion of NH_4 to NO_3 occurs under certain conditions such as an aerobic environment and lack of labile C for microbial utilization of NH_4. The reverse of N mineralization, in which the decomposer organisms assimilate N from other sources in the soil, is referred to as N immobilization. The balance between N mineralization and immobilization depends on quantitative as well as qualitative

aspects of the organic matter and the decomposer organisms (Swift et al. 1979; Jenkinson 1981). Generally, a C:N ratio of around 20-22 is the threshold. Above this ratio and N is immobilized; below this threshold and N mineralization occurs (Harmsen & Schreven, 1955). In comparison with plant biomass, animal biomass usually has a lower C:N because of the large proportion of structural proteins. As high C:N material decomposes, net N mineralization occurs and NH_4^+ accumulates. Even in acidic soils, the alkaline effect of NH_4^+ may lead to increased pH around decomposing animals (Carter & Tibbett, 2006; Hopkins, 2000).

Parmenter & Lamarra (1991) measured the decomposition rate and nutrient loss sequences of rainbow trout (*Oncorhynchus mykiss*) and pinktail duck (*Anas acutas*) carcasses in a Wyoming marsh over a 10-month period. They found that fish carrion decomposed more rapidly than waterfowl carrion. After 10 months, fish carcasses had lost 85% of their initial dry mass, while duck carcasses had lost only 30%. In terms of nutrients, fish carrion lost 95% of N and 60% P while waterfowl carrion lost 65% N and 30% P. The sequence of total element loss rates from carcasses was K > Na > N > S > P > Ca ~ Mg and was similar for both types of carrion. The authors concluded that carrion-derived elements to ecosystems nutrient budget is site-specific, however, since the aquatic environment supports large vertebrate populations, carrion decomposition can contribute significant amounts of important nutrients that ultimately influence the structure and function of the aquatic ecosystem.

Towne (2000) investigated the impact of large ungulate carcasses (*Bos bison, Bos taurus* and *Odocoileus virginianus*) on grassland dynamics (i.e., soil and vegetation response) in northeastern Kansas. The results demonstrated that inorganic N (NH_4-N + NO_3-N), P, and pH were influenced by interactions among animal size, years after death, and distance from the carcass center. Soil K concentration was not different between the center of carcass sites and surrounding soil. Inorganic N and P concentrations were higher in the center of carcasses. Mean pH was significantly lower in the center of carcass site (6.32 ± 0.24) than in the surrounding soil (7.34 ± 0.10).

Parmenter & MacMahon (2009) studied decomposition rates of vertebrate species (e.g., rat carcasses, *R. norvegicus*) in a semi-arid, shrub-steppe environment in Wyoming, USA. They found that decomposition rate varied significantly between microsites (below > surface) and among seasons (spring > summer > autumn ~ winter), with mass loss linearly correlated with ambient air temperature. They also found that energy, K, Na, N, and S being lost to ecosystem more rapidly than skeletal components such as P, Mg, Ca. Furthermore, soil beneath the carcasses showed N, P, and Na increased during decomposition. They concluded that at a landscape scale in the shrub-steppe ecosystem, carrion decomposition constituted < 1 % of the nutrient-cycling budget but contributed significantly to localized soil nutrient dynamics. Similarly, Stokes et al. (2009) investigated the decomposition of small mouse carcasses buried in soil in Western Australia and large pig carcasses placed on the soil surface in southern Ontario. The results showed that regardless of the type of animal model or size of the carcass, nitrogen and phosphorus-based products appeared to offer the greatest potential as forensic tools as there were significant increases in concentrations of these products when compared to the control samples and, with further research, may demonstrate a direct correlation with the postmortem interval.

Carrion decomposition also causes large and lasting effects on soil amino acid and peptide influx, as demonstrated by Macdonald et al. (2014). Two important and overlooked N pools are free amino acid (FAA) and peptide pools that are newly recognized as a source of competition between plants and microorganisms for the N resource. The researchers used kangaroo carcasses (*Macropus giganteus* (Shaw)) and found that there was a significant lasting input of proteins (40 mg/kg) and amino acids (25 mg/kg) into the soil, which increased microbial turnover of these labile N compounds. The authors argued that the immediate removal of carcasses as an ecosystem management tool maybe harmful to the soil nutrient cycles as carrion provide lasting resource islands which influence soil N cycling.

Soils are likely the most valuable to forensic taphonomy (i.e., study of a decaying organism over time and how they may become fossilized) especially after the advanced

decay stage. At this time, the fly larvae have migrated, which greatly reduces their value as forensic tool to estimate PMI. Thus, soil science might be developed as the most accurate means to estimate the extended post mortem interval (PMI) (Carter & Tibbett, 2008). Other than blow fly, several members of the cadaver decomposition food web should be studied to provide robust methods for estimating PMI and for locating clandestine graves. For instance, an estimate of PMI based on insect larval development could be used in conjunction with the concentration of fatty acids in soils and soil bacterial community profiles (i.e., microbial community structures and functions). When used together, multiple aboveground and belowground measures will provide higher confidence to the interpretations of the evidence found at the death scene (Carter & Tibbett, 2008).

Soil chemistry associated with human cadavers has been studied. Aitkenhead-Peterson et al. (2012) used two human corpses to examine the spatial extent of the CDI (one subject was accessible to scavengers and the other was protected from scavenging). They demonstrated that water soluble decomposition products moved off site significantly for pH, electrical conductivity (EC), dissolved organic carbon (DOC), orthophosphate-P, ammonium-N, dissolved organic nitrogen (DON), and K. One of the significant findings was the mapped CDI differed considerably depending upon whether the subject was open to scavenger activity (possibly due to bobcat or vulture). Furthermore, the researchers found that regression analysis with electrical conductivity as the independent variable explained 78% of the variance in soil DON (which is considered a strong positive relationship), suggesting that this method may be an inexpensive way to determine gravesoil in the field particularly between 5469 and 5799 ADD.

Three years later, Aitkenhead-Peterson et al. (2015) examined cold water extractable soil C, N, and P in the CDIs below 14 human cadavers at two sites in Texas. Soil samples were collected from beneath the torso of cadavers at various stage of decomposition. The results indicated that the concentration of soil nitrate-N remained below ambient soil concentration for approximately a year. Nitrate-N is reduced because

under an anaerobic condition beneath the decomposing human corpse, anaerobic bacteria use nitrate as an electron acceptor or an oxygen source. This reducing environment convert NO_3^- to NO or N_2, which are then released as gases. The increase in NO_3-N concentration in CDIs one year after placement of the remains was likely due to burrowing or drilling activities imposed by soil microarthropod communities and plant root growth that causes aeration and oxygenation. Likewise, ammonium-N cannot be nitrified in soil containing purge fluid as the reaction of nitrification requires oxygen (an aerobic process). Hence, ammonium-N is accumulated and remained high in CDIs. However, ammonium-N can be utilized by fungi and soil bacteria (Sagara, 1976), as such, ammonium-N can be expected to decrease due to microbial uptake. As for the DON, it tended to increase in the CDIs and then decrease after 196 days PMI. This is because only certain bacteria can mineralize DON to ammonium-N under anaerobic environment in the CDI. The data indicated both mineralization (conversion of DON to ammonium) and immobilization (conversion of ammonium into DON) may occur after 176 days of decomposition of the remains. The decline in ammonium-N and DON with a subsequent increase in nitrate-N after one year indicates that normal aerobic soil conditions are returned. For DOC, it tends to remain high for about a year. DOC is a substrate for soil microorganisms along with ammonium-N. DOC is expected that it would be eventually mineralized to CO_2 but not until aerobic conditions are restored in the CDIs. The slow breakdown of DOC and potential loss as methane gas under anaerobic condition makes it a good option for predicting PMI (Aitkenhead-Peterson et al. 2015). It should be noted that these are the conditions for surface soils (i.e. 5-7 cm depth) and that there is a high potential for C, N and P movement down the soil profile depending on the specific soil saturated hydraulic conductivity. Recent research showed that C, N and P decomposition products in grave soil are significantly higher than control soil to depths of 30 cm at > 333 d to 680 d PMI (Aitkenhead-Peterson et al. in preparation).

Recent research includes using the human remain detection (HRD) dogs to identify gravesoil. Residual odor from previously decomposing human remains may stay

in the soil and on surfaces long after the remains are gone. The results showed the HRD dogs were able to detect the odor of human remains successfully (75% and 100% accuracy) up to 667 days post-body removal from soil surface (Alexander et al. 2015). Forbes & Perrault (2014) investigated the decomposition odor profiles surrounding vertebrate carrion (pigs, *S. scrofa*) to determine how volatile organic compounds (VOCs) partition between soil and air. They detected 58 compounds that were common to both air and soil samples and demonstrated that soil and air samples produce distinct subsets of VOCs that contribute to the overall decomposition odor.

Pechal et al. (2013; 2014a; 2014b) studied the microbial structure, microbial function, and necrophagous insect community associated with pig carrion experiencing delayed Diptera colonization for a period of five days. Similarly, Kneidel (1984) conducted a blow fly-exclusion experiment to determine whether other dipteran species would colonize mammal carcasses (e.g., *Mus musculus* L.) if blow flies (*Phaenicia caeruleiviridis* Macquart) were excluded for five days.

Soil resilience was introduced into soil science around 22 years ago, mainly to address soil ecology and sustainable land use issues (Blum, 1994). Soil resilience has been defined as the capacity of a soil to recover its functional and structural integrity after a disturbance (Herrick & Wander, 1998; Pimm, 1984). A disturbance is commonly defined as any event that causes a significant change from the normal pattern or functioning of an ecosystem (Forman & Godron, 1986). Whether an event is considered to cause a significant change from the normal function depends on the temporal and spatial scale of interest (Seybold et al. 1999). For example, formation of a single earthworm (Annelida: Megadrilacea) burrow is clearly a disturbance at the scale of the root system but it may be considered part of the normal pattern at the field scale. Other instances of natural disturbances include fires, earthquakes, floods, landslides, and high-intensity storms (Seybold et al. 1999). Some even considered agriculture itself to be one of the greatest stressor through disturbance to the environment via activities such as tillage, application of fertilizers and pesticides, and removal of competitive plant species (e.g., monoculture) (Bezdicek et al. 1996).

The soil capacity to recover from disturbances comprises of two main components: the rate of recovery and the degree of recovery (Herrick & Wander, 1998). The rate of recovery is the amount of time needed for soil to recover to its initial potential after a disturbance while the degree of recovery means the magnitude of recovery to certain stabilized potential relative to its pre-disturbance state. If the disturbance is too drastic, the soil can undergo irreversible degradation in which its capacity to function will not recover within a designated time frame. In this case, the soil resilience capacity has been exceeded, resulting in permanent damage or the need for very expensive restoration measures. The greater the rate or the degree of recovery, the more resilient the soil system is to a specific disturbance (Seybold et al. 1999).

Soil quality is defined as the capacity of a specific kind of soil to sustain plant and animal productivity, maintain and enhance water and air quality, and support human health and habitation (Karlen et al. 1997). Soil resilience is related to soil quality in terms of recovery of soil functions. During a disturbance, soil quality becomes a function of soil resistance. After a disturbance, soil quality becomes a function of soil resilience. Because disturbances are ubiquitous in nature, soil resilience and resistance characteristics become fundamental components of soil quality (Seybold et al. 1999).

Although carrion can be considered as a “disturbance” to the environment (or “functional ecosystem”, it depends on the viewer perpective). Whether carrion is a disturbance depends largely on the scale of the disturbance or the scale of the ecosystem one is interested in. In the case of mass mortality events (MMEs), which are a rapid, catastrophic die-off of organisms, undoubtedly demonstrate their wider scale of ecological importance to the environment (Fey et al. 2015), as the excessive influx of nutrients can turn the soils around the carcasses into a toxic land (due to N toxicity) (Britto & Kronzucker, 2002). In a carrion study conducted by Kneidel (1984), he considered that the scavenging activity on carrion was a “disturbance”, and concluded that disturbance by non-dipteran scavengers such as ants (Hymenoptera: Formicidae), silphids (Coleoptera: Silphidae) and scarab beetles (Coleoptera: Scarabaeidae) helped to maintain the high diversity in small non-mammalian carcasses (e.g., slugs).

As such, the resilience capacity of a soil associated with carrion is vitally important for soil ecosystem health. Soil with high resilience (higher rate and degree of recovery) will recover from excessive nutrients (i.e., disturbance) contributed by carrion over the course of decomposition. A low resilience in soil will result in the shift of soil equilibrium to a new regime of behavior (*sensu* Holling, 1973).

The objective of this study was to investigate soil chemistry profiles of delayed vertebrate decomposition. The pig carcasses were not viewed as disturbances, but the treatment effects were. The treatment effects included a delay of blow fly colonization on pig carrion for a period of 7 and 14 days. I considered the delay onset of blow fly colonization a disturbance. It is well known that blow flies access to carrion can occur immediately after death and lay eggs on carrion within an hour (Anderson & VanLaerhoven, 1996). However, blow fly colonization on a carcass can be delayed due to biotic (e.g., inter-intraspecies competition, intra-guild predation, priority effects, inter-kingdom communication, and quorum quenching, to name a few) and abiotic factors (extreme weather, natural disasters, burial or hidden activity and, concealment) (Campobasso et al. 2001; George et al. 2013). Hence, the absence of the primary decomposers (e.g., necrophagous Diptera) may cause a "disturbance" to the normal function of the decomposition process. It should be noted that the process of cell autolysis and microbial decomposition are still taking place on the carrion which are mostly performed by intestinal and soil microbes (Vass, 2001; Dent et al. 2004), while the blow flies were excluded during the exclusion periods. The pattern of soil chemistry profiles associated with carrion experiencing delayed blow fly colonization compared to the control carrion (i.e., with immediate blow fly colonization) was my major interest coupled with how soil resilience may work in these situations.

METHODS

Site description and experimental design

The decomposition of swine carcass (*S. scrofa*) were studied at the Field Laboratory, Texas A&M University, College Station, Texas, USA (30°33' 18.54'' N

96°25’38.71’’ W, 68 m a.s.l.). The perimeter of the study area was approximately 371 m and the area was approximately 7,943 m^2 (Figure 2.1 and 2.2). Soil at the site was 90% Ships Clay soil series which is a very-fine, mixed, active, themic Chromic Hapludert with a 0-1% slope. The texture of the soil was 70% clay and 8.9% sand with the remainder being silt to a depth of 0-23 cm. The saturated hydraulic conductivity of the soil was 0.756 mm hr^{-1} (http://casoilresource.lawr.ucdavis.edu/gmap/). The vegetation at the study site is considered blackland prairie ecoregion (http://www.texasalmanac.com). Common vegetation found at the study site included Johnsongrass (*Sorgum halepense* L.) (the dominant cover plant, covered approximately 75% at the study site), oak (*Quercus* spp.), annual sunflower (*Helianthus annuus* L.), thistles (*Cirsium* spp. Mill.), Western horse nettle (*Solanum dimidiatum* Raf.), Camphorweed (*Heterotheca subaxillaris* (Lam.)), muskmelon (*Cucumis melo* L.), jujube (*Zizyphus jujube* Miller),wild purple morning glory (*Ipomoea cordatotriloba* Dennst. tievine), pink evening primrose (*Oenothera speciose* Nutt.), poison ivy (*Toxicodendron radicans* (L.) Kuntze) and arrow-wood (*Viburnum dentatum* L.)

Studies were conducted in two consecutive summers from 16, June 2013 and 15, June 2014. Both field trials were followed up with two post-experiment sample collections, which were taken on 14 September (Day 90) and 15 December (Day 180) for the 2013 field session and 13 September (Day 90) and 12 December (Day 180) for the 2014 field session. Soil samples were also collected on Day 245 of each trial for a soil porosity study. The pig carcasses were sex determined and weighed using a hanging scale.

A total of nine pig carrions were obtained for each year and they were purchased from a local farm at Anderson, Texas. The carcasses were double bagged and transported within 1 hour after death to the study site. Time of carcass placement in the field was recorded (around 1700) and carrions were randomly placed minimally 20 m apart along three transects. All carcasses were oriented with heads to cardinal north and dorsal side towards the east. The random placement of pig carcasses in the field was calculated by using Latin Square design and the arrangement of treatments groups were

different between years (Figure 2.3 and 2.4). Besides, the spot of carcass placement were different between trials, which were five meters towards the East in 2014 trial from the original location in summer 2013, albeit the same study site was used. During each field season, three random carcasses were enclosed in individual 1.8 m x 1.8 m x 1.8 m Lumite® screen (18 x 14 mesh size) portable field cages (BioQuip Products, Rancho Dominquez, CA, USA) for seven days to exclude insects, this treatment was designated as Post-7 group. Another three carcasses were enclosed with similar manner as above but enclosed for 14 days, thus were designated as Post-14 group (Figure 2.5). Insects were allowed to access the remaining three carcasses, which were served as Control (Figure 2.6). All carcasses were covered with hand-made anti-scavenging cages (0.6 m height x 0.9 m width x 1.2 m length) constructed of steel frames enclosed with poultry netting. Each anti-scavenging cage was topped with a layer of woven green fabric (Figure 2.6) to prevent direct sunlight and heat on the carcass to mitigate drying too soon during the summer. All cages were then properly labelled according to their designation. Large rocks were placed on top of each cage to prevent scavenger activities. Daily routine observations were conducted every night at approximately 10 pm in the field to check for vertebrate scavenger activity.

Climatological data such as temperature and precipitation were recorded. Three NexSens DS1923 micro-T temperatures loggers (Fondriest Environmental, Inc., Alpha, OH, USA) (Figure 2.7) were placed at the study site 0.3 m above the ground to measure local ambient temperature every 60 min for 40 days continuously. Temperature data were converted into accumulated degree hours (ADH) based on the following formula:

$$\mathrm{ADH} = \sum_{i=1}^{n} (\emptyset - \emptyset_0)$$

where $\emptyset$ is the ambient temperature (in degree Celsius). The minimum threshold temperature is $\emptyset_0$ (Higley & Haskell, 2009). Although it is a species specific value, we set the minimum development temperature threshold as 0°C in this chapter. To obtain

the value of accumulated degree days (ADD), the ADH was divided by 24 (i.e., ADD = ADH / 24). Precipitation during the study period was recorded using a rain gauge that was placed on the wooden stake approximately 1.20 m above the ground, and about 1 m north from the carcass at the study site (Figure 2.8).

Soil samples at the study site were taken five days (Day -5) prior the placement of carrion. These soil samples were then sent to Soil, Water and Forage Testing Laboratory, Texas A&M University, College Station for a baseline soil chemistry study. During the first and subsequent days of the experiment, soil samples were collected from beneath and at the side of pig carcasses (designated as soil lateral, which is approximately 30 cm distant from the location of soil beneath the carrion), along with a control sample which was located five meters away north from the carrion (Figure 2.12) on Days 0, 7, 14, 21, 40, 90 and 180 to evaluate the soil chemistry profiles over time. Soil samples were also collected on Day 243 of each annual trial for soil porosity study. Approximately 400 g soil sample were collected (using a plastic trowel and a steel tin can with 170 g capacity) (Figure 2.13) that were disinfected using Lysol after every single use. After collection, each soil sample was labeled and kept in a Ziploc bag. Note that in the 2014 trial, an additional five soil samples were collected from the highest slope (at the East edge) of the study site (ca. 70 m a.s.l.) on every sampling day (Day 0 until Day 180) to serve as control to all treatments (Figure 3.1). All soil samples were taken to a depth of 10 cm using a 2cm diameter steel soil corer. Collected soil samples were kept in cooler box (L: 70 cm; W: 40 cm; H: 45 cm) filled with ice (~4°C) and transported to the F.L.I.E.S. Facility. Each sample was well mixed and approximately 20 g of soil was then separated from each individual sample using a plastic spoon and then the subsample was kept in a smaller Ziploc bag. These subsamples were stored in the freezer (Kenmore®, USA) at -20 °C until soil extraction.

Soil nutrient extraction and soil chemical analysis

Each 20 g soil subsample was logged into a database and assigned an identification number. All soils were processed as field moist. Samples were defrosted

and then transferred into a stainless steel mesh sieve (2 mm sieve) to remove stones and root debris. The fraction passing through the 2-mm sieve was collected and weighed using a scale (Mettler Toledo®, USA), while the soil fraction more than 2 mm was also weighed and recorded separately. A total of 3 g was then collected from 2 mm sample with a steel spatula, and transferred into a 50 ml HDPE (High Density Polyethylene) centrifuge tube. Approximately 30 ml ultra-pure water (Barnstead Water Purification System; Thermo Scientific Inc.) was added to the centrifuge tube and then weighed to achieve a 1:10 soil:water ratio. The centrifuge tubes were then place on a shaker (VWR®, USA) for 18 hrs at 50 rpm. After 18 hrs, the tubes were centrifuged using a Sorvall™ RC 6 Plus (Thermo Scientific, USA) at 25 °C at 10,000 g-force for 20 minutes. After centrifugation, the supernatant was removed from the tube using syringe and canula, and transferred into a beaker where pH and electrical conductivity (EC) were recorded. Extracts were then filtered under vacuum through a Whatman® GF/F filter (nominal pore size 0.7 μm) to remove any floating organic maerials. Samples were analyzed for nutrients immediately using a chemical analyzer (i.e., SmartChem). Figure 3.2 (A - E) showed the equipment used in NAWA laboratory.

Ammonium-N was analyzed using the phenate hypochlorite method with sodium nitroprusside enhancement (USEPA method 350.1) and nitrate-N was analyzed using Cd-Cu reduction (USEPA method 353.4). Orthophosphate-P was quantified using the ascorbic acid, molybdate blue method (SmartChem 200 Method 410-3651). All colorimetric analytical methods were performed with a SmartChem Discrete Analyzer (Westco Scientific Instrument Inc, Brookfield, CT, USA) (Figure 3.3). For the 2014 trial, two more analyses were added to the chemical analysis: dissolved organic carbon (DOC) and total dissolved nitrogen (TDN). DOC and TDN were measured using high temperature Platinum-catalyzed combustion with a Shimadzu TOC-VCSH and Shimadzu total measuring unit TNM-1 (Shimadzu Corp., Houston, TX, USA). Dissolved organic carbon was measured as non-purgeable organic carbon (NPOC) which entails acidifying the samples (250 μl 2 M HCl) and sparging for 4 min with C-free air. Dissolved organic nitrogen (DON) was estimated as the product of TDN-(NH_4-N +

NO_3-N). National Institute of Standard and Technology (NIST) traceable and control standards plus replicate samples was run every 10^{th} sample on all analyses to monitor instrument precision and for quality assurance and control. A simplified flow chart of soil nutrient extraction techniques is shown in Figure 3.4.

Figure 3.1. Map showing the location of the study site near Snook, Texas. Number in the box indicates elevation at that particular spot. Slope is gradually elevated from West to the East (the upper slope, which is 0.3 – 0.6 m higher). Note that the North direction is indicated in the compass at the top right (Google Map 2013).

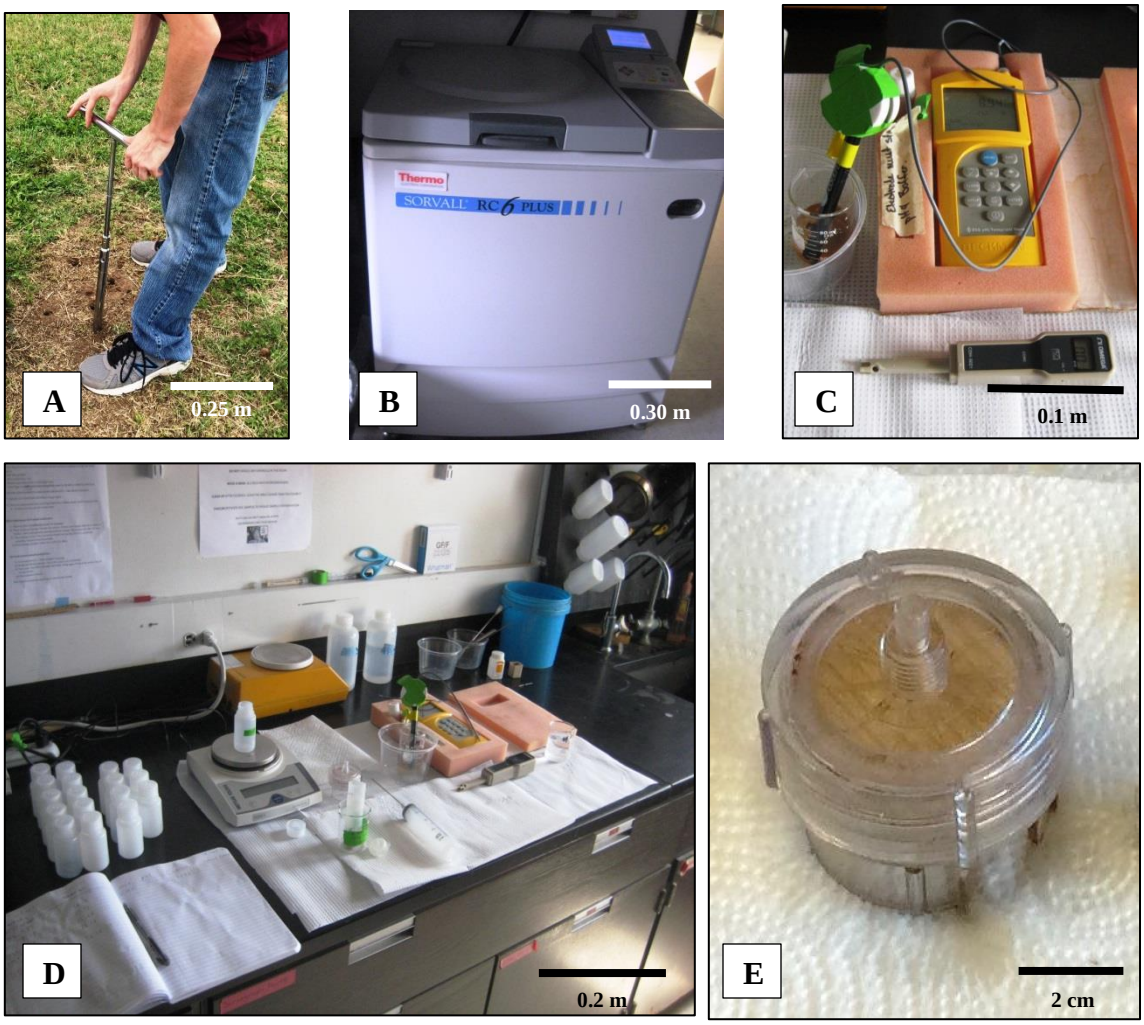

Figure 3.2. Equipment used in the NAWA laboratory for soil collection and soil nutrient extraction for both summers 2013 and 2014 at Snook, Texas. A) a core sampler to collect soil samples, B) centrifuge, C) pH meter and a conductivity meter, D) soil nutrient extraction setup on a working bench in the NAWA laboratory, E) a nucleopore filter.

Figure 3.3. SmartChem Discrete Analyzer (located at the NAWA lab, Texas A&M University) used to measure soil nutrient concentrations (NO_3-N, NH_4-N and PO_4-P) from the soil samples collected in both summers 2013 and 2014 at the field site located at Snook, Texas.

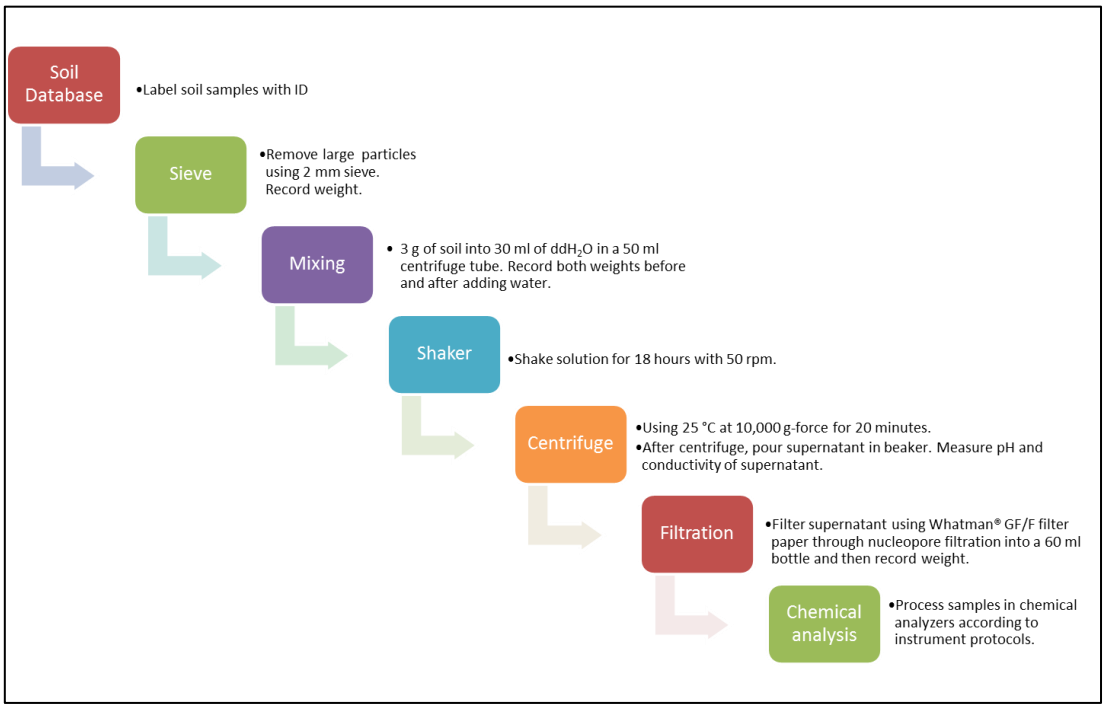

Figure 3.4. Simplified flowchart of soil nutrient extraction techniques performed in the present study. All soil processing works were conducted in the NAWA lab, Texas A&M University.

Soil porosity

Soil samples for the porosity calculation were collected on Day 243 postmortem for both the 2013 and 2014 trials. A total of 30 soil samples (27 soil samples were from the pig locations while three samples from the upper slope of the study site) were collected. Soil samples were collected using a soil core sampler (2 cm in diameter, 10 cm in height). After soil collection, samples were weighed, and then oven dried for 72 hrs at 60 °C. After 72 hours, the weight of the samples was then determined (dry soil mass).

To determine soil porosity, the following formula was used:

$$\text{Porosity} = 1 - \frac{D_b}{D_p}$$

where D_b is soil bulk density and D_p is soil particle density. Soil bulk density (g/cm^3) was measured by the formula:

$$D_b = \frac{\text{mass of dry soil (g)}}{\text{volume of core } (cm^3)}$$

Volume of the core sampler (= volume of solid and pore spaces) was measured by using the formula:

$$V = \pi r^2 h$$

where V = volume (cm^3), r = radius of the core sampler (cm), and h is the height of the core sampler (cm). In the present study, the core volume was 31.4 cm^3.

To obtain soil particle density (D_p), the following formula was used:

$$D_p = \frac{\text{mass of dry soil (g)}}{\text{volume of solid } (cm^3)}$$

To calculate soil particle density, the pycnometer was first filled with water and weighed. It was then partially emptied, a known mass of dry soil (M_{solids}) was added, and it was refilled with water and weighed. The difference in mass of the pycnometer when filled with water and the mass when filled with water and soil minus the mass of the soil was equal to the mass of water displaced. The volume of water displaced is the mass of water displaced divided by the density of water (1 g/cm^3). The volume of water displaced is equal to the volume of soil solids. The mass of solids divided by the volume of solids (V_{solids}) gives the particle density of the solids. The above statements can be expressed in formula as follow:

$$V_{solids} = \frac{M_{pycnometer+water} - (M_{pycnometer+water+solids} - M_{solids})}{\mathcal{P}_{water}}$$

$$= \frac{M_{water\ displaced}}{\mathcal{P}_{water}}$$

To obtain the soil particle density, six soil samples (n = 6) from 2013 trial and 12 soil samples (n = 12) from 2014 trial were used for calculation and the results obtained were 2.15 g cm^{-3} and 2.25 g cm^{-3}, for 2013 and 2014 trials, respectively. Note that the value 2.65 g/cm^3 is always assumed when soil particle density is unknown (which is the average density of a mineral surface soil) (Bruand et al. 1996). The lighter soil particle density in this study may indicate more organic matter presence in the soil at the study site, compared to the general assumed mineral soil.

Statistical analyses

Soil chemistry data were analyzed using statistical program JMP® Pro version 11.0.0 (SAS Institute Inc., NC, USA) for ANOVA, Tukey-Kramer HSD post-hoc test, correlation and multiple regression analyses. Cross validation on each regression model was performed using random KFold in JMP. In addition, R project for statistical computing (R 3.0.2) was employed to analyze soil chemistry data using vegan package (Oksanen et al. 2013). Vegan contains the methods of multivariate analysis (e.g.,

PERMANOVA, NMDS) needed in analyzing ecological communities, and tools for diversity analysis (refer Chapter 2 and Appendix D for details). All statistical results with value $p < 0.05$ were considered having a significant difference.

RESULTS

Weather data in summer 2013

A total of 985 readings were taken by micro-T temperatures loggers that were placed in the field from 16 June 2013 (5 pm) to 27 July 2013 (5 pm). The mean temperature was 30.59 ± 7.81 °C, with maximum 47.67 ± 4.48°C and minimum 15.5 ± 0.0 °C. Total accumulated degree hour (ADH) for the 2013 trial was 29219.70 (base temperature of 0 °C). According to the nearest National Weather Station (KCLL) at Easterwood Field Airport, College Station, Texas (data downloaded from www.wunderground.com), there were nine rain events and five thunderstorms recorded during the study period. Total precipitation recorded from the rain gauge was 39.12 mm.

Weather data in summer 2014

A total of 985 readings were taken by micro-T temperatures loggers that were placed in the field from 15 June 2014 (5 pm) to 26 July 2014 (5 pm). The mean temperature was 29.27 ± 6.49 °C, with maximum 43.00 ± 1.80 °C and minimum 19.00 ± 0.00 °C. Total accumulated degree hour (ADH) for 2014 trial was 28090.70 (base temperature of 0 °C). There were 13 rain events, 11 thunderstorms and two fog events recorded during the study period. Total precipitation recorded from the rain gauge was 171.45 mm.

Weather comparison between summers 2013 and 2014

Generally, the combined data showed mean temperature in summer 2013 was higher than the mean temperature in summer 2014. A two sample, two-tailed T-test was employed to compare the two year temperature data and the results showed a significant difference between the two years with 2013 showing significantly higher temperatures (p

= 0.0004). Table 1 showed the T-test result on weather comparison between summers 2013 and 2014. Figure 2.18 showed the mean temperatures data of both 2013 and 2014 trials and Figure 2.19 showed the amount of precipitation for both summers. Although the summer of 2014 showed a higher volume of precipitation (171.45 mm) compared to summer 2013 (39.12 mm), a two sample, two-tailed T-test indicated no significant difference between the years (p = 0.2725). Table 2.2 showed the comparison of precipitation of both years.

Accumulated degree hours (ADH) and accumulated degree days (ADD)

Accumulated Degree Hours in summer 2013 was significantly higher than for summer 2014 (p = 0.028). Based on the readings obtained from micro-T data logger, ADH and ADD was calculated up to 40 days of experiment with a base temperature of 0 °C. Table 3.1 demonstrated the ADH and ADD during sampling day in the field for 2013 and 2014 trials.

Table 3.1. ADH and ADD (base temperature 0 °C) during sampling days in the field site for summers 2013 and 2014 at Snook, Texas

Sampling Day	2013		2014	
	ADH	ADD	ADH	ADD
0	35.03	1.45	38.33	1.59
7	5213.70	217.23	5010.50	208.77
14	10622.36	442.59	9663.83	402.65
21	15635.53	651.48	14539.83	605.82
40	29219.70	1217.48	28090.66	1170.44

Soil chemistry profiles before the placement of pig carrion

Soil samples were analyzed by the Soil, Water and Forage Testing Laboratory (College Station, Texas) for nine parameters including pH, conductivity, NO_3-N, P, S, Na, K, Ca and Mg.

The samples analyzed at Day -5 (prior to carcass placement) showed no significant difference when comparing 2013 and 2014 except for Na ($p = 0.03$) (Table 3.2). In 2013 trial, mean pH was 7.96 ± 0.05 and in 2014, mean pH was 7.96 ± 0.05 (Figure 3.5). Mean conductivity 351.6 ± 54.9 μmho in 2013 and 264.33 ± 27.46 μmho in 2014 (Figure 3.6); NO_3-N was 2.33 ± 4.04 ppm in 2013 and 0.33 ± 0.57 ppm in 2014 displaying a large but non-significant reduction in concentration (Figure 3.7). Soil P was 29.33 ± 18.33 ppm in 2013 increasing to 37.67 ± 20.81 ppm in 2014 while soil K was 578 ± 184.46 ppm in 2013 increasing to 646.33 ± 111.84 ppm in 2014 (Figure 3.7 and 3.8). Soil Ca was 7998.33 ± 528.26 ppm and soil Mg was 406.67 ± 38.08 ppm in 2013 both increasing to 8547.67 ± 918.65 ppm and 407 ± 43.96 ppm, respectively (Figure 3.8). Soil S was 14 ± 2 ppm in 2013 and 17.66 ± 1.52 ppm in 2014 while soil Na was 12.67 ± 2.08 ppm in 2013 and significantly decreased to 6 ± 0 ppm in 2014 (Figure 3.7). Calcium was the highest concentrated inorganic nutrient found in the soil samples in 2013 trial, as well as in 2014 trial. Nitrate concentration was the lowest in both years. Both pH and Mg were quite consistence between years, while conductivity, NO_3-N, and Na tended to decrease between 2013 and 2014. P, K, Ca, Mg and S tended to increase between 2013 and 2014 (Figure 3.5 - 3.8).

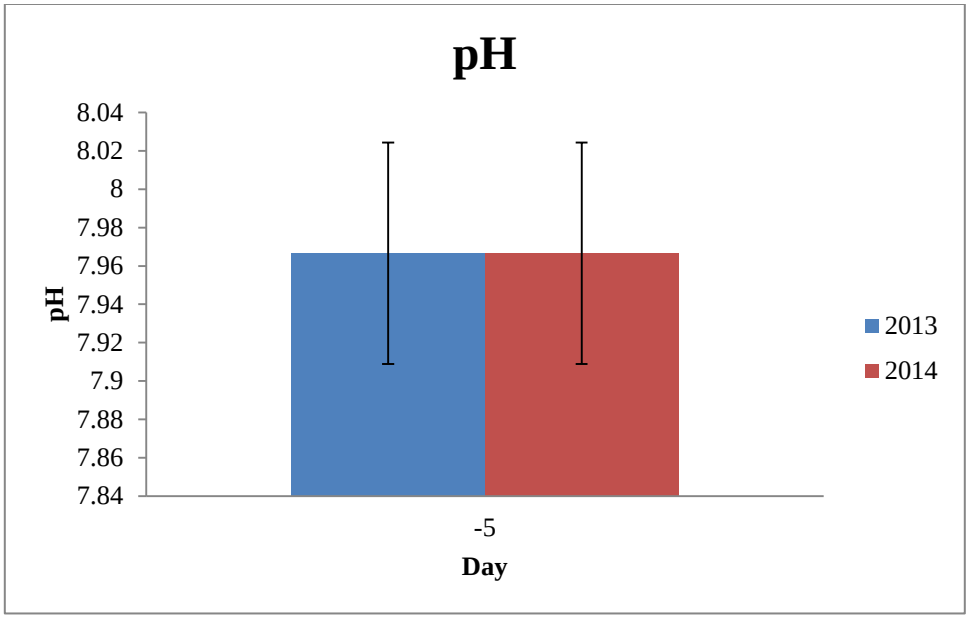

Figure 3.5. Comparison of soil pH value between years (p = 1.00) in the 5 days prior to cadaver placement at the field site located at Snook, Texas (error bar = standard deviation).

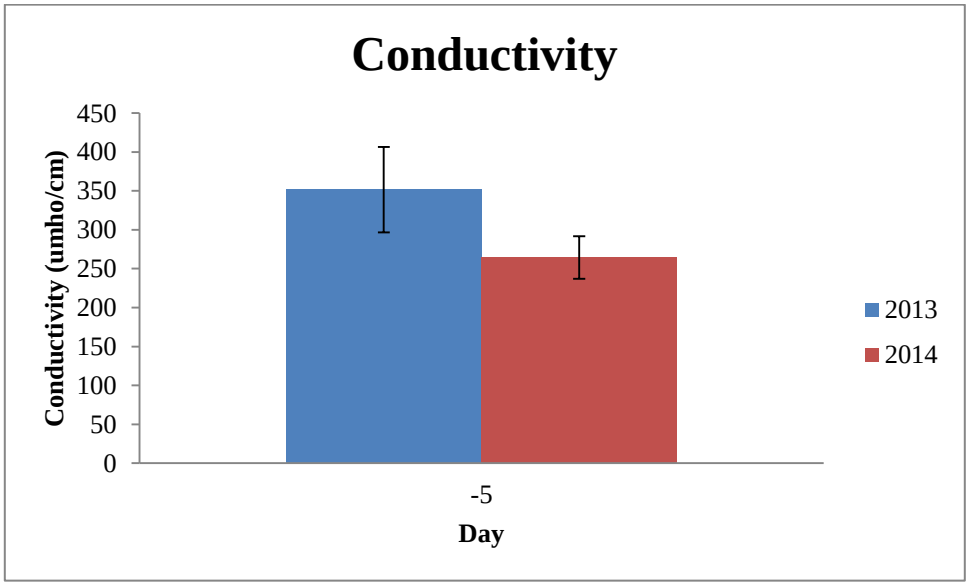

Figure 3.6. Comparison of soil conductivity (µmho) between years (p = 0.09) in the 5 days prior to cadaver placement at the field site located at Snook, Texas (error bar = standard deviation).

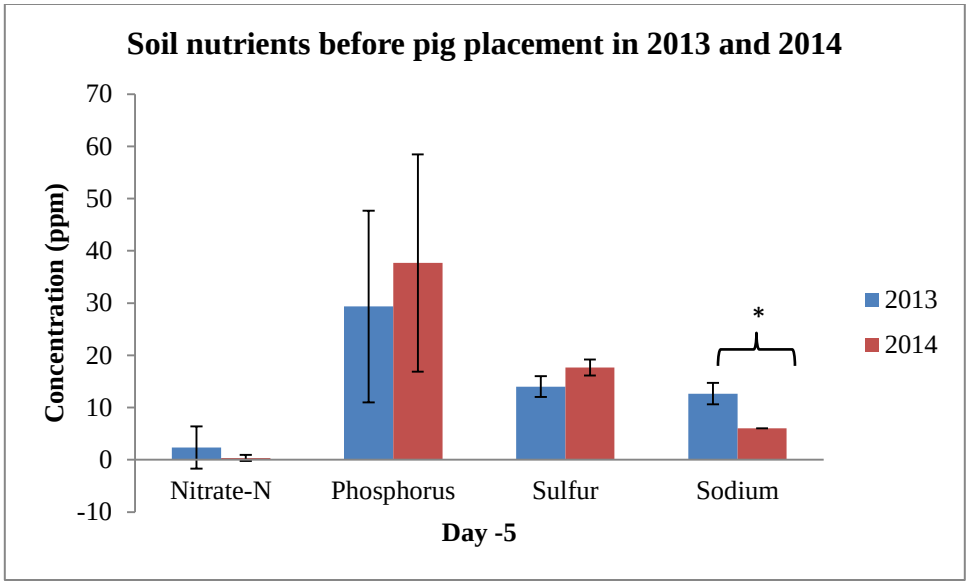

Figure 3.7. Comparison of soil nutrient concentration (ppm) between years in the 5 days prior to cadaver placement at the field site located at Snook, Texas (the only significant difference was found for soil Na concentration, $p = 0.03$). Error bars are standard deviations.

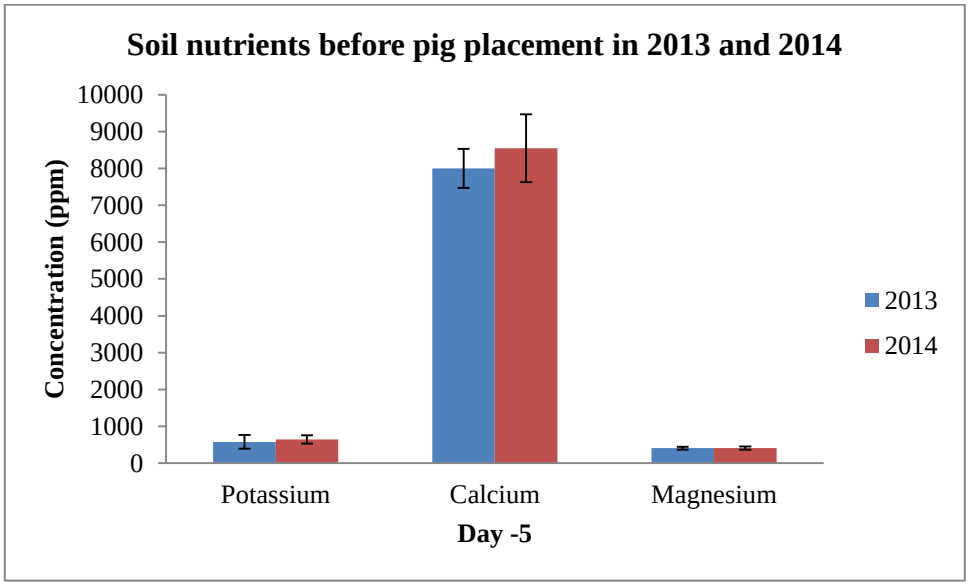

Figure 3.8. Comparison of soil nutrient concentration (ppm) between years years in the 5 days prior to cadaver placement at the field site located at Snook, Texas (all nutrients showed no significant difference between years, $p > 0.05$). Error bars are standard deviations.

Table 3.2. Two-tailed T-Test on soil nutrients comparison on Day -5 between summers 2013 and 2014 at Snook, Texas.

Soil nutrients	Difference	Standard error difference	t Ratio	df	p value
pH	0.00	0.05	0	4	1.0000
Conductivity	-87.33	35.48	-2.46	2.93	0.0925
Nitrate-N	-2.00	2.36	-0.85	2.08	0.4825
Phosphorus	8.33	16.02	0.52	3.93	0.6308
Potassium	68.33	124.55	0.55	3.30	0.6218
Calcium	549.30	611.80	0.90	3.19	0.4318
Magnesium	0.33	33.58	0.00	3.92	0.9926
Sulfur	3.67	1.45	2.52	3.74	0.0769
Sodium	-6.67	1.20	-5.55	2.00	0.0310*

Other than the results generated from the Soil, Water and Forage Testing Laboratory, additional three soil samples (n = 3) were collected from the study site and sent to NAWA laboratory. The soil chemistry results obtained from NAWA laboratory were used in the analysis and are designated as Pre-Treatment. When the experiment was initiated, Pre-Treatment (without pig carrion) was then compared with Control (pig carrion with immediate blow fly access), Post-7 (pig carrion with delayed blow fly access for seven days) and Post-14 (pig carrion with delayed blow fly access for 14 days) in all sampling days (analyzed by one-way ANOVA). Note that Pre-Treatment, in this case, was a constant value throughout the study which serves as a baseline.

Soil chemistry dynamics comparison between years

Permutational multivariate analysis of variance (PERMAVONA) was performed on the combined soil chemistry data of 2013 and 2014 trials using R. Results showed

that there was significant difference between years (F = 2.8756; r^2 = 0.0076; p = 0.044). Due to the difference in year dynamics, soil chemistry data were analyzed separately by year, in other words, the two years data were not pooled together, but analyzed by individual year. Non-metric multidimensional scaling (NMDS) was employed to visualize the level of similarity of individual cases of a dataset. The NMDS plot of stress is demonstrated in Figure 3.9A and NMDS ordinations in Figure 3.9B. The minimum stress value in a given dimensionality was 0.0685, r^2 = 0.9831. This result indicated an excellent representation of data in reduced dimension with an acceptable amount of distortion. Although there was no clear separation among data points in NMDS ordination, the data points of 2014 trial were more concentrated than data points in 2013 trial, which was more scattered in its distribution.

Replicate effect was also tested by PEMANOVA and results showed that there was no significant difference among replicates for either year (F = 0.4173; r^2= 0.0011; p = 0.74). As such, data in all replicates were pooled together and analyzed.

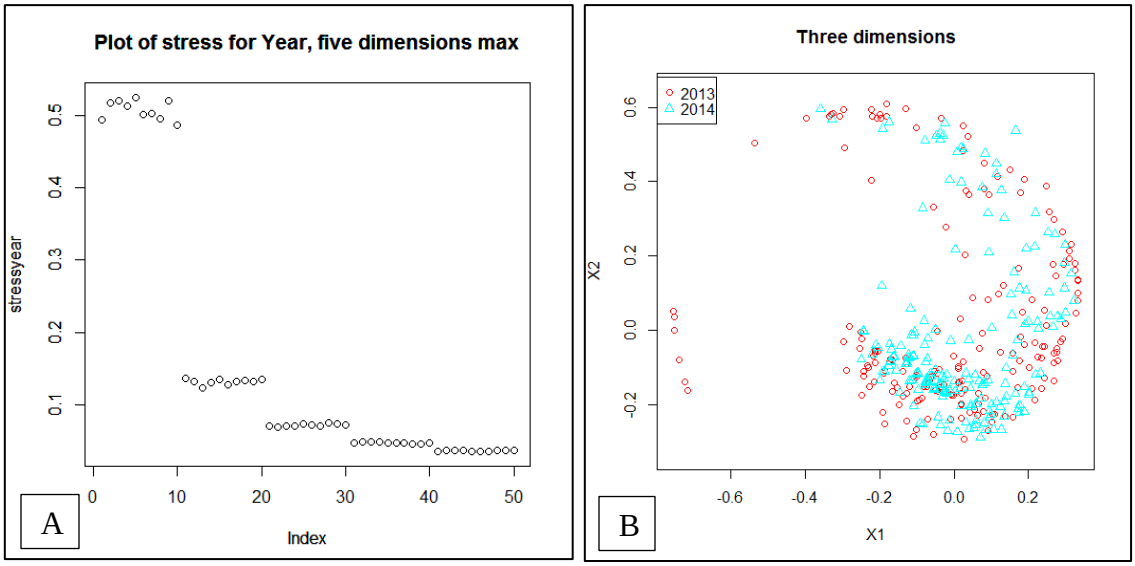

Figure 3.9. A. NMDS plot of stress (stress test 0.0685; r^2 = 0.9831). B. NMDS ordinations of soil data by years (2013 and 2014 trials) collected in the field site located in Snook, Texas. Plots indicate distances between data points and there was significant difference in distances between both trials (p = 0.044).

Soil chemistry profiles in summer 2013

Soil samples collected from beneath, lateral and 5-meter away (regions) from carrion for the Control and two treatments (Post-7 and Post-14) were examined for pH, conductivity, NO_3-N, NH_4-N, and PO_4-P.

When all data in summer 2013 was pooled and analyzed by PERMANOVA in R, the results showed that Day, Treatment, Region were significantly different, as well as interactions among the independent variables (Table 3.3). The NMDS plot of stress and ordination plots of Day, Treatment, and Region for 2013 trial was demonstrated in Figure 65 to show distances between factors and variables. Minimum stress for given dimensionality was 0.0716 and r^2 = 0.9816. This result indicated an excellent representation of data in reduced dimension with an acceptable amount of distortion. In Figure 3.10, there was overlapping in data points for Day and Treatments, but there was a discernable separation in soil regions from the carrion, where total soil chemistry profiles were distinctly different at soil beneath, soil lateral and soil 5 meter.

Table 3.3. Analysis of the overall effects on soil chemistry profiles in summer 2013 at Snook, Texas using PERMANOVA (* indicates significant difference).

Factor	df	SS	MS	F Model	R^2	p value
Day	6	7.466	1.2443	30.272	0.2085	0.001*
Treatment	2	0.258	0.1291	3.140	0.0072	0.008*
Region	3	11.508	3.8361	93.328	0.3215	0.001*
Day x Treatment	12	1.512	0.1260	3.065	0.0422	0.001*
Day x Region	12	6.659	0.5549	13.501	0.1860	0.001*
Treatment x Region	4	0.626	0.1565	3.808	0.0174	0.001*
Day x Treatment x Region	24	2.625	0.1094	2.661	0.0733	0.001*
Residual	125	5.138	0.0411		0.1435	
Total	188	35.792			1.0000	

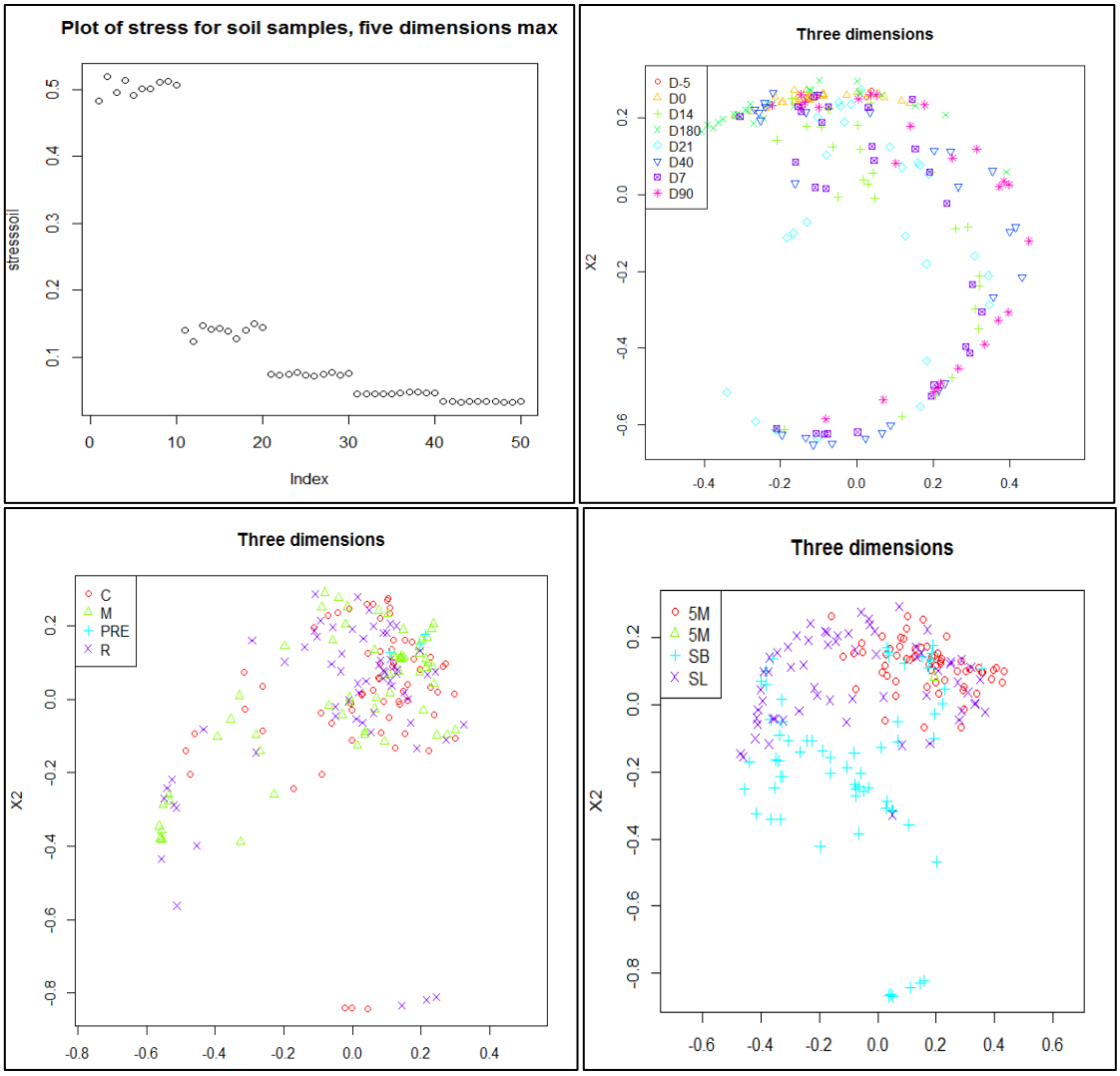

Figure 3.10. NMDS plot of stress (stress test 0.0716; r^2 = 0.9816) (top left) and ordination plots of carrion decomposition days (top right), treatments (bottom left) and soil regions (bottom right) in 2013 trial at Snook, Texas. (Legend: C = Control; M = Post-7; R = Post-14; Pre = Pre-treatment, which is five days before the placement of pig carrion at the study site; SB = soil beneath; SL = soil lateral; 5M = 5 m away from carrion, which is served as the control).

In order to determine soil resilience (ability of recovery to initial value), statistical tests such as ANOVA and Tukey HSD post-hoc tests were employed in JMP

to detect the differences according days of decomposition. Alpha level of 0.05 indicates significant difference.

pH

In general, soil pH decreased over time (Day 0: mean pH 9.44 ± 0.52; Day 90 6.93 ± 0.22), regardless of treatments and soil regions, although there was a slight increased on Day 180 (pH 7.63 ± 0.17) (Figure 3.11). Statistical analysis showed that soil pH was not significantly different by Treatments ($p = 0.0504$). However, pH at the different soil sample regions (beneath, lateral, and 5 m) and different days of decomposition were significantly different (Table 3.4). Figure 3.11 demonstrated mean pH across treatments in three different regions (beneath, lateral, and 5 meter away) over decomposition day (Day -5 to Day 180 postmortem). Table 3.5 lists results for pH at soil beneath, soil lateral and soil 5 meter across treatments over time. There was no significant difference on Day 0, however, as time progressed, soil pH changed significantly according to treatments and soil regions. Results suggested that pH changes significantly from soil beneath to soil lateral, and then to soil 5 m away, and the way pH changes was related to the timing of blow fly colonization treatment although treatment effect was not significantly different in general. Resilience between pre-treatment soil and carrion was not observed even after 180 days of decomposition, indicating a significant change in pH following carrion decomposition to the soil pH regulating system. However, resilience among treatment groups (Control, Post-7, and Post-14) was achieved at least on Day 90.

Table. 3.4. ANOVA on soil pH by treatments, regions and days in summer 2013 at Snook, Texas (* indicates significant difference).

Factor	df	F ratio	p value
Treatment	3	2.6467	0.0504●
Region	3	2.8046	0.0411*
Day	7	81.0124	<0.0001*

● Marginal significant difference.

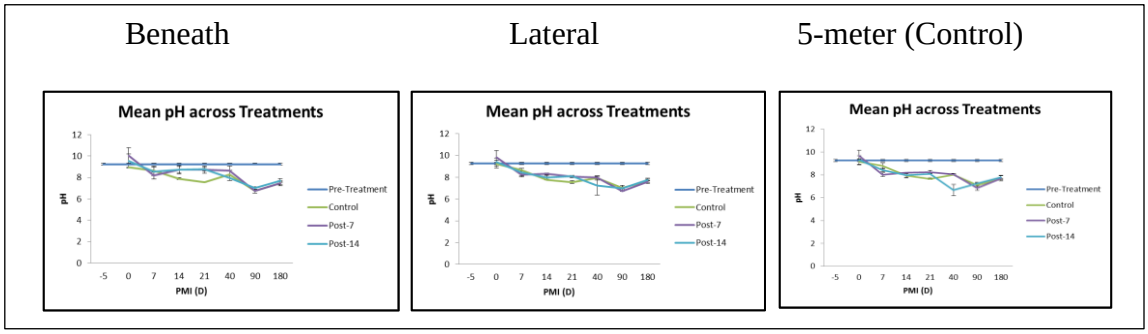

Figure 3.11. Mean soil pH across treatments at three different soil regions over carrion decomposition days in summer 2013 at Snook, Texas.

Table 3.5. Significant difference in pH at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2013 at Snook, Texas (Pre denotes Pre-treatment while C denotes Control).

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
pH	7	Pre x Post-7	0.0072	0.0017	<0.0001
		Pre x Post-14	-	0.0079	0.0014
		C x Post-7	-	-	0.0028
		Pre x C	-	-	0.0310
	14	Pre x C	0.0007	<0.0001	<0.0001
		C x Post-7	0.0150	0.0044	-
		C x Post-14	0.0123	-	-
		Pre x Post-7	-	<0.0001	0.0001
		Pre x Post-14	-	<0.0001	<0.0001
		Post-7 x Post-14	-	0.0440	-
	21	Pre x C	<0.0001	<0.0001	<0.0001
		C x Post-7	0.0004	0.0010	0.0004
		C x Post-14	0.0003	0.0004	0.0037
		Pre x Post-7	-	<0.0001	<0.0001
		Pre x Post-14	-	<0.0001	<0.0001
	40	Pre x C	0.0224	0.0298	0.0015
		Pre x Post-14	0.0037	0.0030	<0.0001
		Pre x Post-7	-	0.0367	0.0018
		C x Post-14	-	-	0.0008
		Post-7 x Post-14	-	-	0.0007
	90	Pre x C	<0.0001	<0.0001	<0.0001
		Pre x Post-7	<0.0001	<0.0001	<0.0001
		Pre x Post-14	<0.0001	<0.0001	<0.0001

Table 3.5 (Continued).

Day	Treatments	p value		
		Beneath	Lateral	5 meter (control)
180	Pre x C	<0.0001	<0.0001	<0.0001
	Pre x Post-7	<0.0001	<0.0001	<0.0001
	Pre x Post-14	<0.0001	<0.0001	<0.0001

"-" No significant difference.

Conductivity

Increased conductivity in the soil persisted quite a long time and a bell shape distribution was observed. No resilience was observed between the pre-treatment soil and the carrion soil after carrion placement even after 180 days of decomposition. However, resilience between blow fly access pig carcasses (Control) and blow fly-delayed carcasses (both Post-7 and Post-14) achieved resilience at least on Day 90. In general, conductivity in soil beneath (mean 830 ± 615 µS/cm) was higher compared to conductivity at soil lateral (mean 238 ± 211 µS/cm) while conductivity at soil 5 meter was lower regardless of treatments (Day 0: mean 157 ± 37 µS/cm; Day 180: mean 84 ± 12 µS/cm). Furthermore, delayed of blow fly colonization on carrion increased conductivity at soil beneath (especially between Control vs Post-14 on Day 21 and Day 40), although statistical analysis showed that conductivity was not significantly different by Treatments ($p = 0.2947$) overall. Conductivity at the three different soil regions and different days of decomposition showed a significant difference ($p < 0.0001$ and 0.0002, respectively) (Table 3.6). Figure 3.12 demonstrated mean conductivity across treatments in three different soil sample regions over decomposition day. Table 3.7 presents significant results in conductivity at soil beneath, soil lateral and soil 5 meter across treatments over time.

Table 3.6. ANOVA on soil conductivity by treatments, regions and days in summer 2013 at Snook, Texas (* indicates significant difference).

Factor	df	F ratio	p value
Treatment	3	1.2452	0.2947
Region	3	42.6280	<0.0001*
Day	7	4.3545	0.0002*

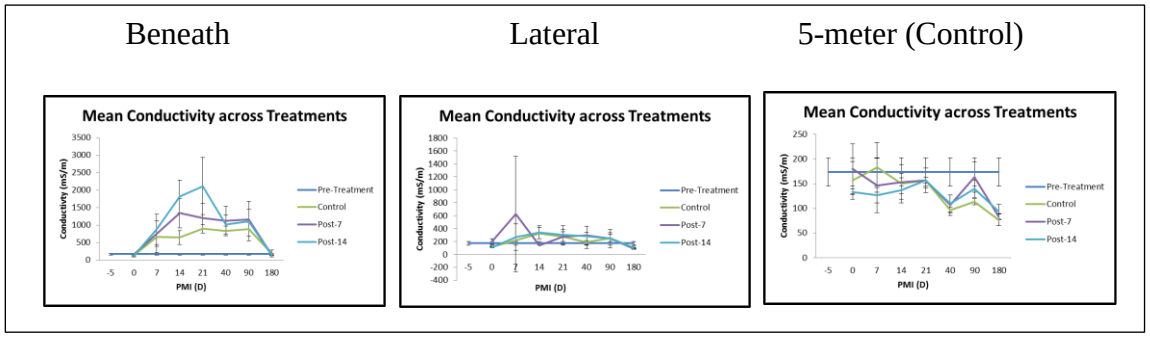

Figure 3.12. Mean soil conductivity across treatments at three different soil regions over carrion decomposition days in summer 2013 at Snook, Texas (Error bar = standard deviation).

Table 3.7. Significant difference in conductivity at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2013 at Snook, Texas (Pre denotes Pre-treatment while C denotes Control).

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
Conductivity	0	C x Post-7	-	0.0284	-
		Post-7 x Post-14	-	0.0233	-
	14	Pre x Post-7	0.0107	-	-
		Pre x Post-14	0.0013	-	-
		C x Post-14	0.0107	-	-
		C x Post-7	-	0.0388	-
		Pre x Post-7	-	-	-
		Pre x Post-14	-	-	-
		Post-7 x Post-14	-	0.0277	-
	21	Pre x C	-	-	-
		C x Post-7	-	-	-
		C x Post-14	0.0483	-	-
		Pre x Post-7	-	-	-
		Pre x Post-14	0.0038	-	-
	40	Pre x C	-	-	0.0067
		Pre x Post-14	0.0162	-	0.0196
		Pre x Post-7	0.0087	-	0.0149
		C x Post-14	-	-	-
		Post-7 x Post-14	-	-	-
	90	Pre x C	-	-	0.0497
		Pre x Post-7	0.0276	-	-
		Pre x Post-14	0.0355	-	-

Table 3.7 (Continued).

Day	Treatments	p value		
		Beneath	Lateral	5 meter (control)
180	Pre x C	-	0.0031	0.0007
	Pre x Post-7	-	0.0068	0.0011
	Pre x Post-14	-	0.0117	0.0023

"-" No significant difference.

Nitrate-N (NO_3-N)

Nitrate-N demonstrated a similar pattern at soil beneath and soil lateral (a sudden peak on Day 90 for soil beneath; Day 40 for soil lateral), although the concentration of nitrate-N between the two sites differed significantly. Although all treatments increased in nitrate-N concentration in soil beneath on Day 90 (Control = 711.90 ± 121.62 μg/kg, Post-7= 751.68 ± 494.05 μg/kg, Post-14 = 330.93 ± 133.81 μg/kg), there was no significant difference among treatments for that day. Overall, nitrate-N was not significantly different by Treatments ($p = 0.6773$), However, nitrate-N at the three different soil regions and different days of decomposition displayed a significant difference ($p = 0.0089$ and < 0.0001, respectively (Table 3.8). Figure 3.13 demonstrated mean nitrate-N across treatments in three different soil regions over decomposition days. Table 3.9 presents significant results in nitrate-N at soil beneath, soil lateral and soil 5 m across treatments over time. There was resilience observed between pre-treatment soil and carrion soils on Day 90 and onwards, and there was resilience observed between treatments (Control, Post-7 and Post-14) on Day 21 where there was no significant difference among them. Note that the tremendous increased in nitrate-N on Day 90 was probably due to the nitrification process where ammonium has been converted into nitrate when an oxygen supply was possibly provided.

Table 3.8. ANOVA on soil nitrate-N by treatments, regions and days in summer 2013 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	3	0.5080	0.6773
Region	3	3.9745	0.0089*
Day	7	12.8482	<0.0001*

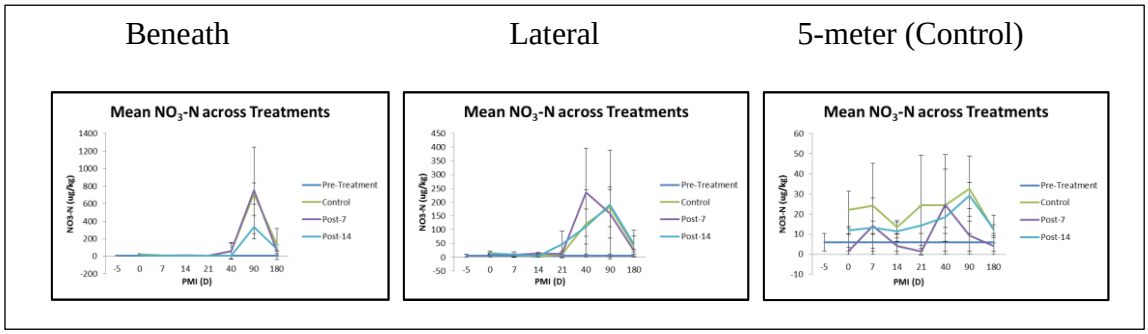

Figure 3.13. Mean soil nitrate-N across treatments at three different soil regions over carrion decomposition days in summer 2013 at Snook, Texas (Error bar = standard deviation).

Table 3.9. Significant difference in nitrate-N at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2013 at Snook, Texas (Pre denotes Pre-treatment while C denotes Control).

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
Nitrate-N	0	C x Pre	-	-	0.0229
		Pre x Post-7	-		0.0124
	14	C x Post-7	-	0.0198	-
		Post-7 x Post-14	-	0.0272	-
	40	Pre x C	0.0444	-	0.0484
		Pre x Post-7	0.0343	-	-

"-" No significant difference.

Ammonium-N (NH_4-N)

Pig carcasses with delayed blow fly colonization for 14 days (Post-14) revealed high concentration of ammonium-N in soil beneath on Day-14 (103409.79 ± 21521.02 μg/kg), and then decreased to 4625.13 ± 2260.83 μg/kg after seven days (Day 21), followed by another peak by Control pigs on Day 21 with 47541.77 ± 6182.33 μg/kg. Ammonium-N was not significantly different by Treatments (p = 0.2730), However, ammonium-N at different soil regions and different days of decomposition were significant difference, with p = 0.0024 and 0.0156, respectively (Table 3.10). Figure 3.14 demonstrated mean ammonium-N across treatments in three different soil regions over decomposition day. Table 3.11 listed significant results in ammonium-N at soil beneath, soil lateral and soil 5 meter across treatments over time. Resilience between treatments occurred on Day 40 while resilience between pre-treatment soil and carrion soils occurred on Day 180.

Table 3.10. ANOVA on soil ammonium-N by treatments, regions and days in summer 2013 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	3	1.3083	0.2730
Region	3	4.9746	0.0024*
Day	7	2.5551	0.0156*

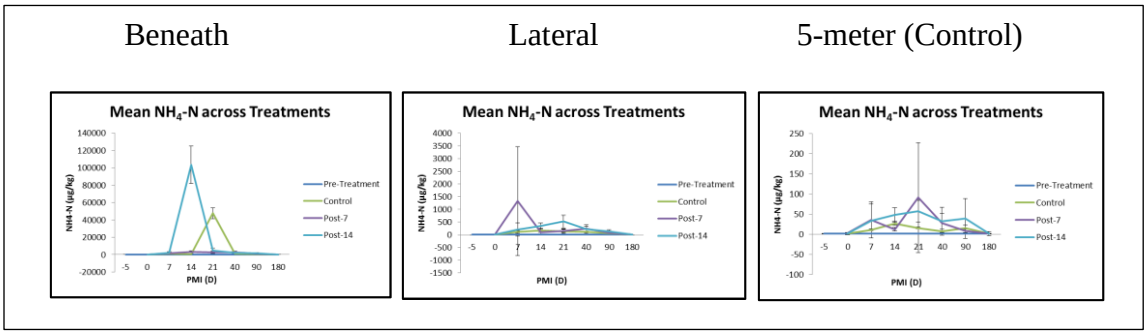

Figure 3.14. Mean soil ammonium-N across treatments at three different soil regions over carrion decomposition days in summer 2013 at Snook, Texas (Error bar = standard deviation).

Table 3.11. Significant difference in ammonium-N at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2013 at Snook, Texas (Pre denotes Pre-treatment while C denotes Control).

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
Ammonium-N	0	C x Post-7	0.0043	0.0367	-
		Pre x Post-7	0.0379	-	-
	14	Pre x Post-14	-		0.0019
		C x Post-14	<0.0001		-
		Pre x Post-14	<0.0001	0.0028	-
		Post-7 x Post-14	<0.0001	0.0232	0.0090
	21	Pre x C	<0.0001		-
		C x Post-7	<0.0001		
		C x Post-14	<0.0001	0.0337	
		Pre x Post-14	-	0.0068	-
		Post-7 x Post-14	-	0.0418	-
	40	Pre x Post-14	0.0496	-	-
		Pre x Post-7	0.0154	-	-
	90	Pre x Post-14	-	0.0311	-

"-" No significant difference

Orthophosphate-P (PO_4-P)

The longer the delay of blow fly colonization on carrion, the higher the orthophosphate-P concentration in the soil. This observation can be seen in soil beneath and soil lateral. Post-14 had the highest concentration of orthophosphate-P from Day 0 until Day 90, with the peak on Day 40 (576.28 ± 167.77 μg/kg). Orthophosphate-P was not significantly different by Treatments ($p = 0.2174$), However, orthophosphate-P at different soil regions and different days of decomposition were significant difference, with p value < 0.0001 and 0.0002, respectively (Table 3.12). Figure 3.15 demonstrated mean orthophosphate-P across treatments in three different soil regions over decomposition day. Table 3.13 presents significant results in orthophosphate-P at soil beneath, soil lateral and soil 5 meter across treatments over time.

Table 3.12. ANOVA on soil orthophosphate-P by treatments, regions and days in summer 2013 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	3	1.4948	0.2174
Region	3	40.8793	<0.0001*
Day	7	4.3496	0.0002*

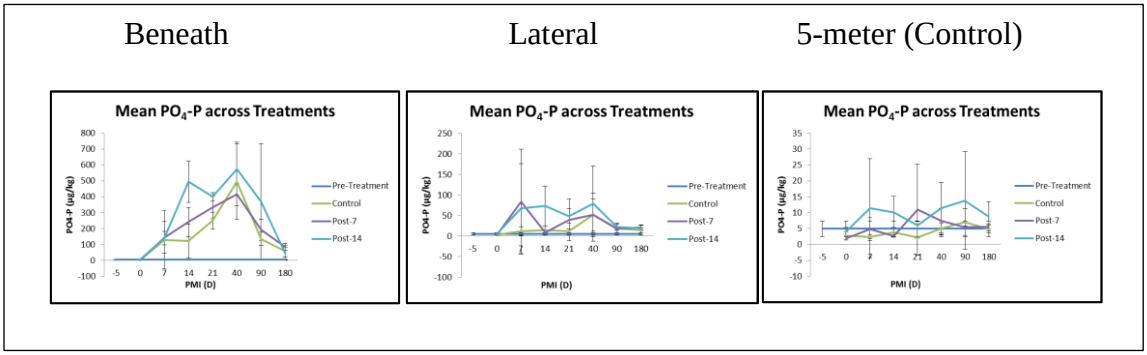

Figure 3.15. Mean soil orthophosphate-P across treatments at three different soil regions over carrion decomposition days in summer 2013 at Snook, Texas (Error bar = standard deviation).

Table 3.13. Significant difference in orthophosphate-P at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2013 at Snook, Texas (Pre denotes Pre-treatment while C denotes Control).

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
Orthophosphate-P	14	Pre x Post-14	0.0011	0.0410	-
		C x Post-14	0.0060	-	-
		Post-7 x Post-14	0.0472	-	-
	21	Pre x C	0.0008	-	-
		C x Post-14	0.0177	-	-
		Pre x Post-14	<0.0001	-	-
		Pre x Post-7	0.0001	-	-
	40	Pre x Post-14	0.0070	-	-
		Pre x Post-7	0.0405	-	-
		C x Pre	0.0163	-	-
	180	Post-7 x Pre	0.0349	0.0268	-

"-" No significant difference

Soil moisture

Post-14 had the highest content of soil moisture underneath the carrion, indicating the longer period of delayed blow fly colonization on carrion, the higher moisture in the soil. This was probably due to the decompositional fluid seeped into the soil. Soil moisture increased on Day 180 regardless of treatment (19.37 ± 3.46%), and this was probably due to precipitation events. Soil moisture was not significantly different by Treatments ($p = 0.1559$), However, soil moisture at different soil regions and different days of decomposition were significant difference, with p value < 0.0001 and < 0.0001, respectively (Table 3.14). Figure 3.16 demonstrated mean soil moisture across treatments in three different soil regions over decomposition day. Table 3.15 listed significant results in soil moisture at soil beneath, soil lateral and soil 5 meter across treatments over time.

Table 3.14. ANOVA on soil moisture by treatments, regions and days in summer 2013 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	3	1.7620	0.1559
Region	3	21.4498	<0.0001*
Day	7	16.2027	<0.0001*

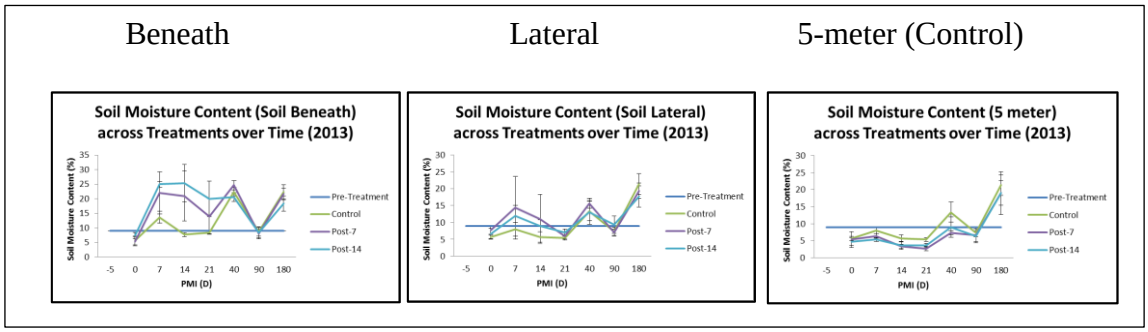

Figure 3.16. Mean soil moisture across treatments at three different soil regions over carrion decomposition days in summer 2013 at Snook, Texas (Error bar = standard deviation).

Table 3.15. Significant difference in soil moisture at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2013 at Snook, Texas (Pre denotes Pre-treatment while C denotes Control).

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
Soil moisture	0	Pre x Post-7	0.0262	-	0.0382
		Pre x C	0.0416	0.0149	0.0050
		Pre x Post-14	-	-	0.0194
	7	Pre x Post-14	0.0037	-	0.0005
		Pre x Post-7	0.0127	-	0.0043
		Pre x C	-	-	0.0020
		C x Post-14	0.0262	-	-
	14	C x Post-14	0.0158	-	-
		Pre x Post-14	0.0238	-	0.0002
		Pre x C	-	-	<0.0001
		Pre x Post-7	-	-	0.0001
	21	Pre x C	-	0.0017	<0.0001
		C x Post-14	0.0427	-	-
		Pre x Post-14	-	0.0497	<0.0001

Table 3.15 (Continued).

Day	Treatments	p value		
		Beneath	Lateral	5 meter (control)
	Pre x Post-7	-	0.0058	<0.0001
40	Pre x Post-14	<0.0001	-	-
	Pre x Post-7	<0.0001	-	-
	C x Pre	<0.0001	-	-
	Post-7 x Post-14	0.0249	-	-
180	Post-7 x Pre	0.0003	0.0038	0.055•
	Post-14 x Pre	0.0024	0.0107	0.055•
	C x Pre	0.0002	0.0012	-

• Marginal significant difference.

"-" No significant difference.

Correlation between soil nutrients in summer 2013

Pearson's pairwise correlation was performed on all variables in soil chemistry. The results showed eight pairs of variables were significantly correlated, either positively or negatively (Table 3.16). The strongest positive correlation was orthophosphate-P and conductivity, with the coefficient of correlation (r) of 0.83. The strongest negative correlation was between nitrate-N and pH, with r = -0.43. Most ionic variables (e.g., nitrate-N, ammonium-N, and phosphate-P) were positively correlated with conductivity.

Table 3.16. Pearson's pairwise correlation between soil chemistry variables from soil samples from all regions collected in summer 2013 at Snook, Texas (* denotes significant difference).

Variable	By Variable	Correlation	p value
Conductivity	pH	0.0520	0.4740
NO_3-N	pH	-0.4273	<0.0001*
NO_3-N	Conductivity	0.2313	0.0012*
NH_4-N	pH	0.0692	0.3404
NH_4-N	Conductivity	0.4654	<0.0001*
NH_4-N	NO_3-N	-0.0594	0.4134
PO_4-P	pH	0.0236	0.7457
PO_4-P	Conductivity	0.8298	<0.0001*
PO_4-P	NO_3-N	0.1096	0.1399
PO_4-P	NH_4-N	0.4163	<0.0001*
Soil moisture	pH	-0.0291	0.6890
Soil moisture	Conductivity	0.4629	<0.0001*
Soil moisture	NO_3-N	-0.0305	0.6741
Soil moisture	NH_4-N	0.2405	0.0008*
Soil moisture	PO_4-P	0.4986	<0.0001*

Multiple regression and cross validation models on soil nutrients in 2013 for PMI predictions

Multiple regression analysis was performed on soil chemistry profiles in 2013 trial. Day of decomposition was treated as Y and other variables (pH, conductivity, NO_3-N, NH_4-N, PO_4-P, and soil moisture) were used as X to construct model effect, and then cross validation test using KFold validation method (number of fold= 5) was conducted to determine RSquare (r^2) and the root-mean-square error (RMSE).

The relationship between day of decomposition and soil chemistry profiles can be determined by using model analysis. The potential application of this model is to predict day of decomposition (or day of carcass placement) and these relationships could be useful in forensic investigations.

Control (Soil beneath)

The model was significantly different ($p < 0.0001$), with high strength of relationship (RSquare = 0.90). Table 3.17 showed significant predictors for soil beneath the Control carrion. A prediction expression is also provided. Figure 3.17 presents the actual by predicted plot of Day.

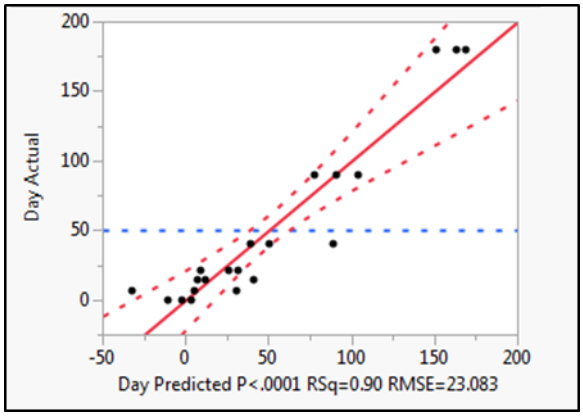

Figure 3.17. Actual by predicted plot of Day in multiple regression model (Soil beneath the Control carrion in summer 2013 at Snook, Texas).

Table 3.17. Parameter estimates in regression model for soil beneath (Control) in summer 2013 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	508.61	127.84	3.98	0.0014*
pH	-58.48	14.11	-4.12	0.0010*
Conductivity	-0.10	0.03	-3.22	0.0061*
NO_3-N	0.03	0.04	0.77	0.4539
NH_4-N	-0.000011	0.0004	-0.02	0.9816
PO_4-P	0.03	0.05	0.63	0.5377
Moisture	4.58	1.13	4.03	0.0012*

Prediction expression: PMI (D) = 508.61 – 58.48*pH – 0.10*Conductivity + 0.03*NO_3-N – 0.00001*NH_4-N + 0.03*PO_4-P + 4.58*Moisture

Cross validation test

Validation test showed a very high RSquare (0.99) and low RMSE (3.10) (Table 3.18), indicating that this model predicted well for day of decomposition (Figure 3.18).

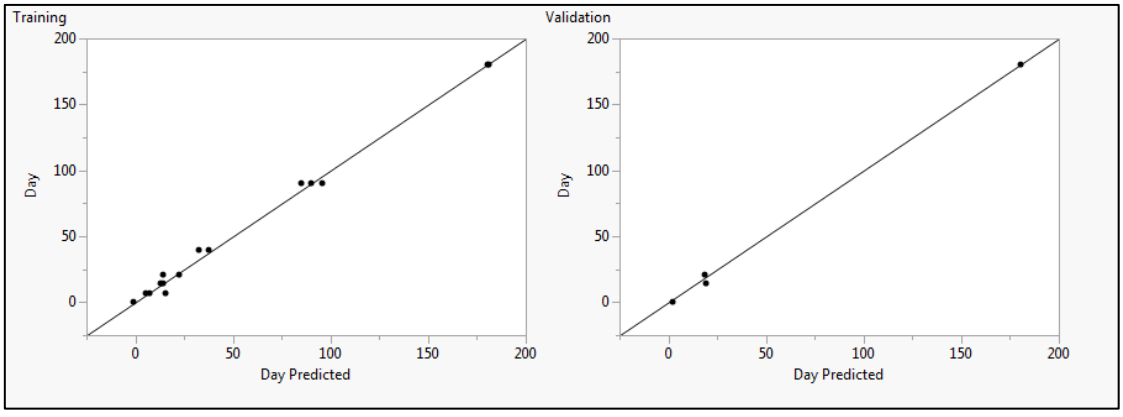

Figure 3.18. Actual by predicted plot of Day in cross validation model (Soil beneath the Control carrion in summer 2013 at Snook, Texas).

Table 3.18. Measures of training and validation models (Soil beneath the Control carrion in summer 2013 at Snook, Texas).

Training		Validation	
RSquare	0.99	RSquare	0.99
RMSE	4.24	RMSE	3.10
Mean Abs Dev	3.07	Mean Abs Dev	2.56
-LogLikelihood	48.70	-LogLikelihood	10.20
SSE	306.52	SSE	38.48
Sum Freq	17	Sum Freq	4

Post-7 (Soil beneath)

The model was significantly different (p = 0.0018), with high strength of relationship (RSquare = 0.74). Table 3.19 showed significant predictors for soil beneath the Post-7 carrion. A prediction expression is also provided. Figure 3.19 presents the actual by predicted plot of Day.

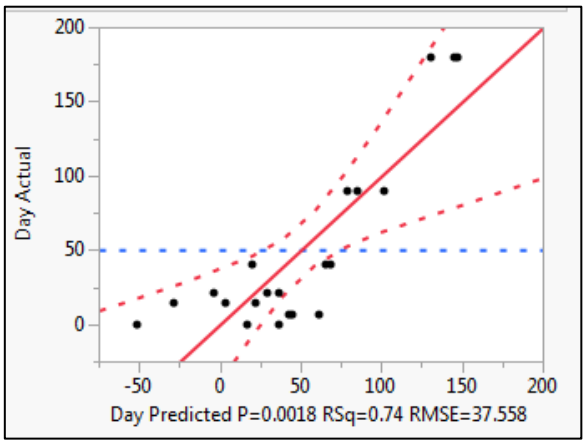

Figure 3.19. Actual by predicted plot of Day in multiple regression model (Soil beneath the Post-7 carrion in summer 2013 at Snook, Texas).

Table 3.19. Parameter estimates in regression model for soil beneath (Post-7) in summer 2013 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	591.97	160.97	3.68	0.0025*
pH	-54.18	14.82	-3.65	0.0026*
Conductivity	-0.27	0.10	-2.66	0.0185*
NO_3-N	0.09	0.05	1.73	0.1063
NH_4-N	0.08	0.04	2.06	0.0589
PO_4-P	0.12	0.10	1.17	0.2624
Moisture	-0.95	2.00	-0.47	0.6435

Prediction expression: PMI (D) = 591.97 – 54.18*pH – 0.27*Conductivity + 0.09*NO3-N – 0.08*NH4-N + 0.12*PO4-P – 0.95*Moisture

Cross validation test

Validation test showed a very high RSquare (0.98) and low RMSE (8.04) (Table 3.20), indicating that this model predicted well for day of decomposition (Figure 3.20).

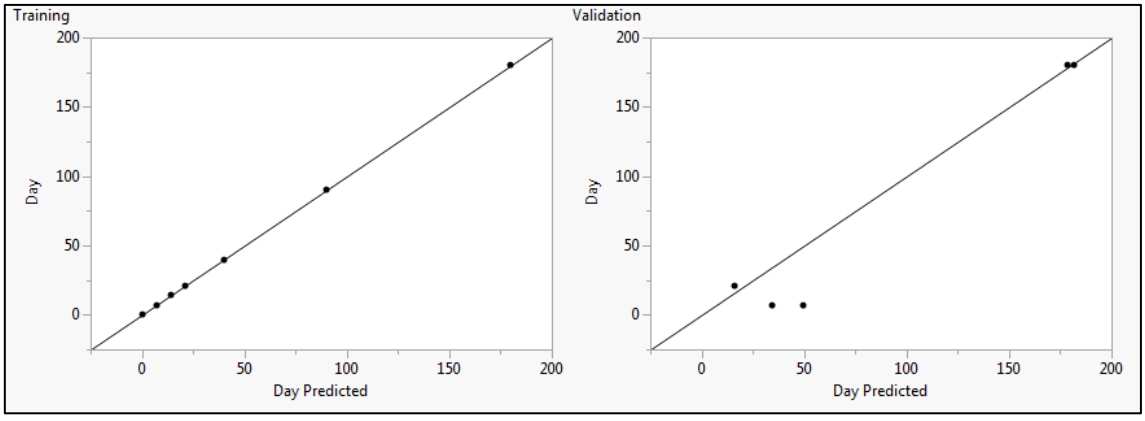

Figure 3.20. Actual by predicted plot of Day in cross validation model (Soil beneath the Post-7 carrion in summer 2013 at Snook, Texas).

Table 3.20. Measures of training and validation models (Soil beneath the Post-7 carrion in summer 2013 at Snook, Texas).

Training		Validation	
RSquare	0.99	RSquare	0.98
RMSE	4.04	RMSE	8.04
Mean Abs Dev	3.32	Mean Abs Dev	6.67
-LogLikelihood	47.8	-LogLikelihood	14.01
SSE	277.47	SSE	258.93
Sum Freq	17	Sum Freq	4

Post-14 (Soil beneath)

The model was significantly different (p = 0.0355), with moderate strength of relationship (RSquare = 0.58). Table 3.21 showed significant predictors for soil beneath the Post-14 carrion. A prediction expression is also provided. Figure 3.21 presents the actual by predicted plot of Day.

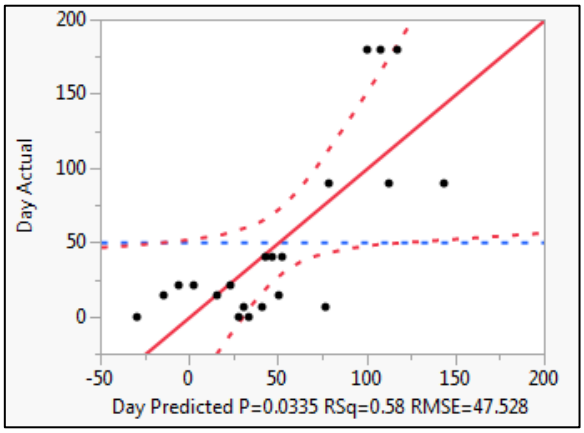

Figure 3.21. Actual by predicted plot of Day in multiple regression model (Soil beneath the Post-14 carrion in summer 2013 at Snook, Texas).

Table 3.21. Parameter estimates in regression model for soil beneath (Post-14) in summer 2013 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	505.46	205.00	2.47	0.0272*
pH	-52.01	21.56	-2.41	0.0302*
Conductivity	-0.009	0.02	-0.41	0.6852
NO_3-N	0.02	0.156	0.14	0.8944
NH_4-N	0.0001	0.0003	0.31	0.7577
PO_4-P	-0.07	0.06	-1.07	0.3021
Moisture	0.35	2.13	0.16	0.8720

Prediction expression: PMI (D) = 505.46 – 52.01*pH – 0.009*Conductivity + 0.02*NO_3-N – 0.0001*NH_4-N - 0.07*PO_4-P + 0.35*Moisture

Cross validation test

Validation test showed a very high RSquare (0.98) and low RMSE (4.16) (Table 3.22), indicating that this model predicted well for day of decomposition (Figure 3.22).

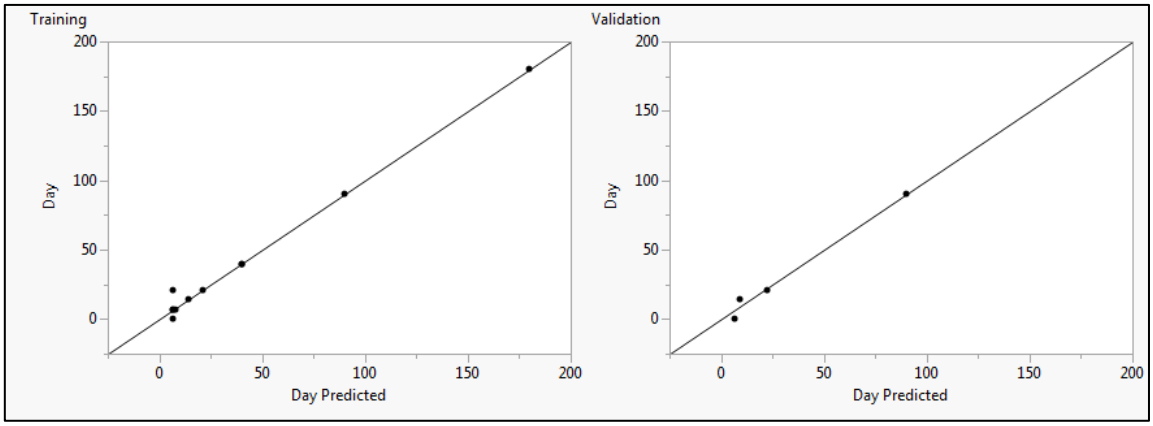

Figure 3.22. Actual by predicted plot of Day in cross validation model (Soil beneath the Post-14 carrion in summer 2013 at Snook, Texas).

Table 3.22. Measures of training and validation models (Soil beneath the Post-14 carrion in summer 2013 at Snook, Texas).

Training		Validation	
RSquare	0.99	RSquare	0.98
RMSE	4.16	RMSE	4.16
Mean Abs Dev	1.81	Mean Abs Dev	3.16
-LogLikelihood	48.39	-LogLikelihood	11.38
SSE	295.41	SSE	69.31
Sum Freq	17	Sum Freq	4

Control (Soil lateral)

The model was significantly different ($p < 0.0001$), with high strength of relationship (RSquare = 0.90). Table 3.23 showed significant predictors for soil lateral the Control carrion. A prediction expression is also provided. Figure 3.23 presents the actual by predicted plot of Day.

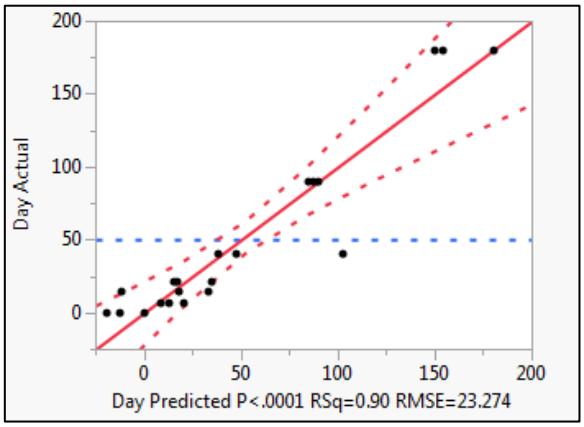

Figure 3.23. Actual by predicted plot of Day in multiple regression model (Soil lateral of the Control carrion in summer 2013 at Snook, Texas).

Table 3.23. Parameter estimates in regression model for soil lateral (Control) in summer 2013 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	P value
Intercept	202.34	109.72	1.84	0.0864
pH	-27.80	11.46	-2.42	0.0295*
Conductivity	-0.04	0.16	-0.27	0.7912
NO_3-N	0.26	0.17	1.53	0.1473
NH_4-N	0.06	0.25	0.24	0.8129
PO_4-P	-1.24	0.69	-1.80	0.0937
Moisture	8.55	1.52	5.61	<0.0001*

Prediction expression: PMI (D) = 202.34 – 27.80*pH – 0.04*Conductivity + 0.26*NO_3-N – 0.06*NH_4-N – 1.24*PO_4-P + 8.55*Moisture

Cross validation test

Validation test showed a very high RSquare (0.99) and low RMSE (7.63) (Table 3.24), indicating that this model predicted well for day of decomposition (Figure 3.24).

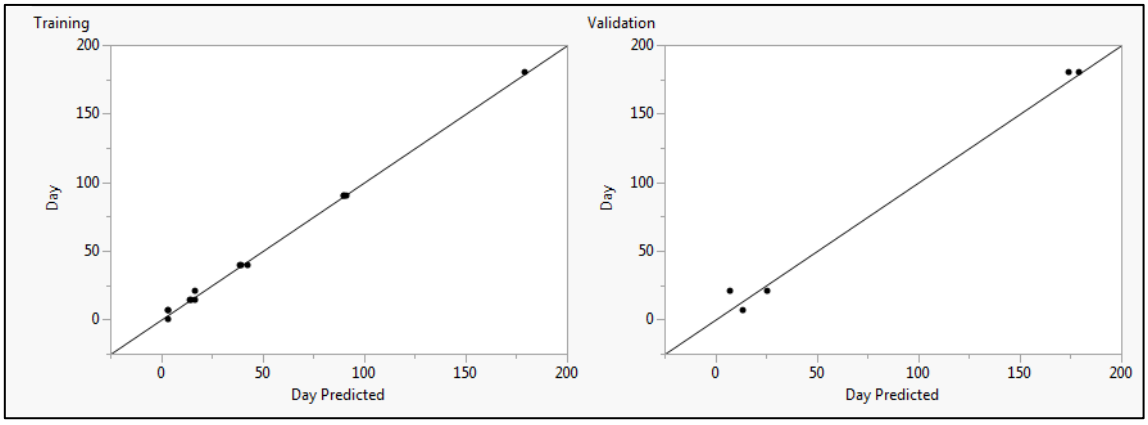

Figure 3.24. Actual by predicted plot of Day in cross validation model (Soil lateral of the Control carrion in summer 2013 at Snook, Texas).

Table 3.24. Measures of training and validation models (Soil lateral of the Control carrion in summer 2013 at Snook, Texas).

Training		Validation	
RSquare	0.99	RSquare	0.99
RMSE	2.35	RMSE	7.63
Mean Abs Dev	1.87	Mean Abs Dev	6.24
-LogLikelihood	36.39	-LogLikelihood	17.25
SSE	88.60	SSE	291.42
Sum Freq	16	Sum Freq	5

Post-7 (Soil lateral)

The model was significantly different ($p < 0.0059$), with high strength of relationship (RSquare = 0.68). Table 3.25 showed significant predictors for soil lateral of the Post-7 carrion. A prediction expression is also provided. Figure 3.25 presents the actual by predicted plot of Day.

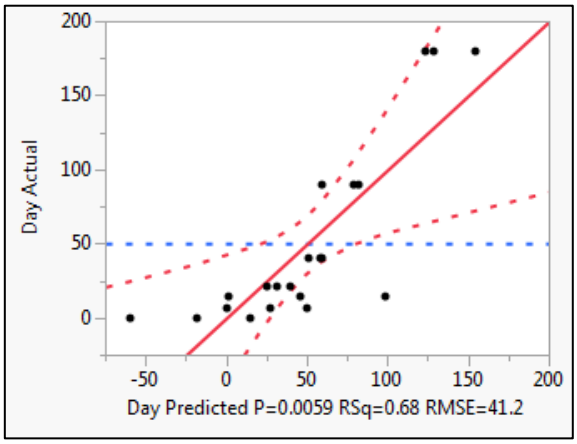

Figure 3.25. Actual by predicted plot of Day in multiple regression model (Soil lateral of the Post-7 carrion in summer 2013 at Snook, Texas).

Table 3.25. Parameter estimates in regression model for soil lateral (Post-7) in summer 2013 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	316.16	98.49	3.21	0.0063*
pH	-41.92	11.73	-3.57	0.0031*
Conductivity	0.11	0.19	0.60	0.5596
NO_3-N	-0.18	0.13	-1.39	0.1852
NH_4-N	-0.08	0.07	-1.07	0.3013
PO_4-P	-0.13	0.47	-0.29	0.7740
Moisture	6.98	2.16	3.23	0.0060*

Prediction expression: PMI (D) = 316.16 – 41.92*pH + 0.11*Conductivity - 0.18*NO_3-N – 0.08*NH_4-N – 1.13*PO_4-P + 6.98*Moisture

Cross validation test

Validation test showed a very high RSquare (0.96) and low RMSE (13.18) (Table 3.26), indicating that this model predicted well for day of decomposition (Figure 3.26).

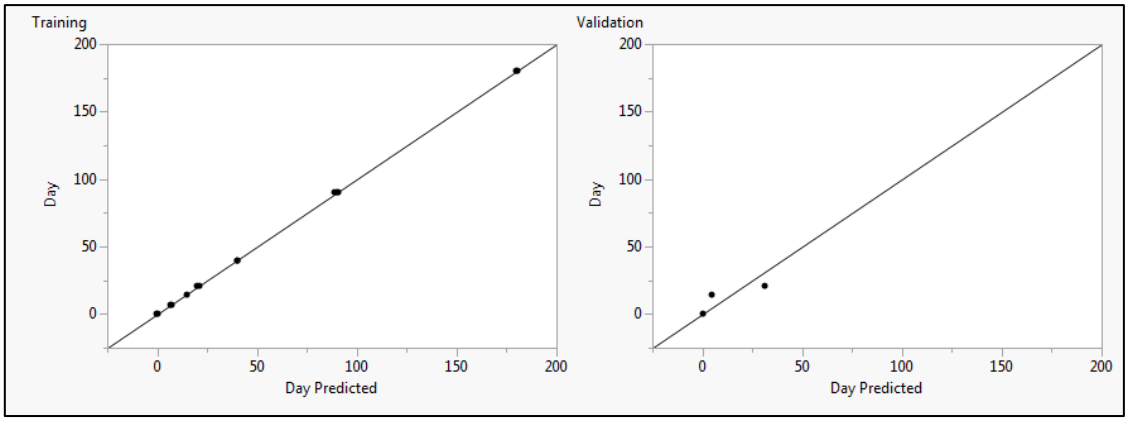

Figure 3.26. Actual by predicted plot of Day in cross validation model (Soil lateral of the Post-7 carrion in summer 2013 at Snook, Texas).

Table 3.26. Measures of training and validation models (Soil lateral of the Post-7 carrion in summer 2013 at Snook, Texas).

Training		Validation	
RSquare	0.99	RSquare	0.96
RMSE	0.52	RMSE	13.18
Mean Abs Dev	0.40	Mean Abs Dev	10.63
-LogLikelihood	13.03	-LogLikelihood	15.99
SSE	4.61	SSE	695.38
Sum Freq	17	Sum Freq	4

Post-14 (Soil lateral)

The model was significantly different ($p < 0.0069$), with high strength of relationship (RSquare = 0.68). Table 3.27 showed significant predictors for soil lateral of the Post-14 carrion. A prediction expression is also provided. Figure 3.27 presents the actual by predicted plot of Day.

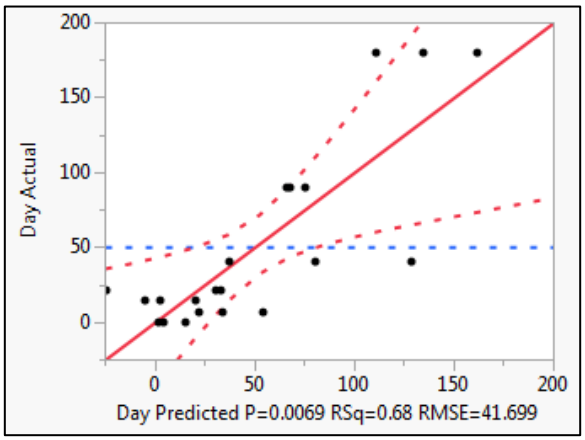

Figure 3.27. Actual by predicted plot of Day in multiple regression model (Soil lateral of the Post-14 carrion in summer 2013 at Snook, Texas).

Table 3.27. Parameter estimates in regression model for soil lateral (Post-14) in summer 2013 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	175.29	138.20	1.27	0.2254
pH	-19.96	15.06	-1.33	0.2062
Conductivity	-0.32	0.15	-2.12	0.0521
NO_3-N	0.15	0.14	1.02	0.3254
NH_4-N	0.05	0.07	0.68	0.5053
PO_4-P	0.11	0.27	0.42	0.6825
Moisture	7.93	2.60	3.05	0.0086*

Prediction expression: PMI (D) = 175.29 – 19.96*pH - 0.32*Conductivity + 0.15*NO_3-N + 0.05*NH_4-N + 0.11*PO_4-P + 7.93*Moisture

Cross validation test

Validation test showed a very high RSquare (0.99) and low RMSE (2.42) (Table 3.28), indicating that this model predicted well for day of decomposition (Figure 3.28).

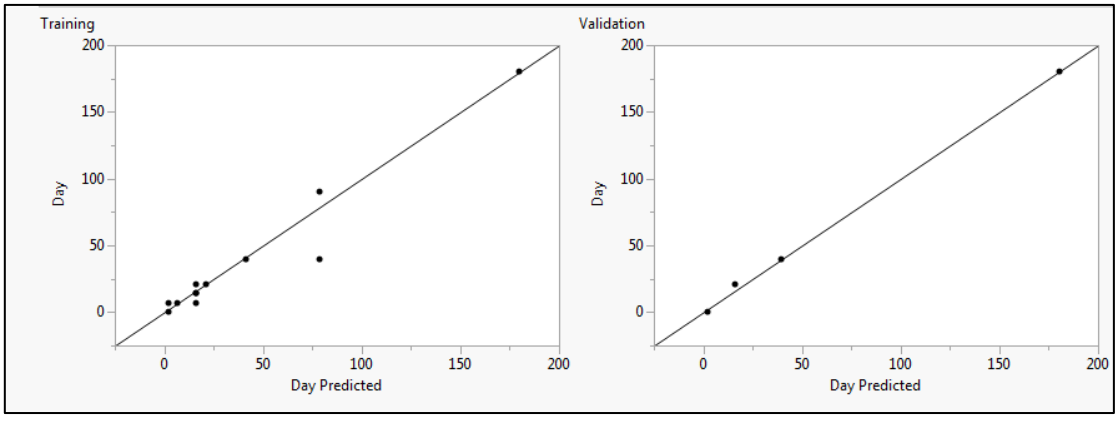

Figure 3.28. Actual by predicted plot of Day in cross validation model (Soil lateral of the Post-14 carrion in summer 2013 at Snook, Texas).

Table 3.28. Measures of training and validation models (Soil lateral of the Post-14 carrion in summer 2013 at Snook, Texas).

Training		Validation	
RSquare	0.94	RSquare	0.99
RMSE	11.26	RMSE	2.42
Mean Abs Dev	6.46	Mean Abs Dev	1.50
-LogLikelihood	61.45	-LogLikelihood	11.51
SSE	2031.85	SSE	29.32
Sum Freq	16	Sum Freq	5

Control (Soil 5 meter)

The model was significantly different ($p < 0.0001$), with high strength of relationship (RSquare = 0.93). Table 3.29 showed significant predictors for soil 5 meter of the Control carrion. A prediction expression is also provided. Figure 3.29 presents the actual by predicted plot of Day.

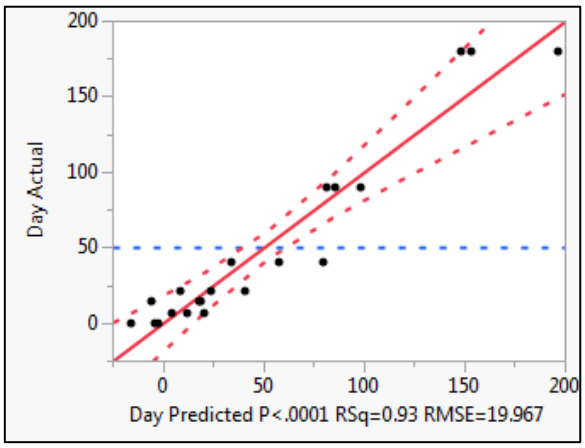

Figure 3.29. Actual by predicted plot of Day in multiple regression model (Soil 5 meter of the Control carrion in summer 2013 at Snook, Texas).

Table 3.29. Parameter estimates in regression model for soil 5 meter (Control) in summer 2013 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	307.48	71.89	4.28	0.0008*
pH	-40.47	8.94	-4.53	0.0005*
Conductivity	0.11	0.18	0.65	0.5258
NO_3-N	-0.53	0.38	-1.39	0.1877
NH_4-N	-1.50	0.66	-2.25	0.0410*
PO_4-P	7.31	3.17	2.30	0.0371*
Moisture	8.37	1.46	5.73	<0.0001*

Prediction expression: PMI (D) = 307.48 – 40.47*pH + 0.11*Conductivity - 0.53*NO_3-N - 1.50*NH_4-N + 7.31*PO_4-P + 8.37*Moisture

Cross validation test

Validation test showed a very high RSquare (0.99) and low RMSE (0.71) (Table 3.30), indicating that this model predicted well for day of decomposition (Figure 3.30).

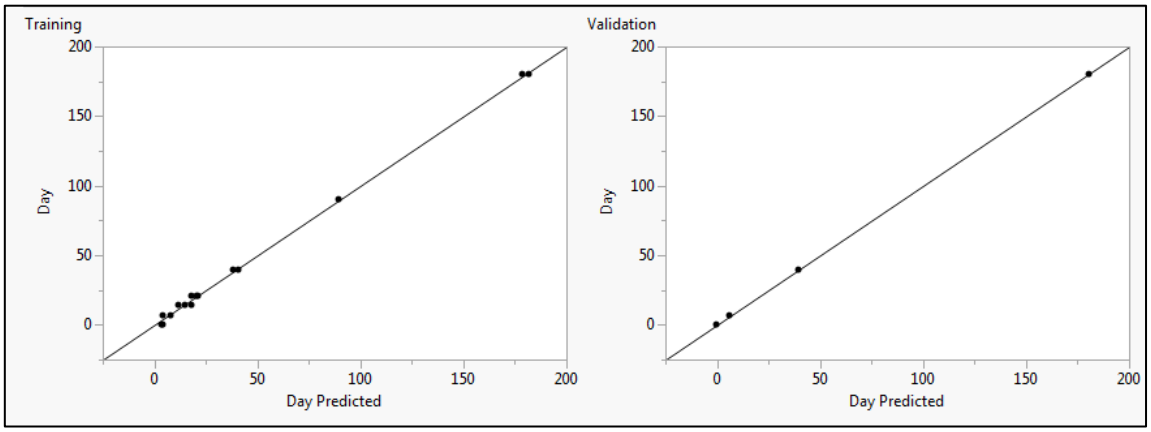

Figure 3.30. Actual by predicted plot of Day in cross validation model (Soil 5 meter of the Control carrion in summer 2013 at Snook, Texas).

Table 3.30. Measures of training and validation models (Soil 5 meter of the Control carrion in summer 2013 at Snook, Texas).

Training		Validation	
RSquare	0.99	RSquare	0.99
RMSE	2.15	RMSE	0.71
Mean Abs Dev	1.73	Mean Abs Dev	0.62
-LogLikelihood	37.16	-LogLikelihood	4.31
SSE	78.86	SSE	2.03
Sum Freq	17	Sum Freq	4

Post-7 (Soil 5 meter)

The model was significantly different ($p < 0.0001$), with high strength of relationship (RSquare = 0.89). Table 3.31 showed significant predictors for soil 5 meter of the Post-7 carrion. A prediction expression is also provided. Figure 3.31 presents the actual data by predicted plot of Day.

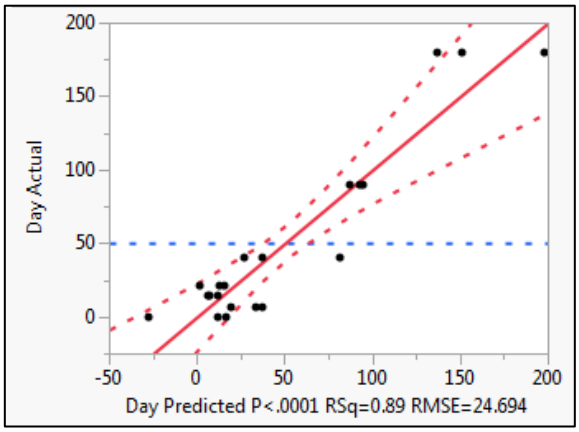

Figure 3.31. Actual by predicted plot of Day in multiple regression model (Soil 5 meter of the Post-7 carrion in summer 2013 at Snook,Texas).

Table 3.31. Parameter estimates in regression model for soil 5 meter (Post-7) in summer 2013 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	148.73	80.52	1.85	0.0860
pH	-23.16	7.97	-2.91	0.0115*
Conductivity	0.17	0.17	1.01	0.3274
NO_3-N	-0.70	0.45	-1.56	0.1405
NH_4-N	-0.98	0.37	-2.60	0.0211*
PO_4-P	9.79	3.68	2.66	0.0186*
Moisture	6.84	1.35	5.05	0.0002*

Prediction expression: PMI (D) = 148.73 – 23.16*pH + 0.17*Conductivity - 0.70*NO_3-N – 0.98*NH_4-N + 9.79*PO_4-P + 6.84*Moisture

Cross validation test

Validation test showed a very high RSquare (0.90) and low RMSE (10.21) (Table 3.32), indicating that this model predicted well for day of decomposition (Figure 3.32).

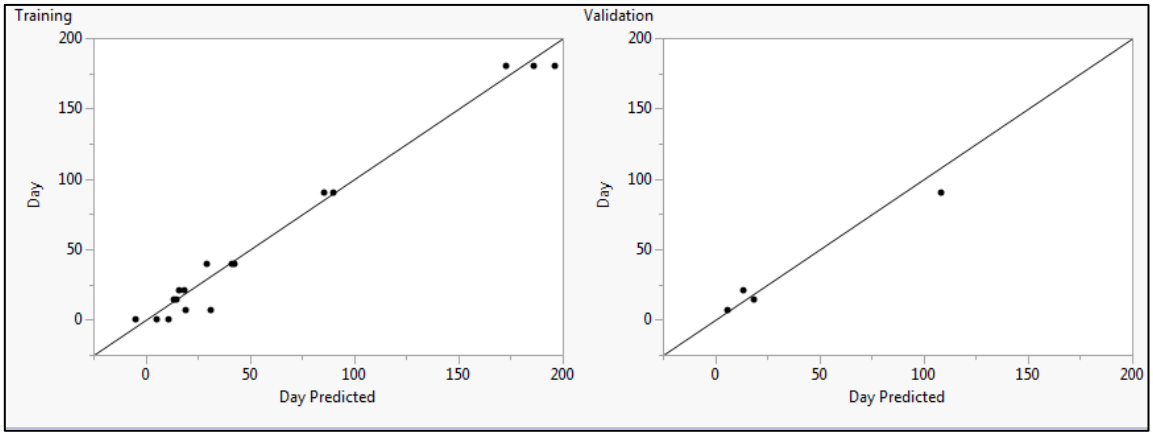

Figure 3.32. Actual by predicted plot of Day in cross validation model (Soil 5 meter of the Post-7 carrion in summer 2013 at Snook, Texas).

Table 3.32. Measures of training and validation models (Soil 5 meter of the Post-7 carrion in summer 2013 at Snook, Texas).

Training		Validation	
RSquare	0.97	RSquare	0.90
RMSE	9.21	RMSE	10.21
Mean Abs Dev	6.78	Mean Abs Dev	7.92
-LogLikelihood	61.88	-LogLikelihood	14.97
SSE	1444.42	SSE	417.16
Sum Freq	14	Sum Freq	4

Post-14 (Soil 5 meter)

The model was significantly different (p = 0.0004), with high strength of relationship (RSquare = 0.79). Table 3.33 showed significant predictors for soil 5 meter of the Post-14 carrion. A prediction expression is also provided. Figure 3.33 presents the actual by predicted plot of Day.

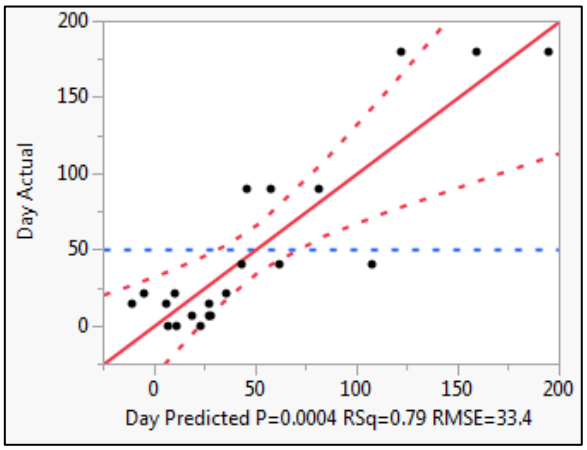

Figure 3.33. Actual by predicted plot of Day in multiple regression model (Soil 5 meter of the Post-14 carrion in summer 2013 at Snook, Texas).

Table 3.33. Parameter estimates in regression model for soil 5 meter (Post-14) in summer 2013 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	-46.44	113.40	-0.41	0.6883
pH	-2.40	12.18	-0.20	0.8466
Conductivity	0.13	0.46	0.29	0.7735
NO_3-N	1.39	1.24	1.12	0.2820
NH_4-N	0.008	0.45	0.02	0.9856
PO_4-P	0.08	1.56	0.05	0.9570
Moisture	10.15	2.06	4.91	0.0002*

Prediction expression: PMI (D) = -46.44 – 2.40*pH + 0.13*Conductivity + 1.39*NO_3-N + 0.008*NH_4-N + 0.08*PO_4-P + 10.15*Moisture

Cross validation test

Validation test showed a very high RSquare (1.00) and low RMSE (1.3165e-9) (Table 3.34), indicating that this model predicted very well for day of decomposition (Figure 3.34).

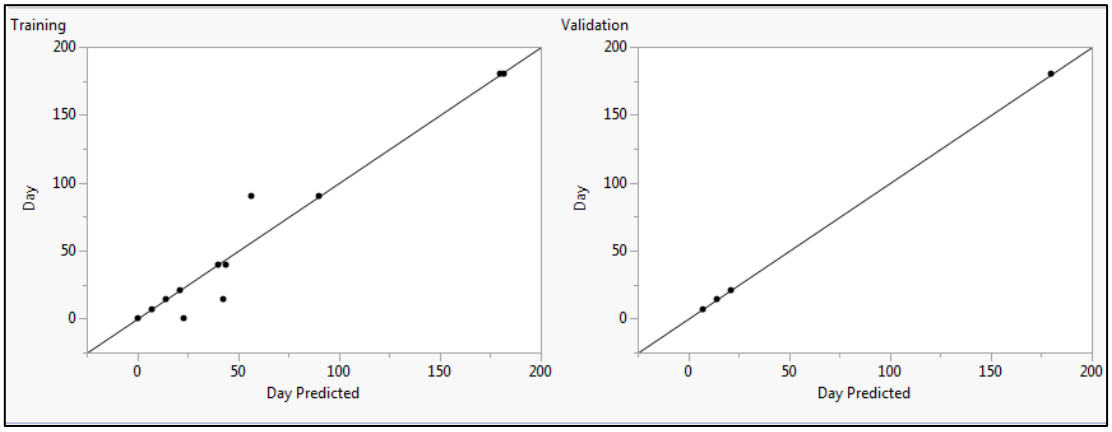

Figure 3.34. Actual by predicted plot of Day in cross validation model (Soil 5 meter of the Post-14 carrion in summer 2013 at Snook, Texas).

Table 3.34. Measures of training and validation models (Soil 5 meter of the Post-14 carrion in summer 2013 at Snook, Texas).

Training		Validation	
RSquare	0.95	RSquare	1
RMSE	12.08	RMSE	1.3165e-9
Mean Abs Dev	5.32	Mean Abs Dev	1.2644e-9
-LogLikelihood	66.48	-LogLikelihood	-76.11
SSE	2484.03	SSE	6.932e-18
Sum Freq	17	Sum Freq	4

Soil chemistry profiles in summer 2014

Two more parameters were added in 2014 trial namely non-purgeable organic carbon (NPOC) and total nitrogen (TN). Additional soil samples (n = 5) were collected in every sampling day (Day -5, 0, 7, 14, 21, 40, 90 and 180) from the upper slope at the study site (East) to serve as overall control. Pre-treatment soils (soil collected five days before pig placement) were also collected and served as the second control. When all data in summer 2014 was pooled and analyzed by PERMANOVA in R, the results showed that Day, Treatment, Site were significant difference, as well as interactions between the independence variables (Table 3.35). The NMDS ordination plots of Day, Treatment, and Region are demonstrated in Figure 3.35 to show distances between factors and variables. Minimum stress for given dimensionality was 0.0473 and r^2 = 0.9934. This result indicated an excellent representation of data in reduced dimension with an acceptable amount of distortion.

Table 3.35. Analysis of the overall effects on soil chemistry profiles in summer 2014 at Snook, Texas using PERMANOVA (* denotes significant difference).

Factor	df	SS	MS	F Model	R^2	p value
Day	6	6.3959	1.066	31.755	0.22081	0.001*
Treatment	2	0.2562	0.1281	3.815	0.00884	0.008*
Region	2	10.0424	5.0212	149.577	0.3467	0.001*
Day x Treatment	12	1.1146	0.0929	2.767	0.03848	0.001*
Day x Region	12	4.6025	0.3835	11.425	0.15889	0.001*
Treatment x Region	4	0.554	0.1385	4.126	0.01913	0.001*
Day x Treatment x Region	24	1.7706	0.0738	2.198	0.06113	0.001*
Residual	126	4.2297	0.0336		0.14602	
Total	188	28.966			1.0000	

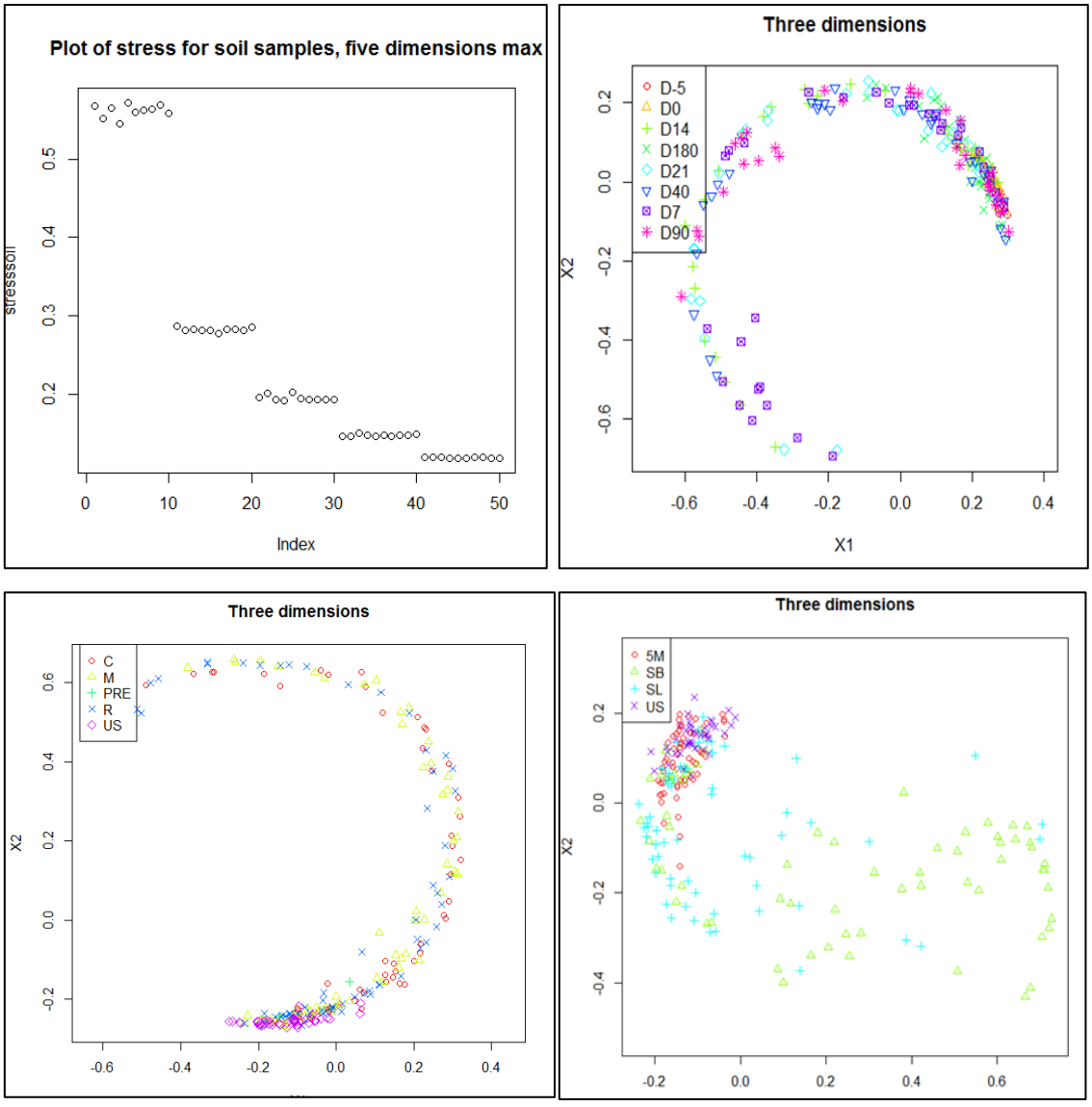

Figure 3.35. NMDS plot of stress (Stress test 0.0473; $r^2 = 0.9934$) (top left) and NMDS ordinations of carrion decomposition days (top right), treatments (legend: C = Control; M = Post-7; R = Post-14; PRE = Pre-treatment; US = Upper slope) (bottom left) and soil regions (bottom right) in summer 2014 at Snook, Texas.

pH

In general, pH increased over decomposition day. Statistic showed that pH was significantly different by Treatments ($p = 0.0048$), soil regions (include soil beneath, lateral, and 5 meter) ($p = 0.0087$) and days of decomposition ($p \leq 0.0001$) (Table 3.36). Figure 3.36 demonstrated mean pH across treatments in three different regions (beneath, lateral, and 5 meter away) over decomposition days (Day -5 to Day 180 postmortem). Table 3.37 presents significant results in pH at soil beneath, soil lateral and soil 5 meter across treatments over time. pH in pre-treatment soil and carrion soils were not the same even after Day 180 of carrion decomposition; however, pH at upper slope and pre-treatment soil was significantly different, indicating soil pH did change over time in soil regardless of the presence of carrion.

Table. 3.36. ANOVA on soil pH by treatments, regions and days in summer 2014 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	4	3.8411	0.0048*
Region	4	3.4843	0.0087*
Day	7	13.8661	<0.0001*

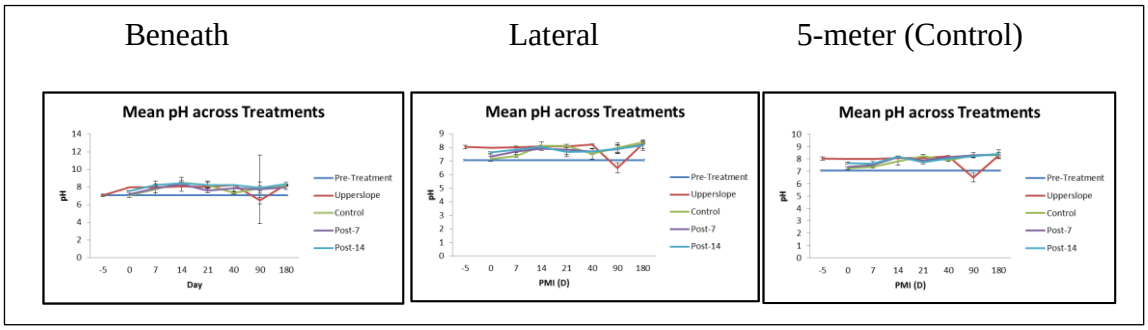

Figure 3.36. Mean soil pH across treatments at three different soil regions over carrion decomposition days in summer 2014 at Snook, Texas (Error bar = standard deviation).

Table 3.37. Significant difference in pH at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2014 at Snook, Texas.

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
pH	0	US x Pre	<0.0001	<0.0001	<0.0001
		US x C	<0.0001	<0.0001	<0.0001
		US x Post-7	<0.0001	<0.0001	<0.0001
		US x Post-14	-	0.0102	0.0075
		Pre x Post-14	0.0014	0.0004	<0.0001
		Pre x Post-7	-	-	0.0264
		C x Post-14	0.0021	0.0020	0.0022
		Post-7 x Post-14	0.0221	0.0398	0.0162
	7	US x Pre	0.0300	0.0002	<0.0001
		Pre x Post-14	0.0127	0.0019	0.0052
		Pre x Post-7	-	0.0088	0.0252
		C x US	-	0.0051	0.0005
		Post-7 x US	-	-	0.0057
		Post-14 x US	-	-	0.0335

Table 3.37 (Continued).

Day	Treatments	p value		
		Beneath	Lateral	5 meter (control)
14	Pre x C	<0.0001	<0.0001	0.0002
	C x Post-14	-	-	0.0416
	Pre x Post-7	<0.0001	0.0001	<0.0001
	Pre x Post-14	<0.0001	<0.0001	<0.0001
	US x Pre	<0.0001	<0.0001	<0.0001
	US x C	0.0033	-	-
	US x Post-14	0.0107	-	-
21	Pre x C	0.0006	0.0005	<0.0001
	C x Post-7	-	-	0.0038
	C x Post-14	-	-	0.0003
	Pre x Post-7	-	0.0051	<0.0001
	Pre x Post-14	0.0005	0.0336	<0.0001
	Post-7 x Post-14	0.0465	-	-
	US x Pre	0.0009	0.0002	<0.0001
	US x Post-14	-	-	0.0043
40	Pre x C	-	-	<0.0001
	Pre x Post-14	<0.0001	0.0093	<0.0001
	Pre x Post-7	0.0004	0.0077	<0.0001
	C x Post-7	0.0145	-	0.0057
	C x Post-14	0.0001	-	-
	US x Pre	<0.0001	<0.0001	<0.0001
	US x C	<0.0001	0.0016	0.0003
	US x Post-14	-	0.0130	0.0291

Table 3.37 (Continued).

Day	Treatments	p value		
		Beneath	Lateral	5 meter (Control)
	US x Post-7	-	0.0161	-
90	Pre x C	-	0.0273	0.0003
	Pre x Post-7	-	-	0.0003
	Pre x Post-14	-	0.0391	0.0004
	Pre x US	-	-	0.0427
	US x Post-14	0.0011	0.0004	<0.0001
	US x Post-7	0.0036	0.0005	<0.0001
	US x C	0.0018	0.0003	<0.0001
180	Pre x C	<0.0001	<0.0001	<0.0001
	Pre x Post-7	<0.0001	0.0001	<0.0001
	Pre x Post-14	<0.0001	0.0002	<0.0001
	US x Pre	<0.0001	<0.0001	<0.0001

"-" No significant difference; Pre = Pre-treatment; C = Control; US = Upper slope.

Conductivity

Statistic showed that conductivity was significantly different by Treatments ($p = 0.0002$), soil regions (include soil beneath, lateral, and 5 meter) ($p < 0.0001$) and days of decomposition ($p < 0.0001$) (Table 3.38). Figure 3.37 demonstrated mean conductivity across treatments in three different soil regions over decomposition day. Table 3.39 presents significant results in conductivity at soil beneath, soil lateral and soil 5 meter across treatments over time. In soil beneath, conductivity was the highest in Post-14 carrion, indicating the longer period of delay in blow fly colonization on carrion, the higher the soil conductivity. However, conductivity in control carrion and Post-7 carrion was not significant difference. At soil lateral, Control carrion showed the highest conductivity compared to other treatment groups, indicating the spread of decomposition fluid and ions to the side of the carrion.

Table. 3.38. ANOVA on soil conductivity by treatments, regions and days in summer 2014 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	4	5.6252	0.0002*
Region	4	40.4524	<0.0001*
Day	7	5.4671	<0.0001*

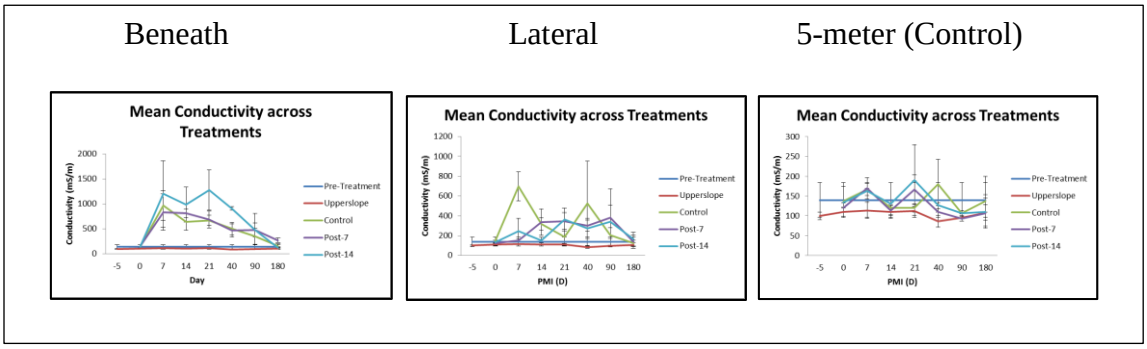

Figure 3.37. Mean soil conductivity across treatments at three different soil regions over carrion decomposition days in summer 2014 at Snook, Texas (Error bar = standard deviation).

Table 3.39. Significant difference in soil conductivity at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2014 at Snook, Texas.

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
Conductivity	7	Pre x Post-14	0.0142	-	-
		C x Pre	-	<0.0001	-
		C x US	0.0286	<0.0001	-
		C x Post-7	-	<0.0001	-
		C x Post-14	-	0.0002	-
		Post-14 x US	0.0054	-	-
	14	Pre x C	0.0195	0.0284	-
		C x Post-14	-	0.0444	-
		Pre x Post-7	0.0022	0.0162	
		Pre x Post-14	0.0003	-	-
		US x C	0.0060	0.0046	-
		US x Post-7	0.0006	0.0025	-
		US x Post-14	<0.0001	-	-
		Post-7 x Post-14	-	0.0254	-

Table 3.39 (Continued).

Day	Treatments	p value		
		Beneath	Lateral	5 meter (control)
21	Pre x C	0.0281	-	-
	C x Post-14	0.0122	0.0465	-
	Pre x Post-7	0.0251	0.0160	-
	Pre x Post-14	<0.0001	0.0094	-
	Post-7 x Post-14	0.0137	-	-
	US x C	0.0097	-	-
	US x Post-7	0.0085	0.0028	-
	US x Post-14	<0.0001	0.0016	-
40	Pre x C	0.0008	-	-
	Pre x Post-14	<0.0001	-	-
	Pre x Post-7	0.0017	-	-
	C x Post-14	0.0003	-	-
	Post-7 x Post-14	0.0001	-	-
	US x C	<0.0001	-	0.0130
	US x Post-14	<0.0001	-	-
	US x Post-7	0.0002	-	-
90	Pre x Post-7	0.0090	-	-
	US x Post-14	0.0434	-	-
	US x Post-7	0.0021	-	-
180	C x Post-7	0.0129	-	-
	Pre x Post-7	0.0039	-	-
	Post-7 x Post-14	0.0015	-	-
	US xPost-7	0.0002	-	-

"-" No significant difference; Pre = Pre-treatment; C = Control; US = Upper slope.

Nitrate-N (NO_3-N)

Similar to 2013 trial, nitrate-N concentration peaked on Day 40 (for Control carrion) and peaked later on Day 90 (for both treatments). This result indicates the delay of blow fly colonization on carrion did significantly impact the nitrification process. Nitrate-N was significantly different by Treatments ($p = 0.0299$), soil regions (include soil beneath, lateral, and 5 meter) ($p < 0.0001$) and days of decomposition ($p < 0.0001$) (Table 3.40). Figure 3.38 demonstrated mean nitrate-N across treatments in three different soil regions over decomposition days. Table 3.41 presents significant results in nitrate-N at soil beneath, soil lateral and soil 5 meter across treatments over time. Resilience between control soil (upper slope) and carrion soil was achieved on Day 180, where all the soil samples showed no significant difference among each other in terms of nitrate-N concentration.

Table. 3.40. ANOVA on soil nitrate-N by treatments, regions and days in summer 2014 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	4	2.8975	0.0299*
Region	4	11.0729	<0.0001*
Day	7	10.0543	<0.0001*

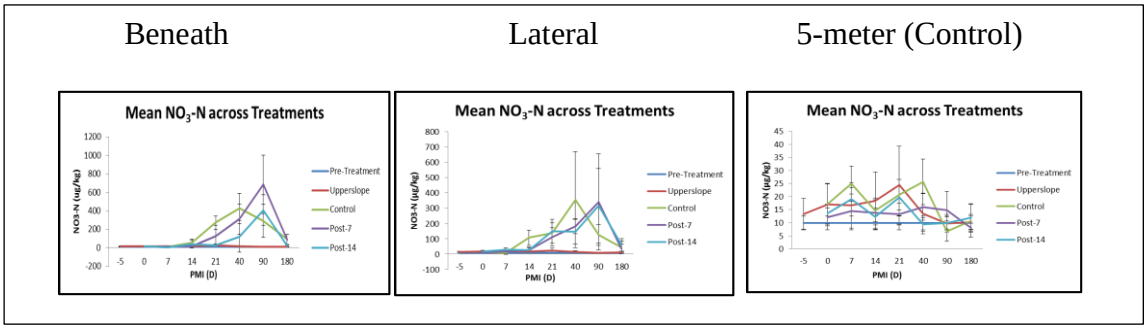

Figure 3.38. Mean soil nitrate-N across treatments at three different soil regions over carrion decomposition days in summer 2014 at Snook, Texas (Error bar = standard deviation).

Table 3.41. Significant difference in soil nitrate-N at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2014 at Snook, Texas.

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
NO_3-N	14	Pre x C	-	0.0023	-
		C x Post-14	-	0.0084	-
		C x Post-7	-	0.0089	-
		US x C	-	0.0020	-
	21	Pre x C	0.0007	0.0493	-
		C x Post-14	0.0012	-	-
		C x US	0.0005	-	-
		Pre x Post-14	-	0.0286	-
		US x Post-14	-	0.0283	-
	40	Pre x C	0.0071	-	-
		C x Post-14	-	-	0.0498
		US x C	0.0033	0.0293	-
		US x Post-7	0.0317	-	-
	90	Pre x Post-7	0.0020	-	-
		US x Post-14	0.0375	-	-

Table 3.41 (Continued).

Day	Treatments	p value		
		Beneath	Lateral	5 meter (control)
	US x Post-7	0.0008	-	-
180	US x Post-7	0.0323	-	-
	C x US	0.0295	-	-

"-" No significant difference; Pre = Pre-treatment; C = Control; US = Upper slope.

Ammonium-N (NH_4-N)

Highest concentration of ammonium-N was observed at soil beneath of Post-14 carrion, followed by Post-7 and Control carrion. Similar observation was also noted at soil lateral. On Day 7, ammonium-N at 5 meter soil of carrion (both control and treatment groups) showed a significant difference with the control soils at upper slope as well as pre-treatment soil, suggesting lateral extension of ammonium-N to the distant of 5 meter. Ammonium-N was significantly different by Treatments ($p = 0.0006$), soil regions (include soil beneath, lateral, and 5 meter) ($p < 0.0001$) and days of decomposition ($p = 0.0008$) (Table 3.42). Figure 3.39 demonstrated mean ammonium-N across treatments in three different soil regions over decomposition days. Table 3.43 presents significant results in ammonium-N at soil beneath, soil lateral and soil 5 meter across treatments over time. Resilience between control soil and carrion soil was not achieved even after 180 days of carrion decomposition.

Table. 3.42. ANOVA on soil ammonium-N by treatments, regions and days in summer 2014 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	4	5.0840	0.0006*
Region	4	21.4324	<0.0001*
Day	7	3.7055	0.0008*

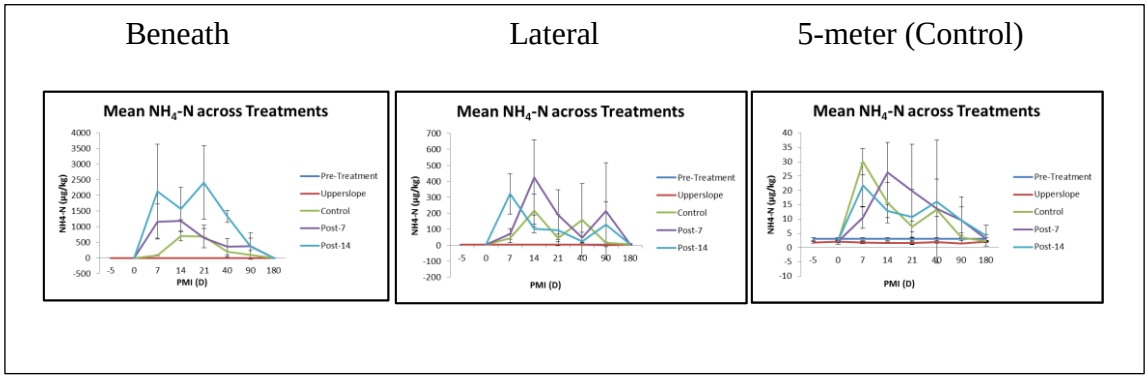

Figure 3.39. Mean soil ammonium-N across treatments at three different soil regions over carrion decomposition days in summer 2014 at Snook, Texas (Error bar = standard deviation).

Table 3.43. Significant difference in soil ammonium-N at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2014 at Snook, Texas.

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
NH_4-N	7	US x Post-14	0.0057	<0.0001	0.0001
		Pre x Post-14	0.0127	<0.0001	0.0007
		C x Post-7	-	-	0.0005
		C x Post-14	0.0165	0.0003	-
		Post-7 x Post-14	-	0.0008	0.0307
		C x US	-	-	<0.0001
		C x Pre	-	-	<0.0001
	14	Pre x Post-14	0.0002	-	-
		Pre x Post-7	0.0022	0.0027	0.0012
		C x Post-14	0.0222	-	-
		Post-7 x Post-14	-	0.0194	-
		US x C	0.0406	-	0.0220
		US x Post-7	0.0008	0.0011	0.0003
		US x Post-14	<0.0001	-	-
	21	C x Post-14	0.0110	-	-
		Post-7 x Post-14	0.0089	-	-
		Pre x Post-14	0.0008	-	-
		US x Post-7	-	0.0352	-
		US x Post-14	0.0003	-	-
	40	Pre x Post-14	<0.0001	-	-
		C x Post-14	<0.0001	-	-
		Post-7 x Post-14	<0.0001	-	-
		US x Post-7	0.0410	-	-
		US x Post-14	<0.0001	-	-

Table 3.43 (Continued).

Day	Treatments	p value		
		Beneath	Lateral	5 meter (control)
180	US x Post-14	<0.0001	0.0004	-
	US x Post-7	<0.0001	0.0057	-
	US x C	<0.0001	0.0098	-
	Pre x Post-14	<0.0001	0.0054	-
	Pre x Post-7	<0.0001	-	-
	Pre x C	<0.0001	-	-
	Pre x US	0.0272	-	-

"-" No significant difference; Pre = Pre-treatment; C = Control; US = Upper slope.

Orthophosphate-P (PO_4-P)

On Day 7, Post-14 had the highest orthophosphate-P concentration among other treatments. However, Control and Post-7 became the highest on Day 14. This phenomenon was observed in soil beneath and soil lateral. Orthophosphate-P was significantly different by Treatments ($p = 0.0242$), soil regions ($p < 0.0001$) and days of decomposition ($p = 0.0138$) (Table 3.44). Figure 3.40 demonstrated mean orthophosphate-P across treatments in three different soil regions over decomposition days. Table 3.45 presents significant results in orthophosphate-P at soil beneath, soil lateral and soil 5 meter across treatments over time. Again, resilience in orthophosphate-P was not achieved between Control and carrion soils. The impact of carrion with delayed colonization on orthophosphate-P still can be observed after Day 180.

Table. 3.44. ANOVA on soil ammonium-N by treatments, regions and days in summer 2014 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	4	2.8627	0.0242*
Region	4	17.5394	<0.0001*
Day	7	2.5882	0.0138*

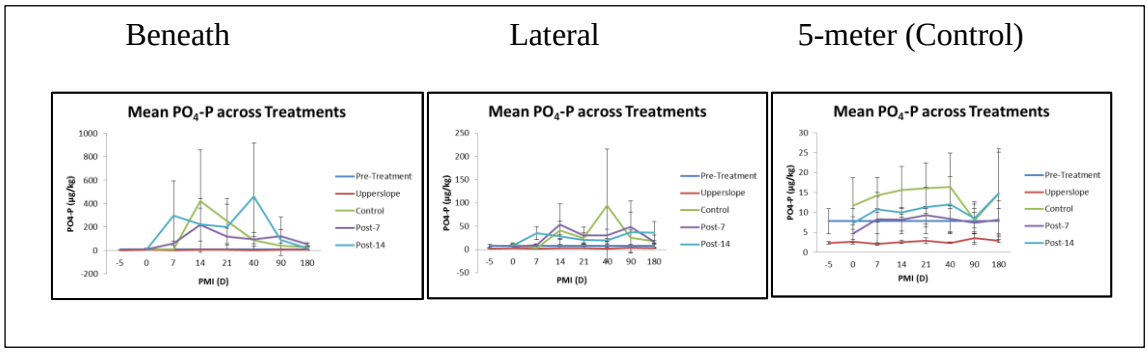

Figure 3.40. Mean soil orthophosphate-P across treatments at three different soil regions over carrion decomposition days in summer 2014 at Snook, Texas (Error bar = standard deviation).

Table 3.45. Significant difference in orthophosphate-P at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2014 at Snook, Texas.

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
PO_4-P	0	US x Post-14	0.0151	0.0146	-
		US x C	0.0176	0.0031	0.0209
	7	US x Post-14	-	<0.0001	-
		Pre x Post-14	-	0.0011	-
		C x Post-14	-	0.0003	-
		Post-7 x Post-14	-	0.0020	-
		C x US	-	-	0.0056
	14	US x C	-	-	0.0006
		US x Post-7	-	0.0299	-
		US x Post-14	-	-	0.0410
	21	C x US	0.0459	-	0.0025
		US x Post-7	-	0.0147	-
	40	Pre x Post-14	<0.0001	-	-
		C x Post-14	<0.0001	-	-
		Post-7 x Post-14	<0.0001	-	-
		US x C	-	-	0.0134
		US x Post-14	<0.0001	-	-
	180	US x Post-14	-	0.0100	-
		US x Post-7	<0.0001	-	-
		US x C	0.0038	-	-
		Pre x Post-7	0.0002	-	-
		Pre x C	0.0335	-	-
		C x Post-7	0.0410	-	-
		Post-7 x Post-14	0.0018	-	-

"-" No significant difference; Pre = Pre-treatment; C = Control; US = Upper slope.

Non-purgeable organic carbon (NPOC)

Carrion with delayed blow fly colonization did significantly change the deposition of NPOC in the soil. NPOC was highly concentrated and persisted for a long time period (until Day 90) in soil beneath, especially at Post-14 carrion. Interestingly, at soil lateral, Control carrion showed the highest concentration of NPOC, suggesting lateral spread of NPOC from the CDIs. Furthermore, on Day 7, soil at 5 meter away from control carrion was significantly higher in NPOC. NPOC was significantly different by Treatments ($p = 0.0317$), soil regions (include soil beneath, lateral, and 5 meter) ($p < 0.0001$) and days of carrion decomposition ($p < 0.0001$) (Table 3.46). Figure 3.41 demonstrated mean NPOC across treatments in three different soil regions (beneath, lateral, and 5 meter away) over decomposition days (Day -5 to Day 180 postmortem). Table 3.47 presents significant results in NPOC at soil beneath, soil lateral and soil 5 meter across treatments over time.

Table. 3.46. ANOVA on soil NPOC by treatments, regions and days in summer 2014 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	4	2.6966	0.0317*
Region	4	12.5712	<0.0001*
Day	7	5.0300	<0.0001*

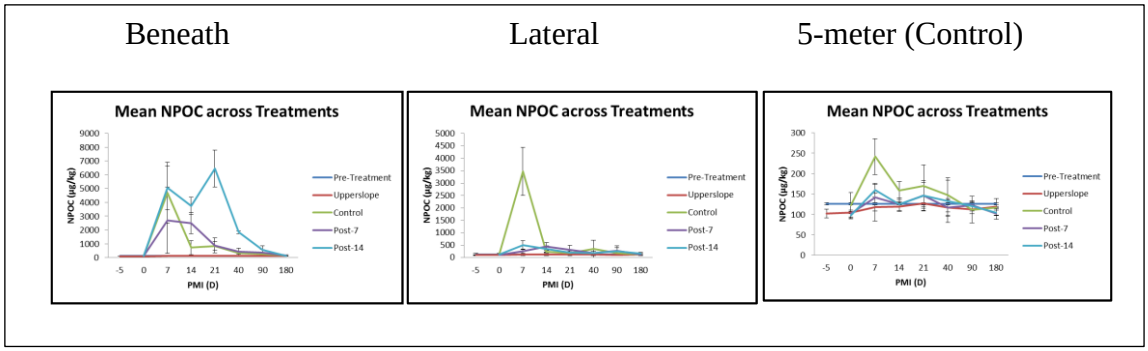

Figure 3.41. Mean soil NPOC across treatments at three different soil regions over carrion decomposition days in summer 2014 at Snook, Texas (Error bar = standard deviation).

Table 3.47. Results of significant difference in NPOC at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2014 at Snook, Texas.

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
NPOC	7	US x Post-14	0.0395	-	-
		C x Post-14	-	<0.0001	-
		C x Post-7	-	<0.0001	0.0354
		C x US	-	<0.0001	0.0040
		C x Pre	-	<0.0001	0.0144
	14	US x Post-7	-	0.0018	-
		US x Post-14	0.0070	0.0247	-
		Pre x Post-14	0.0154	-	-
		Pre x Post-7	-	0.0052	-
		C x Post-14	0.0461	-	-
	21	C x Post-14	0.0264	-	-
		US x Post-14	0.0056	-	-
		Pre x Post-14	0.0124	-	-
		Post-7 x Post-14	0.0279	-	-

Table 3.47

(Continued).

Day	Treatments	p value		
		Beneath	Lateral	5 meter (control)
40	Pre x Post-14	<0.0001	-	-
	C x Post-14	0.0001	-	-
	Post-7 x Post-14	0.0002	-	-
	US x Post-14	<0.0001	-	-
90	US x Post-14	0.0096	-	-
	Pre x Post-14	0.0244	-	-

"-" No significant difference; Pre = Pre-treatment; C = Control; US = Upper slope.

Total nitrogen (TN)

Similar to NPOC, TN was highly concentrated at the soil beneath, with Post-14 group had the highest concentration along the decomposition day (up to Day 40). Lateral movement of TN was suggested based on the evidence at soil lateral and soil 5 meter where the concentration of TN was significantly higher compared with the control soils. TN was significantly different by Treatments ($p = 0.0023$), soil regions ($p < 0.0001$) and days of decomposition ($p < 0.0001$) (Table 3.48). Figure 3.42 demonstrated mean TN across treatments in three different soil regions over decomposition days. Table 3.49 presents significant results in TN at soil beneath, soil lateral and soil 5 meter across treatments over time.

Table. 3.48. ANOVA on soil TN by treatments, regions and days in summer 2014 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	4	4.2934	0.0023*
Region	4	30.6533	<0.0001*
Day	7	4.9405	<0.0001*

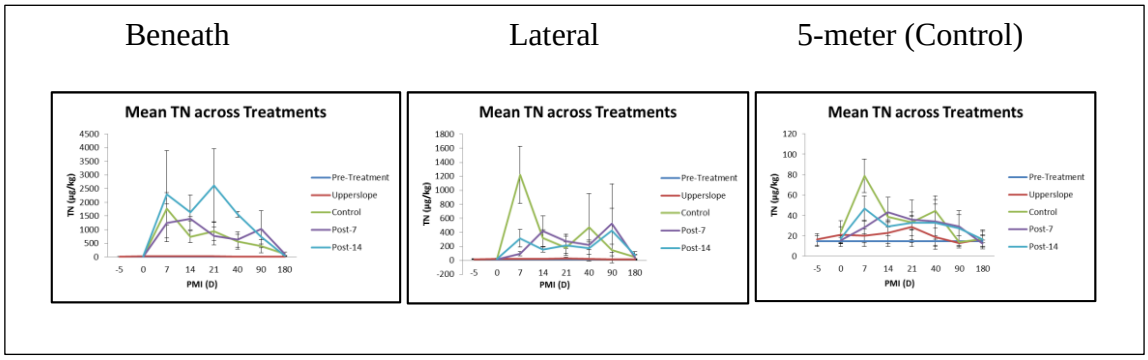

Figure 3.42. Mean soil TN across treatments at three different soil regions over carrion decomposition days in summer 2014 at Snook, Texas (Error bar = standard deviation).

Table 3.49. Significant difference in TN at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2014 at Snook, Texas.

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
TN	7	US x Post-14	0.0096	-	0.0210
		Pre x Post-14	0.0200	-	0.0133
		C x Post-14	-	0.0003	0.0133
		C x Post-7	-	<0.0001	0.0003
		C x US	-	<0.0001	<0.0001
		C x Pre	-	<0.0001	<0.0001
	14	US x Post-7	0.0001	0.0015	-
		US x Post-14	<0.0001	-	-
		Pre x Post-14	<0.0001	-	-
		Pre x Post-7	0.0004	0.0031	0.0191
		C x Post-14	0.0125	-	-
		C x Pre	0.0436	0.0231	-
		C x US	0.0241	0.0133	-
	21	C x Post-14	0.0291	-	-
		US x Post-14	0.0004	0.0462	-
		US x Post-7	-	0.0079	-
		Pre x Post-14	0.0011	-	-
		Pre x Post-7	-	0.0118	-
		Post-7 x Post-14	0.0154	-	-
	40	Pre x Post-14	<0.0001	-	-
		C x Post-14	<0.0001	-	-
		Post-7 x Post-14	0.0001	-	-
		US x C	0.0044	-	-
		US x Post-7	0.0016	-	-
		US x Post-14	<0.0001	-	-

Table 3.49 (Continued).

Day	Treatments	p value		
		Beneath	Lateral	5 meter (control)
	Pre x Post-7	0.0038	-	-
	Pre x C	0.0096	-	-
90	US x Post-14	0.0480	-	-
	US x Post-7	0.0059	-	-
	Pre x Post-7	0.0132	-	-
180	C x US	0.0346	-	-
	Post-7 x US	0.0481	-	-

"-" No significant difference; Pre = Pre-treatment; C = Control; US = Upper slope.

Soil moisture

In general, soil moisture was increasing over time, probably due to the rain events around Day 180. Soil beneath of Post-14 carrion demonstrated higher soil moisture content compared to other treatment groups. Soil moisture was significantly different by Treatments ($p = 0.0081$), soil regions (include soil beneath, lateral, and 5 meter) ($p < 0.0001$) and days of decomposition ($p < 0.0001$) (Table 3.50). Figure 3.43 demonstrated mean soil moisture across treatments in three different soil regions over decomposition days (Day -5 to Day 180 postmortem). Table 3.51 presents significant results in soil moisture at soil beneath, soil lateral and soil 5 meter across treatments over time.

Table. 3.50. ANOVA on soil moisture by treatments, regions and days in summer 2014 at Snook, Texas (* denotes significant difference).

Factor	df	F ratio	p value
Treatment	4	3.5303	0.0081*
Region	4	7.7801	<0.0001*
Day	7	23.9372	<0.0001*

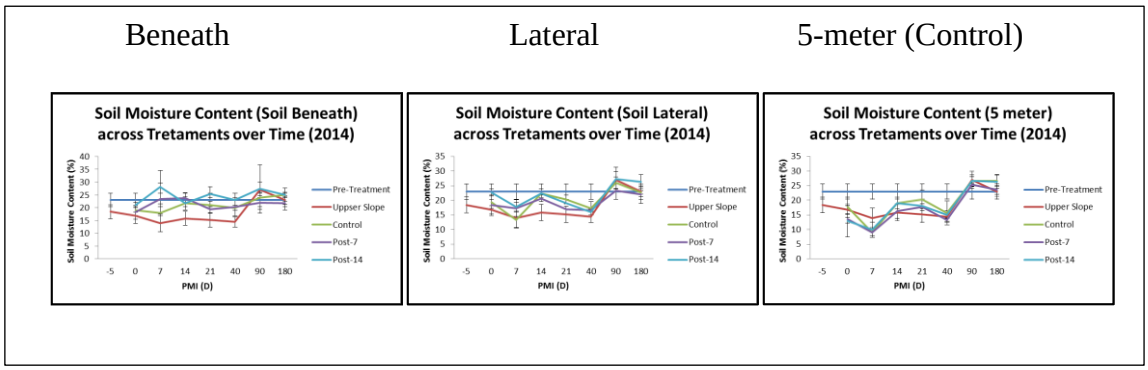

Figure 3.43. Mean soil moisture across treatments at three different soil regions over carrion decomposition days in summer 2014 at Snook, Texas (Error bar = standard deviation).

Table 3.51. Significant difference in soil moisture at soil beneath, soil lateral and soil 5 meter across treatments over time (day) in summer 2014 at Snook, Texas.

Factor	Day	Treatments	p value		
			Beneath	Lateral	5 meter (control)
H_2O	0	Pre x Post-14	-	-	0.0228
	7	US x Post-14	0.0021	-	-
		US x Post-7	0.0478	-	-
		US x Pre	-	0.0232	0.0162
		C x Pre	-	0.0365	0.0003
		Pre x Post-7	-	-	0.0003
		Pre x Post-14	-	-	0.0009
	14	US x Post-7	0.0146	-	-
		US x Post-14	-	0.0306	-
		C x US	-	0.0306	-
	21	US x Post-14	0.0020	-	-
		US x Pre	-	0.0316	-
	40	US x Post-14	0.0056	-	-
		Pre x Post-7	-	-	0.0170
		Pre x US	0.0195	0.0084	0.0169
	90	US x Pre	-	0.0079	0.0035
		C x Pre	-	-	0.0208
		Pre x Post-14	-	0.0205	0.0208
	180	Pre x Post-14	-	0.0263	0.0214
		Pre x C	-	-	0.0147

"-" No significant difference; Pre = Pre-treatment; C = Control; US = Upper slope.

Correlation between soil nutrients in 2014

Pearson's pairwise correlation was performed on all variables in soil chemistry. The results showed 23 pairs of variables were significantly correlated, either positively or negatively (Table 3.52). Six strongest positive correlations ($r > 8.0$) were detected namely ammonium-N and conductivity, NPOC and conductivity, NPOC and ammonium-N, TN and conductivity, TN and ammonium-N, and, TN and NPOC. The strongest negative correlation was between nitrate-N and pH, with $r = -0.12$. Most ionic variables (e.g., nitrate-N, ammonium-N, phosphate-P, NPOC and TN) were positively correlated with conductivity while soil moisture had weak correlation with all variables.

Table 3.52. Pearson's pairwise correlation between soil chemistry variables for soil samples collected from all regions in summer 2014 at Snook, Texas (* denotes significant difference).

Variable	By Variable	Correlation	p value
Conductivity	pH	0.1053	0.1096
NO_3-N	pH	-0.1212	0.0654
NO_3-N	Conductivity	0.3465	<0.0001*
NH_4-N	pH	0.2479	0.0001*
NH_4-N	Conductivity	0.8690	<0.0001*
NH_4-N	NO_3-N	0.0972	0.1398
PO_4-P	pH	0.2223	0.0006*
PO_4-P	Conductivity	0.6784	<0.0001*
PO_4-P	NO_3-N	0.2318	0.0004*
PO_4-P	NH_4-N	0.7134	<0.0001*
NPOC	pH	0.1275	0.0525
NPOC	Conductivity	0.8458	<0.0001*
NPOC	NO_3-N	-0.0507	0.4421
NPOC	NH_4-N	0.8253	<0.0001*
NPOC	PO_4-P	0.4625	<0.0001*
TN	pH	0.1629	0.0130*

Table 3.52 (Continued).

Variable	By Variable	Correlation	p value
TN	Conductivity	0.9735	<0.0001*
TN	NO_3-N	0.2597	<0.0001*
TN	NH_4-N	0.9029	<0.0001*
TN	PO_4-P	0.6586	<0.0001*
TN	NPOC	0.9091	<0.0001*
Soil moisture	pH	0.1440	0.0283*
Soil moisture	Conductivity	0.2344	0.0003*
Soil moisture	NO_3-N	0.1650	0.0119*
Soil moisture	NH_4-N	0.2944	<0.0001*
Soil moisture	PO_4-P	0.2719	<0.0001*
Soil moisture	NPOC	0.1945	0.0029*
Soil moisture	TN	0.2531	<0.0001*

Multiple regression and cross validation models on soil nutrients in 2014 for PMI predictions

Multiple regression analysis was performed on soil chemistry profiles in 2014. Day of decomposition was treated as Y and other variables (pH, conductivity, NO_3-N, NH_4-N, PO_4-P, NPOC, TN and soil moisture) were used as X to construct model effect. A cross validation test using KFold validation method (number of fold = 5) was conducted to determine RSquare (r^2) and the root-mean-square error (RMSE).

Ecologically speaking, the relationship between day of decomposition and soil chemistry profiles can be determined by using model analysis. The potential application of this model is to predict day of decomposition and these relationships may be useful in forensic investigations.

Control (Soil beneath)

The model was significantly different (p = 0.0002), with high strength of relationship (RSquare = 0.88). Table 3.53 showed significant predictors for soil beneath the Control carrion. A prediction expression is also provided. Figure 3.44 presents the actual by predicted plot of Day.

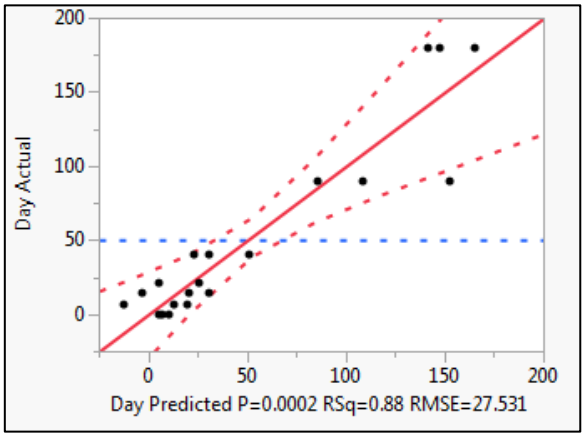

Figure 3.44. Actual by predicted plot of Day in multiple regression model (Soil beneath of Control carrion in summer 2014 at Snook, Texas).

Table 3.53. Parameter estimates in regression model for soil beneath (Control) in summer 2014 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	-640.74	122.07	-5.25	0.0002*
pH	66.57	20.31	3.28	0.0066*
Conductivity	-0.13	0.15	-0.88	0.3948
NO_3-N	0.18	0.10	1.78	0.1007
NH_4-N	-0.06	0.07	-1.00	0.3354
PO_4-P	0.01	0.06	0.17	0.8688
NPOC	0.03	0.03	1.04	0.3208
TN	-0.04	0.09	-0.47	0.6441
Moisture	9.98	3.86	2.58	0.0239*

Prediction expression: PMI (D) = -640.74 + 66.57*pH – 0.13*Conductivity + 0.18*NO_3-N – 0.06*NH_4-N + 0.01*PO_4-P + 0.03*NPOC – 0.04*TN + 9.98*Moisture

Cross validation test

Validation test showed a very high RSquare (0.99) and low RMSE (3.12) (Table 3.54), indicating that this model predicted well for day of decomposition (Figure 3.45).

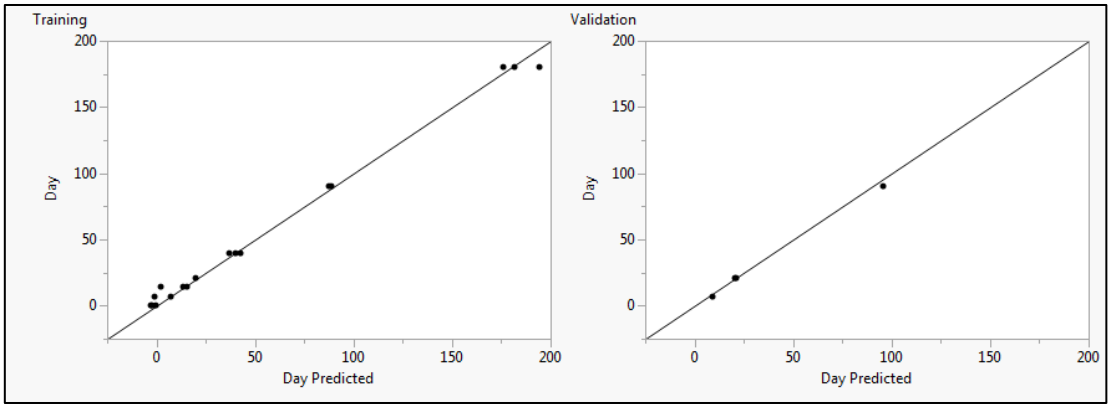

Figure 3.45. Actual by predicted plot of Day in cross validation model (Soil beneath of Control carrion in summer 2014 at Snook, Texas).

Table 3.54. Measures of training and validation models (Soil beneath of Control carrion in summer 2014 at Snook, Texas).

Training		Validation	
RSquare	0.99	RSquare	0.99
RMSE	5.32	RMSE	3.12
Mean Abs Dev	3.46	Mean Abs Dev	2.22
-LogLikelihood	52.54	-LogLikelihood	10.22
SSE	481.40	SSE	38.98
Sum Freq	17	Sum Freq	4

Post-7 (Soil beneath)

The model was significantly different ($p = 0.0015$), with high strength of relationship (RSquare = 0.82). Table 3.55 showed significant predictors for soil beneath the Post-7 carrion. A prediction expression is also provided. Figure 3.46 presents the actual by predicted plot of Day.

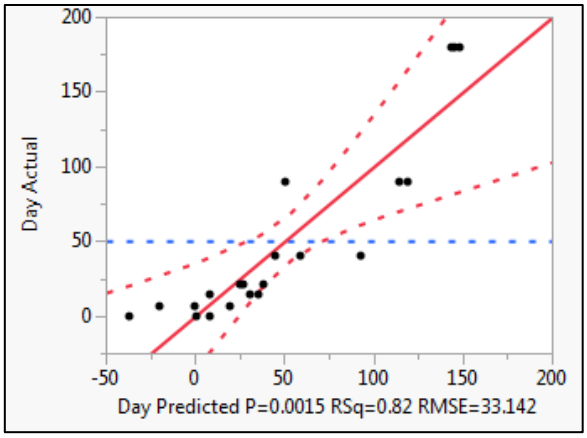

Figure 3.46. Actual by predicted plot of Day in multiple regression model (Soil beneath of Post-7 carrion in summer 2014 at Snook, Texas).

Table 3.55. Parameter estimates in regression model for soil beneath (Post-7) in summer 2014 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	-1138.30	217.03	-5.24	0.0002*
pH	137.91	28.03	4.92	0.0004*
Conductivity	0.37	0.12	2.94	0.0124*
NO_3-N	0.07	0.15	0.48	0.6423
NH_4-N	-0.20	0.16	-1.21	0.2487
PO_4-P	0.04	0.12	0.38	0.7135
NPOC	0.03	0.02	1.70	0.1151
TN	-0.16	0.15	-1.07	0.3072
Moisture	4.11	2.67	1.53	0.1524

Prediction expression: PMI (D) = -1138.30 + 137.91*pH – 0.37*Conductivity + 0.07*NO_3-N – 0.20*NH_4-N + 0.04*PO_4-P + 0.03*NPOC – 0.16*TN + 4.11*Moisture

Cross validation test

Validation test showed a very high RSquare (0.99) and low RMSE (6.64) (Table 3.56), indicating that this model predicted well for day of decomposition (Figure 3.47).

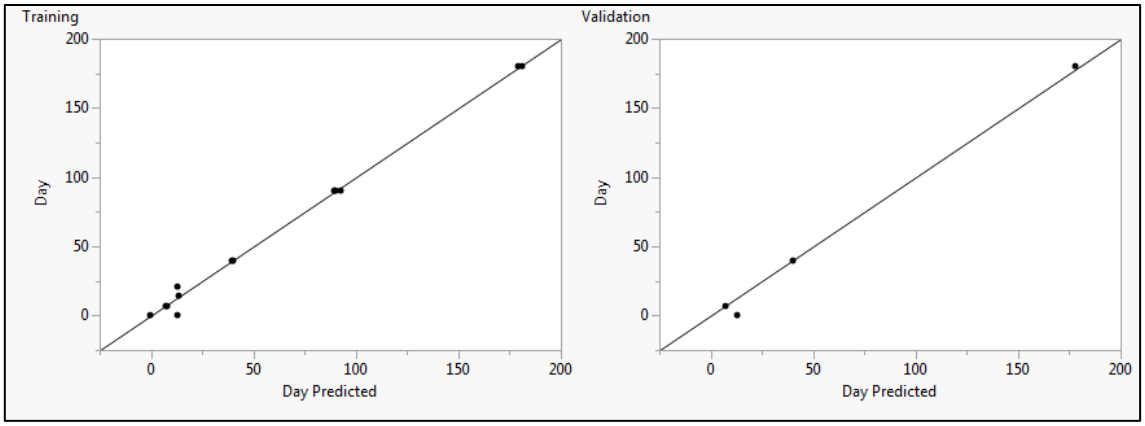

Figure 3.47. Actual by predicted plot of Day in cross validation model (Soil beneath of Post-7 carrion in summer 2014 at Snook, Texas).

Table 3.56. Measures of training and validation models (Soil beneath of Post-7 carrion in summer 2014 at Snook, Texas).

Training		Validation	
RSquare	0.99	RSquare	0.99
RMSE	4.69	RMSE	6.64
Mean Abs Dev	2.74	Mean Abs Dev	3.86
-LogLikelihood	50.40	-LogLikelihood	13.24
SSE	374.30	SSE	176.42
Sum Freq	17	Sum Freq	4

Post-14 (Soil beneath)

The model was significantly different (p = 0.068), with high strength of relationship (RSquare = 0.63). Table 3.57 showed significant predictors for soil beneath the Post-14 carrion. A prediction expression is also provided. Figure 3.48 presents the actual by predicted plot of Day.

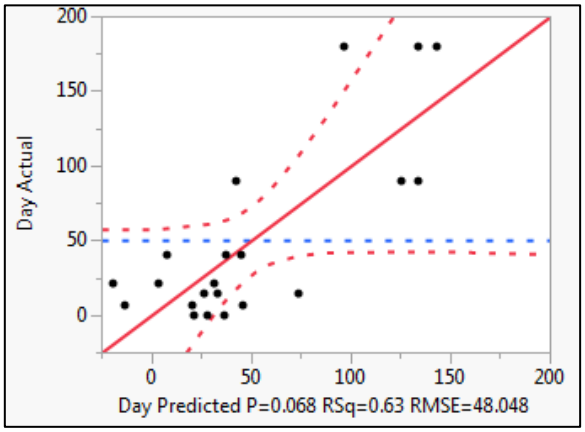

Figure 3.48. Actual by predicted plot of Day in multiple regression model (Soil beneath of Post-14 carrion in summer 2014 at Snook, Texas).

Table 3.57. Parameter estimates in regression model for soil beneath (Post-14) in summer 2014 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	-822.12	310.04	-2.65	0.0211*
pH	100.08	35.20	2.84	0.0148*
Conductivity	-0.01	0.19	-0.10	0.9247
NO_3-N	0.10	0.21	0.47	0.6478
NH_4-N	-0.07	0.28	-0.23	0.8182
PO_4-P	0.04	0.10	0.35	0.7300
NPOC	0.02	0.02	0.92	0.3776
TN	-0.02	0.21	-0.10	0.9189
Moisture	4.44	2.73	1.63	0.1298

Prediction expression: PMI (D) = -822.12 + 100.08*pH – 0.01*Conductivity + 0.10*NO_3-N – 0.07*NH_4-N + 0.04*PO_4-P + 0.02*NPOC – 0.02*TN + 4.44*Moisture

Cross validation test

Validation test showed a very high RSquare (0.99) and low RMSE (7.45) (Table 3.58), indicating that this model predicted well for day of decomposition (Figure 3.49).

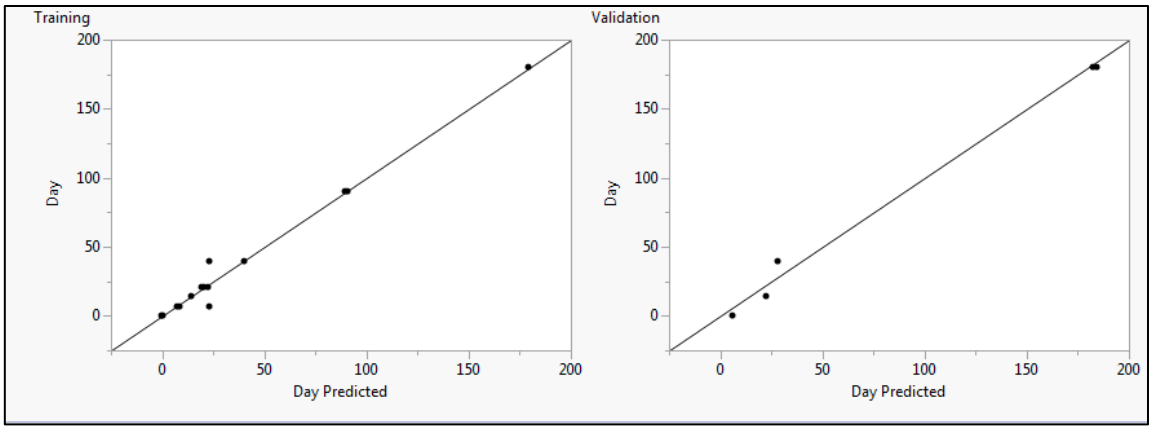

Figure 3.49. Actual by predicted plot of Day in cross validation model (Soil beneath of Post-14 carrion in summer 2014 at Snook, Texas).

Table 3.58. Measures of training and validation models (Soil beneath of Post-14 carrion in summer 2014 at Snook, Texas).

Training		Validation	
RSquare	0.98	RSquare	0.99
RMSE	5.90	RMSE	7.45
Mean Abs Dev	2.59	Mean Abs Dev	6.63
-LogLikelihood	51.12	-LogLikelihood	17.14
SSE	558.34	SSE	278.10
Sum Freq	16	Sum Freq	5

Control (Soil lateral)

The model was significantly different (p = 0.022), with high strength of relationship (RSquare = 0.71). Table 3.59 showed significant predictors for soil lateral of Control carrion. A prediction expression is also provided. Figure 3.50 presents the actual by predicted plot of Day.

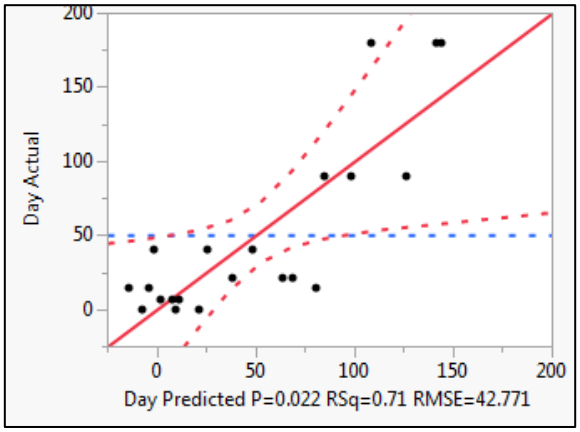

Figure 3.50. Actual by predicted plot of Day in multiple regression model (Soil lateral of Control carrion in summer 2014 at Snook, Texas).

Table 3.59. Parameter estimates in regression model for soil lateral (Control) in summer 2014 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	-779.69	256.45	-3.04	0.0103*
pH	91.50	29.22	3.13	0.0087*
Conductivity	0.16	0.42	0.39	0.7042
NO_3-N	-0.52	0.46	-1.12	0.2834
NH_4-N	-0.76	0.39	-1.94	0.0764
PO_4-P	2.22	1.02	2.16	0.0517
NPOC	-0.05	0.12	-0.41	0.6865
TN	0.11	0.31	0.37	0.7162
Moisture	5.08	3.65	1.39	0.1896

Prediction expression: PMI (D) = -779.69 + 91.50*pH + 0.06*Conductivity - 0.52*NO_3-N – 0.76*NH_4-N + 2.22*PO_4-P - 0.05*NPOC + 0.11*TN + 5.08*Moisture

Cross validation test

Validation test showed a very high RSquare (0.99) and low RMSE (0.88) (Table 3.60), indicating that this model predicted well for day of decomposition (Figure 3.51).

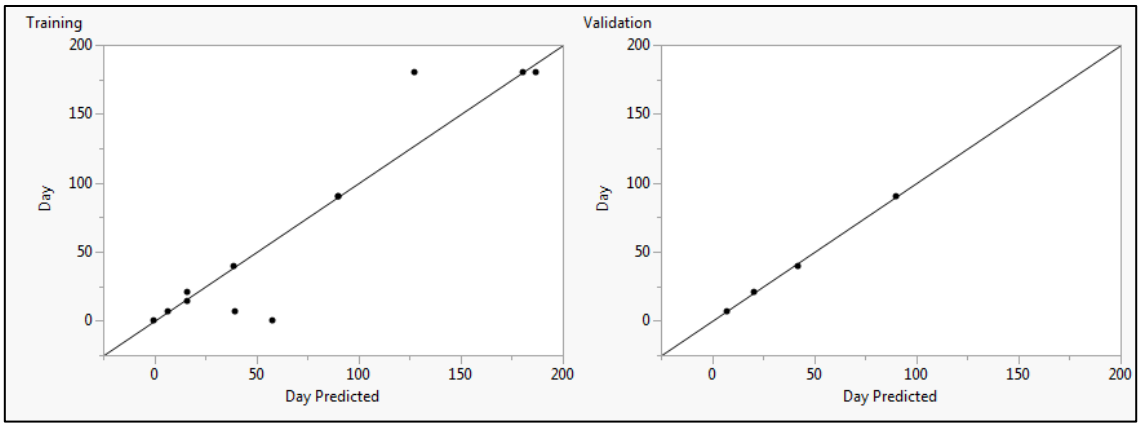

Figure 3.51. Actual by predicted plot of Day in cross validation model (Soil lateral of Control carrion in summer 2014 at Snook, Texas).

Table 3.60. Measures of training and validation models (Soil lateral of Control carrion in summer 2014 at Snook, Texas).

Training		Validation	
RSquare	0.89	RSquare	0.99
RMSE	20.68	RMSE	0.88
Mean Abs Dev	9.88	Mean Abs Dev	0.51
-LogLikelihood	75.62	-LogLikelihood	5.20
SSE	7275.68	SSE	3.15
Sum Freq	17	Sum Freq	4

Post-7 (Soil lateral)

The model was significantly different (p = 0.0013), with high strength of relationship (RSquare = 0.83). Table 3.61 showed significant predictors for soil lateral of Post-7 carrion. A prediction expression is also provided. Figure 3.52 presents the actual by predicted plot of Day.

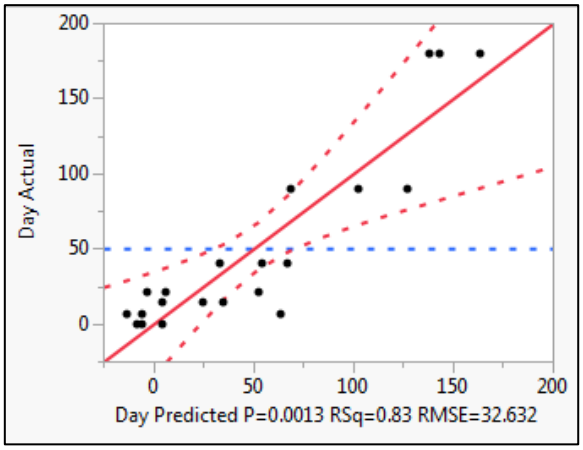

Figure 3.52. Actual by predicted plot of Day in multiple regression model (Soil lateral of Post-7 carrion in summer 2014 at Snook, Texas).

Table 3.61. Parameter estimates in regression model for soil lateral (Post-7) in summer 2014 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	-925.32	190.91	-4.85	0.0004*
pH	104.39	23.39	4.46	0.0008*
Conductivity	-0.17	0.21	-0.78	0.4478
NO_3-N	0.78	0.94	0.83	0.4241
NH_4-N	0.28	0.84	0.33	0.7475
PO_4-P	2.77	0.91	3.02	0.0107*
NPOC	0.11	0.18	0.65	0.5301
TN	-0.79	0.94	-0.84	0.4158
Moisture	8.30	2.73	3.03	0.0104*

Prediction expression: PMI (D) = -925.32 + 104.39*pH – 0.07*Conductivity + 0.78*NO_3-N – 0.28*NH_4-N + 2.77*PO_4-P + 0.11*NPOC – 0.79*TN + 8.30*Moisture

Cross validation test

Validation test showed a low RSquare (0.31) and high RMSE (55.56) (Table 3.62), indicating that this model did not predict well for day of decomposition (Figure 3.53).

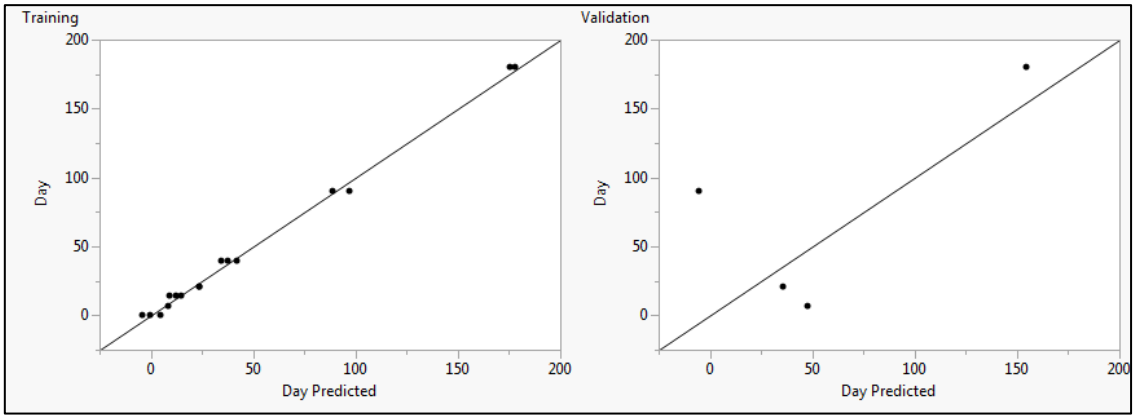

Figure 3.53. Actual by predicted plot of Day in cross validation model (Soil lateral of Post-7 carrion in summer 2014 at Snook, Texas).

Table 3.62. Measures of training and validation models (Soil lateral of Post-7 carrion in summer 2014 at Snook, Texas).

Training		Validation	
RSquare	0.99	RSquare	0.31
RMSE	3.60	RMSE	55.56
Mean Abs Dev	3.07	Mean Abs Dev	47.60
-LogLikelihood	43.21	-LogLikelihood	27.18
SSE	207.91	SSE	15436.41
Sum Freq	16	Sum Freq	5

Post-14 (Soil lateral)

The model was significantly different (p = 0.0389), with high strength of relationship (RSquare = 0.67). Table 3.63 showed significant predictors for soil lateral of Post-14 carrion. A prediction expression is also provided. Figure 3.54 presents the actual by predicted plot of Day.

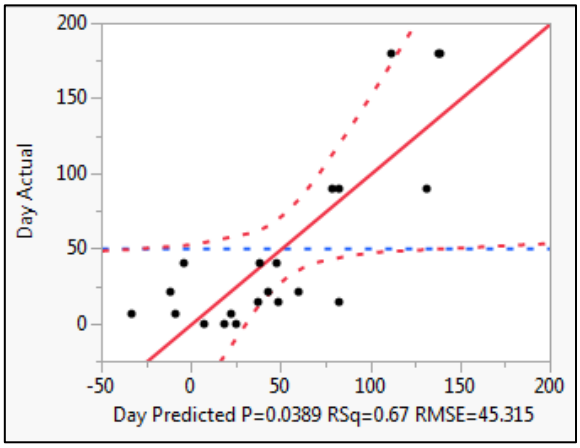

Figure 3.54. Actual by predicted plot of Day in multiple regression model (Soil lateral of Post-14 carrion in summer 2014 at Snook, Texas).

Table 3.63. Parameter estimates in regression model for soil lateral (Post-14) in summer 2014 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	-865.93	382.89	-2.26	0.0431*
pH	103.15	45.46	2.27	0.0425*
Conductivity	0.12	0.23	0.52	0.6154
NO_3-N	0.003	0.52	0.01	0.9943
NH_4-N	-0.19	0.43	-0.46	0.6555
PO_4-P	1.70	0.81	2.10	0.0574
NPOC	-0.06	0.19	-0.32	0.7570
TN	-0.007	0.56	-0.01	0.9894
Moisture	3.18	3.44	0.92	0.3737

Prediction expression: PMI (D) = -865.93 + 103.15*pH + 0.12*Conductivity + 0.003*NO_3-N – 0.19*NH_4-N + 1.70*PO_4-P - 0.06*NPOC – 0.007*TN + 3.18*Moisture

Cross validation test

Validation test showed a very high RSquare (0.99) and low RMSE (0.32) (Table 3.64), indicating that this model predicted well for day of decomposition (Figure 3.55).

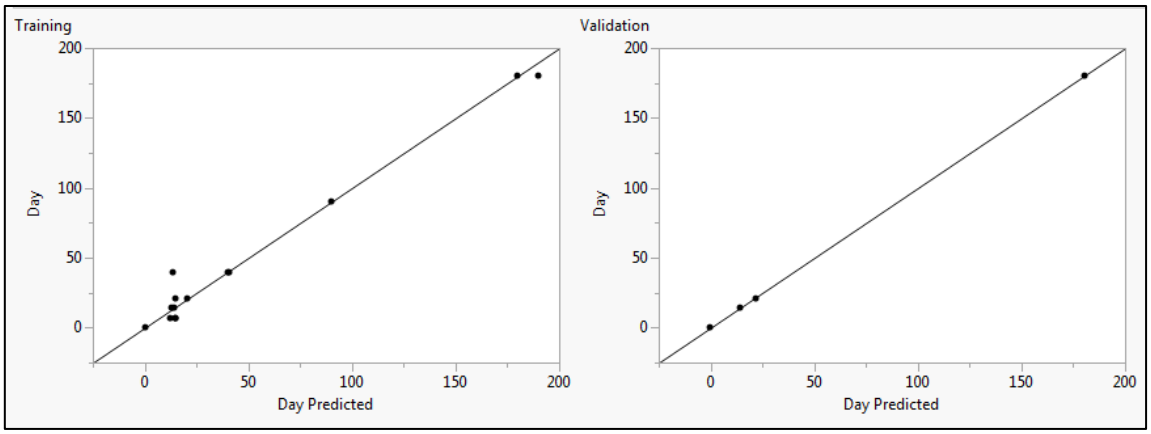

Figure 3.55. Actual by predicted plot of Day in cross validation model (Soil lateral of Post-14 carrion in summer 2014 at Snook, Texas).

Table 3.64. Measures of training and validation models (Soil lateral of Post-14 carrion in summer 2014 at Snook, Texas).

Training		Validation	
RSquare	0.98	RSquare	0.99
RMSE	7.57	RMSE	0.32
Mean Abs Dev	3.90	Mean Abs Dev	0.27
-LogLikelihood	58.55	-LogLikelihood	1.14
SSE	976.59	SSE	0.41
Sum Freq	17	Sum Freq	4

Soil 5 meter (Control)

The model was significantly different (p = 0.0023), with high strength of relationship (RSquare = 0.81). Table 3.65 showed significant predictors for soil 5 meter of Control carrion. A prediction expression is also provided. Figure 3.56 presents the actual by predicted plot of Day.

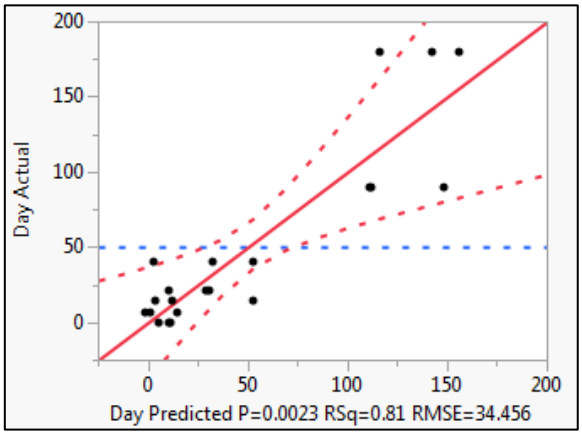

Figure 3.56. Actual by predicted plot of Day in multiple regression model (Soil 5 meter of Control carrion in summer 2014 at Snook, Texas).

Table 3.65. Parameter estimates in regression model for soil 5 meter (Control) in summer 2014 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	-572.44	187.37	-3.05	0.0100*
pH	69.67	25.16	2.77	0.0170*
Conductivity	0.49	0.36	1.38	0.1927
NO_3-N	-11.34	3.57	-3.17	0.0080*
NH_4-N	-13.43	4.90	-2.74	0.0180*
PO_4-P	0.06	1.98	0.03	0.9752
NPOC	-1.35	0.52	-2.55	0.0253*
TN	11.88	3.83	3.10	0.0092*
Moisture	6.53	3.01	2.17	0.0508

Prediction expression: PMI (D) = -572.44 + 69.67*pH + 0.49*Conductivity – 11.34*NO_3-N – 13.43*NH_4-N + 0.06*PO_4-P - 1.35*NPOC + 11.88*TN + 6.53*Moisture

Cross validation test

Validation test showed a very low RSquare (0.19) and high RMSE (74.01) (Table 3.66), indicating that this model did not predict well for day of decomposition (Figure 3.57).

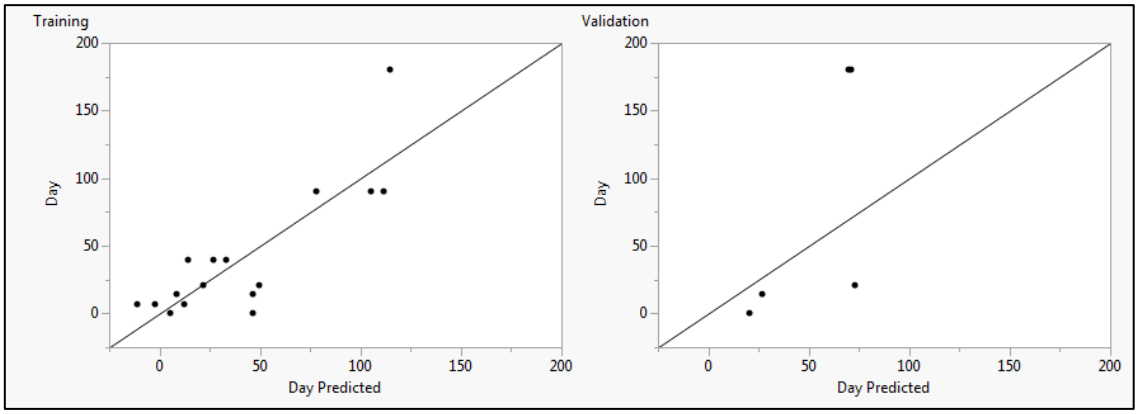

Figure 3.57. Actual by predicted plot of Day in cross validation model (Soil 5 meter of Control carrion in summer 2014 at Snook, Texas).

Table 3.66. Measures of training and validation models (Soil 5 meter of Control carrion in summer 2014 at Snook, Texas).

Training		Validation	
RSquare	0.70	RSquare	0.19
RMSE	25.59	RMSE	74.01
Mean Abs Dev	19.46	Mean Abs Dev	60.95
-LogLikelihood	74.58	-LogLikelihood	28.61
SSE	10484.37	SSE	27390.35
Sum Freq	16	Sum Freq	5

Post-7 (Soil 5 meter)

The model was significantly different (p = 0.0126), with high strength of relationship (RSquare = 0.74). Table 3.67 showed significant predictors for soil 5 meter of Post-7 carrion. A prediction expression is also provided. Figure 3.58 presents the actual by predicted plot of Day.

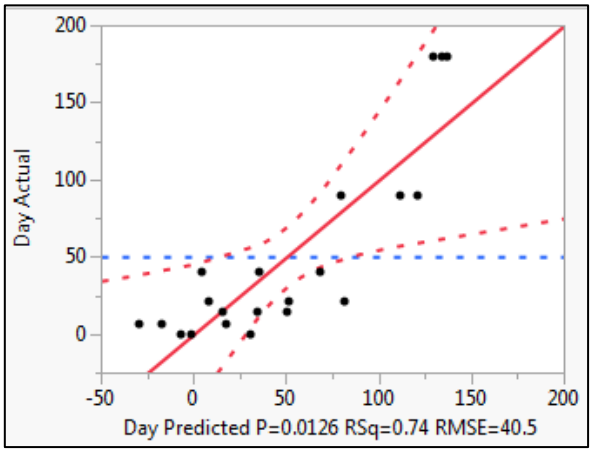

Figure 3.58. Actual by predicted plot of Day in multiple regression model (Soil 5 meter of Post-7 carrion in summer 2014 at Snook, Texas).

Table 3.67. Parameter estimates in regression model for soil 5 meter (Post-7) in summer 2014 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	-536.69	299.83	-1.79	0.0987
pH	65.59	38.25	1.71	0.1122
Conductivity	0.39	0.63	0.61	0.5504
NO_3-N	-2.36	4.53	-0.52	0.6111
NH_4-N	-2.10	4.29	-0.49	0.6331
PO_4-P	2.20	3.42	0.64	0.5314
NPOC	-0.43	1.07	-0.40	0.6937
TN	0.73	4.30	0.17	0.8667
Moisture	5.17	2.78	1.86	0.0878

Prediction expression: PMI (D) = -536.69 + 65.59*pH + 0.39*Conductivity – 2.36*NO_3-N – 2.10*NH_4-N + 2.20*PO_4-P - 0.43*NPOC + 0.73*TN + 5.17*Moisture

Cross validation test

Validation test showed a very high RSquare (0.96) and low RMSE (6.66) (Table 3.68), indicating that this model predicted well for day of decomposition (Figure 3.59).

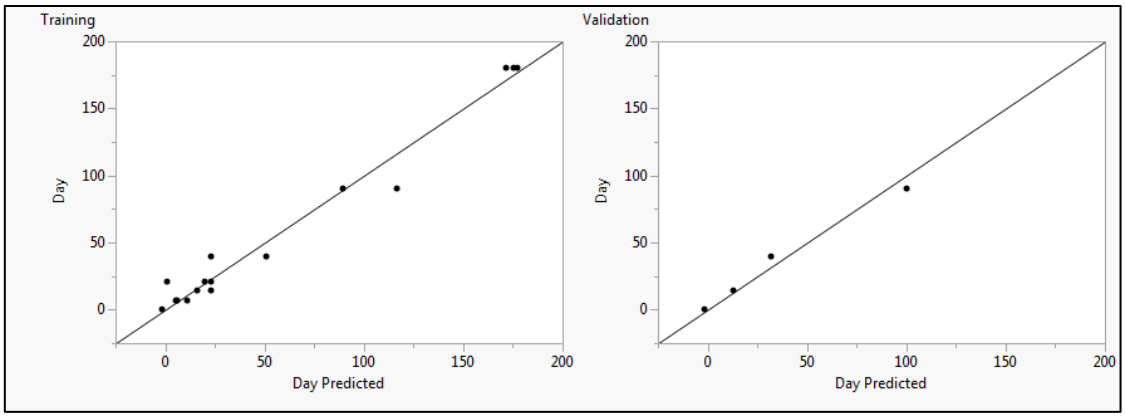

Figure 3.59. Actual by predicted plot of Day in cross validation model (Soil 5 meter of Post-7 carrion in summer 2014 at Snook, Texas).

Table 3.68. Measures of training and validation models (Soil 5 meter of Post-7 carrion in summer 2014 at Snook, Texas).

Training		Validation	
RSquare	0.97	RSquare	0.96
RMSE	10.16	RMSE	6.66
Mean Abs Dev	6.84	Mean Abs Dev	5.30
-LogLikelihood	63.54	-LogLikelihood	13.26
SSE	1757.43	SSE	177.54
Sum Freq	17	Sum Freq	4

Post-14 (Soil 5 meter)

The model was significantly different (p = 0.013), with high strength of relationship (RSquare = 0.74). Table 3.69 showed significant predictors for soil 5 meter of Post-14 carrion. A prediction expression is also provided. Figure 3.60 presents the actual by predicted plot of Day.

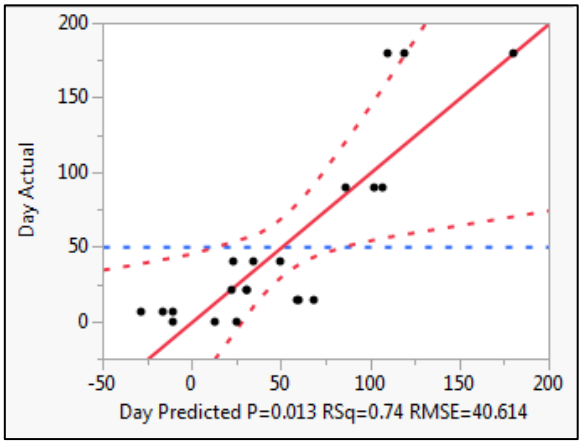

Figure 3.60. Actual by predicted plot of Day in multiple regression model (Soil 5 meter of Post-14 carrion in summer 2014 at Snook, Texas).

Table 3.69. Parameter estimates in regression model for soil 5 meter (Post-14) in summer 2014 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	-418.82	374.71	-1.12	0.2856
pH	50.83	45.48	1.12	0.2856
Conductivity	-0.25	0.36	-0.70	0.4966
NO_3-N	1.95	3.88	0.50	0.6247
NH_4-N	1.35	5.01	0.27	0.7911
PO_4-P	2.88	2.28	1.26	0.2300
NPOC	-0.18	0.66	-0.28	0.7847
TN	-1.31	3.52	-0.37	0.7162
Moisture	4.75	2.07	2.29	0.0406*

Prediction expression: PMI (D) = -418.82 + 50.83*pH - 0.25*Conductivity + 1.95*NO_3-N + 1.35*NH_4-N + 2.88*PO_4-P - 0.18*NPOC - 1.31*TN + 4.75*Moisture

Cross validation test

Validation test showed a very high RSquare (0.75) and low RMSE (17.01) (Table 3.70), indicating that this model predicted well for day of decomposition (Figure 3.61).

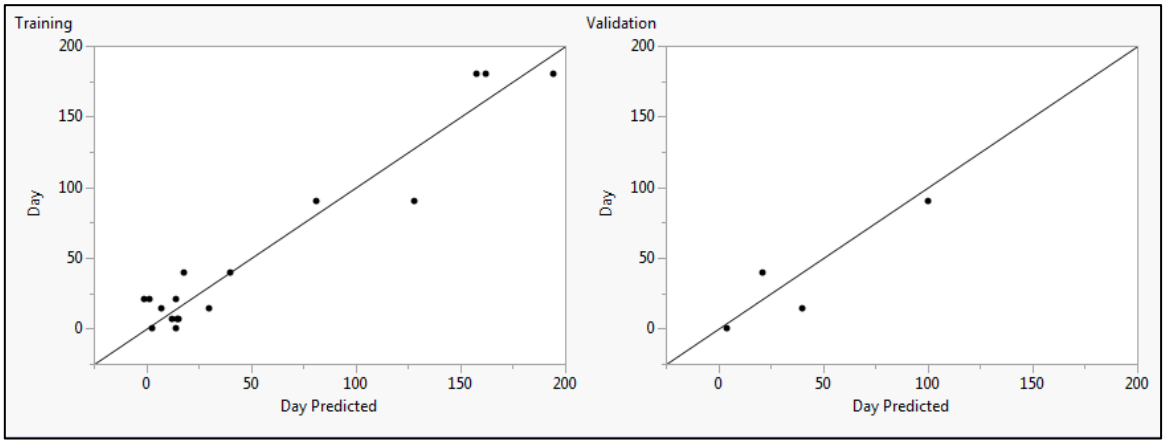

Figure 3.61. Actual by predicted plot of Day in cross validation model (Soil 5 meter of Post-14 carrion in summer 2014 at Snook, Texas).

Table 3.70. Measures of training and validation models (Soil 5 meter of Post-14 carrion in summer 2014 at Snook, Texas).

Training		Validation	
RSquare	0.93	RSquare	0.75
RMSE	16.50	RMSE	17.01
Mean Abs Dev	13.74	Mean Abs Dev	14.78
-LogLikelihood	71.78	-LogLikelihood	17.01
SSE	4630.68	SSE	1158.72
Sum Freq	17	Sum Freq	4

Upper slope

The model was significantly different ($p < 0.0001$), with high strength of relationship (RSquare = 0.70). Table 3.71 showed significant predictors for upper slope soil (as control soil). A prediction expression is also provided. Figure 3.62 presents the actual by predicted plot of Day.

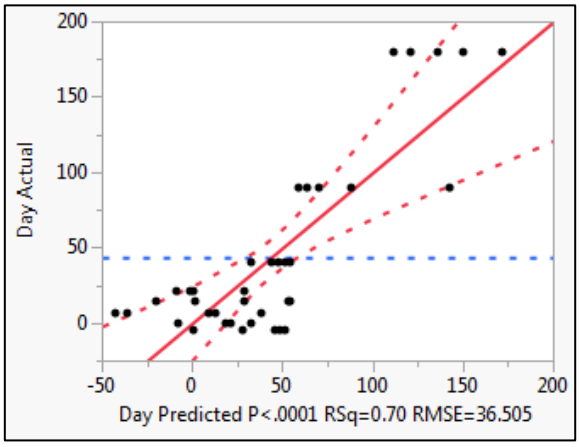

Figure 3.62. Actual by predicted plot of Day in multiple regression model (soil of upper slope in summer 2014 at Snook, Texas).

Table 3.71. Parameter estimates in regression model for soil of upper slope in summer 2014 at Snook, Texas (* denotes significant difference).

Terms	Estimate	Std Error	t Ratio	p value
Intercept	-234.66	179.07	-1.31	0.1997
pH	18.93	19.98	0.95	0.3507
Conductivity	-0.16	0.48	-0.34	0.7362
NO_3-N	-19.75	6.20	-3.18	0.0033*
NH_4-N	-17.23	25.19	-0.68	0.4989
PO_4-P	-9.07	12.27	-0.74	0.4655
NPOC	-0.01	0.47	-0.03	0.9739
TN	18.01	6.46	2.79	0.0089*
Moisture	8.44	1.84	4.58	<0.0001*

Prediction expression: PMI (D) = -418.82 + 50.83*pH - 0.25*Conductivity + 1.95*NO_3-N + 1.35*NH_4-N + 2.88*PO_4-P - 0.18*NPOC - 1.31*TN + 4.75*Moisture

Cross validation test

Validation test showed a very high RSquare (0.99) and low RMSE (5.27) (Table 3.72), indicating that this model predicted well for day of decomposition (Figure 3.63).

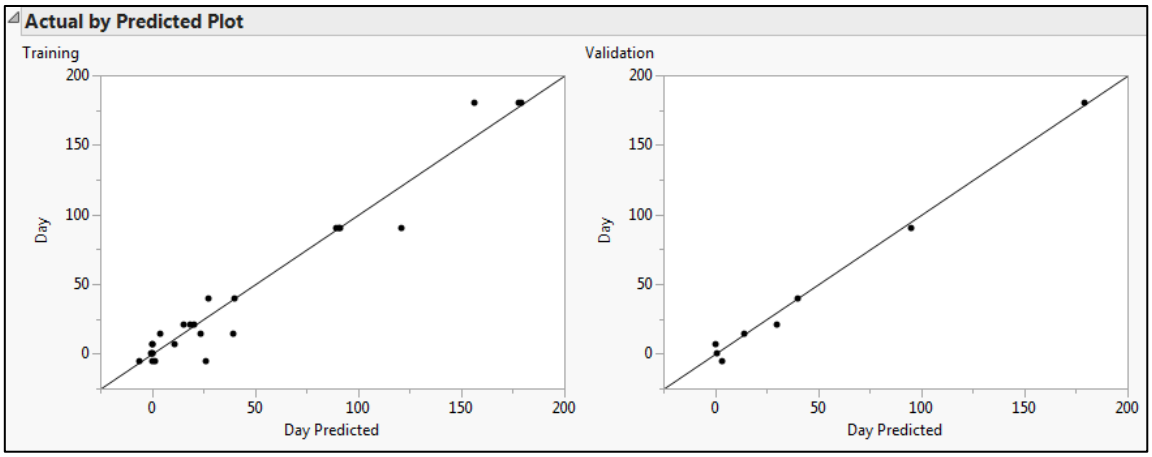

Figure 3.63. Actual by predicted plot of Day in cross validation model (Soil of upper slope in summer 2014 at Snook, Texas).

Table 3.72. Measures of training and validation models (Soil of upper slope in summer 2014 at Snook, Texas).

Training		Validation	
RSquare	0.93	RSquare	0.99
RMSE	14.90	RMSE	5.27
Mean Abs Dev	8.03	Mean Abs Dev	3.95
-LogLikelihood	131.86	-LogLikelihood	24.65
SSE	7109.46	SSE	222.47
Sum Freq	32	Sum Freq	8

Soil porosity

Soil porosity (%) was significantly different between years (p = 0.0042), where 2013 trial had a higher percentage of porosity (mean 66.62 ± 17.19%) compared to 2014 trial (55.23 ± 11.75%) (Figure 3.64).

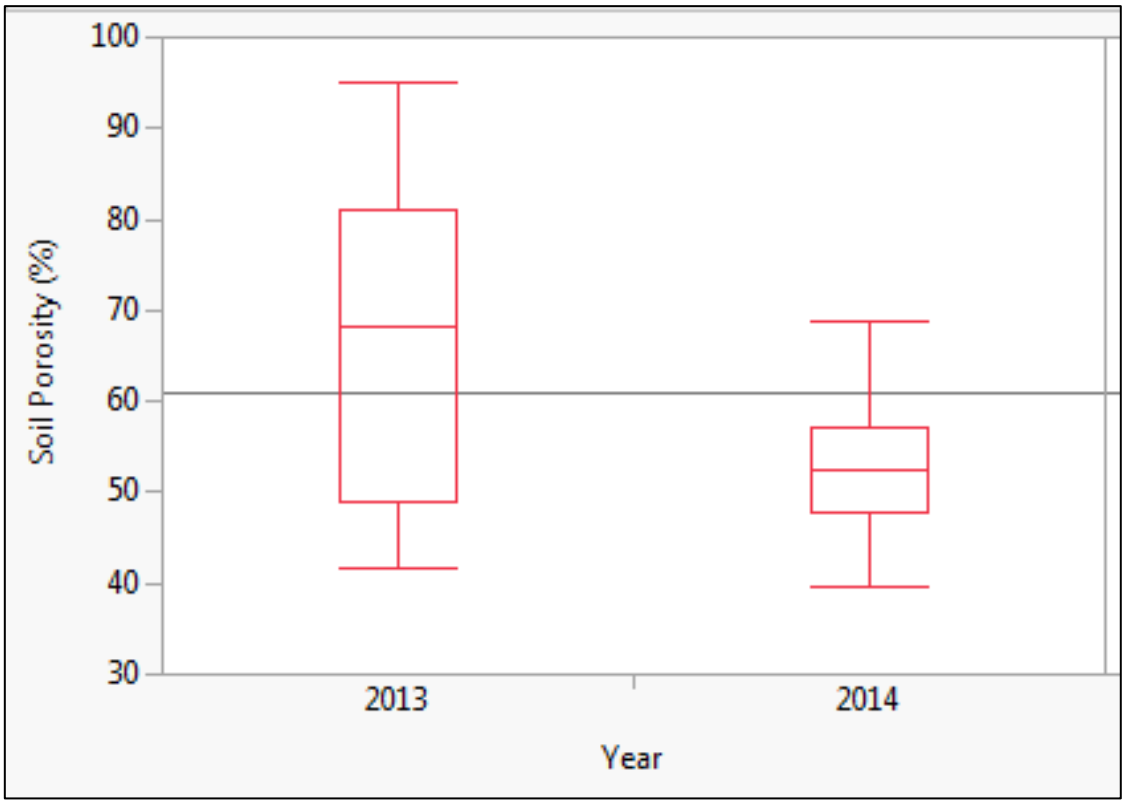

Figure 3.64. Box plots of soil porosity during 2013 and 2014 trials at the field site located at Snook, Texas.

Soil porosity in 2013

Soil porosity was tested for treatment and site effects. ANOVA showed that there was no significant difference in soil porosity between treatments (p = 0.2914) and no significant difference between soil regions (i.e., beneath, lateral, and 5 meter away) (p = 0.2599). Although Control and Upper slope had a higher porosity means compared to

treatment groups (i.e., Post-7 and Post-14) (Figure 3.65) while soil at upper slope and soil lateral had higher mean porosity compared to other regions (Figure 3.66). In other words, delayed of blow colonization on carcasses and soil regions around the pig carcasses did not affect the percentage of soil porosity (Table 3.73 and Table 3.74).

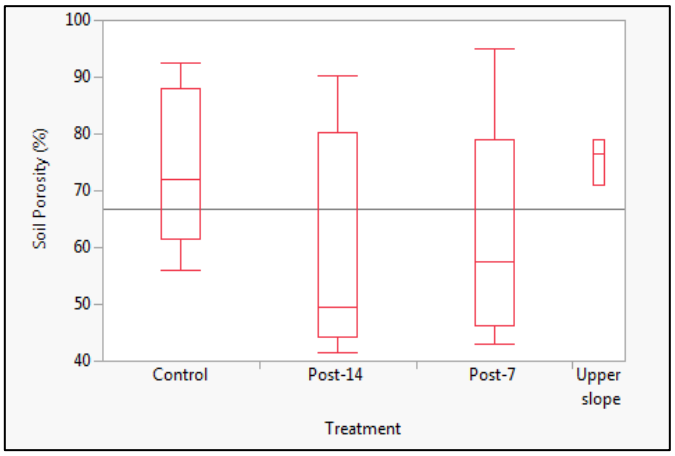

Figure 3.65. Box plots of soil porosity (%) across treatments in 2013 trial at Snook, Texas.

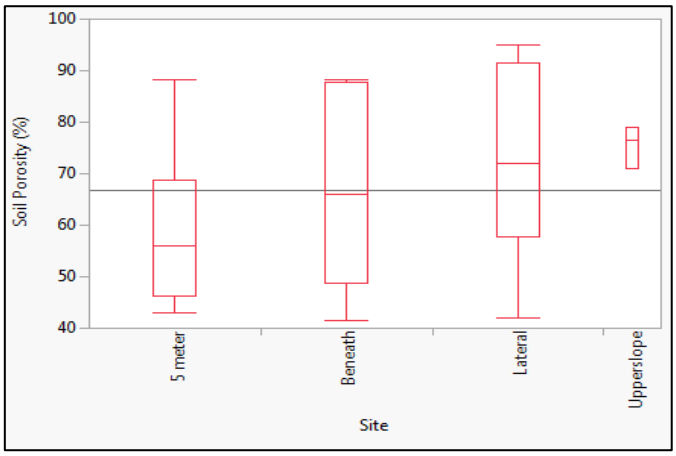

Figure 3.66. Box plots of soil porosity (%) across soil regions in 2013 trial at Snook, Texas.

Table 3.73. ANOVA of soil porosity (%) among treatments in 2013 trial at Snook, Texas.

Source	df	SS	MS	F ratio	P value
Treatment	3	1128.1805	376.060	1.3131	0.2914
Error	26	7446.1503	286.390		
C. Total	29	8574.3308			

Table 3.74. ANOVA of soil porosity (%) among soil regions in 2013 trial at Snook, Texas.

Source	df	SS	MS	F ratio	P value
Region	3	1205.8325	401.944	1.4183	0.2599
Error	26	7368.4983	283.404		
C. Total	29	8574.3308			

Soil porosity in 2014

Soil porosity was tested for treatment and site effects. ANOVA showed that there was no significant difference in soil porosity between treatments ($p = 0.9176$) and no significant difference between soil regions (i.e., beneath, lateral, and 5 meter away) ($p = 0.1647$) (Figure 3.67 and Figure 3.68, respectively). Although soil beneath demonstrated high mean of soil porosity compared to other regions. Again, delayed of blow colonization on carcasses and soil regions around the pig carcasses did not affect the percentage of soil porosity in 2014 (Table 3.75 and Table 3.76).

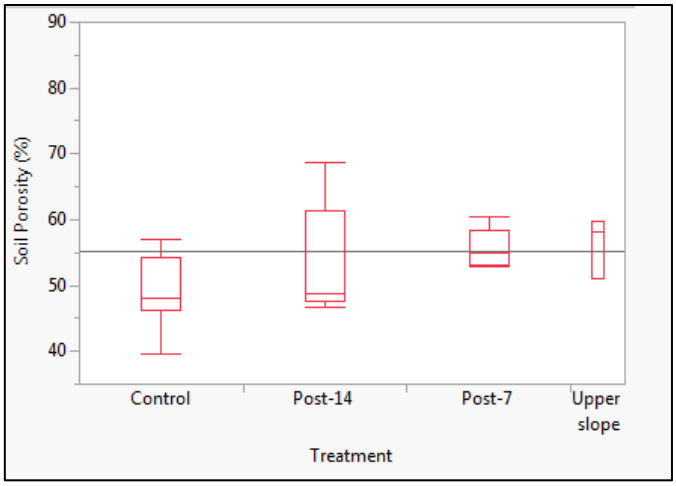

Figure 3.67. Box plots of soil porosity (%) across treatments in 2014 trial at Snook, Texas.

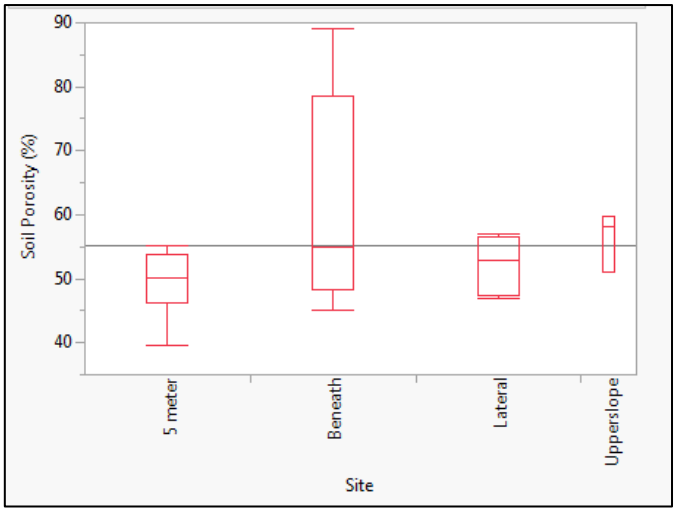

Figure 3.68. Box plots of soil porosity (%) across regions in 2014 trial at Snook, Texas.

Table 3.75. ANOVA of soil porosity (%) among treatments in 2014 trial at Snook, Texas.

Source	df	SS	MS	F ratio	P value
Treatment	3	75.7604	25.253	0.1671	0.9176
Error	26	3930.3551	151.168		
C. Total	29	4006.1155			

Table 3.76. ANOVA of soil porosity (%) among regions in 2014 trial at Snook, Texas.

Source	df	SS	MS	F ratio	P value
Region	3	701.5445	233.848	1.8399	0.1647
Error	26	3304.5709	127.099		
C. Total	29	4006.1155			

Pairwise correlation

Pearson's pairwise correlation between soil porosity in 2013 and 2014 trials demonstrated a very weak negative correlation (r^2 = -0.0080; p = 0.9665). Figure 3.69 presents correlation and regression model of soil porosity according to treatments between 2013 and 2014 trials.

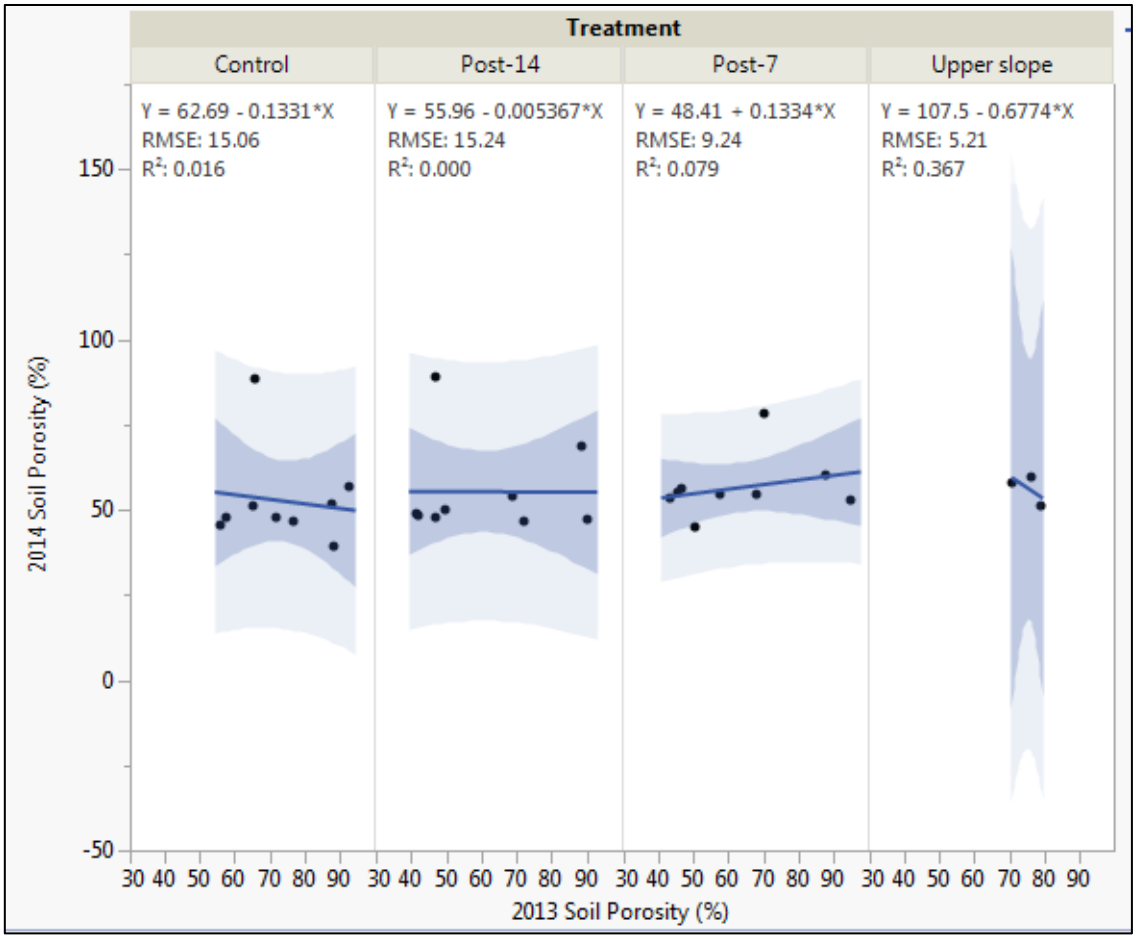

Figure 3.69. Pearson's pairwise correlation of soil porosity (%) according to treatments between 2013 and 2014 trials at the field site located at Snook, Texas.

Comparison between years

Comparison of important statistical results over the two trials was presented at Table 3.77. Note that temperature, ADH, overall of soil chemistry profiles, and soil porosity were significant difference between trials. Most importantly, treatment (i.e., delay of blow colonization on carrion) did significantly impact soil chemistry in 2013 and 2014 trials, although pH was marginally significant difference in 2013. Furthermore, treatments significantly affect the soil chemistry profiles in day of decomposition as well as soil region. The means and standard deviations of soil nutrients for each trial were provided in the Appendix F.

Table 3.77. Comparison of important statistical results in soil chemistry profiles between 2013 and 2014 trials at Snook, Texas.

Factor	2013	2014
Ambient temperature (°C)	Significant difference between years	
Precipitation (mm)	No significant difference between years	
ADH	Significant difference between years	
Overall soil chemistry profiles	Significant difference between years	
Factors that were significant within year	Day	Day
	Treatment	Treatment
	Region	Region
	Day x Treatment	Day x Treatment
	Day x Region	Day x Region
	Treatment x Region	Treatment x Region
	Day x Treatment x Region	Day x Treatment x Region
pH	Day	Day
	Treatment•	Treatment
	Region	Region
Conductivity	Day	Day
	Region	Treatment
		Region
Nitrate-N	Day	Day
	Region	Treatment
		Region
Ammonium-N	Day	Day
	Region	Treatment
		Region
Orthophosphate-P	Day	Day
	Region	Treatment
		Region

Table 3.77 (Continued).

Factor	2013	2014
NPOC	NA	Day Treatment Region
TN	NA	Day Treatment Region
Soil moisture	Day Region	Day Treatment Region
Significant correlation between parameters	NO_3-N x pH (r^2= -0.4273) NO_3-N x Conductivity (r^2= 0.2313) NH_4-N x Conductivity (r^2= 0.4654) PO_4-P x Conductivity (r^2= 0.8298) PO_4-P x NH_4-N (r^2= 0.4163) Soil moisture x Conductivity (r^2= 0.4629) Soil moisture x NH_4-N (r^2= 0.2405) Soil moisture x PO_4-P (r^2= 0.4986)	NO_3-N x Conductivity (r^2= 0.3465) NH_4-N x pH (r^2= 0.2479) NH_4-N x Conductivity (r^2= 0.8690) PO_4-P x pH (r^2= 0.2223) PO_4-P x Conductivity (r^2= 0.6784) PO_4-P x NO_3-N (r^2= 0.2318) PO_4-P x NH_4-N (r^2= 0.7134) NPOC x Conductivity (r^2= 0.8458) NPOC x NH_4-N (r^2= 0.8253) NPOC x PO_4-P (r^2= 0.4625)

Table 3.77 (Continued).

Factor	2013	2014
		TN x pH (r^2= 0.1629)
		TN x Conductivity (r^2= 0.9735)
		TN x NO_3-N (r^2= 0.2597)
		TN x NH_4-N (r^2= 0.9029)
		TN x PO_4-P (r^2= 0.6586)
		TN x NPOC (r^2= 0.9091)
		Soil moisture x pH (r^2= 0.1440)
		Soil moisture x Conductivity (r^2= 0.2344)
		Soil moisture x NO_3-N (r^2= 0.1650)
		Soil moisture x NH_4-N (r^2= 0.2944)
		Soil moisture x PO_4-P (r^2= 0.2719)
		Soil moisture x NPOC (r^2= 0.1945)
		Soil moisture x TN (r^2= 0.2531)
Soil porosity		
Year	Significant difference between years	
Soil porosity		
Treatment	No significant difference	No significant difference
Region	No significant difference	No significant difference

• Marginal significant difference; NA= not available.

DISCUSSION

This study represents the first experimental study on soil chemistry changes associated with delayed insect colonization of carrion up to 14 days. Soil chemistry profiles were affected significantly by treatments, days of decomposition, and soil regions (see Table 3.77). Soil properties and nutrients such as conductivity, ammonium-N, orthophosphate-P, soil moisture, non-purgeable organic carbon and total nitrogen were associated with higher concentration in carrion with delayed blow fly colonization. In addition, soil regions demonstrated significant differences among different sites where soil beneath the carrion represents the highest nutrient concentrations, followed by soil lateral, and soil 5 meter. Transport of nutrients from beneath the carrion to the lateral extension has been observed in nutrients such as conductivity, NPOC and TN. Similarly, soil nutrient was significantly different along the decomposition stages and days, as reported by many previous studies on grave soil (Benninger et al. 2008; Pringle et al. 2010; Stokes et al. 2013; Macdonald et al. 2014).

The soil chemistry profiles were significantly difference between trials (see Figure 3.9). This observation could be due to the significant difference in ambient temperatures and ADH during the study period (Day 0 - 40) between the two years. Although the amount of precipitation was not significantly different, but more rain had been received in 2014 trial (+ 132.33 mm) compared to 2013 trial. Many researches have been published regarding the relationship of temperature and soil chemistry profiles, especially dealing with climate change topics (Davidson & Janssens, 2006; Emmett et al. 2004; Schmidt et al. 1999). A study found that a warmer climate will increase the average soil temperature by 0.9 - 1.5°C at 10 cm depth (Mellander et al. 2007). Soil temperature enhancement by approximately 2°C in general increased, or tended to increase net N and P mineralization (Schmidt et al. 1999). Emmett et al. (2004) concluded that soil process such as respiration and net nitrogen mineralization were affected by changes in rainfall pattern and temperature. Furthermore, the effect of changing temperature and water content will affect C content in soil as well as soil enzyme activities such as soil protease (Sardans et al. 2008). In addition to that, soil

organisms will be affected by changing temperatures which indirectly change the soil nutrients. The soil nematode community was strongly affected by increased soil temperature and the increasing nematode density had an important impact on soil microbial biomass and turnover rates (Ruess et al. 1999). Also, elevated soil temperature will lead to increased grazing on microorganisms, contributing to net N and P mineralization rates and plant nutrient availability (Ruess et al. 1999).

Soil beneath the carrion was the most suitable soil region to represent CDI, although lateral movement of soil nutrients to the adjacent soil (i.e., soil lateral and even soil 5 meter) has been observed in this study (e.g., see TN concentrations in Figure 3.42). Note that the soil samples collected in this study were approximately 10 cm from the soil surface. Hence, it is not evaluated in this study on how deep that the soil nutrients can move downwards into the soil. This remains a research question worth to look into in the future. The soil beneath the carrion has been examined in many previous studies. Parmenter & MacMahon (2009) collected cub-carcass soil and measured the associated nutrients and found soil N, P, and Na increased during carrion decomposition. Moreover, Benninger et al. (2008) investigated the dynamics of C, N, and P compounds in soil beneath pig (*S. scrofa*) cadavers and found significant increases in soil pH, TN, and P concentrations. The lateral extent of a CDI during advance decay stage of decomposition has been reviewed by Carter et al. (2006). In general, the degree of lateral extent of a CDI is depend of the maggot mass migration and soil texture while vertical extent of a CDI depends on cadaver size and types of soil (Carter et al. 2006). For instance, Coe (1978) observed that CDI in sandy loam soil associated with elephant (*Loxodonta africana* Blumenbach) (~1620 kg) decomposition extending to 40 cm below the cadaver, 35 cm at 1 m from the cadaver, and 8 cm at 2 m from the carcass. Conversely, the CDI associated with the decomposition of a 633 kg elephant carcass on quartz gravel extended to 1.5 m below the soil surface (Coe, 1978). In comparison with a 620 g guinea pig (*Cavia porcellus* L.), CDI was extended to 14 cm below the cadaver in sandy soil (Bornemissza, 1957). Aitkenhead-Peterson et al. (2012) examined the lateral extent of decomposition products to a depth of 7 cm soils beneath two human cadavers.

The spatial extent for DOC and DON for both bodies (one was protected from scavengers and one was exposed to scavengers activities) was large but similar suggesting some movement off site for both compounds. The results further showed that pH was lower and electrical conductivity was higher in the soil under both decomposing cadavers relative to control soils (Aitkenhead-Peterson et al. 2012). These findings were in agreement with the present study where there was lateral movement of soil nutrients to the adjacent soil (which was approximately 30 cm from soil beneath the carrion) such as conductivity, NPOC and TN.

In the present study, pH of the soil beneath the carcasses decreased temporally (from Day 0 to Day 21) regardless of treatments in 2013 trial (see Figure 3.11). Although in 2014, pH increased from Day 0 to Day 14, and then decreased from Day 21 to Day 40, and increased again from Day 90 to Day 180. The stochastic events with pH in the 2014 trial could be due to various factors such as changes in temperatures and soil moistures (due to precipitation) which in turn influence soil respiration (CO_2 efflux) and perhaps contributed to the changes in soil pH (Reth et al. 2005). Towne (2000) recorded mean pH was significantly lower in the center of carcass site than in the surrounding soil and found that there was no significant change in soil pH at all distance intervals over time. In another decomposition study, Stokes et al. (2013) found pH increased rapidly before decreasing to initial pH levels and eventually below basal pH. Aitkenhead-Peterson et al. (2012) found that pH was significantly lower in the CDI associated with human cadavers compared to the control soil. In fact, pH has been shown to increase and decrease in soil below human and other mammal remains. Vass et al. (1992) reported an increase in pH under human remains within a few ADD, peaking at 750 ADD, and then declining to control (~3750 ADD) and even below basal soil value up to 4500 ADD. Fiedler et al. (2004) found lower pH in graves that were approximately 27 years old relative to the control soils. Benninger et al. (2008) found significant fluctuations in gravesoil pH throughout the trial. A significant increase in gravesoil pH was observed on Days 14 and 23, which was followed by a decrease to a level that was significantly lower than control soil pH on Day 30, and then it increased significantly again on Day 43 and

another decreased on Day 72 and 100. Wilson et al. (2007) reported increased pH from 4.6 to 7.2 of buried pigs up to 378 days. A study was conducted to understand long term effects of continuous human decomposition on the soil environment at the University of Tennessee Anthropology Research Facility (ARF). Analyses revealed increased pH readings, presumably resulting from ammonification of the soil, were observed in areas of high decomposition (Damann et al. 2012). Also, Pringle et al. (2010) observed so much variability in pH among decomposition islands under pigs and concluded that they were not confident in using it as a tool for forensic investigations.

Gravesoil has been shown to be able to detect electrically by resistivity surveys in criminal investigations (Cheetham, 2005), graveyards (Matias et al. 2004) and controlled experiments (Pringle et al. 2008). An elevated conductivity level relative to control has been successfully detected at the target site (Jervis et al. 2009). Increased conductivity at a murdered victim deposition sites have also been reported (Harrison & Donnelly, 2009). In the present study, conductivity was increased over time regardless of treatments, with the peak on Day 21, and then returned to basal level on Day 180 of carrion decomposition (see Figure 3.12 and 3.37). The interesting observation was that the treatment groups had higher conductivity compared with the control pigs. In other words, the effect of delayed blow fly colonization on carrion had impacted the dynamics of soil conductivity significantly. The longer period of the delay blow fly colonization was, the higher the soil conductivity beneath the carrion, as well as soil lateral and even soil 5 meter away from carrion. This observation indicates substantially that there was lateral extension of decomposition fluid containing ionic compounds to the soil 5 meter away from carrion. Similar observation was noted in Pringle et al. (2010) where the authors found a temporal rapid increase of the conductivity of a buried pig after one-year post-burial, and slowly increased until two years. Aitkenhead-Peterson et al. (2012) mapped the CDIs of two human cadavers and found that the conductivity was significantly higher than the control soils, but there was no significantly different between the two cadavers, although scavenger-access cadaver had a larger spread of mapped conductivity. Macdonald et al. (2014) used kangaroo (*M. giganteus*) carcass to

examine soil nutrient changes and found that the electrical conductivity was significantly higher than the control soil by week 12 and week 24 after death.

In 2013 trial, nitrate-N peaked on Day 90 regardless of treatments, however, the lowest concentration of nitrate-N was observed on Post-14 (330.9 ± 133.8 μg/kg) compared to Post-7 and Control pigs (751.7 ± 494.1 μg/kg and 711.9 ± 121.6 μg/kg, respectively), although statistically all these groups had no significant difference (see Figure 3.13). In 2014 trial, the peak of nitrate-N (425.91 ± 163.08 μg/kg) was observed on Day 40 on Control pigs (which were 50 days earlier than 2013 trial). This condition could be due to differences in abiotic and biotic factors between trials as discussed earlier. However, similar observation was noted in the concentration of nitrate-N between treatment groups on Day 90 where soils beneath the Post-7 pigs had higher concentration of nitrate-N than Post-14 pigs (688.07 ± 315.38 μg/kg and 408.10 ± 166.21 μg/kg, respectively) (see Figure 3.38). Again, this observation demonstrated that delay of blow fly colonization did change the concentration of soil nitrate-N, that the concentration of nitrate-N was inversely proportionate with the period of delay blow fly colonization, although no statistical difference was found between these two treatments on Day 90. Carcass decomposition did increase the concentration of nitrate as demonstrated by many studies. Aitkenhead-Peterson et al. (2012) found significant higher DON and nitrate-N concentration of the mapped human cadavers CDIs than the upslope control soils. Similarly, Macdonald et al. (2014) also found significant difference in DON between carcass-treated soils and control soils on both weeks 12 and 24, reflecting the decrease of C:N ratio of the treated soil. The introduction of mammalian skeletal muscle tissues to soil causes an initial lag phase in nitrification, before rapid nitrification begins on days 8 (for human tissue) and days 12 (for pork, beef and lamb tissues) (Stokes et al. 2013). The rate of nitrification process could be affected by soil type. Nitrification appeared to start almost immediately in the sandy clay loam, whereas a lag in the loamy sand soil was observed prior to a rapid increase in nitrate concentration (Stokes et al. 2009). As the soil type in the present study was clay soil, it was expected that there was a lag phase in nitrification process. Nitrate is produced by

the microbial oxidation of ammonium by a specialized microbe group called chemoautotrophic archaebacteria, and is part of the normal soil nitrogen cycle (Stevenson & Cole, 1999). Hopkins et al. (2000) reported no nitrification for a pig carcass burial in heavy clay soil while Melis et al. (2007) observed rapid nitrification in temperate forest soils below a decomposing bison (*Bison bonasus* L.) carcass in Poland. Other than soil types, nitrification could be adversely impacted by several factors such as anaerobic conditions, low population of the nitrifying community in soil system, soil pH (Kyveryga et al. 2004; Nugroho et al. 2007) and high concentration of ammonia which inhibit the nitrite conversion step (Balmelle et al. 1992). In terms of soil nutrient correlation, a significant negative correlation was found between nitrate and pH in this study. This result was in lined with Meyer et al. (2013) who also found negative correlation between these two parameters.

In 2013 trial, ammonium-N at soil beneath the control pigs peaked on Day 21 (47541.78 ± 6182.33 μg/kg). However, both treatments pigs showed peaks on Day 14 (seven days earlier than Control), with Post-14 being the highest (103409.80 ± 21521.02 μg/kg) followed by Post-7 (2831.04 ± 1430.05 μg/kg). Both treatments remained higher concentration of ammonium-N than Control carcasses even on Day 90. The concentration of ammonium-N of all groups then decreased and returned to basal level on Day 180 (Figure 3.14). Similarly, in 2014 trial, Control pigs achieved peak on Day 21 but the treatment groups achieved the peak one week earlier with the highest concentration being Post-14 and then Post-7. Resilience occurred on Day 180 where all three groups achieved similar basal level without significant difference in concentration (see Figure 3.39). The impact on ammonium-N by delayed blow fly colonization on carrion is evident. This observation again, confirmed that the concentration of ammonium-N is proportionate to the period of delayed blow fly access to swine carcasses. Aitkenhead-Peterson et al. (2012) found that human cadaver without scavenger access had significantly higher ammonium-N relative to control soils and cadaver with scavenger access. Stokes et al. (2013) observed that the interment of mammalian skeletal muscle tissues caused a rapid flush in ammonium-N concentration,

followed by a rapid decrease after peaks. Stokes et al. (2009) found that all soils that contained a cadaver showed a significant increase in the concentration of ammonium extracted from the soil. However, the concentration of ammonium declined rapidly over time in the sandy clay loam and loamy sand soils. At high pH, ammonium is released more readily as volatile gaseous compounds such as ammonia, which was a possible source of odor detected in the loamy sand soil (Stokes et al. 2009). Hopkins et al. (2000), Stokes et al. (2013) and Meyer et al. (2013) also found a significant positive relationship between ammonium-N concentrations with pH. However, Stokes et al. (2009) argued that as the initial pH of the soil system increased, the correlation decreased. Sandy clay loam showed a weaker correlation whereas loamy sand showed no relationship between pH and ammonium concentration. Thus, the correlation of pH and ammonium can only be applied to acidic soil and an alternative model is required for alkaline system. Likewise, in the present study, rapid declined after peak was also observed in 2013 trial where the ammonium-N of Post-14 group peaked from 103409.80 μg/kg on Day 14 and dropped to 4625.13 μg/kg on Day 21. Furthermore, significant positive correlation between ammonium and pH was also noted, although the correlation is weak (r^2 = 0.2479), perhaps it was due to initial soil pH in this study, which was more or less neutral (pH 7.42 ± 0.21). As the soil pH was alkaline in 2013 trial (pH 9.36 ± 0.41), there was no correlation observed between ammonium and pH, as suggested by Stokes et al. (2009). Nevertheless, in 2014 trial, ammonium-N is strongly correlated ($r^2 > 0.8$) with conductivity, TN and NPOC.

Total nitrogen is the sum of the organic and inorganic nitrogen (NO_3- and NH_4+) in soil (Stokes et al. 2009). It was measured only in 2014 trial (see Figure 3.42). Hence, comparison between trials was not possible. TN in the present study showed two peaks along the decomposition process, which was on Day 7 and Day 21, regardless of treatments, although Post-7 achieved the its highest peak on Day 14. However, Post-14 pigs significantly rendered the highest TN concentration during the peaks compared to Control. Resilience occurred on Day 90 where all three groups showed no significant difference among each other. Furthermore, TN concentrations in carrion soils (Control

and Post-7 pigs) were significantly higher than the control soils at upper slope. Lateral movement of TN to soil lateral and soil 5 meter has been observed on Day 7 and Day 14. The possible reason why TN had two peaks (Day 7 and Day 21) during the carrion decomposition was due to mineralization (process which microbes decompose organic N from carrion to ammonium, the rate of mineralization may vary with soil temperature, moisture and aeration, or due to delayed carrion decomposition) and nitrification processes, which is the process by which microorganisms convert ammonium to nitrate to obtain energy. Nitrate is the most plant available form of N, but it is highly susceptible to leaching losses. Stokes et al. (2009) findings are similar with the present study. Stokes et al. (2009) found that there was a significant increase in the TN content of gravesoils when compared to the control samples in the first 14 days of the decomposition trial, a second smaller peak was observed between days 21 and 42 of the decomposition period. The authors also observed that the TN level returned to basal level as decomposition period extended. Macdonald et al. (2014) studying kangaroo decomposition in Australia also found significant difference in TN on week 12 and week 24. Benninger et al. (2008) investigated the dynamics of TN in soil beneath pig carcasses in Canada and found significant difference in soil TN concentration. Based on their results, the authors proposed that a significant increase in the concentration of gravesoil nutrients represented a maximum PMI of 72 days based on TN concentration and the control soil. In contrast, Damann et al. (2012) found no significant difference of the mean percentage of TN content per gram of soil for all samples within the University of Tennessee Anthropology Facility, USA.

Orthophosphate-P increased over decomposition days, peaked on Day 40 (in 2013 for all treatments, and only Post-14 in 2014) and Day 14 (in 2013 trial, for Control and Post-7 only) and then decreased to basal level on Day 180 (except Post-7, which was still significantly different with soil beneath the Control pig, in other words, there was treatment effect in this case). However, if compared with upper slope soil and pre-treatment soil, orthophosphate-P concentration was still significant difference on Day 180. Note that delayed blow fly colonization on carrion, especially Post-14, had the

higher orthophosphate concentration compared to other groups from Day 14 to Day 90 in 2013 trial (Figure 3.15). However, in 2014 trial, Post-14 had higher orthophosphate concentration on Day 7 and Day 40 only (see Figure 3.40). Skeletal muscle tissue decomposition study by Stokes et al. (2013) found an increase in phosphate concentration. Aitkenhead-Peterson et al. (2012) reported that orthophosphate-P in the mapped CDIs for two human cadavers were significantly higher than orthophosphate-P in the control soils, and there was no significant difference in orthophosphate-P between the two CDI's. In the current study, lateral movement was noted on Day 7 where orthophosphate-P concentration was significantly different at soil lateral between Control pig and Post-14 pigs. This finding were in agreement with Aitkenhead-Peterson et al. (2012) where they found lateral spread of orthophosphate-P for both human subjects were large. Benninger et al. (2008) found a significant increase in soil-extractable phosphorous over the 100 day cycle and concluded that it did not return to basal levels during the experimental period. Similar in this study, the non-carrion soils which include the upper slope soil and pre-treatment soil showed a significant difference with the carrion soil (only at soil beneath), suggesting no resilience in this case. However, this only applied to soil beneath the carrion, while soil lateral and soil 5 meter showed resilience (no significant difference) between carrion soil and non-carrion soil. Therefore, location of soil is vitally important when studying deposition and movement of soil nutrients associated with ephemeral resources or during the sampling process for forensic application as this could be a factor contributing to error or misinterpretations. It is noteworthy to mention that carrion (with or without delayed of blow fly colonization) gave rise to 110-154 folds (in 2013 trial) and 28-46 folds (in 2014 trial) of orthophosphate-P concentration compared to the initial level of orthophosphate-P on Day 0. Macdonald et al. (2014) found that the addition of the carcass caused a significant and lasting 20-fold increase in plant available P relative to the control. Similar to Benninger et al. (2008), Stokes et al. (2009) also reported a remarkable large and significant P increase in soil extractable phosphorus over 100 day cycle and did not return to basal level. Towne (2000) detected a significant increase in P concentrations

beneath a decomposing ungulate (*B. bison*) carcass and confirmed that P concentrations remained significantly higher at the carcass sites when compared to the control sites, up to 3 years postmortem.

In the body, the P store is found in nucleic acid and coenzymes, sugar phosphates and phospholipids (Dent et al. 2004). Other than *in-vivo* resource, animals can acquire P through the predation and herbivory. Therefore, a decomposing carcass will release a large pulse of P into the surrounding soil. The residual P signatures in the current study maybe due to two factors as suggested by Stokes et al. (2009). Firstly, incomplete mineralization of cadaver-derived P from the organic to inorganic forms that contribute to the supply of the extractable fraction, and secondly, the incomplete uptake of the enhanced soil mineral P pool by plants and microorganisms.

Note that non-purgeable organic carbon (NPOC) was measured only in 2014 trial. NPOC is obtained when soil sample was sparged with a small amount of acid; the inorganic carbon (IC) in the sample is converted into CO_2. This CO_2 is then removed, and the total organic carbon (TOC) is obtained by measuring the total carbon (TC) in the treated sample. When the CO_2 from the IC is removed, purgeable organic carbon (POC) may also be lost. As such, the TOC obtained with this method is referred as NPOC (Florescu et al. 2013).

The results showed Control and Post-7 achieved highest concentration of NPOC on Day 7, while Post-14 exhibited two peaks during the decomposition process on Day 7 and Day 21, with day 21 being the highest peak (see Figure 3.41). This observation again confirmed that delayed blow fly colonization on carrion did change the NPOC dynamics significantly different than Control pig with immediate blow fly access. Another explanation to this phenomenon was due to the decomposer activities. The first peak on Day 7 was probably due to microbial respiration and decomposition on Post-14 carrion. The insect-exclusion cage was then removed on Day 14 which allowed blow fly colonization on pig carcasses. As such, on Day 21, a second peak of NPOC was observed as the direct result from maggot and microbial consumption on pig carrion (i.e., byproducts of metabolism and excretion, redistribution of decompositional products

by insect activities, etc.). As usual, Post-14 group had the highest NPOC concentration compared to Control and Post-7 from Day 7 until Day 90. Lateral movement of NPOC to soil lateral and soil 5 meter was obvious in Control pigs on Day 7, where there was significant difference in NPOC concentrations between Control pigs and treatment groups. This could be due to fly larvae migration on Control pigs on day 7 that help spreading the NPOC to other locations. For Post-7 and Post-14 group, NPOC was mainly deposited *in-situ* and the lateral spread was not significantly different between treatments. On Day 40, treatment effect was still able be detected where Control pigs and Post-14 pigs was significantly different. On Day 90, carrion soil and non-carrion control soils (upper slope soil and pre-treatment soil) was significantly different, suggesting no resilience between carrion and non-carrion soil can be seen at this day, but there was resilience between control pigs and treatment pigs (no significant difference in soil NPOC concentration between these groups). However, on Day 180, non-carrion soils and carrion soils all had achieved resilience (returned to basal level) without statistical difference with each other.

There are a wide variety of carbon forms present in soils including leaf and branch litter, as well as highly decomposed form such as humus (Schumacher, 2002). Previous reports have indicated an increase in total C values in gravesoils sampled beneath pig carcasses (Hopkins et al. 2000). It is assumed that most of the carbon released from the carcass was lost to the atmospheric environment as volatile gases (mainly CO_2). The pattern of CO_2 release in summer and autumn months has been directly attributed to the activity of fly larvae on a carcass (Putman 1978a; 1978b). Macdonald et al. (2014) found the carrion soil TOC increased and was significantly different at week 12 from the control soil, but not at the week 24, suggesting resilience of NPOC between carrion-treated soil and control soil. In contrast, some studies did not see significant difference in total carbon between carrion soils and control soils (Benninger et al. 2008; Damann et al. 2012). Aitkenhead-Peterson et al. (2012) measured dissolved organic carbon (DOC) found that the control soil had a significant lower value compared to CDIs. They also found lateral spread of mapped CDI for DOC

was quite extensive for both human cadavers. Similar to this study, lateral spread of NPOC was detected on Day 7 to the distant of 5 meter away from the carrion.

Soil moisture in 2013 trial showed significantly different between Control pigs and Post-14 pigs on Day 7, Day 14, and Day 21, indicating more watery content found in soil beneath of Post-14 pigs (see Figure 3.16), while in 2014, although Post-14 pigs had higher soil water content than the other groups throughout the decomposition process, but there was no significant difference among groups (see Figure 3.43). The reason that the Post-14 had higher water content was probably due to more purge fluid (e.g., blood) seeped into soil beneath during the blow fly exclusion period. Again, this may demonstrate that insect activities do play a role in regulating water cycles from carrion back into the soil ecosystem during carrion decomposition process. As for Control pigs (those with immediate insect access), blood and other decomposition fluid maybe taken up or ingested by insects and other arthropods, which contribute to the loss of water content beneath the Control carcasses. Another possible explanation is that the insect exclusion cages (in both Post-7 and Post-14 groups) may serve as another extra layer to prevent water loss from direct sunlight (evaporation). However, this hypothesis has little evidence as there was no significant difference found between ambient temperatures inside the exclusion cages (the treatment groups) and outside of exclusion cages (the Control pigs) ($df = 2$; $F = 0.0000$; $p = 1.0000$). In general, soil moisture content in 2014 trial was higher than 2013 trial. This was due to the differences in precipitation amount received in both years where 2014 received higher amount of rain than 2013 trial. Due to this reason, soil moisture can be affected by water content derived from carrion (e.g., saliva, mucus, blood, urine) or from the atmospheric (i.e., precipitation). Note that microbial and soil organism respiration also contribute to the soil moisture. Hence, the relationship between microbial metabolism function and soil moisture during carrion decomposition should be determined.

Damann et al. (2012) found higher moisture content within the Anthropology Facility used for study human decomposition, but this phenomenon could be explained by topography, shade, and land use, rather than just decomposition events (Damann et al.

2012). In contrast, Benninger et al. (2008) found no significant difference in soil moisture content between gravesoil and control soil. Wilson et al. (2007) found that soil moisture levels varied considerably with season, pit depth and location both within and between buried sites of pig carrion. Their results showed that the soils surrounding the pigs were in general slightly wetter than the controls. Furthermore, Wilson et al. (2007) also found that soil moisture was fluctuated during the summer months, responding to antecedent weather conditions and amount of precipitation. Also, they found rapid fluctuation in redox potential correspond with rainwater flushing through the site after periodic downpours.

CONCLUSIONS

The results generated from this study demonstrated that there was a significant change in soil chemistry dynamics in response to delayed vertebrate decomposition. Hence, hypothesis null was rejected. It was evident that there was impacts on the soil chemistry profiles temporally and spatially following delayed blow fly colonization on carrion. Although the associated impacts can be *in-situ* by microscale standard or being highly localized (within several meters radius from carrion), the impacts could be significant especially during mass mortality event across a larger landscape in which delayed in carrion decomposition could occur simultaneously due to the biotic limiting factor such as low decomposer population. Such events will contribute huge perturbation to the ecosystem and could potentially alter soil nutrient recycling processes and shift the equilibrium of soil ecosystem. Soil resilience has been demonstrated through this study and its efficiency is depend on, for examples, degree of perturbation (i.e., period of delayed carrion decomposition), types of carrion (e.g., C:N ratio), type of soil, soil temperature, initial soil chemo-physical properties (e.g., pH), soil microbiota, type of nutrient examined (whether it is a recalcitrant soil nutrient), and external supply (e.g., amount of precipitation or application of fertilizer). Higher soil resilience is represented with swift recovery, indicating better soil quality. However, in soil chemistry associated with carrion decomposition, I suggest soil resilience should be addressed specifically to

a particular soil nutrient, as soil ecosystem responds differently towards the influx of different soil nutrient. Besides, soil chemistry resilience during carrion decomposition could be addressed in two dimensions, namely resilience between treatments (with and without delayed in insect colonization), and resilience before and after perturbations (soil with and without carrion). As such, soil resilience could be explained in a more detail approach on how the soil ecosystem deals with the sudden influx of nutrients from an/multiple ephemeral resource(s).

In term of application, soil chemistry profiles associated with carrion with delayed blow fly colonization could impact the accuracy and reliability of forensic soil chemistry. Currently, most of the models using soil chemistry to predict mPMI were developed from decomposition studies under exposed or buried environment. However, these models should not be used when an unburied cadaver has been wrapped, where insect colonization has been deterred for a certain period of time. In this study, several models have been developed and validated statistically to address such problems when dealing with cadaver found on ground with a history of delayed insect colonization.

There are many potential research opportunities in soil chemistry associated with carrion decomposition, either ecologically or forensically. Future studies should look into what kind of soil structure and function determine soil resilience, factors that affect efficiency in nutrient recycling, dynamics of soil nutrients under different circumstances and environments, CDI's horizontal and vertical movement, as well as developing standard operating procedures (SOP) or framework on how to use soil chemistry in forensic applications, for instance, the determination of mPMI, location of death or perhaps in the future, identifying the victim (whether it was a human, or animal carcass, size of the body, age, gender, ethinicity etc.) based on the deposited soil chemistry profiling.

CHAPTER IV

SOIL ARTHROPOD COMMUNITY IN ASSOCIATION WITH DELAYED VERTEBRATE DECOMPOSITION

INTRODUCTION

Detritus, or any source of nonliving organic matter, is considered the basal trophic level of many food webs (Lindeman, 1942; Odum, 1969). It is estimated that 99% of the organic matter in a terrestrial ecosystem are of plant-origin (e.g., leaf litter, root exudates, stems) (Swift et al. 1979). Hence, decomposition of detritus has been intensively studied for many decades to provide evidence that this process is fundamental for persistence of an ecosystem (Swift et al. 1979). Although it is vital to understand the decomposition of plant-origin detritus, which dominates the Earth's detrital pool, there has been very limited research on animal-origin detritus (i.e., carrion).

Carrion is defined as dead and decaying vertebrate animal remains (Oxford Dictionary, 2016). Although carrion represents only a small part of the total detritus pool in most large-scale ecosystems (Swift et al. 1979; Parmenter & MacMahon, 2009), it plays significant roles in nutrient recycling that differ from plant detritus: (i) carrion is nutrient rich (low C:N ratio) whereas plant litter is usually low in nutrients with a high C:N ratio (Swift et al. 1979), and (ii) carrion decomposes much faster compared to plant litter, perhaps up to three orders of magnitude faster (Parmenter & MacMahon, 2009) (see Table 1.1 for comparison between plant and carrion decomposition). These two qualities make carrion a unique resource in quality and a distinct component of the detritus pool in an ecosystem (Barton et al. 2013a).

Carrion decomposition results in the release of the chemical components of the remains through autolysis and putrefaction (Dent et al. 2004). The release of chemical substituents creates localized island of increased soil fertility, subsequently influencing local plant and soil invertebrate community structure, thereby contributing to spatiotemporal heterogeneity of organism assemblages, and eventually driving the

evolution of scavengers and decomposers (DeVault et al. 2003). The quantitative understanding of carrion decomposition in various ecosystems has been studied. For example, nutrient recycling in a freshwater habitat has been demonstrated by Parmenter & Lamarra (1991) where they measured decomposition rate and nutrient loss sequences of rainbow trout (*Oncorhynchus mykiss*) and pinktail duck (*Anas acutas*) carcasses in Wyoming marsh over a 10-month period. They determined fish carrion decomposed more rapidly than waterfowl carrion. After 10 months, fish carcasses had lost 85% of their initial dry mass, while duck carcasses had lost only 30%. In terms of nutrients, fish carrion lost 95% of N and 60% P while waterfowl carrion lost 65% N and 30% P. The sequence of total element loss rates from carcasses was K > Na > N > S > P > Ca ~ Mg and was similar for both types of carrion. The impact of large ungulate carcasses (*Bos bison, Bos taurus* and *Odocoileus virginianus*) on grassland dynamics (i.e., soil and vegetation response) in northeastern Kansas, USA has also been examined. The results demonstrated that inorganic N (NH_4-N + NO_3-N), P, and pH were influenced by interactions among animal size, years after death, and distance from the carcass center. Soil K concentrations were not different between the center of carcass sites and surrounding soil. Inorganic N and P concentrations were higher in the center of carcasses. Mean pH was significantly lower in the center of carcass site (6.32 ± 0.24) than in the surrounding soil (7.34 ± 0.10). And, Parmenter & MacMahon (2009) examined decomposition rates of vertebrate species (e.g., rat carcasses, *R. norvegicus*) in a semiarid, shrub-steppe environment in Wyoming, USA. They found that decomposition rate varied significantly between microsites (below > surface) and among seasons (spring > summer > autumn ~ winter), with mass loss linearly correlated with ambient air temperature. They also found that energy, K, Na, N, and S were decreased more quickly than skeletal components (e.g., P, Mg, Ca). Furthermore, soil beneath the carcasses experienced increased N, P, and Na during decomposition. They concluded that at a landscape scale in the shrub-steppe ecosystem, carrion decomposition constituted < 1% of the nutrient-cycling budget but contributed significantly to localized soil nutrient dynamics.

These nutrients are predominately recycled by soil arthropods, which are quite diverse. In fact, large numbers of microarthropods (e.g., mites and collembolans) are found in most types of soils and soil mites usually outnumber collembolans (Coleman et al. 2004). In rich forest soil, a 100 g samples may contain as many as 500 mites representing almost 100 genera (Wallwork, 1983). Four important groups of mites occur frequently in soil. These mites represent namely the Oribatida, the Prostigmata, the Mesostigamata, and the Astigmata. Oribatids are the most common mites found in soil. These mites are usually fungivores and detritivores. Mesostigmatid mites are typically predators on other small fauna or insect eggs, although some species are fungivores. Acarid mites are found associated with rich, decomposing nitrogen sources and are abundant in agricultural soils or stored products, while the Prostigmata contains a broad diversity of mites with a variety of feeding habits and strategies (Coleman et al. 2004; Labandeira et al. 1997; Koehler, 1997; OConnor, 1994).

Oribatid mites can influence organic litter decomposition and nutrient dynamics in forest floor. They are known to graze on microbial populations or fragmenting plant detritus (Peterson & Luxton, 1982). Oribatid mites can store and process a significant portion of the Ca input in forest litter (Gist & Crossley, 1975). Some families of Prostigmata mites are predaceous, microbial feeders, plant feeders or parasites (Kethley, 1990). In general, the larger predaceous Prostigmata feed upon other arthropods or their eggs, the smaller species are Nematophagous (Kethley, 1990). The Mesostigmata contains fewer soil inhabiting species than do Oribatida and Prostigmata. Many of the mesostigs are parasitic on vertebrates and invertebrates (Krantz, 1978). The true soil species are almost all predators; only a few species (e.g., Uropodidae) are polyphagous, feeding on fungi, nematodes and juveniles insects (Gerson et al. 2008). Many species of Mesostigmata have a close association with other insects or arthropods for dispersal purposes (phoretic relationship) (Hunter & Rosario, 1988). The Astigmata are the least common of the soil mites, although they may become abundant in certain habitats (Luxton, 1981). The free-living Astigmata prefer moist environments high in organic matter and most of the astigmatans are microbial feeders (Andrén et al. 1995). Those

with chelate chelicerae are able to chew vegetable materials, fungi, and algae (Krantz & Lindquist, 1979). The Astigmata are specialists in patchy or ephemeral habitats, and they are able to reproduce in a relatively short time; hence, many species can build up large populations on concentrated resource patches within days (OConnor, 2009b). Astigmatans are the most successful group of mites in establishing symbiotic relationship with both vertebrates and invertebrates (Houck & OConnor, 1991; OConnor, 1994). One of the reasons why Astigmata is the most successful group of mites is their ability to disperse via phoresy (OConnor, 1982; OConnor, 1994). Phoresy (In Greek, *phoras*, means bearing) is a common form of commensalism and is applied to interspecific relationships in which one organism (the phoretic) attaches to another (the host) for the implied purpose of dispersal (Houck & OConnor, 1991). Astigmatid deutonymphs (heteromorphic deutonymphs, or known as hypopi) most commonly occur in association with beetles (Coleoptera), ants and wasps (Hymenoptera), as well as flies (Diptera) (Houck & OConnor, 1991). Several major families of astigmatid mites are almost exclusively composed of arthropod associates, for example, the Histiostomatoidea, Hemisarcoptoidea, Canestrinioidea, and Acaroidea (Houck & OConnor, 1991). Host specific relationships are most common where beetle species exploit rich temporary habitat such as carrion (e.g., *Pelzneria* sp. on *Nicrophorus* beetle) and vertebrate dung (e.g., *Rhopalanoetus* sp. on sacarab beetles) (Houck & OConnor, 1991). The Diptera are common hosts of phoretic Astigmata because they are frequent visitors to ephemeral resources such as dung, carrion, sap-fluxes, and phytotelmata (Houck & OConnor, 1991). The histiostomatid genera such as *Myianoetus*, *Copronomia*, *Ameronoetus* and *Xenanoetus* are restricted to dipteran associations (Houck & OConnor, 1991). Reviews on host association and phoretic mites have been provided by Hunter & Rosario (1988) on Mesostigmata, Norton (1980) on phoretic Oribatida, OConnor (2009a) on Astigmata phoretic mites, Eickwort (1990) on phoretic mites with social insects and Linquist (1975) on mites with arthropods found in forest floor habitats,.

Mites associated with carrion have been documented (Perotti & Braig, 2009; Perotti et al. 2010). Perotti & Braig (2009) studied phoretic mites associated with human

and animal decomposition. More than 212 phoretic mites species associated with carcasses have been reported in the literature (Perotti & Braig, 2009). Among these, Mesostigmata form the dominant group, represented by 127 species where 25 species belongs to Parasitidae and 48 species to Macrochelidae. Most of these mesostigmatids are associated with particular species of flies or carrion beetles. Astigmata mites are more frequently found on dried remains of vertebrate carrion. Of those identified, 52 species were phoretic on scavengers such as hide beetles (Coleoptera: Trogidae), skin beetles (Coleoptera: Dermestidae) and moths (Lepidoptera) (Perotti & Braig, 2009). Barton et al. (2014) examined and compared the changes in abundance, species richness, and composition of mite and beetle assemblages sampled at kangaroo (*M. giganteus*) carcasses in a grassy eucalypt woodland near Canberra, Australia. They found a majority of mites were phoretic, with the mesostigmatid genera *Uroseius* (Uropodidae), *Macrocheles* (Macrochelidae) and *Parasitus* (Parasitidae) the most abundant taxa (excluding astigmatid mites). Abundance and richness patterns of mites and beetles were very different, with mites reaching peak abundance and richness at week 6 and 12, and beetles at week 1 and 6. The results from Barton et al. (2014) showed that mesostigmatid mite assemblages experienced a delay in peak abundance and richness relative to beetle assemblages, suggesting differences in dispersal and reproductive traits of arthropods contribute to the contrasting diversity dynamics of carrion arthropod communities, and further highlight the role of carrion as a driver of diversity and heterogeneity in ecosystem.

Mites associated with decomposing carrion have been investigated for their potential in forensic investigations. The first reports of modern forensic acarology was reported by Brouardel in 1879, where a case of a newborn child that was found mummified and the time of death was independently estimated based on caterpillars and mites present (Perotti et al. 2009). Mégnin was consulted regarding the mite specimens and calculated back the number of generations that would have been required to account for the number of mites present on the corpse. Mégnin concluded that the estimate for the time of death was around seven to eight months before the autopsy (Mégnin (1894),

as cited in Perotti et al. (2009)). Since then, few studies have mentioned mites associated with carrion (Braack, 1986; Goff, 1989). However, in 2009, the journal *Experimental and Applied Acarology* published 12 articles on forensic acarology as a special issue with the goal of boosting interest by acarologists (Perotti et al, 2009a, 2009b; Perotti & Braig, 2009; Desch, 2009; Solarz, 2009; Braig & Perotti, 2009; OConnor, 2009a; Proctor, 2009; Baker, 2009; Turner, 2009; Goff, 2009). In Europe, Saloña et al. (2010) collected arthropods from soil at a body recovery site and determined dominant necrophagous fauna included mites from the families Ascidae: *Proctolaelaps epuraeae* (Hirschmann), and Laelapidae: *Hypoaspis (Gaeolaelaps) aculeifer* (Canestrini). They also reported mites from the families Acaridae: *Sancassania berlesei* (Michael), Ascidae: *Zerconopsis remiger* (Kramer) and Urodinychidae: *Uroobovella pulchella* (Berlese) and Macrochelidae: *Glyptholaspis americana* (Berlese) for the first time in the Iberian Peninsula. Mašán et al. (2013) reported an unusual mesostigmatid mite from the family Melicharidae, *Proctolaelaps euserratus* Karg, found in association with decaying matter of animal and human decomposition in various European countries such as Slovakia, Spain and the United Kingdom. This mite species is thus considered a potential marker for later stages of decomposition, namely butyric fermentation and dry decomposition. Saloña-Bordas & Perotti (2014) reported a case of a hanged corpse in Spain. They recovered four species of phoretic mites namely *Poecilochirus carabi* s.s. G. & R. Canestrini (Parasitidae), *Poecilochirus (Physoparasitus) davydovae* Hyatt (Parasitidae), *Pelzneria crenulata* (Oudemans) (Histiostomatidae), *Pelzneria necrophori* (Dujardin) (Histiostomatidae) on carrion beetles (Silphidae: *Necrodes* and *Nicrophorus*) and rove beetles (Staphylinidae: *Creophilus maxillosus* (L.)), which were used for interpreting the case. In Asia, Silahuddin et al. (2015) documented 11 families of mites associated with rabbit (*Oryctolagus cuniculus* (L.)) carcasses in Malaysia. Similarly, Hanifah et al. (2015) identified *Macrocheles scutatiformis* (Macrochelidae) found on dung beetles (*Phaeochroops freenae* Kuijten) and soil beneath monkey and rabbit carcasses placed in a secondary forest in Malaysia.

In the United States, OConnor (2009a) provided an excellent review on Astigmata of forensic interest, with mite specimens that are deposited in the University of Michigan Museum of Zoology, Ann Arbor, MI, USA. These mites include Acaridae (genera *Acarus, Tyrophagus,* and *Sancassania*), Lardoglyphidae (*Lardoglyphus*), and Histiostomatidae (*Spinanoetus, Pelzneria, Myianoetus, Histiostoma*). Recently, Pimsler et al. (2016) reported that the muscid fly, *Synthesiomyia nudiseta* (van de Wulp) (Diptera: Muscidae) was collected during three indoor medicolegal forensic entomology cases in Texas, USA. In each case, mites were found in association with the sample and subsequently identified as *Myianoeus muscarum* (L.) (Acariformes: Histiostomatidae). This report suggested that this mite is of potential value in forensic investigations, as it lends new insights into the community structure of colonizers on human remains in indoor environments.

Other than mites, arthropods that are commonly found in the soil are quite diverse. Examples of other arthropods commonly encountered in soil include Diplura, Isopoda, Diplopoda, Chilopoda, Araneae, Coleoptera (adults and larvae), Hymenoptera (e.g., ants), Diptera (larvae), Isoptera, Psocoptera, and Oligochaeta (earthworms); all these soil organisms are considered as macrofauna (Coleman et al. 2004).

Soil biota, which includes these arthropods and microbes, play important roles in soil processes and soil quality. For example, microfloras (i.e., protozoa) catabolize organic matter, mineralize and immobilize nutrients. The mesofauna (e.g., nematodes, mites, collembola) and macrofauna (e.g., beetles, earthworms) create fecal pellets, and produce biopores of various sizes, which affect water movement and storage as well as root growth and proliferation (Coleman et al. 2004). Seastedt & Crossley (1988) provided an excellent account on soil arthropods and their roles in litter decomposition and mineralization processes.

Bornemissza (1957) examined arthropod succession on guinea pig (*Cavia porcellus* (L.)) carrion and the effects of decomposition on the soil fauna. Five different stage of decomposition were recognized, and each stage affected the underlying soil fauna differently. The liquefied decomposition products during butyric fermentation

stage destroyed the underlying plants and soil fauna. The fauna beneath the carcass differed greatly from the control, and intermediate zones (i.e., the belt surrounding the carrion 10 cm wide). Bornemissza (1957) concluded that the decomposition of carrion had a significant effect on the soil fauna to a depth of 14 cm, but this was less drastic than in the upper soil layers. The reinvasion or recolonization of the carrion soil by arthropods in the lateral and non-exposed regions was not recovered fully even after a year. In other words, the soil arthropod community was not resilient following carrion decomposition, which may act as a perturbation to the ecosystem.

Carrion as a rich ephemeral resource is fiercely contested by a variety of consumers such as vertebrate scavengers, arthropods and microbes (Hanski, 1987b; Barton et al. 2013), and it is well documented that immediate insects access and colonization on exposed carrion have been documented in many field studies (Anderson, 2001; Matuszewski et al. 2008). Necrophagous insects such as blow flies could be seen on animal carcasses within minutes and oviposit their eggs within hours (Greenberg, 1991). The almost immediate colonization of insects on carrion also gives opportunities to forensic entomologists to estimate the time of colonization (TOC), which could provide clues in the forensic investigations as to when the individual died. However, immediate insect access to carrion is not always the case, and not necessarily true in all condition (Bourel et al. 1999).

Many abiotic and biotic factors could contribute to the delay of insect access to carrion and consequently delay decomposition process (Campobasso et al. 2001). Abiotic factors that delay insect colonization on carrion include weather and location origins such as seasons, temperatures, rainfalls, snows, thunderstorms, tornados, beside a busy highway and high altitudes (e.g., highland or high-rise building) which could deter immediate insect access and oviposition activities (Campobasso et al. 2001; Mahat et al. 2009). Another abiotic factors include burial activities or being hidden, and these could happen either naturally by animal behaviors (e.g., dogs burying bones or carcass hid by scavengers), or artificially (e.g., in criminal cases where human cadavers have been wrapped, buried or hidden in concealed container, or in some rare cases where

insecticide was applied on human corpse to prevent insect colonization) (Anderson, 2001). Third, time could be another abiotic factor, for example, animals that die during night time where blow flies are not active and oviposition is likely to occur in the next morning (Introna et al. 1998; Reibe & Madea, 2010).

A number of biotic factors can impact arthropod colonization of vertebrate carrion. Predation of blow fly eggs and larvae by other organisms (e.g., ants, mites, beetles, or other species of flies) is well documented (Norris, 1965). Second, quorum sensing by bacteria on vertebrate carrion results in microbial volatile organic compounds (MVOCs) to attract or repel certain blow flies could be one of the reasons that cause delay in insect arrival time (Ma et al. 2012; Tomberlin et al. 2012; Davis et al. 2013). Ecological interactions (e.g., competition, priority effects, facilitation effects, inhibition effects) may play a role in deterring insect arrival and colonization or altering insect succession sequence although empirical studies are needed to confirm these observations (Connell & Slatyer, 1977). Changes in insect community structure and function following ecosystem perturbation could deter initial insect colonization on carrion. For example, during mass mortality events (MMEs), where hundreds or thousands of vertebrates die during the same period of time (e.g., salmon runs, locust outbreak, large scale population die-off such as livestock population due to disease or draught), may cause large amount of nutrients flux into the ecosystems (Richey et al. 1975; Barton, 2015). Not only the introduction of large volume of carrion materials into the soil ecosystem, which could cause devastating effects such as nitrogen toxicity which kill most soil flora and fauna in that particular landscape (Bornemissza, 1957; Goyal & Huffaker, 1984), but the MMEs may also cause disturbance to the ecosystem function such as the efficiency of decomposition by the local necrophagous communities. It is assumed that the number or abundance of necrophagous arthropods, such as blow flies, is predetermined based on the current spatiotemporal equilibrium dynamics in a given habitat. In other words, each habitat is able to support a limited but sufficient number of decomposers to perform an optimum ecological function (i.e., decomposition) in maintaining the stability of the ecosystem. However, when an unexpected ecological

disaster occurs, such as MMEs, there could be a shortage in the number of necrophagous "workers" to decompose all the resources available simultaneously, eventually leads to delay in carrion decomposition. It is possible that large-magnitude perturbation (i.e., MMEs) could change the equilibrium state of the disturbed habitat either temporarily or permanently, depending on the degree of resilience and the quality of its internal properties (e.g., network connectivity, community richness, diversity and functions etc.) that are inherited in the ecosystem (Gunderson, 2000).

The ultimate consequences of delayed carrion decomposition could be seen from two perspectives, namely ecological and application. Ecologically speaking, delayed carrion decomposition could have two sides: beneficial and deleterious effects. The benefits of delayed carrion decomposition is carrion could serve as a unique resource pool and alternative habitat for vast variety of organisms for an extended period of time. Furthermore, availability of carrion for a longer period could result in species diversity (especially necrophagous guilds) being maintained while enriching the soil ecosystem (Barton et al. 2013). The negative impacts of delayed carrion decomposition are potentially the contamination of the environment with pathogenic bacteria (Houston & Cooper, 1975). Furthermore, carrion serves as a breeding ground for vast variety of insect vectors as well as provides food for scavenger animals, which could serve as pathogen reservoirs (Busvine, 2012; Jennelle et al. 2009; Jones & Pybus, 2001).

From the perspective of application science, delayed in carrion decomposition could impact the applications of forensic entomology (Pechal et al. 2014b), microbiology (Pechal et al. 2013, 2014a) (see Chapter 2), soil chemistry (see Chapter 3) and perhaps acarology (see the current Chapter). Delayed insect colonization could impose errors when estimating minimum time of insect colonization based on the entomological evidence (e.g., traditional insect succession models), as it is evident that delay carrion decomposition up to five days could change the insect community structure and its successional trajectories (Pechal et al. 2014b). Similar concepts also applies to microbial community succession on carrion, changes in soil chemistry dynamic due to vertical and horizontal movement of soil nutrients following delayed

carrion decomposition, as well as changes in soil arthropods community structure and function when there is absence of primary colonizers on carrion. All these are interesting yet important research questions to be answered by empirical experiment and field studies.

Due to the fact that no previous study had been done on the soil arthropod community associated with carrion experiencing delayed Diptera colonization, the objectives of this study were to examine the impact of delayed primary arthropod colonization of carrion on the successional trajectories of soil arthropod community structure and function. Secondly, this study determined if soil arthropod community succession trajectories, based on structure and function, of carrion experiencing delayed primary arthropod colonization converged over time indicating recovery of succession trajectories as related to what was observed in the controls.

METHODS

Site description and experimental design

Swine carcass (*S. scrofa* L.) decomposition were studied at a site belonging to the Field Laboratory, Texas A&M University, College Station, Texas, USA (30°33’ 18.54’’ N 96°25’38.71’’ W, 68 m a.s.l.). The perimeter of the study area was approximately 371 m and the area was about 7,943 m^2 (Figure 2.1 and 2.2). The soil at the study site was characterized as ship clay (Vertisol). There was a stream located at the north of the study site. The east and south edges were steep cliffs (~6 m) above the stream.

Vegetation at the study site is considered part of the blackland prairie ecoregion (http://www.texasalmanac.com). Common vegetation found at the study site included Johnsongrass (*Sorgum halepense* L.) (dominant cover plant, covered approximately 75% of the study site), oak (*Quercus* spp.), annual sunflower (*Helianthus annuus* L.), thistles (*Cirsium* spp. Mill.), Western horse nettle (*Solanum dimidiatum* Raf.), Camphorweed (*Heterotheca subaxillaris* (Lam.)), muskmelon (*Cucumis melo* L.), jujube (*Zizyphus jujube* Miller),wild purple morning glory (*Ipomoea cordatotriloba* Dennst. tievine), pink

evening primrose (*Oenothera speciose* Nutt.), poison ivy (*Toxicodendron radicans* (L.) Kuntze) and arrow-wood (*Viburnum dentatum* L.).

Studies were conducted in two consecutive summers during June 2013 and 2014. A total of nine pig carcasses purchased from a local pig farmer in Anderson, Texas were obtained for each year replicate. Sex and weight of each pig carcass was determined prior to placement in the field. The animals were deceased at the time of acquisition; therefore, the Texas A&M University Institutional Animal Care and Use Committee required no animal use protocol. The carcasses were double bagged and transported within one hour after death to the study site. Carcasses were placed in the field at approximately 1700 hr. Carcasses were randomly placed minimally 20 m apart along three transects. All carcasses were oriented with heads to cardinal north and dorsal side towards the east. The placement of pig carcasses in the field was calculated by using a Latin Square design, and the arrangement of treatments groups were different between years (Figure 2.3 and 2.4). Each location was only used once. Subsequent locations were never less than five meters from a previous site used. During each field seasons, three random carcasses were enclosed in an individual 1.8 m^3 Lumite® screen (18 x 14 mesh size) portable field cages (BioQuip Products, Rancho Dominquez, CA, USA) for seven days, this treatment was designated as Post-7 group. Another three random carcasses were enclosed with similar manner as above but it was enclosed for 14 days, thus were designated as Post-14 group (Figure 2.5), while all insects were allowed access the remaining three carcasses, which served as control (Figure 2.6). All carcasses were covered with hand-made anti-scavenging cages (0.6 m height x 0.9 m width x 1.2 m length) constructed of steel frames enclosed with poultry netting. Each anti-scavenging cage was topped with a layer of woven green fabric (Figure 2.6) to prevent direct sunlight and heat on the carcass. All cages were then properly labeled according to their designation. Stones were placed on top of each cage to increase weight in order to prevent the movement of cage by extreme wind or scavenger activities. Furthermore, observations for vertebrate scavenging were made daily at approximately 2200 hours.

Climatological data such as temperatures and rainfall were recorded. NexSens DS1923 micro-T temperatures loggers (Fondriest Environmental, Inc., Alpha, OH, USA) (Figure 2.7) were placed at the study site 0.3 m above the ground on the exclusion cages to measure local ambient temperature every 60 min for 40 days continuously. Temperature data were converted into accumulated degree hours (ADH) based on the following formula:

$$\mathrm{ADH} = \sum_{i=1}^{n} (\emptyset - \emptyset_0)$$

where $\emptyset$ is the ambient temperature (in degree Celsius), while the minimum threshold temperature is $\emptyset_0$ (Higley & Haskell, 2009). The minimum development temperature threshold was set as 10°C for this chapter as that is the minimum used for blow flies common on vertebrate carrion during the summer months in Texas, USA. To obtain the value of accumulated degree days (ADD), the ADH was divided by 24 (i.e., ADD = ADH/24). Precipitation during the study period was recorded daily with a rain gauge attached to a wooden stake approximately 1.3 m above the ground and 1 m north from one of the carcasses (Figure 2.8).

Soil samples at the study site were taken five days (Day -5) prior the placement of carrion. These soil samples were then sent to Soil, Water and Forage Testing Laboratory, Texas A&M University, College Station for baseline soil chemistry study. During the initial day of experiment, soil samples were collected from beneath and at the side of pig carcasses (designated as soil lateral, which is approximately 30 cm distant from the location of soil beneath the carrion), along with a control sample which was located 5 m away north from the carrion (Figure 2.12) on Days 0, 7, 14, 21, 40, 90 and 180 to determine the soil arthropod community structure and function over time. Note that soil samples were also collected on Day 243 of each trial to determine soil porosity (methods see Chapter 3). Approximately 400 g soil sample were collected using a plastic trowel and a steel tin can with 170 g capacity (Figure 2.13) that were disinfected using

Lysol between uses. After collection, each soil sample was placed in a Ziploc bag and labeled. These soil samples were kept in a cooler box (L: 70 cm; W: 40 cm; H: 45 cm) filled with ice (~4°C) and transported to the F.L.I.E.S. Facility. For each soil sample, approximately 20 g was then separated from each individual sample, transferred to a freezer bag, and stored in the freezer (Kenmore®, USA) at -20°C for soil chemistry analysis. The remaining soil sample was placed in Berlese funnels (Figure 4.1) for soil arthropod extraction. After a soil sample had been transferred into the bucket, the inside of the soil sample bag was washed with 70% ethanol to detach and collect all remaining arthropods. The ethanol-arthropod mixture was then transferred directly into the mason jar (served as specimen jar) containing 100 ml 70% ethanol, which were used to collect extracted specimens via Berlese funnels.

Berlese funnels were used to process the samples for 72 hours to extract soil arthropods. The bucket used was a utility pail made of plastic (25 cm diameter, 25 cm height, diameter at base 18 cm), with a round shaped hardware cloth (gauze size 23, mesh size 0.6 cm) placed at the bottom of the bucket. Soil samples were placed on three pieces of filter papers (diameter 9 cm, qualitative P8, Fisherbrand®) located on top of hardware cloth located at the base of a funnel (diameter 20 cm, height 18 cm, and 1.4 liter in volume). A 40-watt, 470 lumens, soft white in color (Philips® A19) light bulb was used to dry the soil sample. Soil arthropods moved away from the dried soil would fall into the funnel and be collected in the mason jar assigned to the sample. It should be noted that four Berlese funnels used were a different design (Figure 4.2). These Berlese funnels relied on 60-watt light bulbs with 860 lumens (Philips® A19) due to needing more heat generated for the depth of the funnels.

Samples in each mason jar was examined for the presence of soil arthropods under a dissecting stereo microscope (EMZ-8TR (0.7x – 4.5x), Trinocular Zoom Stereo, Meiji, Japan) (Figure 4.3). The content of the mason jar was shake gently before poured into a plastic petri dish (diameter 90 mm, BD Falcon™). A piece of grid paper (divided into 16 squares) was attached to the bottom of the petri dish for the purpose of mite counting and sampling protocols (Figure 4.4). All extracted soil arthropods were

counted, recorded and identified to the lowest taxonomical rank possible using Triphehorn & Johnson (2005) and Stehr (1987). Once counted, soil arthropods and the excess ethanol were transferred to a 400 ml beaker (Pyrex®). From there, a piece of Grade 3 filter paper (15 cm diameter; Whatman®) labeled with specimen details was placed inside the funnel to trap soil arthropods while the excess ethanol was removed via filtration through a Büchner funnel (Figure 4.5). After filtration, a piece of wax paper (Reynolds®) was used to wrap the filter paper, along with the trapped arthropods, sealed and then stored in a plastic container in a freezer as specimen records.

Mites recovered from the soil samples were counted and divided to two categories namely Oribatida and Non-oribatids. To identify the mites to the family, subsampling of mites were performed. Four distinct non-overlapped numbers were generated using random number generator online (https://www.random.org/) for every specimen jar. For the mites that fell randomly into these four numbers on the 16-square grid paper were then picked up using a loop modified from minuten pin and transferred into a small petri dish (35 mm x 10 mm) filled with several drops of specimen clearing fluid (Bioquip®). Mite specimens were immersed in the fluid for 24 hours at room temperature placed in a fume chamber. The clearing duration could be extended depending on the degree of clearing. This clearing fluid is principally a lacto-phenol solution with glacial acetic acid. Note that this clearing fluid did not clear guanine produced by Acari. After clearing, the specimens were transferred to a glass slide (size 76 x 25 mm; Hamilton Bell Co., Inc.) with a small drop of polyvinyl alcohol (PVA) mounting medium (Bioquip®). Note that PVA medium contained Elvanol and lactophenol and is usually used as a substitute for Hoyer's solution. The limitation of PVA medium is that it can be crystalized after a while and could be problematic to remount the specimen from the slide when re-mounting is needed. A square cover slip (22 mm; Bioquip®) was then placed on top of the mite specimens (a slide may contain up to 10 mites from the same specimen jar). The slide was labeled accordingly. Slides were then air-dried for 3-4 days and stored in a microscope slide box (76 x 25 mm; Bioquip®) in an upright position under laboratory conditions. When encountered during

processing of soil arthropods, phoretic mites were collected from the arthropods (e.g., underneath the elytra of a beetle) and processed as in the methods described.

Mites mounted on slides were examined under a light microscope with 40x magnification (Nikon, Japan) (Figure 4.6) and identification was carried out to Family level using Krantz & Walter (2009). Confirmations of Astigmata and other groups of mites were made by Dr. Barry O'Connor at University of Michigan Museum of Zoology (UMMZ) while Oribatida were made by Dr. Roy Norton (retired professor of State University of New York). Undescribed phoretic mite species and several other mite specimens that were found in soil samples were vouchered at the TAMU Insect Collection with accession number #722.

Figure 4.1. Left. Berlese funnels and the racks used to extract soil arthropods from soil samples collected from the field site at Snook, Texas during summers 2013 and 2014. Right. Mason jar containing 70% ethanol to preserve extracted soil arthropods from Berlese funnels.

Figure 4.2. Four modified steel cans served the same function as Berlese funnels. These funnels are located at the F.L.I.E.S Facility, College Station, Texas.

Figure 4.3. The stereo microscope (Meiji, Japan) used in sorting and identifying soil arthropods collected from the field site at Snook, Texas in summers 2013 and 2014.

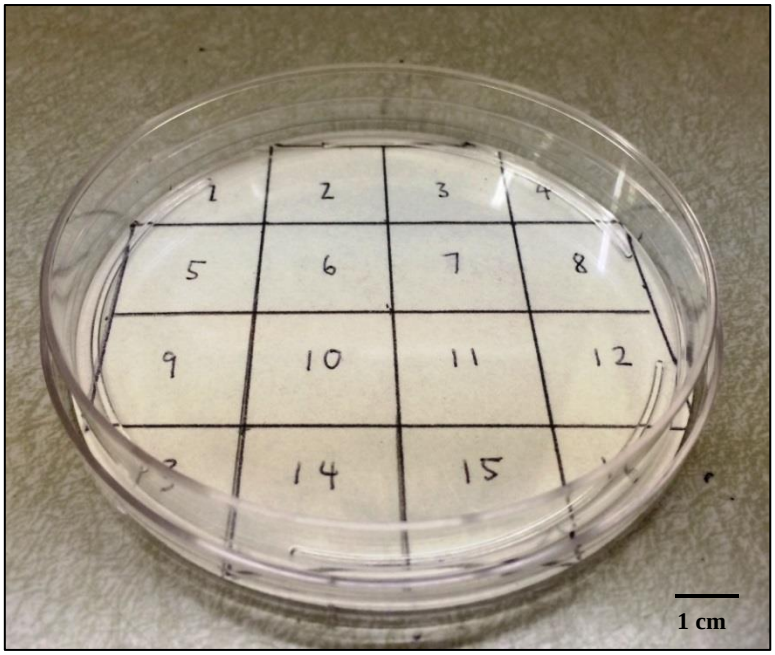

Figure 4.4. Petri dish with a self-designed 16-square grid paper used for counting and subsampling of mite specimens for slide mounting purposes.

Figure 4.5. Büchner funnel used for filtration purpose (i.e., to separate soil arthropods from the ethanol effluent). The extracted arthropods on the filter paper were then kept in the freezer as records.

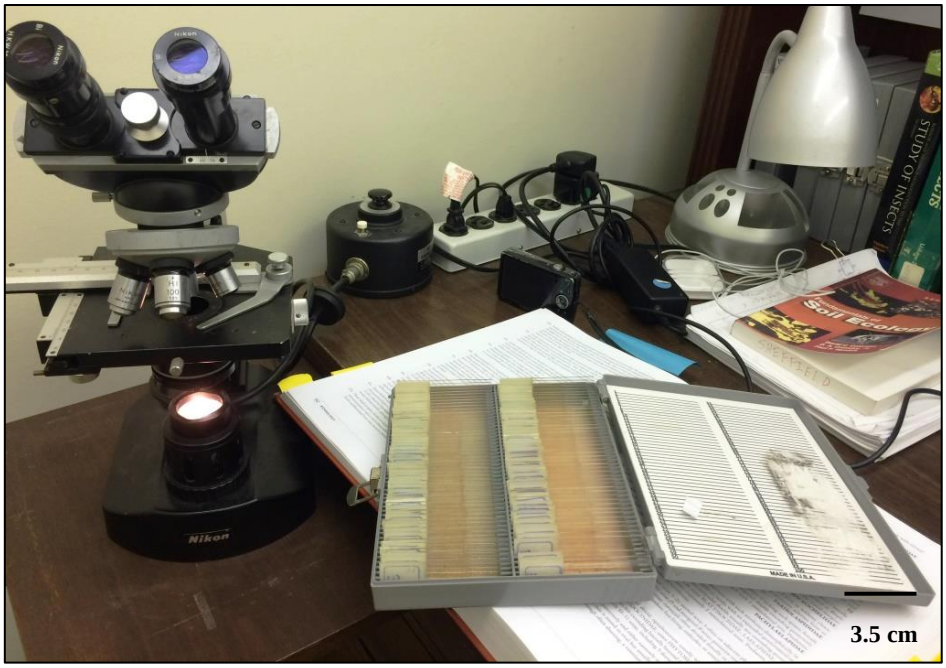

Figure 4.6. The compound microscope (Nikon, Japan) used to identify mite specimens extracted from the soil samples to the lowest possible taxonomic rank (e.g., Order or Family). A slide box was demonstrated beside the compound microscope.

Statistical analyses

Soil arthropod community data (separated by Order, Family, Genus and species, and Function) were analyzed using statistical program JMP® Pro version 11.0.0 (SAS Institute Inc., NC, USA) for analysis of variance (ANOVA) and Tukey-Kramer HSD post-hoc test. Soil arthropod community structure data were also calculated for ecological indices such as species richness (S), Dominance (D_i), Simpson's diversity index (D), Shannon-Wiener's Diversity Index (H'), and Evenness (E). A diversity index is a quantitative measure that reflects how many different species there are in a dataset, and at the same time, taking into account how evenly the basic entities are distributed among those species.

Species Richness, S, simply quantifies how many different species of the dataset contained while Dominance (D_i) was calculated according to the equation below:

$$D_i = \frac{n_i}{N} \times 100$$

where n_i is the number of individual species collected, and N is the total number of specimen collected. Species dominant is classified according to Tischler's scale: eudominant $10\% \leq D_i \leq 100\%$, dominant $5\% \leq D_i \leq 10\%$, subdominant $2\% \leq D_i \leq 5\%$, recedent $1\% \leq D_i \leq 2\%$ and subrecedent $0\% \leq D_i \leq 1\%$ (Tischler, 1949).

Simpson's Index (D) measured both richness and proportion of each species and is calculated using this formula:

$$D = \sum_{i=1}^{S} P_i^2$$

where Pi is the proportion of species i. In brief, Simpson's index is the sum of proportion of each species in the community and represented the probability of two randomly selected individuals in the community belong to the same species. Shannon-Wiener Index (H') is similar with Simpson's Index where the measurement takes species richness and proportion of species into account, and is calculated based on the following formula:

$$H' = -\sum_{i=0}^{n} P_i(\ln P_i)$$

In general, Shannon-Wiener Index is the negative sum of multiply products between species proportion (P_i) and natural log of species proportion ($\ln P_i$).

Evenness (E) is an indicator of similarity in abundance of different species. Evenness is measured on the scale from 0 to 1 where zero represents more variations in communities whereas one represents complete evenness. Evenness is defined as:

$$E = \frac{H'}{\ln S}$$

Evenness is the number obtained via dividing the value of Shannon-Wiener Index by natural log of species richness (S).

When Shannon-Wiener's Index was converted to Effective Number of Species (ENS), which is EXP (H').

$$ENS = EXP\ (H')$$

If the ENS value is close to 1, this indicates that the arthropod community has an equivalent diversity as a community with 1 equally-common species.

In addition, R project for statistical computing (R 3.0.2) was employed to analyze soil arthropod community data using vegan package (Oksanen et al. 2013). Vegan contains the methods of multivariate analysis (e.g., Permutational Analysis of Variance, or PERMANOVA) needed in analyzing ecological communities, and tools for diversity analysis. Bonferroni corrections were used to test for significance of pair-wise comparisons without an increased probability of rejecting the null when it was actually true (Type I error) (Cabin & Mitchell, 2000).

Non-metric multidimensional scaling (NMDS) was used to evaluate soil arthropod community structure and function between treatments over days in package Vegan function Adonis in R. It is an analysis of variance using distance matrices; for partitioning distances matrices among sources of variation and fitting linear models to distance matrices. It uses a permutation test with pseudo-F ratio. Generally, NMDS is a nonparametric ordination technique that avoids assuming linearity among community variables (McCune et al. 2002).

Multi-response permutation procedures (MRPP) was used for testing statistical differences between overlay groups of soil arthropod communities within the ordination using methods described elsewhere (Biodini et al. 1985). Indicator species analysis

(ISA) completed MRPP by assigning significant indicator values to carbon substrates that were indicative of community functional separation among treatments and over decomposition day (McCune & Grace, 2002). The indicator value described which arthropod Order/Family/Genus or species was the best indicator among the arthropod community based on the abundance data, with 0 representing no indication and 100 being a perfect indication for each grouping. All statistical results with value $p < 0.05$ were considered significant difference.

RESULTS

Weather data in summer 2013

A total of 985 readings were taken by three micro-T temperature loggers that were placed in the field from 16 June 2013 (5 pm) to 27 July 2013 (5 pm). The overall mean temperature was 30.59 ± 7.81 °C, with maximum 47.67 ± 4.48°C and minimum 15.50 ± 0.00 °C. Total accumulated degree hour (ADH) for 2013 trial was 29209.70 (base temperature of 10 °C). According to the nearest National Weather Station (KCLL) at Easterwood Field Airport, College Station, Texas (data downloaded from www.wunderground.com), there were nine rain events and five thunderstorms recorded during the study period. Total precipitation recorded from the rain gauge throughout the study period was 39.12 mm.

Weather data in summer 2014

A total of 985 readings were taken by three micro-T temperature loggers that were placed in the field from 15 June 2014 (5 pm) to 26 July 2014 (5 pm). The overall mean temperature was 29.27 ± 6.49 °C, with maximum 43.00 ± 1.80°C and minimum 19.00 ± 0.00 °C. Total accumulated degree hour (ADH) for 2014 trial was 28080.67 (base temperature of 10 °C). There were 13 rain events, 11 thunderstorms and two fog events recorded during the study period. Total precipitation recorded from the rain gauge throughout the study period was 171.45 mm.

Weather comparison between summers 2013 and 2014

Generally, combined data showed overall mean temperature in summer 2013 was higher than mean temperature in summer 2014. The two-tailed T test was employed to compare temperature data between years and the results showed a significant difference ($p = 0.0004$). Table 2.1 showed the T-test result on weather comparison between summers 2013 and 2014. Figure 2.18 showed the mean temperature data of both 2013 and 2014 and Figure 2.19 showed the amount of precipitation for both summers. Although summer 2014 showed a higher amount of precipitation (171.45 mm) compared to summer 2013 (39.12 mm), the two-tailed T-test showed not significance between these two years ($p = 0.2725$). Table 2.2 compared precipitation of both years.

Accumulated degree hours (ADH) and accumulated degree days (ADD)

Accumulated Degree Hours (with base temperature 10 °C) for summer 2013 and 2014 was demonstrated in Figure 4.7, where the ADH in summer 2013 was significantly greater than during summer 2014. The T-test result demonstrated that there was significant difference in ADH between the two trials ($t (1964.141) = -2. 1944$, $p = 0.0283$). Based on the readings obtained from micro-T data logger, ADH and ADD was calculated up to 40 days of experiment. Table 4.1 demonstrated the ADH and ADD during field sample date for the 2013 and 2014 trials.

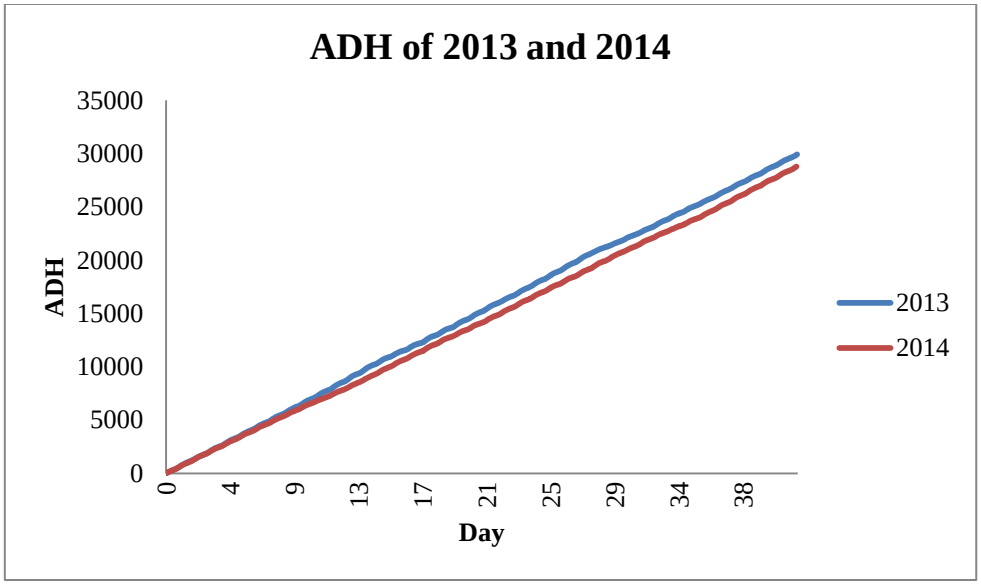

Figure 4.7. Average ADH (Base temperature 10 °C) during summers 2013 and 2014 at the field site located at Snook, Texas.

Table 4.1. ADH and ADD of field sampling days in summers 2013 and 2014 in the field site at Snook, Texas.

Sampling Day	2013		2014	
	ADH	ADD	ADH	ADD
0	25.03	1.19	28.33	1.18
7	5203.70	216.82	5000.50	208.35
14	10612.36	442.18	9653.83	402.24
21	15625.53	651.06	14529.83	605.40
40	29209.70	1217.07	28080.66	1170.02

Year effect

There was a significant year effect (df = 1; F = 11.509; p = 0.001) between two trials by Order of arthropods (Figure 4.8 showed NMDS plot between years). Furthermore, when Function of soil arthropods was analyzed for Year effect, the results showed that there was significant difference between year (df = 1; F = 11.321; p = 0.001). Hence, data were analyzed separately.

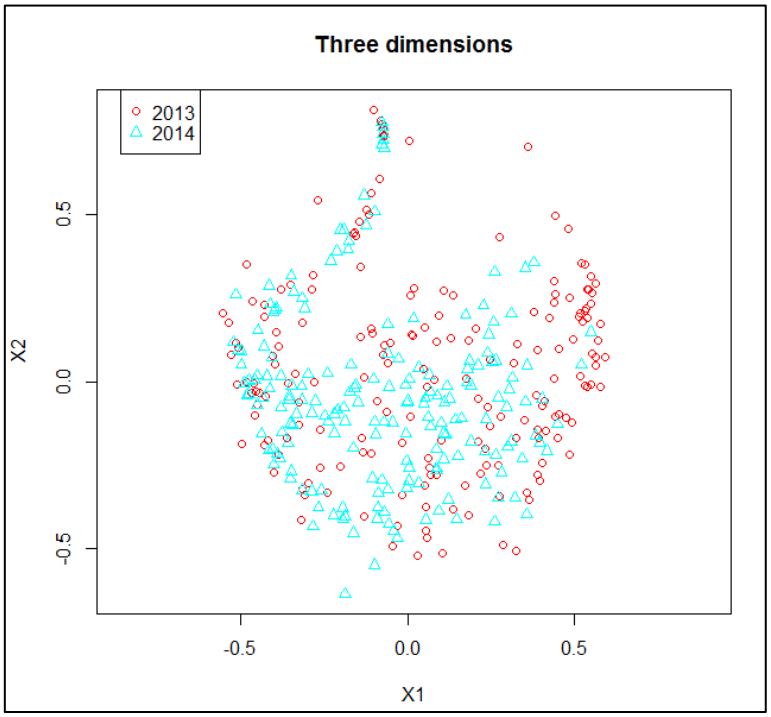

Figure 4.8. NMDS ordinations showing data distribution between years (summers 2013 and 2014) by arthropod Orders in Snook, Texas (minimum stress = 0.1537; r^2 = 0.8761).

Replicate effect

There was no replicate effect (df = 2; F = 1.1204; p = 0.295) among the replicates by Order of soil arthropods. Also, when replicate effect was tested on Function of soil arthropods, the result showed that there was no significant difference (df = 1, F = 0.3213; p = 0.936). Therefore, all data in the replicates were pooled and analyzed.

Community structure and function of soil arthropods in 2013

Total Order in 2013

A total of 13 Orders in the Class Insecta, one Order in the Class Malacostraca, one suborder of the Subclass Acari (Oribatida), two Classes (Diplopoda and Chilopoda), two Phylums (Nematoda and Annelida) and one group of mites (morphospecies from the Order Mesostigmata, Suborder Prostigmata and Cohort Astigmatina) were identified in 2013 trials. Table 4.2 showed the Orders and other taxonomic ranks identified in 2013 trial. The most dominant group was the non-Oribatida mite group (93.27%).

Table 4.2. Total abundance and dominance of Orders in the Class Insecta and other taxonomic ranks identified from all soil samples in summer 2013 at Snook, Texas.

No.	Taxonomic rank		Total abundance	Dominance
1.	Group*	Non-Oribatida mites	155144	93.27
2.	Order	Diptera	4170	2.51
3.	Suborder	Oribatida	3595	2.16
4.	Order	Coleoptera	1492	0.90
5.	Order	Hymenoptera	816	0.49
6.	Order	Collembola	390	0.23
7.	Order	Hemiptera	321	0.19
8.	Order	Psocoptera	281	0.17
9.	Order	Thysanoptera	49	0.03
10.	Order	Isopoda	30	0.02
11.	Order	Araneae	24	0.01
12.	Order	Orthoptera	13	0.01

Table 4.2 (Continued).

No.	Taxonomic rank		Total abundance	Dominance
13.	Order	Protura	1	0.00
14.	Order	Blattodea	2	0.00
15.	Order	Diplura	8	0.00
16.	Order	Lepidoptera	2	0.00
17.	Phylum	Annelida	5	0.00
18.	Phylum	Nematoda	2	0.00
19.	Class	Diplopoda	1	0.00
20.	Class	Chilopoda	1	0.00
		Total	166347	100

* Non-Oribatida mites were assigned as a group of morphospecies that contained members from the Order Mesostigmata, Suborder Prostigmata, Suborder Endeostigmata and Cohort Astigmatina.

Total Family in 2013

A total of 46 families in the Class Insecta, three families in the Class Arachnida (note that Families in the Subclass Acari are treated in a different section of result in this Chapter), and one family in the Class Malacostraca were identified in 2013 trial. Table 4.3 showed the families of soil arthropods (excluding mite families) identified in 2013 trial. The most dominant family was Muscidae, where the muscid larvae were the most encountered Insecta in soil samples.

Table 4.3. Total abundance and dominance of Families of soil arthropods identified from all soil samples in summer 2013 at Snook, Texas.

No.	Order	Family	Total abundance	Dominance
1.	Diptera	Muscidae	3339	48.13
2.	Hymenoptera	Formicidae	812	11.71
3.	Diptera	Calliphoridae	645	9.30
4.	Coleoptera	Tenebrionidae	572	8.25
5.	Coleoptera	Staphylinidae	411	5.92
6.	Coleoptera	Dermestidae	213	3.07
7.	Collembola	Entomobryidae	93	1.34
8.	Psocoptera	Psocidae	91	1.31
9.	Collembola	Sminthuridae	78	1.12
10.	Diptera	Stratiomyidae	62	0.89
11.	Coleoptera	Anthicidae	59	0.85
12.	Diptera	Sarcophagidae	55	0.79
13.	Collembola	Isotomidae	52	0.75
14.	Collembola	Hypogastruridae	52	0.75
15.	Hemiptera	Anthocoridae	49	0.71
16.	Thysanoptera	Thripidae	44	0.63
17.	Collembola	Bourlettilliedae	44	0.63
18.	Coleoptera	Latridiidae	37	0.53
19.	Coleoptera	Scarabaeidae	35	0.50
20.	Psocoptera	Liposcelididae	28	0.40

Table 4.3 (Continued).

No.	Order	Family	Total abundance	Dominance
21.	Coleoptera	Histeridae	20	0.29
22.	Hemiptera	Aphididae	18	0.26
23.	Diptera	Fanniidae	17	0.25
24.	Araneae	Araneidae	15	0.22
25.	Coleoptera	Nitidulidae	14	0.20
26.	Hemiptera	Rhyparochromidae	11	0.16
27.	Coleoptera	Corylophidea	9	0.13
28.	Coleoptera	Curculionidae	7	0.10
29.	Diplura	Japygidae	7	0.10
30.	Hemiptera	Cicadellidae	6	0.09
31.	Coleoptera	Erotylidae	5	0.07
32.	Coleoptera	Silvanidae	5	0.07
33.	Coleoptera	Ptilidae	5	0.07
34.	Coleoptera	Cleridae	3	0.04
35.	Coleoptera	Trogidae	3	0.04
36.	Coleoptera	Silphidae	2	0.03
37.	Coleoptera	Monotomidae	2	0.03
38.	Coleoptera	Carabidae	2	0.03
39.	Coleoptera	Chrysomelidae	2	0.03
40.	Hemiptera	Lasiochilidae	2	0.03

Table 4.3 (Continued).

No.	Order	Family	Total abundance	Dominance
41.	Araneae	Salticidae	2	0.03
42.	Protura	Eosentomidae	1	0.01
43.	Blattodea	Blattidae	1	0.01
44.	Coleoptera	Elateridae	1	0.01
45.	Hemiptera	Reduviidae	1	0.01
46.	Malacostraca	Armadillidiidae	1	0.01
47.	Hemiptera	Rophalidae	1	0.01
48.	Araneae	Philodromidae	1	0.01
49.	Thysanoptera	Phlaeothripidae	1	0.01
50.	Araneae	Thomisidae	1	0.01
		Total	6937	100

Total Genus and species in 2013

A total of 26 genera and species of soil arthropods have been identified in 2013 trial (Table 4.4). The most abundance genus/species encountered was the larvae of *Hydrotaea* sp. (formerly known as *Ophyra* sp.) (Diptera: Muscidae). Note that the mites (Acari) were excluded from this analysis.

Table 4.4. Total abundance and dominance of Genera and species of soil arthropods identified from all soil samples in summer 2013 at Snook, Texas.

No.	Family	Genus and species	Total abundance	Dominance
1.	Muscidae	*Hydrotaea* sp.	3123	67.06
2.	Formicidae	*Solenopsis invicta*	670	14.39
3.	Calliphoridae	*Chrysomya rufifacies*	622	13.36
4.	Stratiomyidae	*Hermetia illucens*	57	1.22
5.	Dermestidae	*Dermestes* sp.	42	0.90
6.	Sarcophagidae	*Sarcophaga* sp.	30	0.64
7.	Scarabaeidae	*Ataenius* sp.	17	0.37
8.	Fanniidae	*Fannia* sp.	16	0.34
9.	Anthicidae	*Vacusus* sp.	15	0.32
10.	Calliphoridae	*Cochliomyia macellaria*	14	0.30
11.	Formicidae	*Brachymyrmex* sp.	12	0.26
12.	Formicidae	*Strumigenys* sp.	8	0.17
13.	Liposcelidae	*Liposcelis* sp.	6	0.13
14.	Corylophidae	*Sericoderus* sp.	6	0.13
15.	Cleridae	*Necrobia rufipes*	3	0.06
16.	Trogidae	*Omorgus suberosus*	3	0.06
17.	Nitidulidae	*Omosita* sp.	3	0.06
18.	Scarabaeidae	*Ataenius platensis*	2	0.04
19.	Anthicidae	*Vacusus vicinus*	1	0.02
20.	Silvanidae	*Ahasverus* sp.	1	0.02

Table 4.4 (Continued).

No.	Family	Genus and species	Total abundance	Dominance
21.	Elateridae	*Aeolus* sp.	1	0.02
22.	Fanniidae	*Fannia scalaris*	1	0.02
23.	Chrysomelidae	*Altica* sp.	1	0.02
24.	Monotomidae	*Monotoma* sp.	1	0.02
25.	Philodromidae	*Tibellus* sp.	1	0.02
26.	Curculionidae	*Baris* sp.	1	0.02
		Total	4657	100

Total function in 2013

Five functional groups of soil arthropods have been identified in 2013 trial (Table 4.5). The most abundance functional group (~63%) was the detritivores (e.g. Collembola, Psocoptera etc.), followed by Predator/Parasite group (~22%) such as Reduviidae, Asilidae or Order Aranea. The third was the necrophagous group, which feed on the carrion (13%) directly such as the fly larvae. This group composed of Family Calliphoridae and Sarcophagidae. For the complete reference of functional groups to all soil arthropods collected in this study, see Appendix G.

Table 4.5. Total abundance and dominance of Functions of soil arthropods identified from all soil samples in summer 2013 at Snook, Texas.

No.	Functional group	Total Abundance	Dominance
1	Detritivore	4680	63.00
2	Predator/Parasite	1632	21.97
3	Necrophagous	968	13.03
4	Herbivore	91	1.22
5	Fungivore	58	0.78
	Total	7429	100

Replicate in 2013

There was no replicate effect detected among soil arthropod data (by Order) in 2013 trial. Hence, replicates were pooled and analyzed together.

Order in 2013

PERMANOVA was performed on soil arthropod data by Order level. Results showed that there was Day effect and Region effect, however, Treatment was not significant. There was also an interaction between Day and Region (Table 4.6).

Table 4.6. Analysis of the soil arthropod community structure (by Order) in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	16.4166	0.0001*
Treatment	2	0.9902	0.450
Region	2	13.7261	0.001*
Day x Treatment	2	1.1307	0.300
Day x Region	2	4.5034	0.0001*
Treatment x Region	4	0.9203	0.575
Day x Treatment x Region	4	0.6778	0.926

Further analyses were carried out on factor Day and Region. For soil regions, all soil regions were significantly different from each other, indicating soil arthropod community structure changes according to location (Table 4.7). All day to day comparisons were significantly different, except Day 14 x Day 21 (Table 4.8). The NMDS plot of stress for soil arthropod community structure (Figure 4.9) and NMDS ordinations for Day and Region were provided for visualization (Figure 4.10 and 4.11, respectively). Minimum stress for given dimensionality was 0.1701 with $r^2 = 0.8314$. The MRPP analysis for soil region showed a significant difference (A value = 0.0653; Significant of Delta = 0.001 based on 999 permutations) and MRPP for day also showed a significant difference with A value 0.109 and Significant of Delta 0.001.

Table 4.7. Pairwise comparisons between Regions on soil arthropod community structure (by Order) in summer 2013 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F Model	R^2	P value
Beneath x Lateral	Region	1	2.242	2.2419	7.2473	0.0554	0.001*
	Residual	124	38.217	0.3082		0.9446	
	Total	125	40.459			1.0000	
Beneath x 5 m	Region	1	5.326	5.3262	17.307	0.1225	0.001*
	Residual	124	38.161	0.3077		0.8775	
	Total	125	43.487			1.0000	
Lateral x 5 m	Region	1	3.098	3.0982	12.437	0.0912	0.001*
	Residual	124	30.891	0.2491		0.9088	
	Total	125	33.989			1.0000	

Table 4.8. Pairwise comparisons of soil arthropod community structure (by Order) between carrion decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
7	0.001*	-	0.036*	0.003*	0.001*	0.001*	0.001*
14	0.001*	0.036*	-	0.659	0.001*	0.001*	0.001*
21	0.001*	0.003*	0.659	-	0.001*	0.001*	0.001*
40	0.001*	0.001*	0.001*	0.001*	-	0.001*	0.028*
90	0.001*	0.001*	0.001*	0.001*	0.001*	-	0.002*
180	0.001*	0.001*	0.001*	0.001*	0.028*	0.002*	-

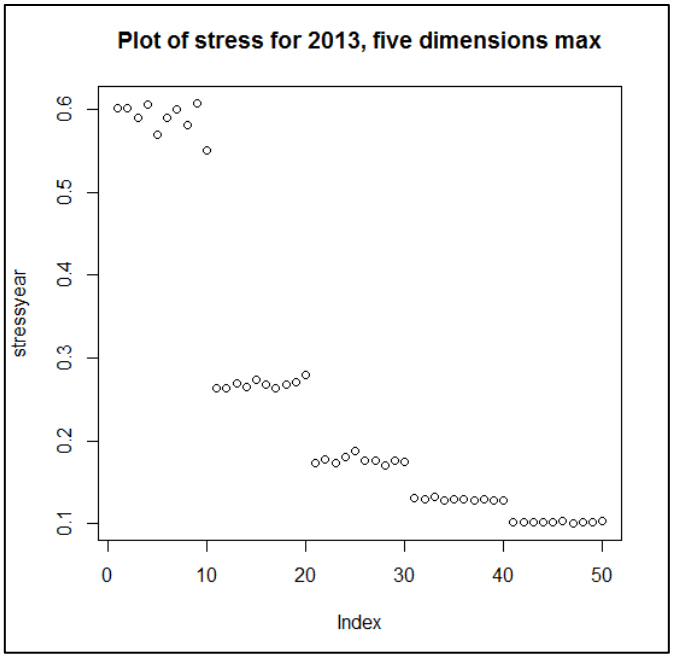

Figure 4.9. NMDS plot of stress for soil arthropod community structure (by Order) in summer 2013 at Snook, Texas (Stress test 0.1701 with $r^2 = 0.8314$).

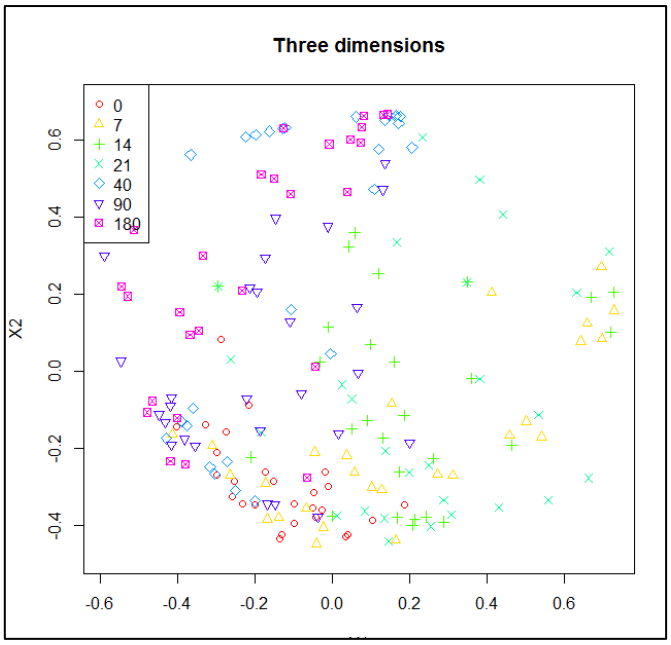

Figure 4.10. NMDS ordinations for soil arthropod community structure (by Order) according to carrion decomposition days in summer 2013 at Snook, Texas.

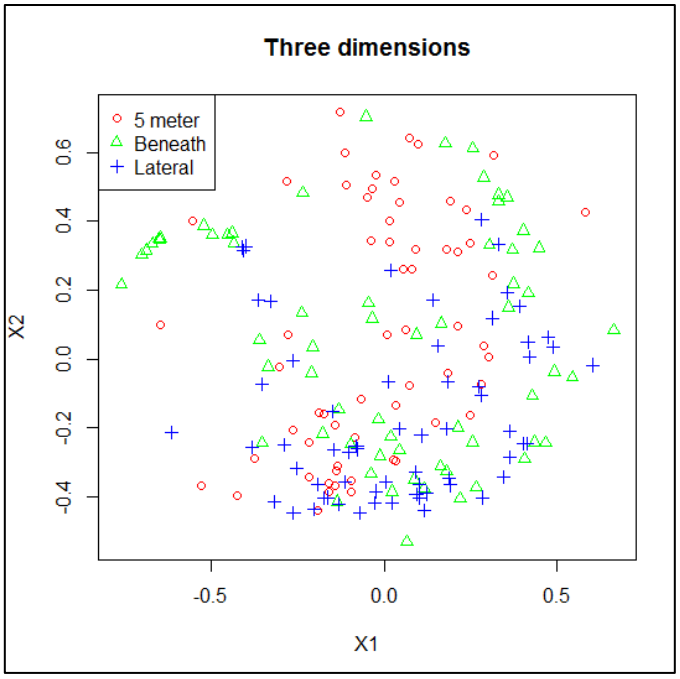

Figure 4.11. NMDS ordinations for soil arthropod community structure (by Order) according to soil regions in 2013.

For ISA, six indicator Orders among soil arthropods in summer 2013 were significant. They were Thysanoptera, Diptera, Collembola, Orthoptera, Psocoptera and Megadrilacea (Table 4.9).

Table 4.9. Indicator species analysis for soil arthropods by Order in summer 2013 at Snook, Texas.

Type	Order	Indicator value	P value
All soils	Thysanoptera	0.1333	0.046*
	Diptera	0.4338	0.001*
	Collembola	0.1005	0.031*
	Orthoptera	0.4103	0.011*
	Psocoptera	0.3594	0.002*
	Megadrilacea (Isopoda)	0.2000	0.029*

Abundance of soil arthropod community structure (by Order) according to soil regions (excluding mites) in 2013

Soil beneath

Soil arthropod community beneath the Control pigs showed higher abundance of Coleoptera from Day 0 to Day 40. However, for Post-7 and Post-14, Diptera larvae were the dominant group of soil arthropods on Day 14 and Day 21, although statistically insignificant compared to the Control (p= 0.556) (Figure 4.12). The abundance of the Orders Diptera and Coleoptera were specifically highlighted (as they are the major necrophagous Orders) at the bottom of Figure 136. The only significant difference detected was on Day 90 of the Order Coleoptera, where Control x Post-7 and Post-7 x Post-14 were significant different p = 0.0238 and 0.0103, respectively).

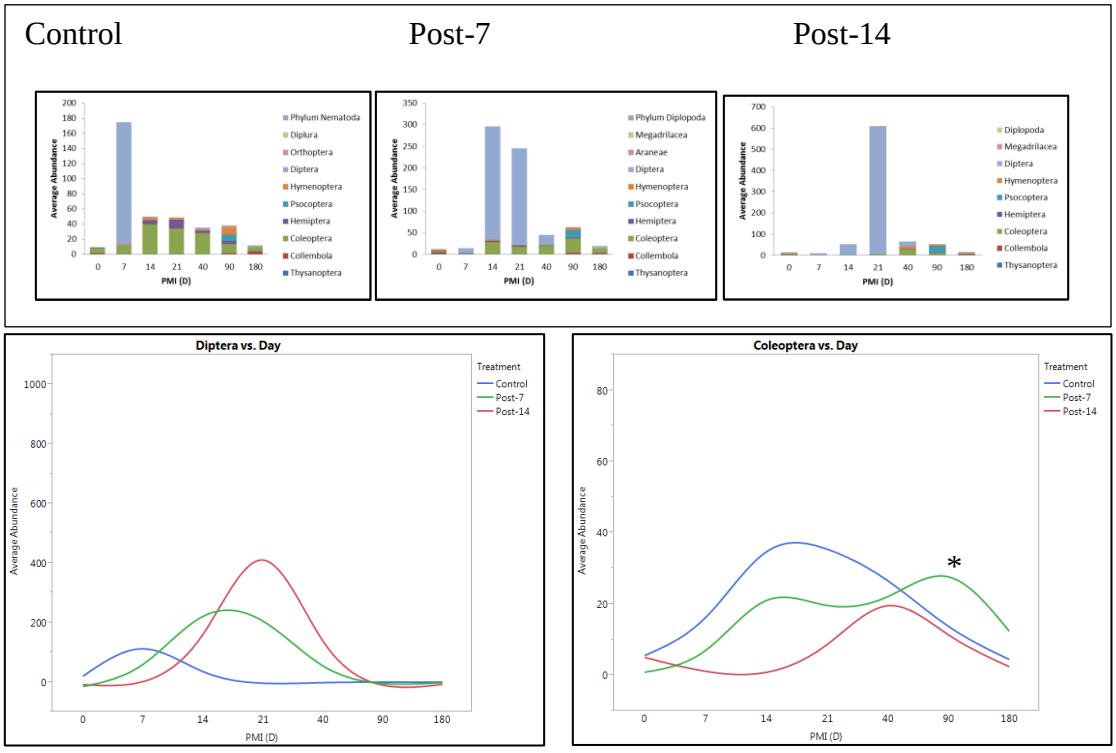

Figure 4.12. Above. Soil arthropod community abundance (by Order) beneath the pig carrion according to Treatments over days in summer 2013 at Snook, Texas. Bottom Left. Abundance of Diptera at soil beneath the carrion across Treatments over day. Bottom Right. Abundance of Coleoptera at soil beneath the carrion across Treatments over day (* indicates significant difference).

Soil lateral

Soil arthropod community beside the Control pigs showed greater abundance of Coleoptera from Day 7 to Day 40, although Hemiptera was almost equally abundant as Coleoptera on Day 21. For Post-7 and Post-14 groups, Hymenoptera (e.g., ants) and Coleoptera were the dominant group of soil arthropods on Day 7 to Day 40. However, there was no significant difference in community structure between Treatments ($p = 0.364$) (Figure 4.13).

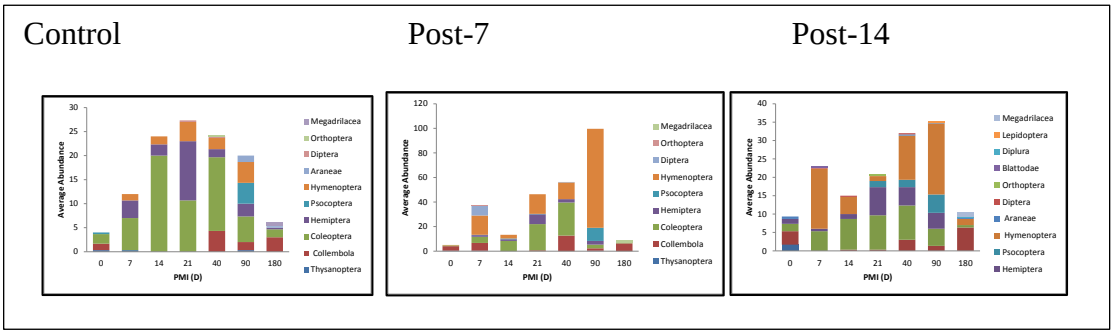

Figure 4.13. Soil arthropod community abundance (by Order) beside the pig carrion according to Treatments over days in summer 2013 at Snook, Texas.

Soil 5 m

Soil arthropod community at the soil 5 m away (served as control soil) from all carrion regardless of Treatment showed varied abundance of soil arthropods, mainly Collembola and Coleoptera. This observation indicates that the soil arthropod community structure at 5 m away was different from those in soil beneath and soil beside the carcasses ($p = 0.001$) (Figure 4.14). However, there was no significant difference found in the community structure between Treatments at soil 5 m ($p = 0.879$).

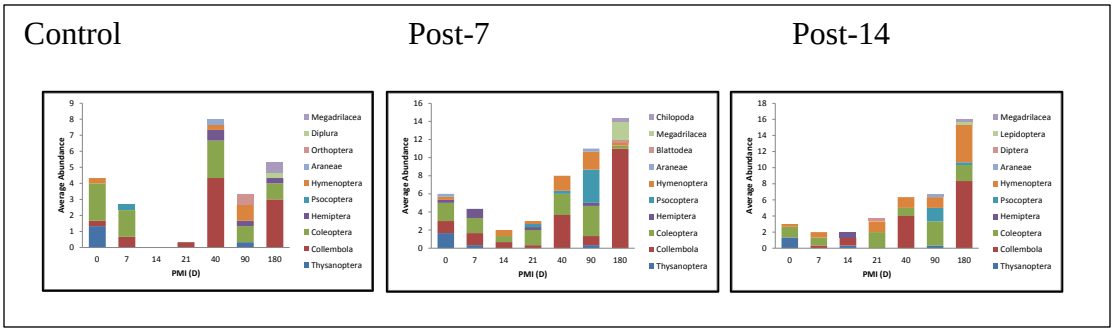

Figure 4.14. Soil arthropod community abundance (by Order) at soil 5 m away from the pig carrion according to Treatments over carrion decomposition days in summer 2013 at Snook, Texas.

Abundance

The full model indicated a significant interaction between Day x Region with $p < 0.0001$. No significant difference was detected for Treatments ($p = 0.7169$). No significant difference was found in abundance between treatments across day or soil region ($p > 0.05$). Figure 4.15 showed soil arthropod community abundance across treatments over day according to soil regions. Resilience was tested only for soil beneath for all treatments. Resilience was observed on Day 90 for Control and Post-14 carcasses ($p > 0.05$) (Table 4.10).

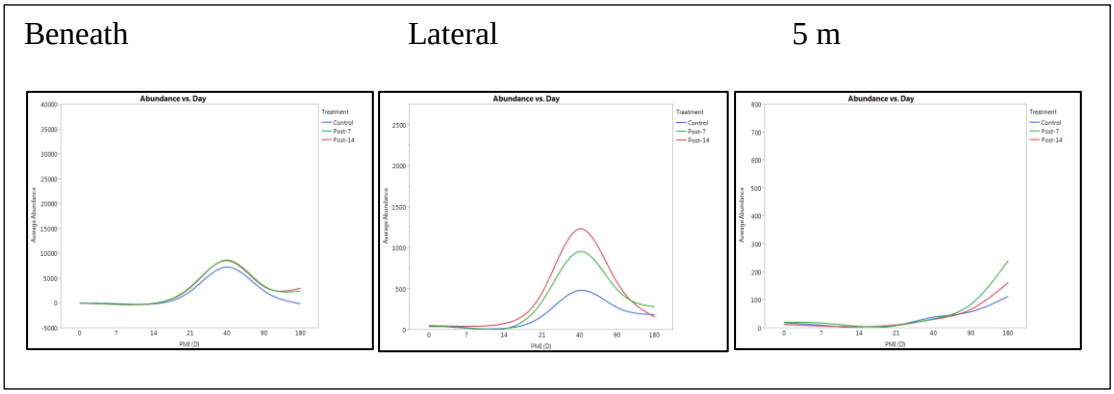

Figure 4.15. Soil arthropod community abundance (by Order) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.10. Resilience for soil arthropod community abundance (by Order) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 40	0.0006*	90
Post-7	None	0.3247	Resistance
Post-14	0 x 40	0.0002*	90

Richness

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) and an interaction between Day x Region with $p = 0.0003$. No significant difference was detected for Treatment ($p = 0.5574$). No statistical difference was detected between Treatments over sampling day at all soil regions ($p > 0.05$). In general, richness at soil beneath of Control carcasses showed higher species richness compared to delayed carcasses. However, richness of Control carcasses in soil lateral and soil at 5 m showed lower richness from Day 7 to Day 21 as compared to other Treatment groups (Figure 4.16). Resilience was observed on Day 180 for Control and on Day 14 for Post-7 carcasses (Table 4.11).

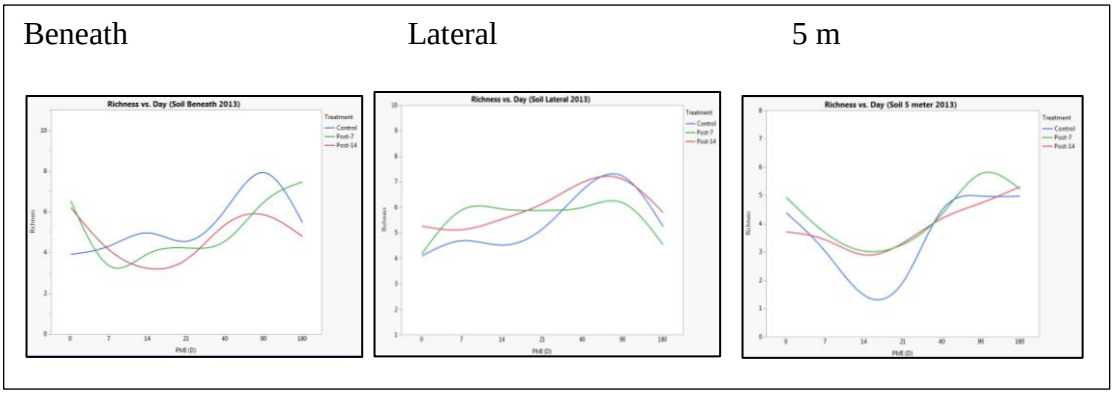

Figure 4.16. Soil arthropod community richness (by Order) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.11. Resilience for soil arthropod community richness (by Order) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference on	P value	Resilience on Day
Control	0 x 90	0.0184*	180
Post-7	0 x 7	0.0134*	14
Post-14	None	0.0267*	Resistance

Simpson's diversity index

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) and three interactions namely Day x Region, Treatment x Region and Day x Treatment x Region with $p < 0.0003$, $p = 0.0020$, and $p = 0.0087$, respectively. No significant difference was detected for Treatment ($p = 0.6653$). Simpson diversity index varied across Treatments over time although there was no significant difference detected at soil beneath and soil lateral (stable community without divergence observed). At soil beneath, the Post-14 group had the highest Simpson's index (~0.80) from Day 14 to Day 40, followed by Post-7 group. At soil lateral, Post-14 carcasses had the lowest diversity compared to other groups from Day 7 to day 40. However, a significant difference was detected at Soil 5 m on Day 14 and Day 21, where ANOVA detected a significant difference between the treatment groups and the Control ($p < 0.05$) (Figure 4.17). Resilience of the soil beneath was observed on Day 14 and 90 for Control and on Day 21 and 90 for Post-14 carcasses (Table 4.12).

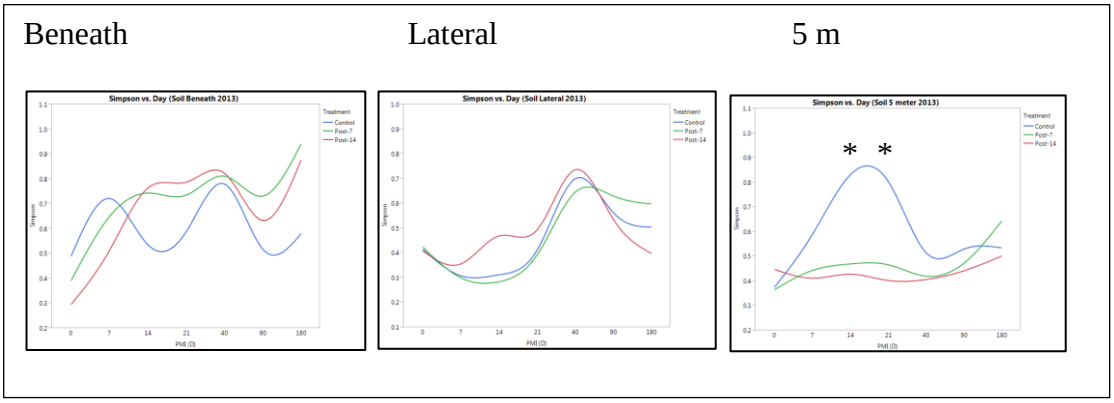

Figure 4.17. Simpson's diversity index of soil arthropods (by Order) across Treatments over time at different soil regions in summer 2013 at Snook, Texas (* indicates significant difference).

Table 4.12. Resilience for soil arthropod community (by Order) by Simpson's diversity index for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference on	P value	Resilience on Day
Control	0 x 7	0.0288*	14
	0 x 40	0.0036*	90
Post-7	None	0.0727	Resistance
Post-14	0 x 14	0.0090*	21
	0 x 40	0.0015*	90

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$). Significant interactions for Day x Region, Treatment x Region and Day x Treatment x Region with $p < 0.0001$, $p = 0.0082$, and $p = 0.0167$ were determined, respectively. There was no statistical difference of Shannon-Wiener's diversity index found between Treatments over every sampling day in soil beneath and soil lateral (no divergence was observed). However, diversity was decreasing for Post-7 and Post-14 carcasses from Day 0 to Day 40, while for Control carcasses, diversity increased from Day 7 to Day 14, and then deceasing on Day 21 and Day 40, and increased again on Day 90 before decreased on Day 180. At soil 5 m, a significant difference was found on Day 21 where Control x Post-7 and Control x Post-14 were statistically different from each other, with p value 0.0300 and 0.0110, respectively (Figure 4.18). Resilience was observed on Day 14 and 90 for Control carcasses. No resilience was observed on Day 180 for Post-7 and Post-14 carcasses (Table 4.13).

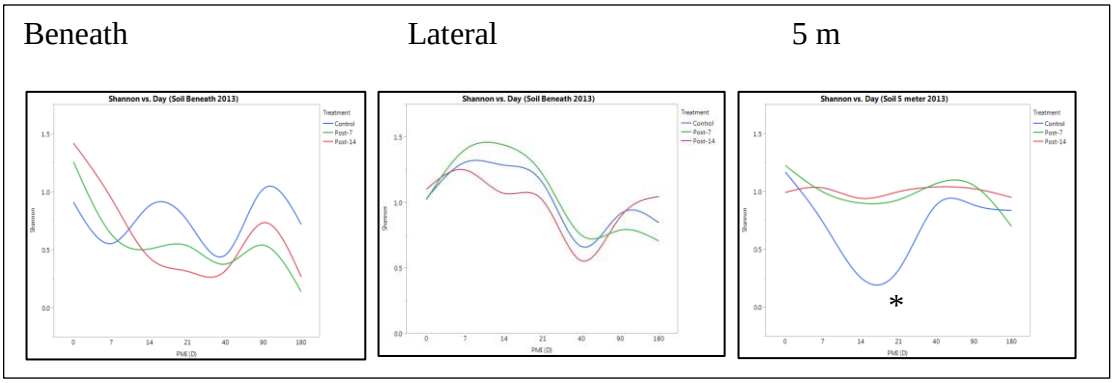

Figure 4.18. Shannon-Wiener's diversity index of soil arthropods (by Order) across Treatments over time at different soil regions in summer 2013 at Snook, Texas (* indicates significant difference).

Table 4.13. Resilience for soil arthropod community (by Order) by Shannon-Wiener's diversity index for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference on	P value	Resilience on Day
Control	0 x 7	0.0397*	14
	0 x 40	0.0026*	90
Post-7	0 x 180	0.0356*	No resilience on Day 180
Post-14	0 x 14	0.0026*	90 and no resilience on Day 180
	0 x 21	0.0123*	
	0 x 40	0.0003*	
	0 x 180	0.0009*	

Evenness

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) and three interactions namely Day x Region, Treatment x Region and Day x Treatment x Region with $p < 0.0001$, $p = 0.0003$, and $p = 0.0248$ were determined, respectively. There was no statistical difference of evenness found between Treatments on every sampling day in soil beneath and soil lateral (no divergence was observed). In general, evenness decreased in Post-7 and Post-14 groups from Day 0 to Day 40 at soil beneath. At soil lateral, evenness increased from Day 0 to Day 7 for all groups, and then decreased all the way to Day 40, and increased thereafter, with Post-14 being the most uneven among other groups. At soil 5 m, a significant difference was detected on Day 21, where Control x Post-14 was found to be significantly different ($p = 0.0378$) (Figure 4.19). Resilience was observed on 90 for Control. For Post-7 carcasses, it was resistant. However, for Post-14 carcasses, there was resilience on Day 21 and 90, but lost the resilience again on Day 180 (Table 4.14).

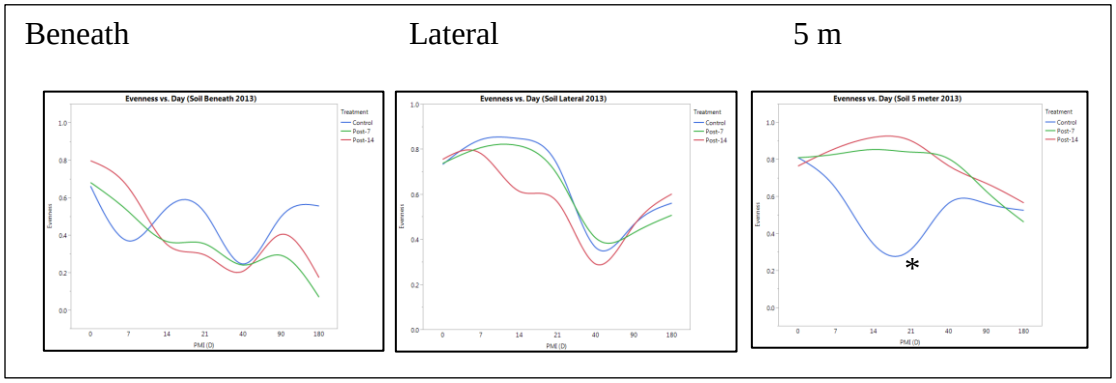

Figure 4.19. Evenness of soil arthropods (by Order) across Treatments over time at different soil regions in summer 2013 at Snook, Texas (* indicates significant difference).

Table 4.14. Resilience for soil arthropod community evenness (by Order) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference on	P value	Resilience on Day
Control	0 x 40	0.0083*	90
Post-7	None	0.1382	Resistance
Post-14	0 x 14	0.0235*	21
	0 x 40	0.0019*	90
	0 x 180	0.0063*	No resilience on Day 180

Effective number of species

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) and an interaction namely Day x Region with $p < 0.0001$. There was no statistical difference for effective number of species (at the Order level) found between Treatments by sampling day in all soil regions (hence, no divergence between treatments by ENS). In general, the effective number of species (Order) decreased from Day 0 to Day 40 for Post-7 and Post-14 groups, while Control carcasses showed an unstable trend (decreased on Day 7, increased on Day 14, decreased on Day 21 and Day 40, and increased on Day 90). The trends of effective number of species in soil lateral and soil 5 m were also different from each other, suggesting high sensitivity of different soil region to the community structure of soil arthropod (Figure 4.20). Resilience was tested only for soil beneath for all treatments and resilience was observed on 90 for Control. For Post-7 carcasses, it was in a resistant state while for Post-14 carcasses, there was no resilience even on Day 180 (Table 4.15).

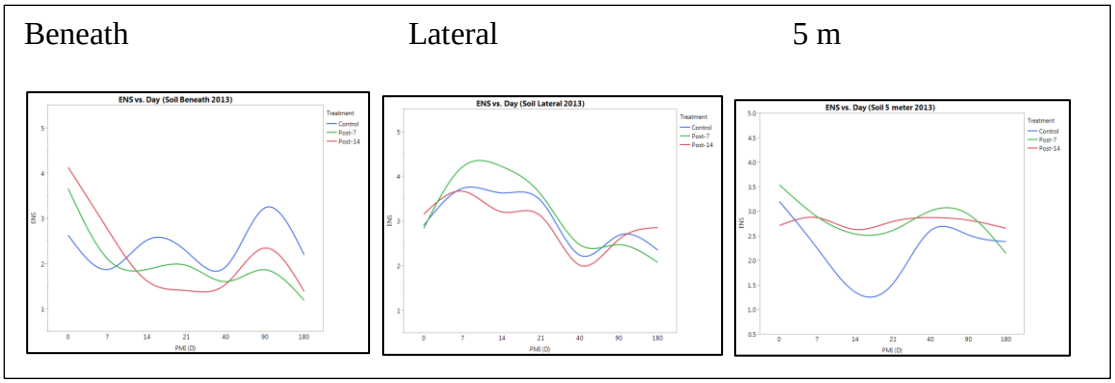

Figure 4.20. Effective number of species (by Order) of soil arthropods across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.15. Resilience for soil arthropod community ENS (by Order) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference on	P value	Resilience on Day
Control	0 x 40	0.0246*	90
Post-7	None	0.0620	Resistance
Post-14	0 x 14	0.0015*	90 and no resilience
	0 x 21	0.0045*	on Day 180
	0 x 40	0.0005*	
	0 x 180	0.0007*	

Family in 2013

PERMANOVA was performed on soil arthropod data by Family level. Results showed that there was Day, Treatment and Region effect ($p < 0.05$). There was also an interaction between Day and Region (Table 4.16).

Table 4.16. Analysis of the soil arthropod community structure (by Family) in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	13.0235	0.001*
Treatment	2	1.4671	0.050*
Region	2	7.3503	0.001*
Day x Treatment	2	0.9265	0.557
Day x Region	2	2.3774	0.001*
Treatment x Region	4	0.9967	0.457
Day x Treatment x Region	4	1.0440	0.373

There was a significant effect in Day, Treatment and Region, therefore further analyses were conducted. All soil regions were significantly from each other, indicating soil community structure changes according to region, although soil beneath and soil lateral was just 30 cm away (Table 4.17). As for day of decomposition, most of the pairwise comparisons between days of decomposition were significantly different, except three pairs namely Day 7 x Day 14, Day 7 x Day 21, and Day 14 x Day 21 where there were no significant differences detected (Table 4.18). In other words, the comparison of those days have no difference in terms of soil arthropod community structure by Families. For Treatments, pairwise comparison showed Control x Post-14 was significantly different ($p = 0.027$) (Table 4.19). The NMDS plot of stress for soil arthropod community structure (Figure 4.21) and NMDS ordinations for Day, Region and Treatment were provided for visualization (Figure 4.22, 4.23 and 4.24, respectively). Minimum stress for a given dimensionality was 0.2440 with $r^2 = 0.5740$ (the strength of correlation was moderate in this case). The MRPP analysis for soil region showed a significant difference (A value = 0.03858; Significant of Delta = 0.001 based on 999 permutations), the MRPP for day also showed a significant difference with A value

0.0753 and Significant of Delta 0.001 while the MRPP for treatments was A = 0.0017 with Significant of Delta 0.147.

Table 4.17. Pairwise comparisons between Regions on soil arthropod community structure by Family in summer 2013 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	1.489	1.4894	4.0556	0.0317	0.001*
	Residual	124	45.540	0.3672		0.9683	
	Total	125	47.029			1.0000	
Beneath x 5 m	Region	1	3.487	3.4874	9.2634	0.0695	0.001*
	Residual	124	0.3765			0.9305	
	Total	125				1.0000	
Lateral x 5 m	Region	1	2.455	2.4550	6.9573	0.0531	0.001*
	Residual	124	43.756	0.3528		0.9469	
	Total	125	46.211			1.0000	

Table 4.18. Pairwise comparisons of soil arthropod community structure by Family between carrion decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.002*	0.001*	0.001*	0.001*	0.001*	0.001*
7	0.002*	-	0.1	0.066	0.003*	0.001*	0.001*
14	0.001*	0.1	-	0.865	0.002*	0.001*	0.001*
21	0.001*	0.066	0.865	-	0.001*	0.001*	0.001*
40	0.001*	0.003*	0.002*	0.001*	-	0.001*	0.001*
90	0.001*	0.001*	0.001*	0.001*	0.001*	-	0.001*
180	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	-

Table 4.19. Pairwise comparisons between Treatments on soil arthropod community structure by Family in summer 2013 at Snook, Texas after Bonferroni's correction.

Region			df	SS	MS	F model	R2	P value
Control	x	Treatment	1	0.404	0.4037	1.031	0.0080	0.432
Post-7		Residual	124	48.554	0.3915		0.9920	
		Total	125	48.957			1.0000	
Control	x	Treatment	1	0.718	0.7182	1.888	0.0150	0.027*
Post-14		Residual	124	47.149	0.3802		0.9850	
		Total	125	47.867			1.0000	
Post-7	x	Treatment	1	0.361	0.3614	0.9298	0.0074	0.495
Post-14		Residual	124	48.208	0.3887		0.9926	
		Total	125	48.569			1.0000	

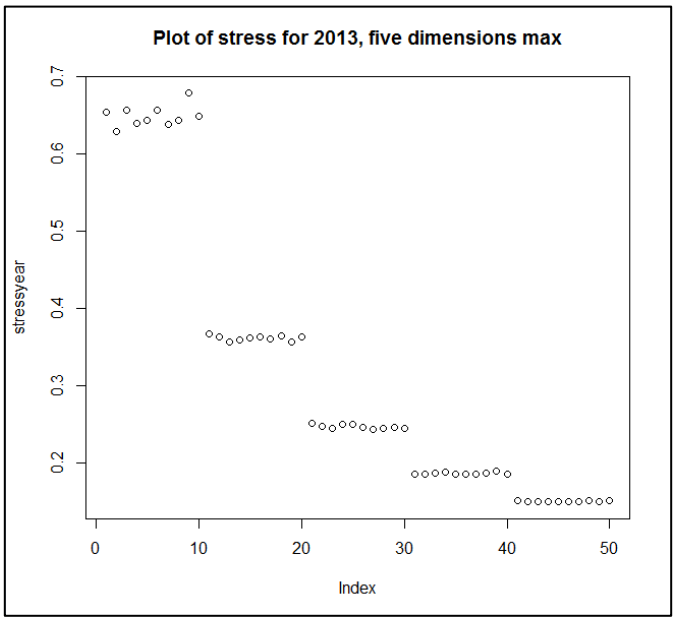

Figure 4.21. NMDS plot of stress for soil arthropod community structure (by Family) in summer 2013 at Snook, Texas (Stress test 0.2440 with $r^2 = 0.5740$).

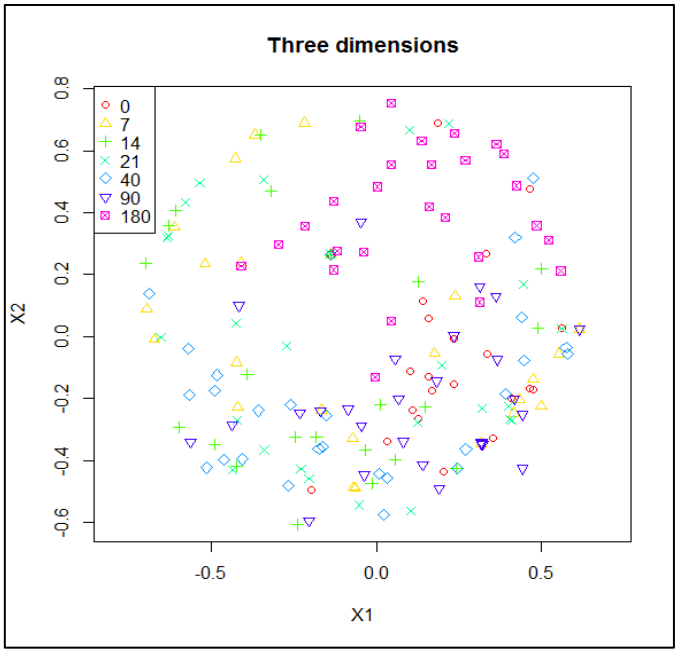

Figure 4.22. NMDS ordinations for soil arthropod community structure (by Family) according to carrion decomposition days in summer 2013 at Snook, Texas.

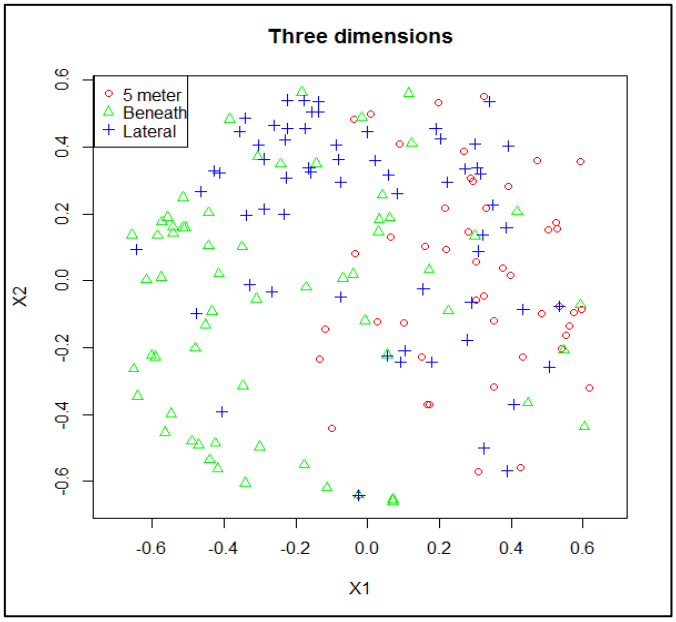

Figure 4.23. NMDS ordinations for soil arthropod community structure (by Family) according to soil regions in summer 2013 at Snook, Texas.

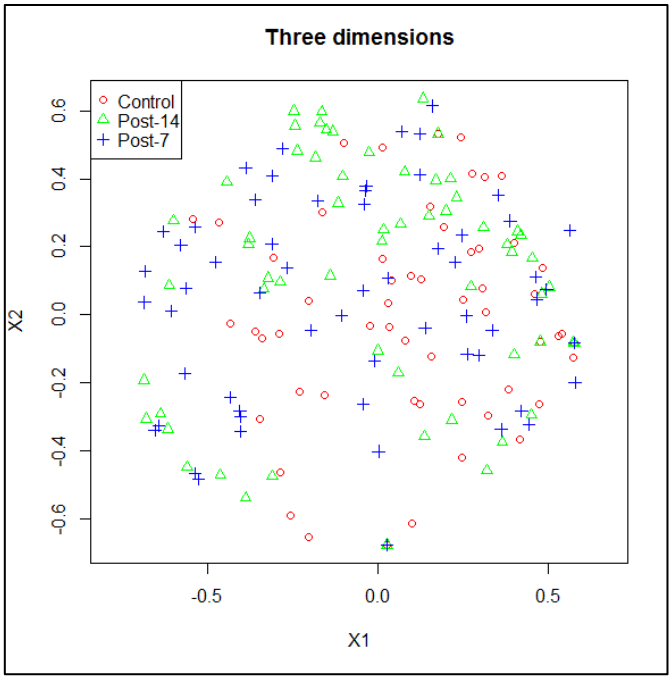

Figure 4.24. NMDS ordinations for soil arthropod community structure (by Family) according to treatments in summer 2013 at Snook, Texas.

Results of ISA showed six family indicators among soil arthropods for pig carcasses exposed in summer 2013. They were Calliphoridae, Dermestidae, Sarcophagidae, Muscidae, Erotylidae and Sminthuridae (Table 4.20). Note that three families (Calliphoridae, Sarcophagidae, Muscidae) are in the Order Diptera, one family in the Order Coleoptera and one in Collembola. All dipteran indicators are necrophagous, the erotylid beetle is a fungivore, and the sminthurid is detritivorous collembolan.

Table 4.20. Indicator species analysis by Family for soil arthropods in summer 2013 at Snook, Texas.

Type	Family	Indicator value	P value
All soils	Calliphoridae	0.6930	0.003*
	Dermestidae	0.2676	0.003*
	Sarcophagidae	0.6545	0.012*
	Muscidae	0.4683	0.001*
	Erotylidae	0.4444	0.036*
	Sminthuridae	0.1781	0.030*

Abundance of soil arthropod community structure (by Family) according to soil regions (excluding mites) in 2013

Soil beneath

Soil arthropod community beneath the Control pigs showed higher abundance of Calliphoridae larvae on Day 7, suggesting an active decomposition stage on pig carrion. However, for Post-7 and Post-14, Muscidae larvae were the dominant group of soil arthropod on Day 14 and Day 21. The shift in soil arthropod community (by family) beneath the carrion in different Treatments was observed between Control x Post-14, $p = 0.027$ (Figure 4.25). The abundance of the Family Calliphoridae, Muscidae, Dermestidae and Staphylinidae was specifically highlighted (as they are the major necrophagous families) in Figure 4.25.

At the soil beneath, there was no significant difference in abundance in each Family between Treatments on every sampling day. However, abundance of Dermestidae was marginally significant difference on Day 14 ($p = 0.0693$) by ANOVA test as well as Family Staphylinidae on Day 21 ($p = 0.0519$), where the Control group was more abundant than the treatment groups.

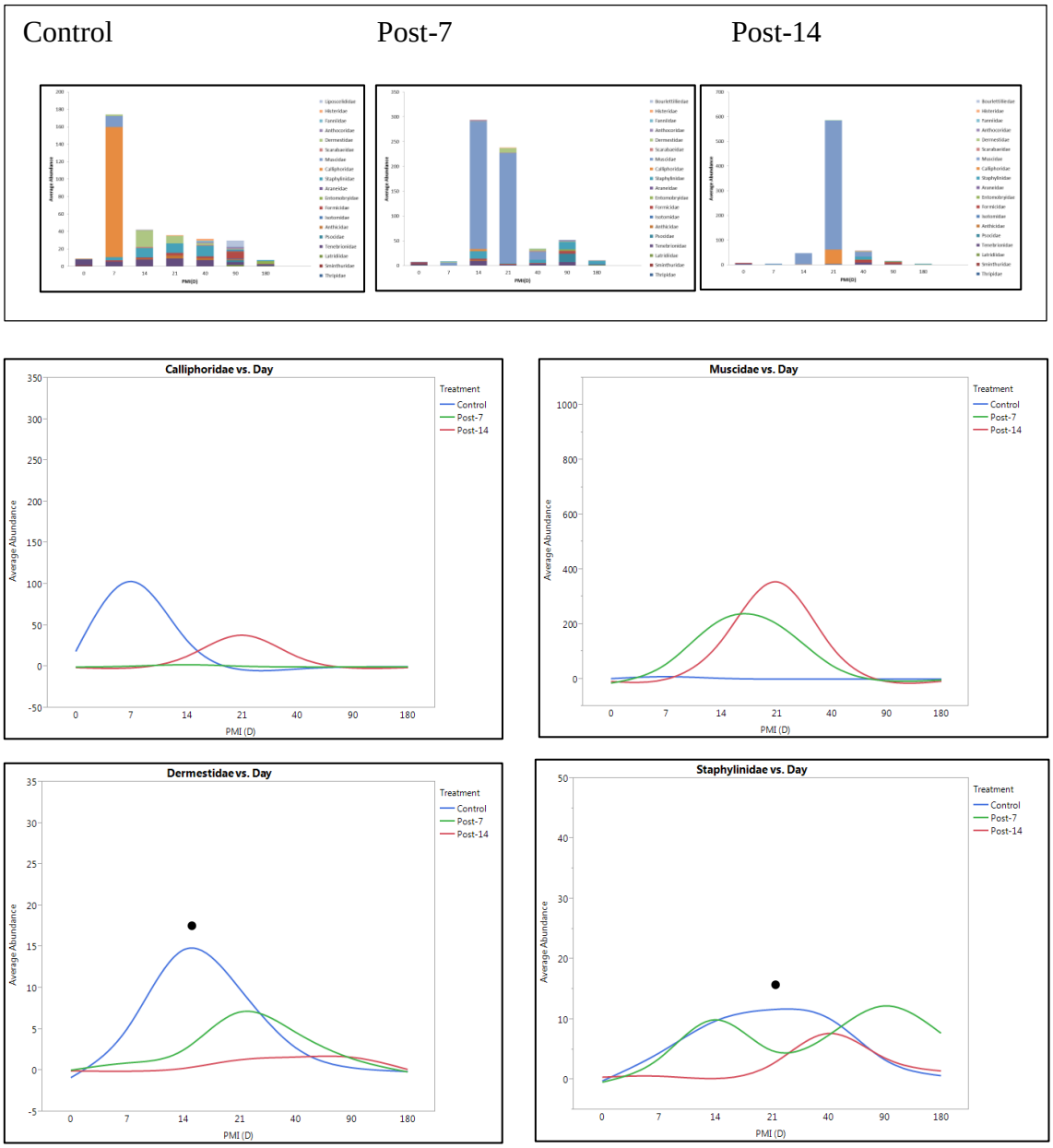

Figure 4.25. Above. Soil arthropod community abundance (by Family) beneath the pig carrion according to Treatments over carrion decomposition days in summer 2013 at Snook, Texas. Middle Left. Abundance of Calliphoridae (larvae). Middle Right. Abundance of Muscidae (larvae). Lower Left. Abundance of Dermestidae (adults and larvae). Lower Right. Abundance of Staphylinidae (adults) across treatments over time at soil beneath the carrion. (•denotes marginal significant difference).

Soil lateral

Soil arthropod community beside the Control pigs showed higher abundance of Family Tenebrionidae (Coleoptera) from Day 14 to Day 40 as the function of tenebrionid beetles are detritivores. Several individuals of Calliphoridae larvae can be seen on Day 7. The ants (Formicidae) can be collected throughout the decomposition process and were most abundant on Day 21. For Post-7 carcasses, Formicidae was the dominant group in the soil arthropod community on Day 7, Day 14, and Day 90 while for Post-14, ants were dominant on Day 7, Day 14, Day 40 and Day 90. There was a contrasting pattern in family distribution of soil arthropods between Control and treatments at the soils beside to swine carcasses, although statistically insignificant ($p = 0.296$) (Figure 4.26).

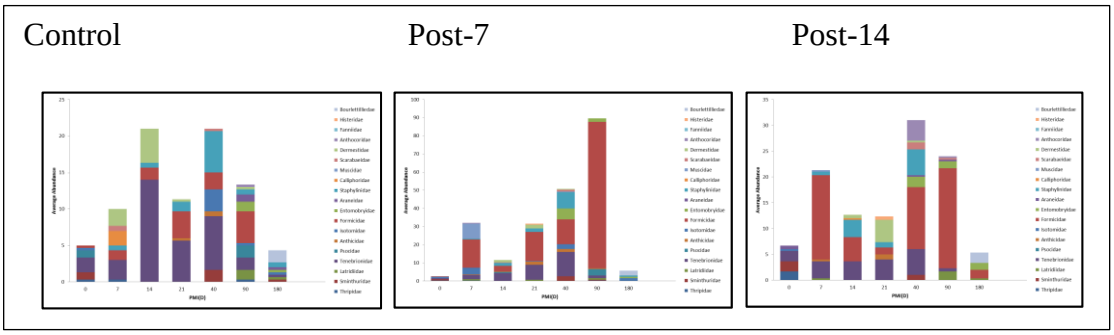

Figure 4.26. Soil arthropod community abundance (by Family) beside the carrion according to Treatments over carrion decomposition days in summer 2013 at Snook, Texas.

Soil 5 m

The soils at 5 m away from carrion were served as control to the soils collected from beneath and lateral of the carrion. The arthropod community structure at soil 5 m was different compared to soil beneath and soil lateral. For all treatment groups, beetle (Tenebrionidae) and thrips (Thripidae) were dominant on Day 0 and Day 7 while Collembola (Sminthuridae) became dominant on Day 40. Moreover, two collembolan

families (Entomobryidae and Bourletiellidae) were present and quite abundant on Day 180 at all soil regions, this observation may indicate succession of collembolan families (they all were categorized as detritivores) over time. There was no significant difference between Treatments at soil 5 m ($p = 0.672$) (Figure 4.27).

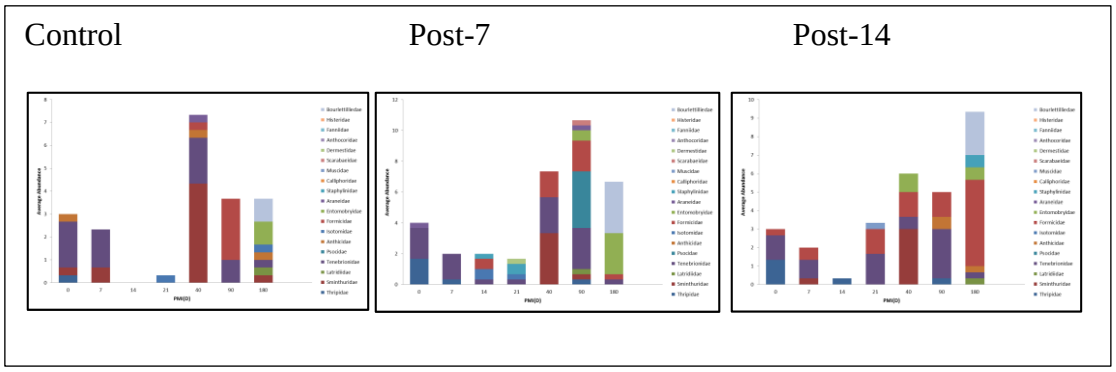

Figure 4.27. Soil arthropod community abundance (by Family) at soil 5 m away from the carrion according to Treatments over carrion decomposition days in summer 2013 at Snook, Texas.

Abundance

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) and three interactions include Day x Treatment with $p = 0.0004$, Day x Region with $p < 0.0001$, and Day x Treatment x Region, with $p < 0.0001$. No significant difference was detected for Treatment ($p = 0.1407$). No significant difference was found in abundance between treatments by sampling day at every soil region ($p > 0.05$), except on Day 14 at soil beneath ($p = 0.0460$) and on Day 180 at soil 5 m (Control x Post-14, $p = 0.0438$) where divergence occurred. For soil beneath, convergence occurred on Day 21 (Figure 4.28). Resilience was tested only for soil beneath for all treatments and resilience was observed on Day 40 for Post-14 carcasses while Control and Post-7 carcasses demonstrated a stable abundance over time (Table 4.21).

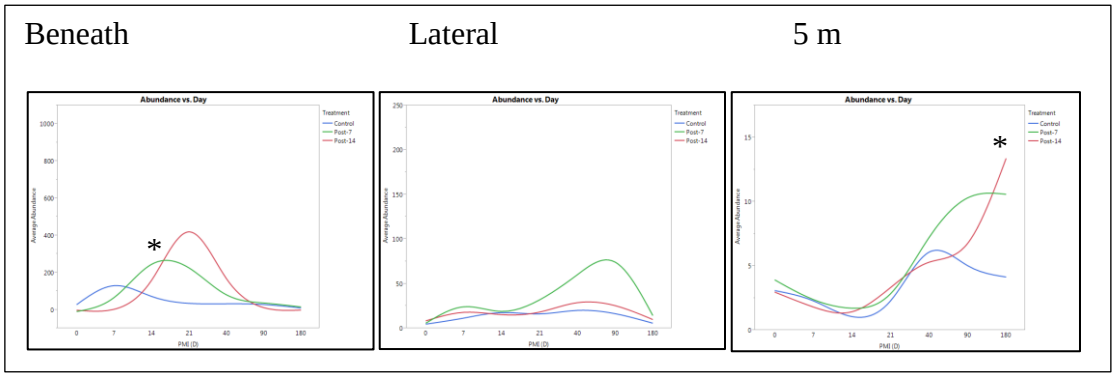

Figure 4.28. Soil arthropod community abundance (by Family) across Treatments over time at different soil regions in summer 2013 at Snook, Texas (* indicates significant difference).

Table 4.21. Resilience for soil arthropod community abundance (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1516	Resistance
Post-7	None	0.1061	Resistance
Post-14	0 x 21	0.0020*	40

Richness

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) and an interaction Day x Region with $p = 0.0053$. No significant difference was detected for Treatments ($p = 0.7126$). No statistical difference was detected between Treatments by sampling day at all soil regions (no divergence observed, the community was resistant by richness). In general, family-level richness at soil beneath of Post-14 carcasses showed lower richness compared to Control and Post-7 carcasses on Day 14 and Day 21. Conversely, richness of Control carcasses at soil lateral was lower compared to treatment groups from Day 0 to Day 21. As for soil at 5 m, all groups showed similar trend in family richness from Day 0 to Day 40, except Post-7 had the highest richness on Day 90, and then declined on Day 180. Note that the pair of Control

x Post-7 on Day 90 at soil 5 m had a p value of 0.0507, which was almost significant (Figure 4.29). Resilience was observed on Day 180 for Control carcasses while Post-7 and Post-14 carcasses demonstrated a stable richness over time (Table 4.22).

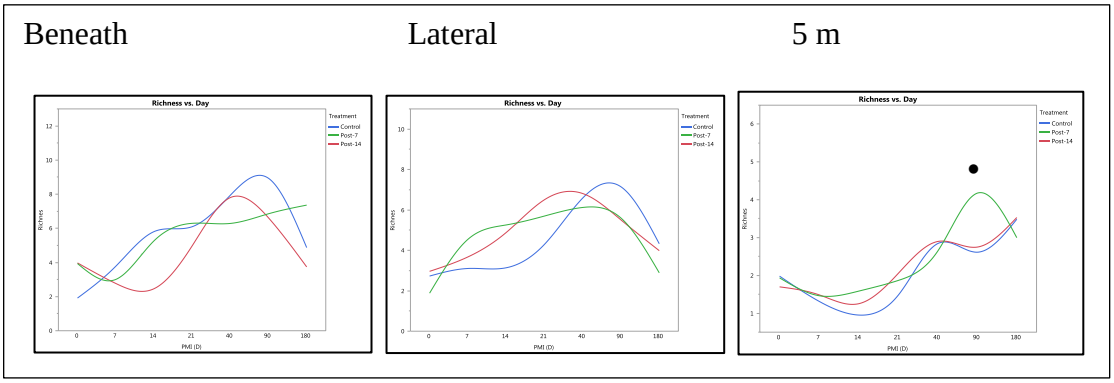

Figure 4.29. Soil arthropod community richness (by Family) across Treatments over time at different soil regions in summer 2013 at Snook, Texas (• denotes marginal significant difference).

Table 4.22. Resilience for soil arthropod community richness (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 90	0.0050*	180
Post-7	None	0.3206	Resistance
Post-14	None	0.0053*	Resistance[#]

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Simpson's diversity index

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) with an interaction Day x Treatment x Region ($p = 0.0037$). No significant difference was detected for Treatment ($p = 0.9539$). Simpson's diversity index varied across Treatments over time at different soil region. At soil beneath, significant differences (divergence) were found at Control x Post-7 and Control x Post-14 on Day 0, with $p = 0.0027$ and 0.0044, respectively. This may due to the heterogeneity (or stochastic) of soil arthropod community in the soil during the initial day of experiment. Convergence occurred on Day 7, indicating that perturbation in ecosystem (i.e., introduction of carrion) "reset" the soil arthropod community diversity. Soil arthropod family diversity increased for Control carcasses from Day 0 to Day 40 (indicating active decomposition process attracted a more diverse of arthropod families), and the diversity decreased gradually on Day 180, while the Post-7 and Post-14 group had higher Simpson's index (means lower diversity in family) from Day 7 to Day 40. At soil lateral, Post-7 and Post-14 carcasses had significantly higher diversity compared to Control carcasses on Day 14 ($p = 0.0317$ and 0.0423, respectively), suggesting delayed insect colonization (treatment effect) on carrion did attract more diverse soil arthropods even at soil lateral. At soil 5 m, a decreasing trend of diversity over time was observed in all groups from Day 0 to Day 40 in general, however, significant differences in family diversity were observed on Day 21 between Control and Post-7 ($p = 0.0451$) (Figure 4.30). Resilience was not observed even on Day 180 for Control carcasses while Post-7 and Post-14 carcasses demonstrated a stable Simpson's diversity over time (Table 4.23).

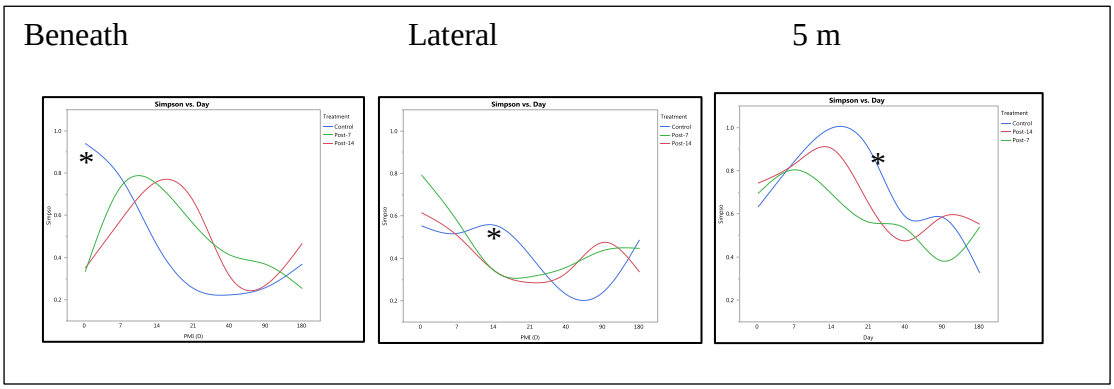

Figure 4.30. Simpson's diversity index of soil arthropod community (by Family) across Treatments over time at different soil regions in summer 2013 at Snook, Texas (* indicates significant difference).

Table 4.23. Resilience for soil arthropod community by Simpson's diversity (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 14	0.0087*	No resilience on Day
	0 x 21	0.0005*	180
	0 x 40	0.0006*	
	0 x 90	0.0006*	
	0 x 180	0.0044*	
Post-7	None	0.1200	Resistance
Post-14	None	0.0613	Resistance

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) with an interaction Day x Treatment x Region ($p = 0.0045$). No significant difference was detected for Treatment ($p = 0.9659$). At soil beneath, significant difference (divergence) was found on Control x Post-7 and Control x Post-14 on Day 0 with $p = 0.0110$ and 0.0169, respectively. The phenomenon could be due to the stochastic arthropod community in the soil on the first day of experiment. Note that

convergence occurred on Day 7, indicating the introduction of carrion into the ecosystem "reset" the soil arthropod diversity. Diversity in arthropod family was decreased on Post-7 and Post-14 carcasses on Day 7 and Day 14, respectively. This indicates delayed insect colonization on carrion decreased soil arthropod diversity at soil beneath the carrion. While for Control carcasses, diversity increased from Day 0 to Day 90, and then deceased on Day 180. Higher diversity was noted at soil lateral for Post-7 and Post-14 groups from Day 7 to Day 21, possibly due to the impact of delayed Diptera colonization on these treatment groups. On Day 14, the Post-7 group had a significant higher diversity with Control group ($p = 0.0259$), although Post-14 almost had a significant higher diversity than Control ($p = 0.0516$). At soil 5 m, diversity decreased for all groups from Day 0 to Day 14, and then increased until Day 40 (Figure 4.31). Resilience was observed on Day 180 for Control carcasses while Post-7 and Post-14 carcasses demonstrated a stable Shannon's diversity over time (Table 4.24).

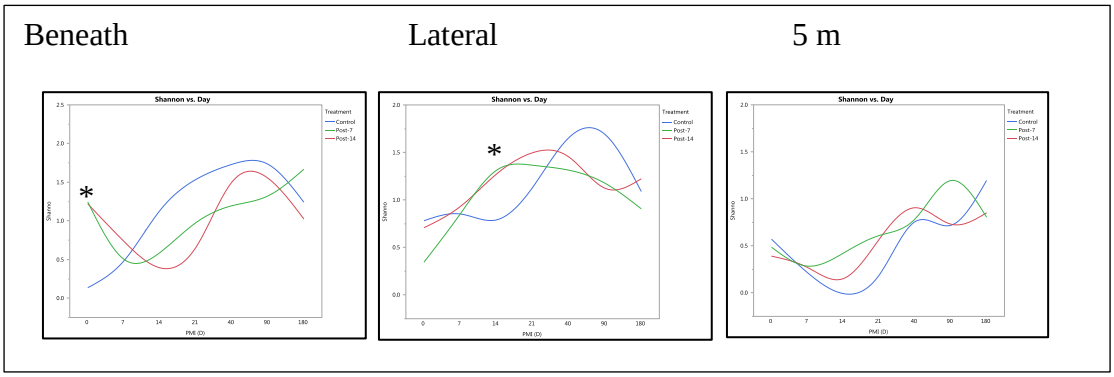

Figure 4.31. Shannon-Wiener's diversity index of soil arthropod community (by Family) across Treatments over time at different soil regions in summer 2013 at Snook, Texas (* indicates significant difference).

Table 4.24. Resilience for soil arthropod community by Shannon's diversity (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 21	0.0106*	180
	0 x 40	0.0044*	
	0 x 90	0.0018*	
Post-7	None	0.1813	Resistance
Post-14	None	0.0171*	Resistance[#]

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Evenness

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p = 0.0213$) with an interaction Day x Treatment x Region ($p = 0.0081$). Statistical difference (divergence) of family evenness found between Treatments on Day 0 at soil beneath (Control x Post-7 and Control x Post-14, $p = 0.0015$ and 0.0023, respectively). Convergence occurred on Day 7, indicating that introduction of carrion "reset" the soil arthropod diversity. At soil beneath, there was a big gap in evenness between Treatments on Day 0. For Control carcasses, evenness increased from Day 0 to Day 21 while evenness decreased in Post-7 and Post-14 groups from Day 0 to Day 14 at soil beneath. At soil lateral, a contrast pattern was observed where evenness increased from Day 0 to Day 7 for Post-7 and Post-14 carcasses, and then decreased all the way to Day 90, and increased again on Day 180. At soil 5 m, Control and Post-14 carcasses achieved the lowest evenness value on Day 14, whereas Post-7 carcasses were on Day 7. The family evenness then increased until Day 40. Significant difference in evenness was observed between Control and Post-7 on Day 21, with $p = 0.0223$ (Figure 4.32). Resilience was not observed even on Day 180 for Control carcasses while Post-7 and Post-14 carcasses demonstrated a stable evenness over time (Table 4.25).

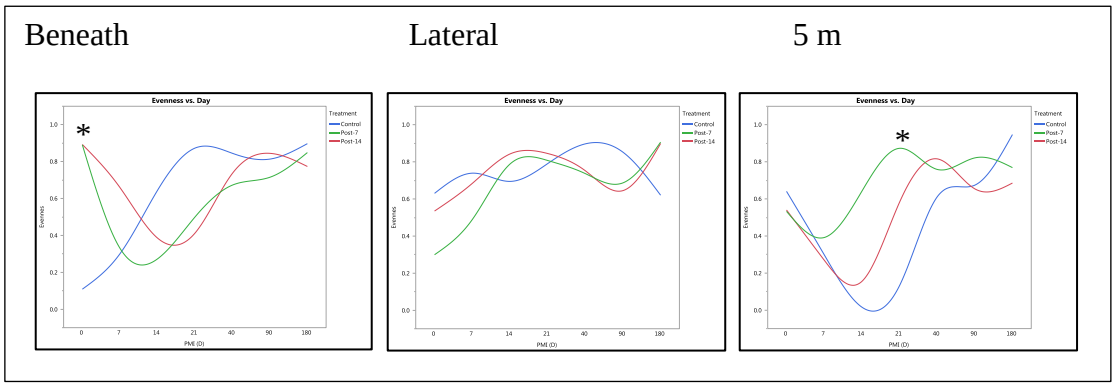

Figure 4.32. Evenness of soil arthropod families across Treatments over time at different soil regions in summer 2013 at Snook, Texas (* indicates significant difference).

Table 4.25. Resilience for soil arthropod community evenness (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 14	0.0039*	No resilience on Day
	0 x 21	<0.0001*	180
	0 x 40	0.0003*	
	0 x 90	0.0004*	
	0 x 180	<0.0001*	
Post-7	None	0.0551	Resistance
Post-14	None	0.1320	Resistance

Effective number of species

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) with an interaction Day x Treatment x Region ($p = 0.0083$). There was statistical difference (divergence) of effective number of species (ENS) found between Treatments on Day 0 at soil beneath between Control x Post-7 ($p = 0.0360$). Convergence occurred on Day 7 indicating the introduction of carrion "reset" the soil arthropod community structure. In general, ENS decreased from Day 0 to Day 7 for

Post-7, and decreased from Day 0 to Day 14 for Post-14 groups, while the Control carcasses increased in effective number of family from Day 0 to Day 90. At soil lateral, a contrast pattern was observed where the ENS values for Post-7 and Post-14 increased from Day 0 to Day 14. Post-7 achieved a plateau from Day 14 to Day 90. As for Control carcasses at soil lateral, ENS decreased from Day 0 to Day 14 (on Day 14, the Control group was significantly different with the Post-7, $p = 0.0402$), and then increased tremendously on Day 90. On the other hand, the trends of ENS in soil 5 m were similar among all treatment groups, except on Day 90, where Post-7 and Control had a marginal significant difference ($p = 0.0510$). Soil beneath and soil lateral demonstrated contrasting pattern of ENS among treatments, suggesting high sensitivity of different soil region to the community structure of soil arthropods (Figure 4.33). Resilience was tested only for soil beneath for all treatments and resilience was observed on Day 180 for Control carcasses while Post-7 and Post-14 carcasses demonstrated a stable community by ENS over time (Table 4.26).

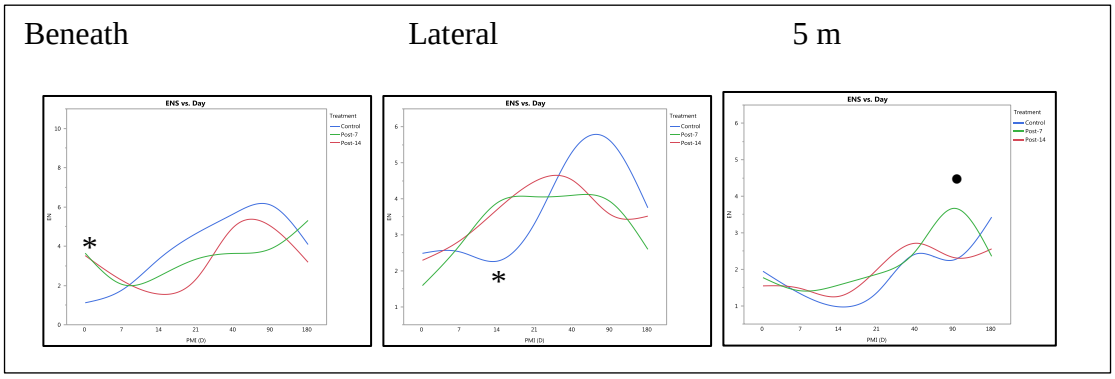

Figure 4.33. Effective number of family of soil arthropod community across Treatments over time at different soil regions in summer 2013 at Snook, Texas (• denotes marginal significant result; * represents significant difference).

Table 4.26. Resilience for soil arthropod community by ENS (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 90	0.0154*	180
Post-7	None	0.2925	Resistance
Post-14	None	0.0162*	Resistance[#]

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Genus in 2013

PERMANOVA was performed on soil arthropod data by Genus level. Results showed that there was Day effect and Region effect (p = 0.042 and 0.001, respectively) (Table 4.27).

Table 4.27. Analysis of the soil arthropod community structure (by Genus) in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	2.1759	0.042*
Treatment	2	0.0129	0.143
Region	2	15.4633	0.001*
Day x Treatment	2	0.7627	0.691
Day x Region	2	1.0080	0.375
Treatment x Region	4	1.0850	0.323
Day x Treatment x Region	4	0.4611	0.992

There was a significant effect in Day and Region, further analyses were carried out. For soil regions, all soil regions were significantly from each other (p = 0.001),

indicating soil community structure changes according to region, although soil beneath and soil lateral was just 30 cm away (Table 4.28). As for day of decomposition, most of the pairwise comparisons between days of decomposition were significantly different, except eight pairs namely Day 7 x Day 14, Day 7 x Day 21, Day 7 x Day 40, Day 14 x Day 21, Day 14 x Day 40, Day 21 x Day 90, Day 40 x Day 90, and Day 0 x Day 180 where there were no significant differences detected (Table 4.29). In other words, the comparisons of those days have no difference in terms of soil arthropod community structure by genus. The NMDS plot of stress for soil arthropod community structure (Figure 4.34) and NMDS ordinations for Day and Region were provided for visualization of data distribution (Figure 4.35 and 4.36, respectively). Minimum stress for given dimensionality was 0.1420 with r^2 = 0.9428. The MRPP analysis for soil region showed a significant difference (A value: 0.118; Significant of Delta = 0.001 based on 999 permutations) while MRPP for day also showed a significant difference with A value 0.0603 and Significant of Delta 0.001.

Table 4.28. Pairwise comparisons between Regions on soil arthropod community structure by Genus in summer 2013 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	2.469	2.4689	8.0377	0.0609	0.001*
	Residual	124	38.090	0.3071		0.9391	
	Total	125	40.559			1.0000	
Beneath x 5 m	Region	1	5.738	5.7379	25.671	0.1715	0.001*
	Residual	124	27.716	0.2235		0.8285	
	Total	125	33.454			1.0000	
Lateral x 5 m	Region	1	2.0724	2.0724	15.582	0.1116	0.001*
	Residual	124	16.4920	0.1330		0.8884	
	Total	125	18.5644			1.0000	

Table 4.29. Pairwise comparisons of soil arthropod community structure by Genus in summer 2013 at Snook, Texas between carrion decomposition days after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.002*	0.017*	0.003*	0.001*	0.001*	0.143
7	0.002*	-	0.13	0.157	0.216	0.023*	0.010*
14	0.017*	0.13	-	0.622	0.284	0.022*	0.014*
21	0.003*	0.157	0.622	-	0.371	0.051•	0.003*
40	0.001*	0.216	0.284	0.371	-	0.169	0.002*
90	0.001*	0.023*	0.022*	0.051•	0.169	-	0.001*
180	0.143	0.010*	0.014*	0.003*	0.002*	0.001*	-

• denotes marginal significant difference

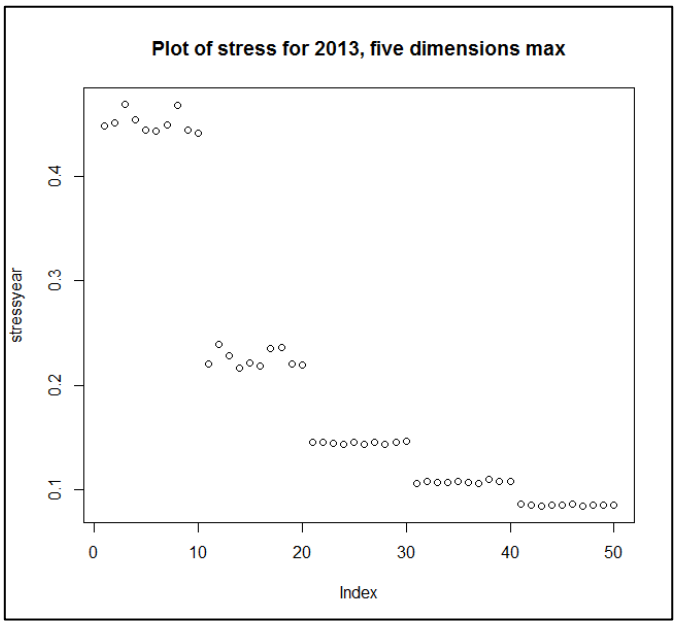

Figure 4.34. NMDS plot of stress for soil arthropod community structure (by Genus) in summer 2013 at Snook, Texas (Stress test 0.1420; $r^2 = 0.9428$).

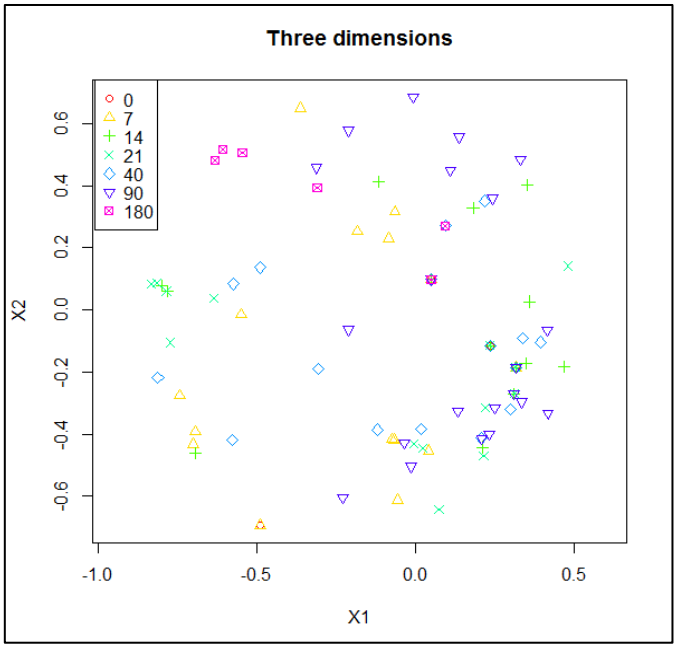

Figure 4.35. NMDS ordinations for soil arthropod community structure (by Genus) according to carrion decomposition days in summer 2013 at Snook, Texas.

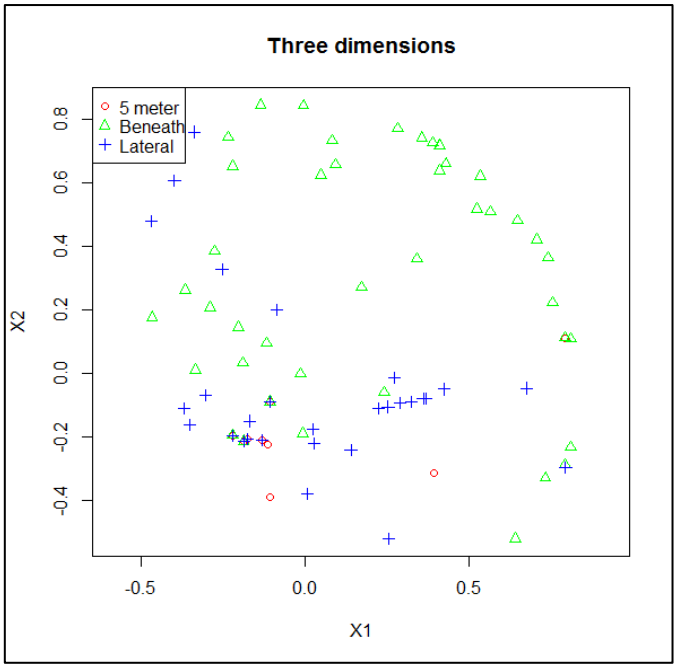

Figure 4.36. NMDS ordinations for soil arthropod community structure (by Genus) according to soil regions in summer 2013 at Snook, Texas.

The ISA results showed six genera of soil arthropods were the significant indicators in summer 2013. They were *Ch. rufifacies* (Calliphoridae), *Co. macellaria* (Calliphoridae), *Hydrotaea* sp. (Muscidae), *Omosita* sp. (Nitidulidae), *Liposcelis* sp. (Liposcelidae) and *Dermestes* sp. (Dermestidae) (Table 4.30). Note that three genera (*Chrysomya*, *Cochliomyia*, *Hydrotaea*) are in the Order Diptera, two genera in the Order Coleoptera (*Omosita* and *Dermestes*) and one genus (*Liposcelis*) in the Order Psocoptera. All dipteran and coleopteran indicators are necrophagous, while the book lice (*Liposcelis* sp.) are soil detritivores.

Table 4.30. Indicator species analysis by Genus for soil arthropods in summer 2013 at Snook, Texas.

Type	Genus and species	Indicator value	P value
All soils	*Chrysomya rufifacies*	0.7026	0.002*
	Cochliomyia macellaria	0.5714	0.016*
	Hydrotaea sp.	0.5008	0.004*
	Omosita sp.	0.6667	0.010*
	Liposcelis sp.	0.6667	0.011*
	Dermestes sp.	0.5079	0.028*

Abundance of soil arthropod community structure (by Genus) according to soil regions (excluding mites) in 2013

Soil beneath

Soil arthropod community beneath the Control pigs showed a very high abundance of *Ch. rufifacies* larvae on Day 7, suggesting these larvae was a dominant species underneath the swine carrion. The second dominant species found at soil beneath on Day 7 of Control carrion was *Hydrotaea* sp. However, for Post-7 and Post-14, *Hydrotaea* larvae were the dominant group of soil arthropods on Day 14 and Day 21. The shift in soil arthropod community (by Genus) beneath the carrion in different Treatments was obvious, although statistically no different between Treatments ($p = 0.209$) (Figure 4.37). The abundance of the *Ch. rufifacies, Hydrotaea sp., Co. macellaria*, and *Dermestes* sp. were specifically highlighted (as they are the major necrophagous genera) at the bottom of Figure 4.37.

At the soil beneath, there was no significant difference in abundance of each Genus between Treatments on every sampling day ($p > 0.05$). However, an interesting trend can be seen. For instance, at soil beneath, *Ch. rufifacies* was dominant on Day 7 on Control pigs, and then slowly decreased over days. Due to the delay of Diptera colonization, *Ch. rufifacies* became dominant on Day 21 for Post-14 group, but with a lower peak (lower abundance) compared to the Control group. This observation suggests that *Ch. rufifacies* oviposited lesser on the delayed carcasses, perhaps it is related to resource quality, or due to the difference in preference in resource utilization pattern by this invasive species.

For *Hydrotaea* larvae, Control group only had a few of these larvae. However, for treatment groups (Post-7 and Post-14), more abundant of *Hydrotaea* larvae can be seen underneath those carcasses. Evidently, some interesting behaviors by necrophagous species could be demonstrated through this field experiment, where the delayed dipteran colonization on carrion did impact the distribution and composition of certain transient soil-dwelling necrophagous species.

For *Co. macellaria*, due to the fact that most of these larvae were presence on the carcasses rather than in the soil, very few of *Co. macellaria* can be seen. However, the larvae were the most abundance on Day 14 in Post-7 group, perhaps this was due to the migrating *Co. macellaria* larvae to the adjacent soil for pupation.

For *Dermestes* sp., adults and larvae were collected from the soil samples. Again, Post-7 group had the highest abundance of *Dermestes* sp. recovered in soil beneath, especially on Day 40, Day 90 and Day 180. This observation suggests that the effect of delay dipteran colonization on carrion did impact the abundance and distribution of *Dermestes* beetles on pig carrion, either temporally and spatially.

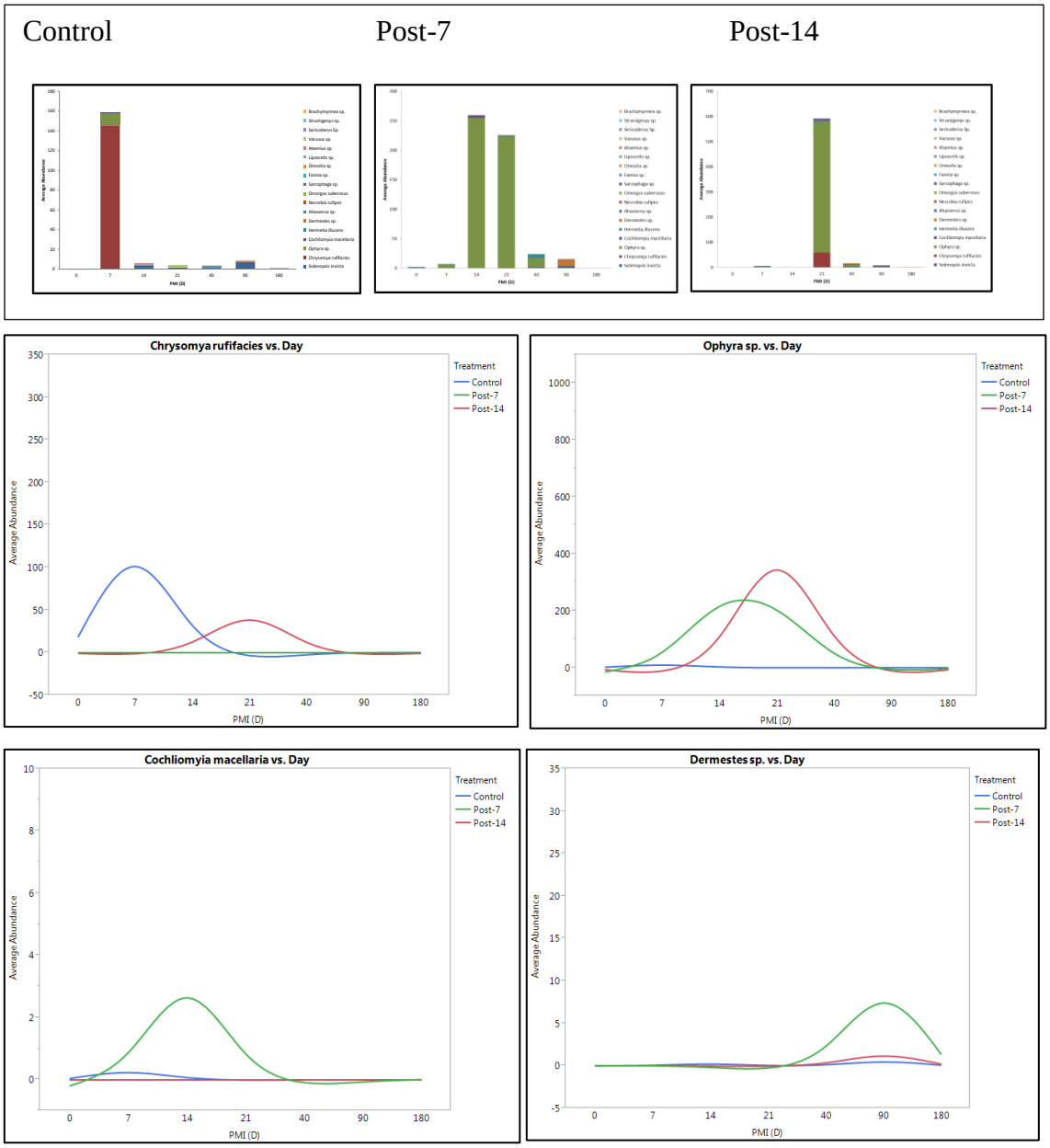

Figure 4.37. Above. Soil arthropod community abundance (by Genus) beneath the carrion according to Treatments over decomposition days in summer 2013 at Snook, Texas. Middle Left. Abundance of *Ch. rufifacies* (larvae) at soil beneath the carrion across treatments over time. Middle Right. Abundance of *Hydrotaea* sp. (larvae) at soil beneath the carrion across treatments over time. Lower Left. Abundance of *Co. macellaria* (larvae) at soil beneath the carrion across treatments over time. Lower Right. Abundance of *Dermestes* sp. (adults and larvae) at soil beneath the carrion across treatments over time.

Soil lateral

Soil arthropod community beside the Control pigs showed higher abundance of red imported fore ants, *Solenopsis invita* Buren (Formicidae) from Day 14 to Day 90. These ants are predators in the soil. The larvae of *Ch. rufifacies* can be seen on Day 7 at the soil beside the Control carcasses, possibly at the dispersal stage. Several individuals of flesh fly larvae, *Sarcophaga bullata* (Parker) (Sarcophagidae) and the adult antlike flower beetles, *Vacusus* sp. (Anthicidae) can be seen on Day 21. Several scarab beetles, *Ataenius* sp. (Scarabaeidae) can be seen on Day 40. On Day 90, *Liposcelis* sp. (Liposcelidae) became abundant in soil, and several individuals of *Dermestes* larvae were recovered on this day. For Post-7 and Post-14 carcasses, *S. invicta* was the dominant group of soil arthropod community on Day 7 to Day 90. Figure 4.38 demonstrated that the soil beside the Control carcasses had higher diversity compared to swine carcasses with delayed dipteran colonization. Again, no significant difference was detected in soil arthropod community structure between Treatments at soil lateral ($p = 0.188$).

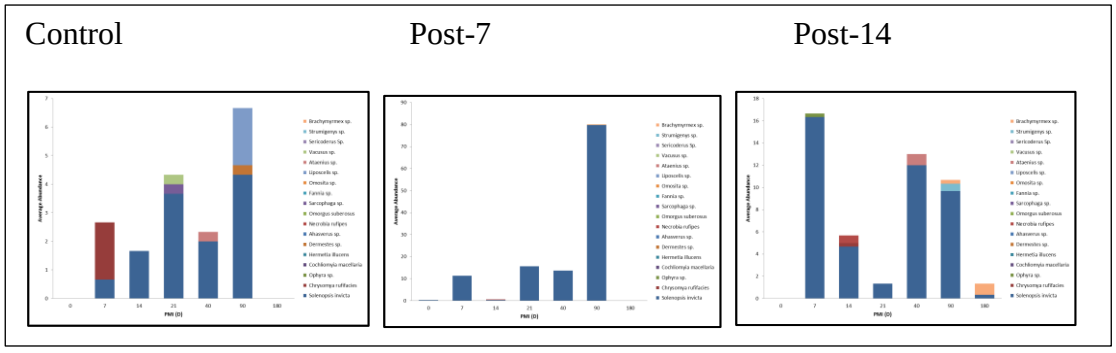

Figure 4.38. Soil arthropod community abundance (by Genus) beside the carrion according to Treatments over carrion decomposition days in summer 2013 at Snook, Texas.

Soil 5 m

The soils at 5 m away from carrion served as the control to the soils collected from beneath and lateral of the carrion. The arthropod community structure at soil 5 m was much different compared to soil beneath, and more similar to soil lateral, where *S. invicta* was the dominant arthropod throughout the sampling days. For Control group, antlike flower beetles, *Vacusus* sp. can be seen on Day 0 while *Strumigenys* sp. (Formicidae) was collected on and Day 40. For Post-7, *S. invicta* was dominant on Day 14, 40, 90 and 180. For Post-14, *S. invicta* was dominant throughput all sampling day, however, *Co. macellaria* 3rd instar were collected at soil 5 m on Day 21 due to maggot dispersal. This indicates that the *Co. macellaria* larvae can migrate up to 5 m away from carrion. On Day 180, ants in the genus *Brachymyrmex* sp. were collected and their abundance outcompeted *S. invicta*, and this may suggest a succession of ant species in soil ecosystem. However, no significant difference in soil arthropod community structure between Treatments was detected at soil 5 m ($p = 0.848$) (Figure 4.39).

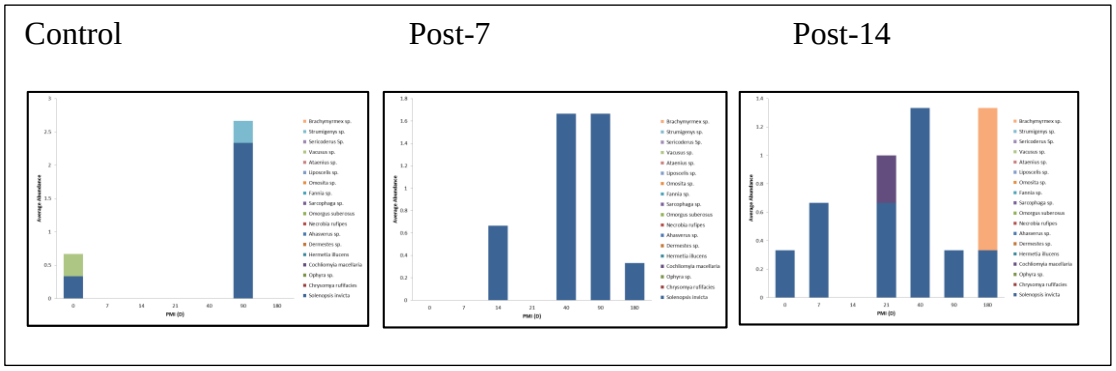

Figure 4.39. Soil arthropod community abundance (by Genus) at soil 5 m away from the carrion according to Treatments over carrion decomposition days in summer 2013 at Snook, Texas.

Abundance

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) and three interactions include Day x Treatment with $p = 0.0004$, Day x Region with $p < 0.0001$, and Day x Treatment x Region, with $p < 0.0001$. No significant difference was detected for Treatment ($p = 0.1581$). No significant difference was found in abundance between treatments by sampling day at every soil region ($p > 0.05$) (Figure 4.40). Resilience was tested only for soil beneath for all treatments and resilience was observed on Day 40 for Post-14 carcasses while Control and Post-7 carcasses demonstrated a stable abundance over time (Table 4.31).

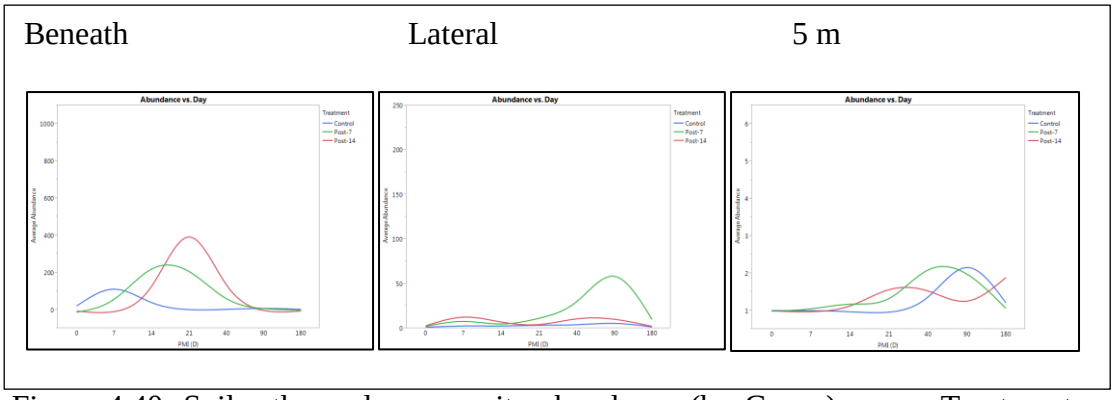

Figure 4.40. Soil arthropod community abundance (by Genus) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.31. Resilience for soil arthropod community abundance (by Genus) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0538	Resistance
Post-7	None	0.1893	Resistance
Post-14	0 x 21	0.0023*	40

Richness

The full model showed a significant difference in Day ($p = 0.0020$) and Region ($p < 0.0001$) without any significant interaction detected. Treatment was not significant difference ($p = 0.3430$. No statistical difference was detected between Treatments by sampling day at all soil regions, indicating a stable community by richness. In general, genus richness at soil beneath of Control carcasses showed higher richness compared to Post-7 and Post-14 carcasses on Day 7 and Day 14. However, Post-7 and Post-14 had more genus richness than Control carcasses on Day 21 and Day 40. On Day 90 and Day 180, richness increased in Control carcasses. At soil lateral, richness of Post-14 group was higher on Day 7 and Day 14 compared to other groups, but the richness in Control carcasses increased from Day 21, peaked on Day 90, then decreased on Day 180. As for soil at 5 m, richness of Post-14 peaked on Day 21 and the second peak on Day 180, while Control group showed a single peak on Day 90 (Figure 4.41). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable richness over time (Table 4.32).

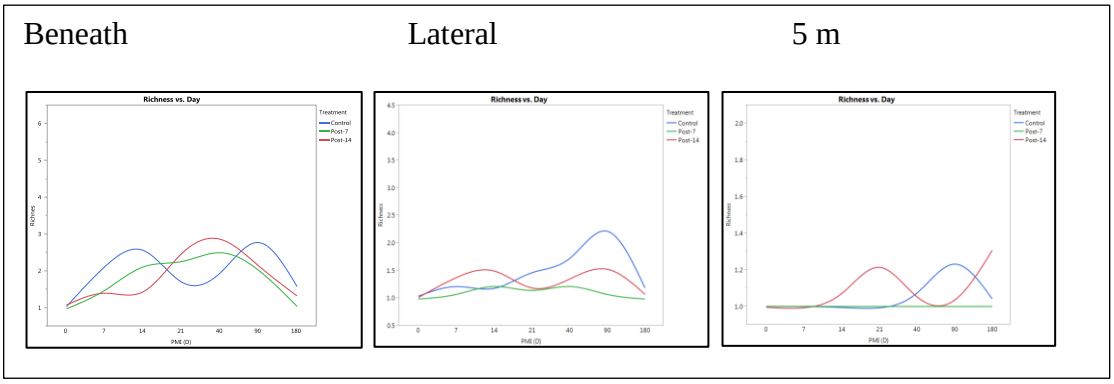

Figure 4.41. Soil arthropod community richness (by Genus) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.32. Resilience for soil arthropod community richness (by Genus) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0965	Resistance
Post-7	None	0.4560	Resistance
Post-14	None	0.3150	Resistance

Simpson's diversity index

The full model showed a significant difference in Day ($p = 0.0015$) and Region ($p < 0.0001$) without any significant interaction detected. Treatment had no significant difference ($p = 0.2414$). No significant difference in Simpson's diversity index across Treatments over every sampling time at all soil regions. Again, this means treatment did not impact soil arthropod at genus level, and the community diversity was under a stable dynamic. At soil beneath, Control carcasses showed increased in soil arthropod genus diversity from Day 0 to Day 14 (indicating active decomposition process attracted many different genera of arthropods), and the diversity decreased gradually on Day 21, and then increased again on Day 90. Post-7 and Post-14 groups had higher Simpson's index (means lower diversity in family) from Day 7 to Day 14, probably due to treatment effect. At soil lateral, Post-14 carcasses had higher diversity compared to Control carcasses on Day 7 and Day 14, suggesting delayed in insect colonization on carrion did increase diversity of soil arthropods. Interestingly, Post-7 had the lowest diversity from Day 20 to Day 180. At soil 5 m, there was very low diversity observed in all groups from Day 0 to Day 180 in general, except Day 21 where Post-14 had a higher diversity compared to other groups (Figure 4.42). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable diversity over time (Table 4.33).

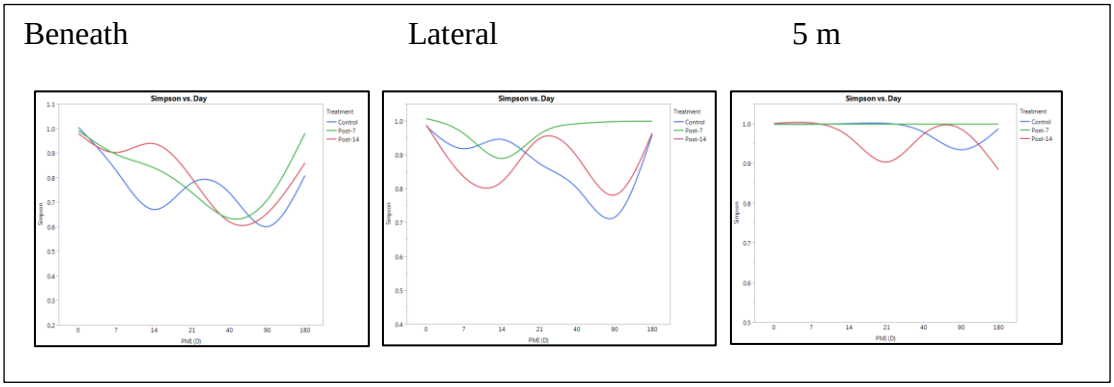

Figure 4.42. Simpson's diversity index of soil arthropod (by Genus) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.33. Resilience for soil arthropod Simpson's diversity (by Genus) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2919	Resistance
Post-7	None	0.4048	Resistance
Post-14	None	0.3434	Resistance

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p = 0.0024$) and Region ($p < 0.0001$) without any significant interaction detected. Treatment had no significant difference ($p = 0.2449$). There was no statistical difference (no divergence) of Shannon-Wiener's diversity index found between Treatments by sampling day in all soil regions, suggesting a stable soil arthropod diversity by Shannon's index. At soil beneath, lower diversity in arthropod was noted on Post-7 and Post-14 carcasses on Day 7 and Day 14. This indicates delayed insect colonization on carrion decreased soil arthropod diversity. While for Control carcasses, diversity increased from Day 0 to Day 14, and then deceased on Day 21, and increased again on Day 90. Higher diversity was noted at soil lateral for Post-14 group from Day 7 to Day 14, possibly due to the impact of delayed

Diptera colonization on this treatment group. At soil 5 m, low diversity was observed for all groups from Day 0 to Day 180, except Post-14 carcasses had a peak on Day 21 and Day 180 (Figure 4.43). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable diversity over time (Table 4.34).

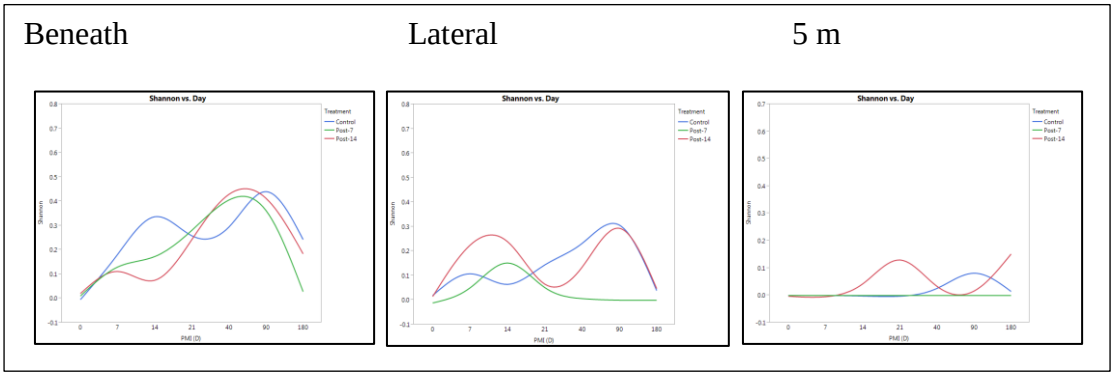

Figure 4.43. Shannon-Wiener's diversity index of soil arthropod (by Genus) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.34. Resilience for soil arthropod Shannon-Wiener's diversity (by Genus) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4289	Resistance
Post-7	None	0.3782	Resistance
Post-14	None	0.3851	Resistance

Evenness

The full model showed a significant difference in Day ($p = 0.0177$) and Region ($p = 0.0003$) without any significant interaction detected. Treatment had no significant difference ($p = 0.2332$). There was no statistical difference (no divergence) of genus evenness found between Treatments by sampling day in all soil regions. Again, this

indicates that treatment had no impact on evenness at the genus level. At soil beneath, evenness of Control carcasses increased from Day 0 to Day 14 while evenness decreased in Post-7 and Post-14 groups from Day 0 to Day 14. Both Post-7 and Post-14 groups peaked on Day 40. At soil lateral, Post-14 carcasses had the highest evenness in genus level on Day 7, Day 14 and Day 90. At soil 5 m, Post-14 carcasses achieved the highest evenness value on Day 21, whereas Control carcasses were on Day 90 (Figure 4.44). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable evenness over time (Table 4.35).

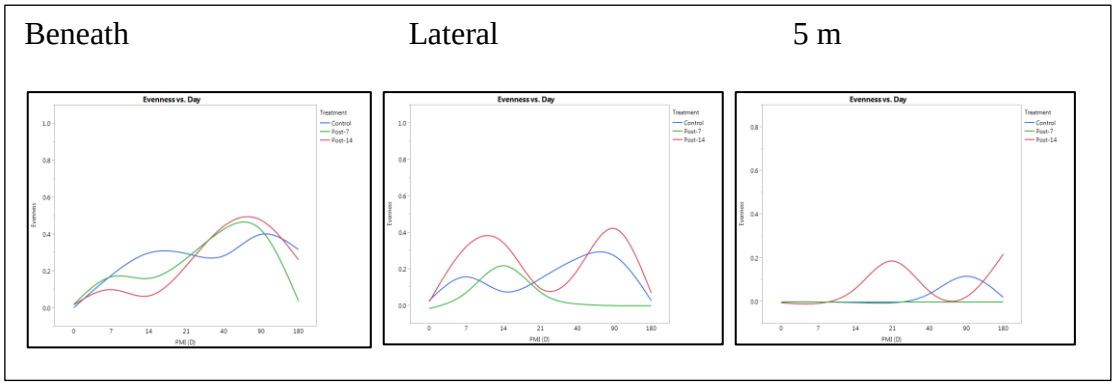

Figure 4.44. Evenness of soil arthropod (by Genus) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.35. Resilience for soil arthropod community evenness (by Genus) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.7694	Resistance
Post-7	None	0.4160	Resistance
Post-14	None	0.4682	Resistance

Effective number of species

The full model showed a significant difference in Day ($p = 0.0026$) and Region ($p < 0.0001$) without any significant interaction detected. Treatment had no significant difference ($p = 0.2587$). There was no statistical difference (no divergence) of effective number of species (ENS) found between Treatments by sampling day in all soil regions. In general, ENS increased from Day 0 to Day 40 for both Post-7 and Post-14 groups while the Control carcasses, despite being higher in ENS than Post-7 and Post-14, increased in effective number of genus from Day 0 to Day 14, and then decreased on Day 40, and increased again on Day 90. At soil lateral, ENS for Post-7 and Post-14 increased from Day 0 to Day 14. As for Control carcasses at soil lateral, ENS increased from Day 0 to Day 7, and then increased slightly on Day 14, and increased all the way up to Day 90 (highest value of ENS). On the other hand, the trends of ENS in soil 5 m were similar among all treatment groups, except on Day 21, where Post-14 had a higher ENS on Day 21 and Day 180 (Figure 4.45). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable ENS over time (Table 4.36).

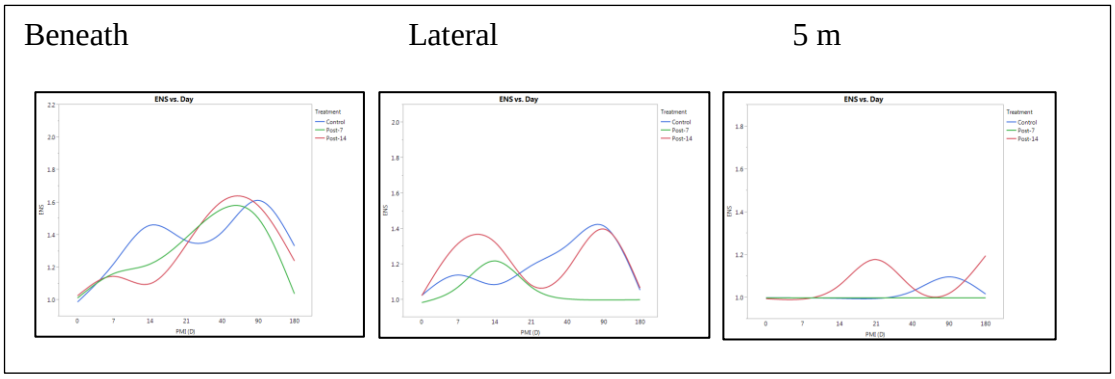

Figure 4.45. Effective number of genus of soil arthropods across Treatments over time at different soil regions in summer 2013 at Snook, Texas

Table 4.36. Resilience for soil arthropod community ENS (by Genus) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4388	Resistance
Post-7	None	0.3715	Resistance
Post-14	None	0.3643	Resistance

Function

PERMANOVA was performed on soil arthropod data by function. Results showed that there was Day effect and Region effect (both p value = 0.001). Moreover, there was an interaction between Day and Region (p = 0.003) (Table 4.37). Again, Treatment was not significantly difference by soil arthropod community function (p = 0.131).

Table 4.37. Analysis of the soil arthropod community function in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	6.1515	0.001*
Treatment	2	1.4632	0.131
Region	2	15.5860	0.001*
Day x Treatment	2	0.4371	0.951
Day x Region	2	2.6878	0.003*
Treatment x Region	4	1.3605	0.103
Day x Treatment x Region	4	1.4341	0.075•

• Marginal significant difference.

There was a significant effect in Day and Region, further analyses were carried out. For soil regions, all soil regions were significantly from each other (p = 0.001),

indicating soil community structure changes according to region, although soil beneath and soil lateral was just 30 cm away (Table 4.38). As for day of decomposition, most of the pairwise comparisons between days of decomposition were significantly different, except four pairs namely Day 7 x Day 14, Day 7 x Day 21, Day 14 x Day 21, and Day 40 x Day 90 where there were no significant difference detected ($p < 0.05$) (Table 4.39), in other words, the comparison of those days have no difference in terms of soil arthropod community structure by function. The NMDS plot of stress for soil arthropod community structure (Figure 4.46) and the NMDS ordinations for Day and Region were provided for visualization about data distribution (Figure 4.47 and 4.48, respectively). Minimum stress for given dimensionality was 0.1465 with $r^2 = 0.8665$. The MRPP analysis for soil region showed a significant difference (A value: 0.0779; Significant of Delta: 0.001 based on 999 permutations) while the MRPP for day also showed a significant difference with A value 0.0654 and Significant of Delta 0.001.

Table 4.38. Pairwise comparisons between Regions on soil arthropod community function in summer 2013 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	1.1238	1.1238	4.6564	0.0362	0.001*
	Residual	124	29.9269	0.2413		0.9638	
	Total	125	31.0507			1.0000	
Beneath x 5 m	Region	1	5.018	5.0178	19.736	0.1373	0.001*
	Residual	124	31.526	0.2542		0.8627	
	Total	125	36.544			1.0000	
Lateral x 5 m	Region	1	4.2046	4.2046	20.09	0.1394	0.001*
	Residual	124	25.9522	0.2093		0.8606	
	Total	125	30.1569			1.0000	

Table 4.39. Pairwise comparisons of soil arthropod community function in summer 2013 at Snook, Texas between decomposition days after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.004*	0.001*	0.001*	0.001*	0.001*	0.001*
7	0.004*	-	0.291	0.185	0.005*	0.007*	0.02*
14	0.001*	0.291	-	0.972	0.002*	0.005*	0.001*
21	0.001*	0.185	0.972	-	0.007*	0.016*	0.001*
40	0.001*	0.005*	0.002*	0.007*	-	0.186	0.003*
90	0.001*	0.007*	0.005*	0.016*	0.186	-	0.001*
180	0.001*	0.02*	0.001*	0.001*	0.003*	0.001*	-

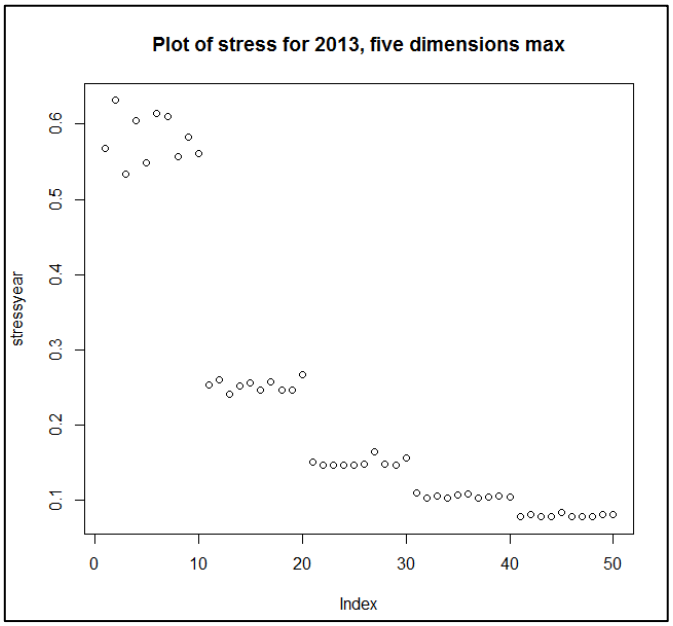

Figure 4.46. NMDS plot of stress for soil arthropod community function in summer 2013 at Snook, Texas (Stress test 0.1465; $r^2 = 0.8665$).

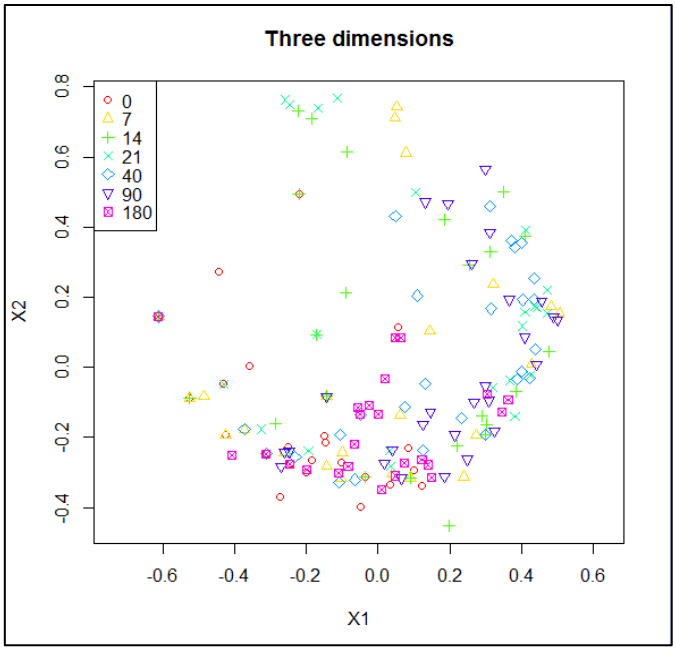

Figure 4.47. NMDS ordinations for soil arthropod community function according to carrion decomposition days in summer 2013 at Snook, Texas.

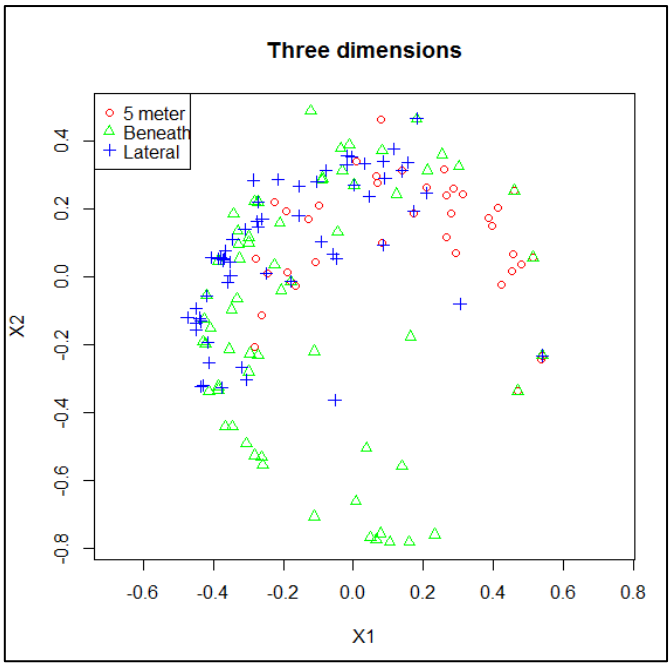

Figure 4.48. NMDS ordinations for soil arthropod community function according to soil regions in summer 2013 at Snook, Texas.

The ISA results showed two functional groups of soil arthropods were the indicators in summer 2013 (Table 4.40). They were necrophagous guild and detritivore guild. These two guilds are important players during decomposition process.

Table 4.40. Indicator species analysis by function for soil arthropods in summer 2013 at Snook, Texas.

Type	Functional group	Indicator value	P value
All soils	Necrophagous	0.4618	0.005*
	Detritivore	0.3373	0.008*

Abundance of soil arthropod community structure (by Function) according to soil regions (excluding mites) in 2013

Soil beneath

The abundance of soil arthropod community by function was different at soil beneath according to treatments (Figure 4.49), although statistically there was no significant different ($p = 0.225$). For the Control carcasses, generalist detritivores can be seen from Day 0 to Day 180. However, necrophagous guild was abundance on Day 7, followed by the increased in the abundance of predators. In contrast, both Post-7 and Post-14 groups had higher detritivores compared to other functional groups throughout the decomposition days. For Post-7, highest abundance of detritivore occurred on Day 14 while Post-14 achieved its peak abundance of detritivores on Day 21. In general, predators usually become more abundance with the increasing numbers of detritivores or necrophagous communities.

Each functional group was highlighted individually (Figure 4.49). Interestingly, carcasses with delayed Diptera colonization (Post-7 and Post-14 groups) had higher abundance of detritivores than Control group from Day 7 to Day 40, although no statistical difference ($p > 0.05$) was found between treatments on every sampling day. For predator / parasite guild, a bell-shaped distribution was observed on Control

carcasses, with the peak abundance on Day 21, which was significantly different from Post-7 and Post-14 ($p = 0.0491$ and 0.0233, respectively). Even on Day 0, there was significant difference between Control and Post-7 group ($p = 0.0435$), possibly due to the natural population of predators existing in the soil on the initial day of experiment. Post-7 carcasses had two peaks of abundance of predators which were on Day 14 and Day 90. For Post-14, highest abundance of predators was recorded on Day 40, which was remarkably delayed compared to Control and Post-7 groups.

For necrophagous guild, there was an increased for Control carcasses from Day 0 to Day 7 (the peak), and then decreased on Day 14, while for Post-14, a peak of abundance was observed only on Day 21. Nevertheless, this peak was lower compared to the one in Control. For herbivores in general, the abundance was high at the initial day of experiment regardless of treatments, decreased during the decomposition process (Day 7 to Day 21), and then gradually increased from Day 40 to Day 90. For fungivore, the abundance was low or absent in all treatment groups, remained stable until Day 40, and slowly increased thereafter up to Day 180, possibly due to the presence of fungi on the pig skeletons.

Resilience was tested only for all functional groups of soil arthropods at soil beneath for all treatments. The results showed detritivores and necrophagous groups were stable over time (Table 4.41).

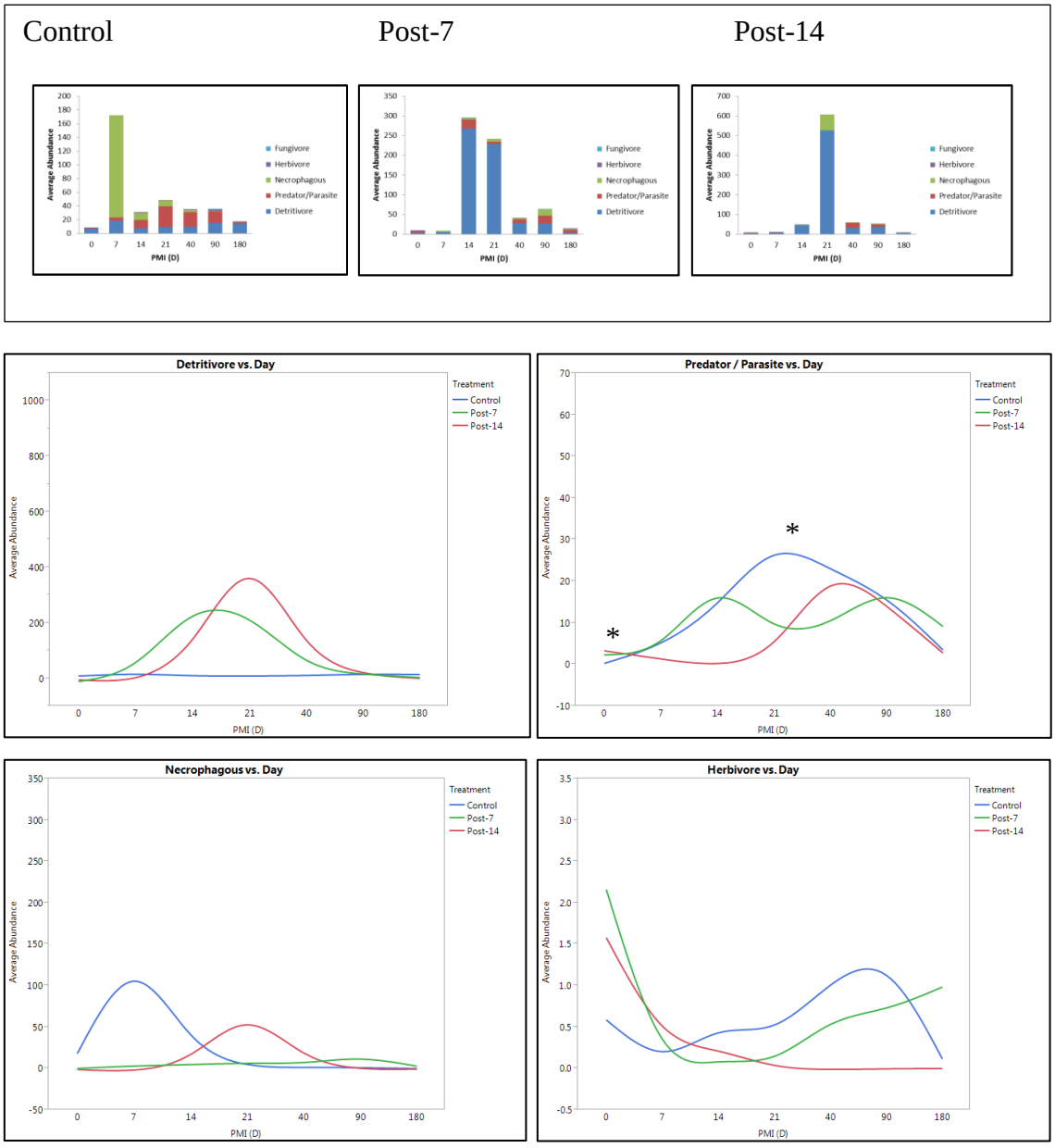

Figure 4.49. Above. Soil arthropod community abundance (by Function) beneath the carrion according to Treatments over carrion decomposition days in summer 2013 at Snook, Texas. Upper Left. Abundance of detritivores at soil beneath the carrion across treatments over time. Upper Right. Abundance of predator / parasite at soil beneath the carrion across treatments over time. Middle Left. Abundance of necrophagous at soil beneath the carrion across treatments over time. Middle Right. Abundance of herbivore at soil beneath the carrion across treatments over time. Lower Left. Abundance of fungivore at soil beneath the carrion across treatments over time.

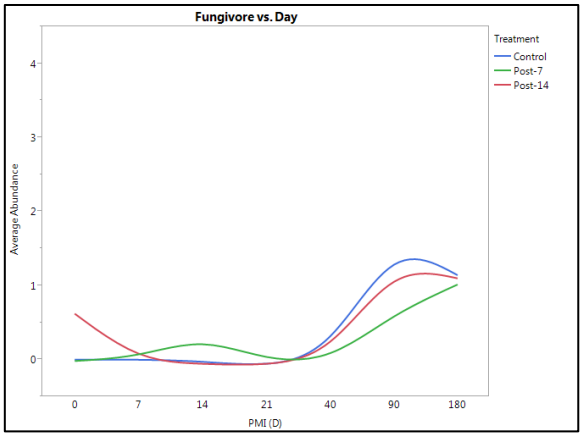

Figure 4.49 (Continued).

Table 4.41. Resilience of soil arthropod community functions for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Function	Treatment	Significant difference	P value	Resilience on Day
Necrophagous	Control	None	0.0953	Resistance
	Post-7	None	0.5396	Resistance
	Post-14	None	0.0629	Resistance
Detritivore	Control	None	0.9145	Resistance
	Post-7	None	0.2770	Resistance
	Post-14	None	0.0506	Resistance
Predator	Control	0 x 21	0.0138*	40
	Post-7	None	0.3576	Resistance
	Post-14	0 x 40	0.0125*	90
Fungivore	Control	0 x 90	0.0220*	180
	Post-7	None	0.3015	Resistance
	Post-14	None	0.0405*	Resistance[#]

Table 4.41 (Continued).

Function	Treatment	Significant difference	P value	Resilience on Day
Herbivores	Control	None	0.0945	Resistance
	Post-7	None	0.0648	Resistance
	Post-14	0 x 7	0.0093*	14 and 90, and
		0 x 21	0.0210*	no resilience
		0 x 40	0.0040*	on Day 180
		0 x 180	0.0040*	

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Soil lateral

Soil arthropod community function at soil lateral consisted of two majority functional groups namely predator and detritivore (Figure 4.50), and therefore, statistically no different between the Treatments ($p = 0.195$). Necrophagous guild can be seen on Day 7 and Day 14 on the Control carcasses, possibly due to the dispersal of fly larvae. It is noteworthy to mention that the predator abundance at soil lateral was much higher on Day 90 for both Post-7 and Post-14 carcasses.

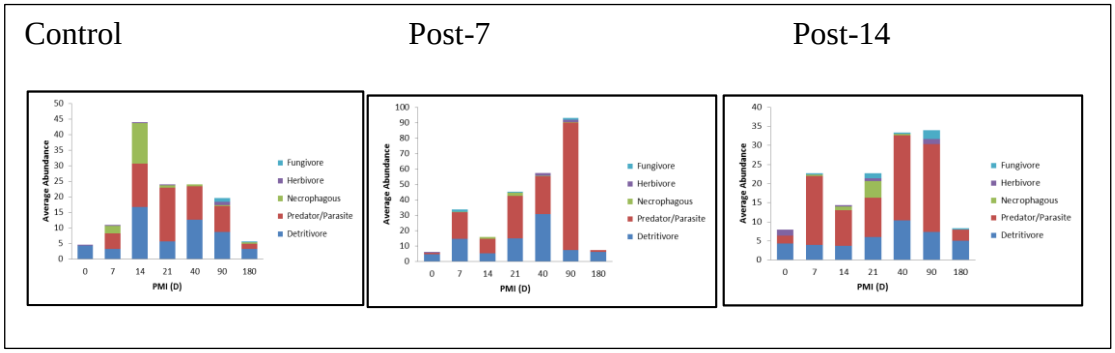

Figure 4.50. Soil arthropod community abundance (by Function) beside the carrion according to Treatments over carrion decomposition days in summer 2013 at Snook, Texas.

Soil 5 m

The soils at 5 m away from carrion served as the control to the soils collected from beneath and lateral of the carrion (Figure 4.51). Likewise, there was no significant difference in soil arthropod function between Treatments ($p = 0.217$) at soil 5 m. The two major functional components found at soil 5 m were detritivores and predators. Several individuals of necrophagous insects (e.g., fly larvae) can be collected on Day 21 from Post-7 and Post-14 groups. Again, this demonstrates that the dispersal of fly larvae could reach the radius of 5 m away from carrion.

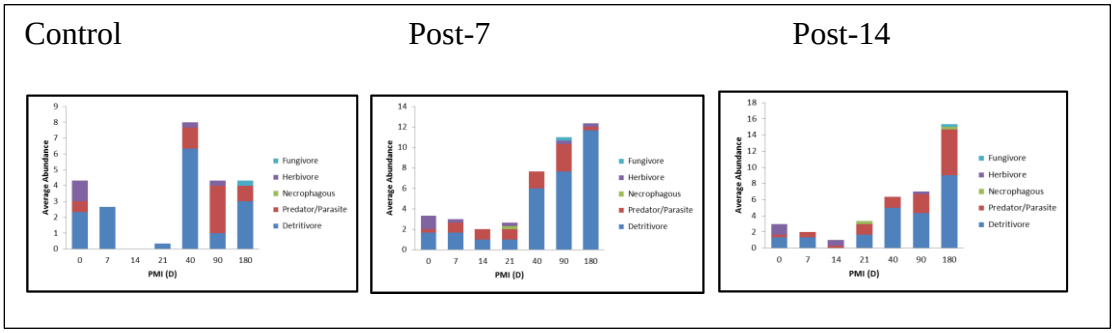

Figure 4.51. Soil arthropod community abundance (by Function) at soil 5 m away from the carrion according to Treatments over carrion decomposition days in summer 2013 at Snook, Texas.

Community structure and function of soil arthropods in 2014

Total Order in 2014

A total of nine Orders in the Class Insecta, one Order in Class Arachnida (Araneae), one Order in the Class Malacostraca, one suborder of the Subclass Acari (Oribatida), two Classes (Symphyla and Chilopoda), and one group of mites (Non-Oribatida; members include Mesostigmata, Prostigmata, Astigmatina) were identified in 2014 trials. Table 4.42 showed the Orders and other taxonomic ranks identified in 2014 trial. The most dominant group was the non-Oribatida mite group (91.77%).

Table 4.42. Total abundance and dominance of Orders in the Class Insecta and other taxonomic ranks identified from all soil samples in summer 2014 at Snook, Texas.

No.	Taxonomic rank		Total abundance	Dominance
1.	Group*	Non-Oribatida mites	108065	91.77
2.	Suborder	Oribatida	4140	3.51
3.	Order	Coleoptera	2165	1.83
4.	Order	Hymenoptera	1180	1.00
5.	Order	Collembola	1079	0.91
6.	Order	Diptera	639	0.54
7.	Order	Hemiptera	306	0.26
8.	Order	Diplura	79	0.06
9.	Class	Symphyla	35	0.02
10.	Order	Thysanoptera	16	0.01
11.	Order	Psocoptera	12	0.01
12.	Order	Araneae	22	0.01

Table 4.42 (Continued).

Taxonomic rank		Total abundance	Dominance
13. Order	Orthoptera	6	0.0051
14. Order	Megadrilacea	3	0.0025
15. Class	Chilopoda	1	0.0008
	Total	117721	100

*= Non-Oribatida mites are assigned as a group of morphospecies that contained members from the Order Mesostigmata, Suborder Prostigmata and Cohort Astigmatina.

Total Family in 2014

A total of 36 families in the Class Insecta, two families in the Class Arachnida (spiders) (note that Families in the Subclass Acari were treated in a different section of result in this Chapter), and one family in the Order Megadrilacea were identified in 2013. Table 4.43 showed the families of soil arthropods (excluding mite families) identified in 2014. The most dominant family was Formicidae, with dominance 22.98%.

Table 4.43. Total abundance and dominance of Families of soil arthropods identified from all soil samples in summer 2014 at Snook, Texas.

No.	Order	Family	Total abundance	Dominance
1.	Hymenoptera	Formicidae	1199	22.98
2.	Coleoptera	Staphylinidae	868	16.64
3.	Coleoptera	Tenebrionidae	798	15.30
4.	Collembola	Entomobryidae	781	14.97
5.	Coleoptera	Dermestidae	303	5.81
6.	Diptera	Muscidae	286	5.48
7.	Diptera	Stratiomyidae	207	3.97
8.	Collembola	Sminthuridae	191	3.66
9.	Collembola	Hypogastruridae	79	1.51
10.	Diplura	Japygidae	79	1.51
11.	Hemiptera	Aphididae	72	1.38
12.	Coleoptera	Scarabaeidae	58	1.11
13.	Diptera	Sarcophagidae	57	1.09
14.	Coleoptera	Latridiidae	36	0.69
15.	Coleoptera	Nitidulidae	35	0.67
16.	Diptera	Fanniidae	29	0.56
17.	Coleoptera	Monotomidae	27	0.52
18.	Coleoptera	Ptilidae	18	0.35
19.	Araneae	Araneidae	16	0.31
20.	Psocoptera	Liposcelidae	12	0.23

Table 4.43 (Continued).

	Order	Family	Total abundance	Dominance
21.	Coleoptera	Elateridae	9	0.17
22.	Thysanoptera	Thripidae	8	0.15
23.	Coleoptera	Curculionidae	7	0.13
24.	Coleoptera	Histeridae	7	0.13
25.	Araneae	Lycosidae	7	0.13
26.	Coleoptera	Anthocoridae	4	0.08
27.	Coleoptera	Anthicidae	3	0.06
28.	Coleoptera	Corylophidae	3	0.06
29.	Coleoptera	Carabidae	3	0.06
30.	Hemiptera	Rhyparochromidae	2	0.04
31.	Coleoptera	Trogidae	2	0.04
32.	Megadrilacea	Lumbricidae	2	0.04
33.	Orthoptera	Gryllidae	2	0.04
34.	Diptera	Sciaridae	2	0.04
35.	Collembola	Isotomidae	1	0.02
36.	Coleoptera	Silphidae	1	0.02
37.	Hemiptera	Cicadellidae	1	0.02
38.	Coleoptera	Coccinellidae	1	0.02
39.	Diptera	Tipulidae	1	0.02
		Total	5217	100

Total Genus and species in 2014

A total of 14 genera and species of soil arthropods have been identified in 2014 trial (Table 4.44). The most abundance species encountered was the red imported fore ants, *S. invicta* (Hymenoptera: Formicidae). Note that the mites were excluded from this analysis.

Table 4.44. Total abundance and dominance of Genera and species of soil arthropods identified from all soil samples in summer 2014 at Snook, Texas.

No.	Family	Genus and species	Total abundance	Dominance
1.	Formicidae	*Solenopsis invicta*	920	50.27
2.	Muscidae	*Hydrotaea* sp.	284	15.52
3.	Formicidae	*Leptogenys* sp.	281	15.36
4.	Stratiomyidae	*Hermetia illucens*	193	10.55
5.	Sarcophagidae	*Sarcophaga bullata*	57	3.11
6.	Scarabaeidae	*Ataenius* sp.	52	2.84
7.	Fanniidae	*Fannia* sp.	28	1.53
8.	Corylophidae	*Sericoderus* sp.	3	0.16
9.	Nitidulidae	*Omosita* sp.	3	0.16
10.	Anthicidae	*Vacusus* sp.	3	0.16
11.	Trogidae	*Omorgus suberosus*	2	0.11
12.	Curculionidae	*Baris* sp.	2	0.11
13.	Formicidae	*Strumigenys* sp.	1	0.05
14.	Silphidae	*Nicrophorus marginalis*	1	0.05
		Total	1830	100

Total function in 2014

Five functional groups of soil arthropods have been identified in 2014 trial (Table 4.45). The most abundance functional group (~46%) was the detritivores (e.g. Collembola, Psocoptera etc.), followed by Predator/Parasite group (~43%) such as Reduviidae, Asilidae or Order Aranea. The third was the necrophagous group, which feed on the carrion (~8%) directly such as the fly larvae. This group composed of Family Calliphoridae and Sarcophagidae. For the complete arthropod functional group assignment, see Appendix G.

Table 4.45. Total abundance and dominance of Functions of soil arthropods identified from all soil samples in summer 2014 at Snook, Texas.

No.	Functional group	Total Abundance	Dominance
1	Detritivore	2528	46.11
2	Predator/Parasite	2367	43.17
3	Necrophagous	432	7.88
4	Herbivore	114	2.08
5	Fungivore	42	0.77
	Total	5483	100

Order in 2014

PERMANOVA was performed on soil arthropod data by Order level. Results showed that there was Day effect and Region effect, however, Treatment was not significant difference. There was also an interaction between Day and Region, while interaction between Day and Treatment was on marginal significant difference (Table 4.46).

Table 4.46. Analysis of the soil arthropod community structure (by Order) in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	21.3096	0.0001*
Treatment	2	1.0874	0.358
Region	2	12.5928	0.001*
Day x Treatment	2	1.6137	0.088•
Day x Region	2	3.1601	0.002*
Treatment x Region	4	0.8535	0.630
Day x Treatment x Region	4	0.5213	0.974

• Marginal significant difference.

Since there was significant effect in Day and Region, further analyses were carried out. For soil regions, soil beneath x soil 5 m and soil lateral x soil 5 m were significantly different from each other (p = 0.0001), indicating soil community structure changes according to locations. However, soil beneath x soil lateral was only marginally significant different, with p value 0.074, this may indicate movement and co-occurrence of similar soil arthropod Orders between soil beneath the carrion and soil at the side of carrion (Table 4.47). As for day of decomposition, all day to day comparisons were significantly different, except Day 14 x Day 21, Day 40 x Day 90, Day 40 x Day 180, and Day 90 x Day 180 where there were no significant difference detected between these days (Table 4.48). The NMDS plot of stress for soil arthropod community structure (Figure 4.52) and NMDS ordinations for Day and Region were provided for visualization about data distribution (Figure 4.53 and 4.54, respectively). Minimum stress for given dimensionality was 0.1231 with $r^2 = 0.9294$. The MRPP analysis for soil region showed a significant difference (A value = 0.0608; Significant of Delta = 0.001 based on 999 permutations) while the MRPP for day also showed a significant difference with A value 0.1107 and Significant of Delta 0.001.

Table 4.47. Pairwise comparisons between Regions on soil arthropod community structure by Order in summer 2014 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	0.4898	0.4898	1.9825	0.0157	0.074•
	Residual	124	30.6352	0.2470		0.9843	
	Total	125	31.1250			1.0000	
Beneath x 5 m	Region	1	4.0755	4.0755	21.108	0.1455	0.0001*
	Residual	124	23.9416	0.1931		0.8545	
	Total	125	28.0172			1.0000	
Lateral x 5 m	Region	1	2.2881	2.2881	13.23	0.0964	0.0001*
	Residual	124	21.4462	0.1729		0.9036	
	Total	125	23.7343			1.0000	

• = Marginal significant difference

Table 4.48. Pairwise comparisons of soil arthropod community structure by Order in summer 2014 at Snook, Texas between carrion decomposition days after Bonferroni's correction in 2014.

Day x Day	0	7	14	21	40	90	180
0	-	0.001*	0.001*	0.002*	0.001*	0.001*	0.001*
7	0.001*	-	0.013*	0.009*	0.001*	0.001*	0.001*
14	0.001*	0.013*	-	0.469	0.002*	0.001*	0.001*
21	0.002*	0.009*	0.469	-	0.002*	0.001*	0.001*
40	0.001*	0.001*	0.002*	0.002*	-	0.294	0.112
90	0.001*	0.001*	0.001*	0.001*	0.294	-	0.313
180	0.001*	0.001*	0.001*	0.001*	0.112	0.313	-

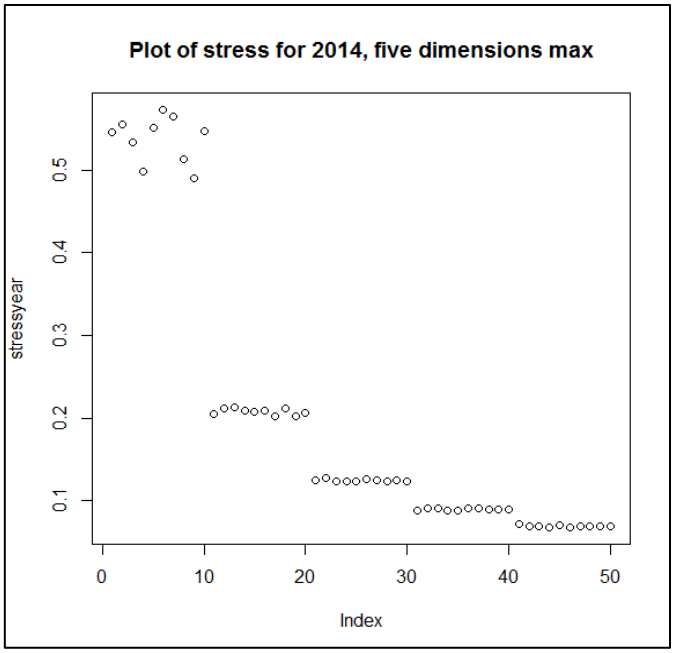

Figure 4.52. NMDS plot of stress for soil arthropod community structure (by Order) in summer 2014 at Snook, Texas (Stress test 0.1231; $r^2 = 0.9294$).

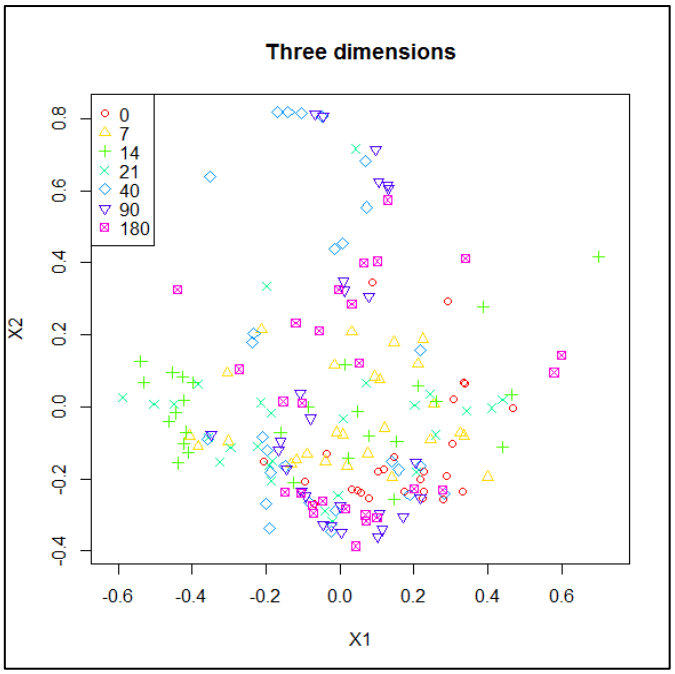

Figure 4.53. NMDS ordinations for soil arthropod community structure (by Order) according to carrion decomposition days in summer 2014 at Snook, Texas.

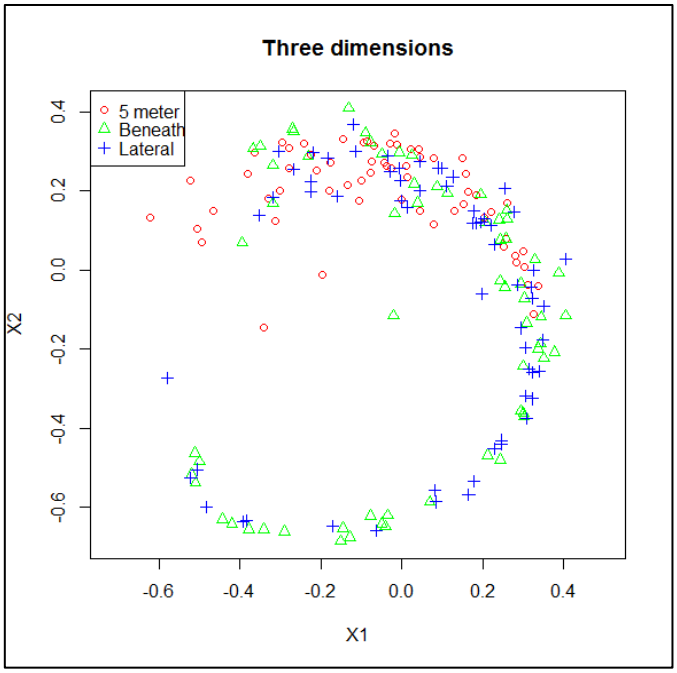

Figure 4.54. NMDS ordinations for soil arthropod community structure (by Order) according to soil regions in summer 2014 at Snook, Texas.

The ISA results showed eight significant indicators (by Order) among soil arthropods in 2014 trial. They were Diplura, Coleoptera, Diptera, Hemiptera, Collembola, Class Symphyla, Psocoptera, Collembola and Oribatida (Table 4.49).

Table 4.49. Indicator species analysis by Order for soil arthropods in summer 2014 at Snook, Texas.

Type	Order	Indicator value	P value
All soils	Diplura	0.1772	0.021*
	Coleoptera	0.1122	0.019*
	Diptera	0.2660	0.002*
	Hemiptera	0.1732	0.047*
	Class Symphyla	0.2095	0.025*
	Psocoptera	0.4444	0.025*
	Collembola	0.0918	0.041*
	Oribatida	0.0857	0.034*

Abundance of soil arthropod community structure (by Order) according to soil regions (excluding mites) in 2014

Soil beneath

Soil arthropod community beneath the Control pigs showed higher abundance of Coleoptera from Day 7 to Day 21. However, for Post-7 and Post-14, Diptera larvae were the dominant group of soil arthropod on Day 14 and Day 21, respectively. However, soil arthropod community structure beneath the pig carrion was statistically insignificant among treatments ($p = 0.455$) (Figure 4.55). The abundance of the Order Diptera and Coleoptera were specifically highlighted (as they are the major necrophagous Orders) at the bottom of Figure 4.55. Significant difference was detected on Day 7 of the Order Coleoptera, where Control x Post-7 and Post-7 x Post-14 were significant difference, with p value 0.0045 and 0.0034, respectively. For Order Diptera, significant differences were detected on Day 14 (Control x Post-7 and Post-7 x Post-14, $p = 0.0434$ and 0.0229, respectively), Day 21 (Control x Post-14 and Post-7 x Post-14, $p = 0.0317$ and 0.0426, respectively) and Day 40 (Control x Post-14 and Post-7 x Post-14, $p = 0.0233$ and 0.0137, respectively).

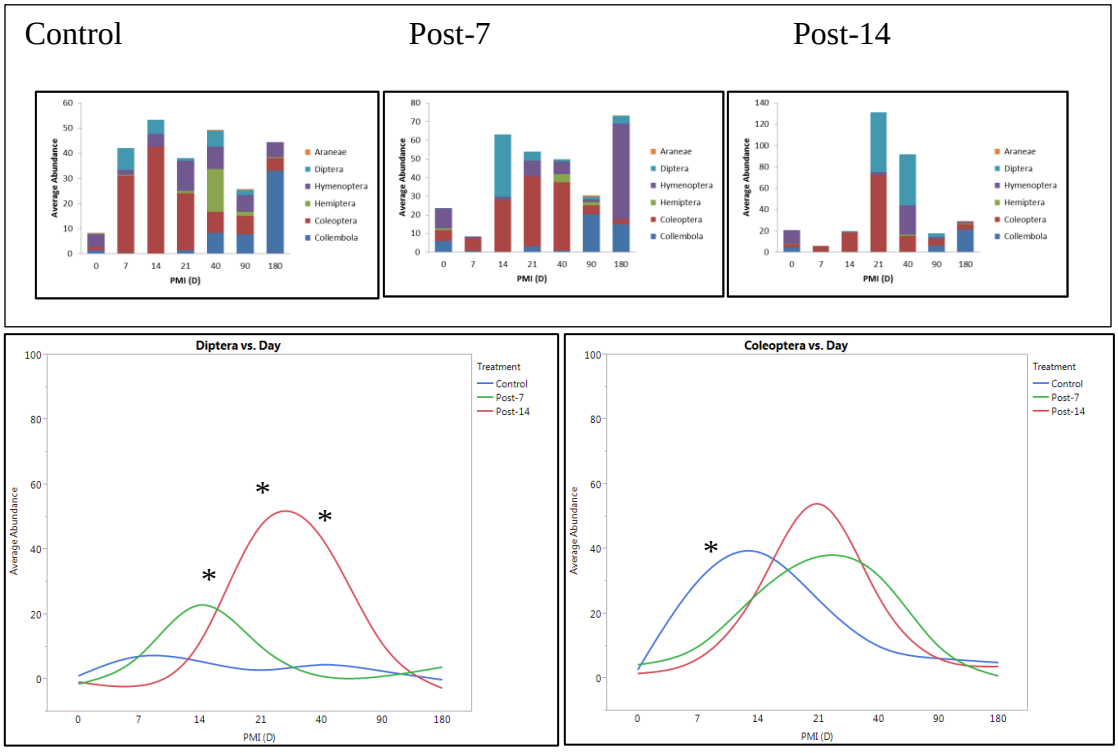

Figure 4.55. Above. Soil arthropod community abundance (by Order) beneath the carrion according to Treatments in summer 2014 at Snook, Texas. Bottom Left. Abundance of Diptera at soil beneath the carrion across Treatments over day. Bottom Right. Abundance of Coleoptera at soil beneath the carrion across Treatments over day (* indicates significant difference).

Soil lateral

Soil arthropod community beside the Control pigs showed higher abundance of Coleoptera from Day 7 to Day 14, and Collembola was dominant on Day 90 and 180. However, for Post-7, Coleoptera was dominant on Day 7, 14 and 21. Hemiptera became dominant on Day 40, and Collembola was the dominant group from Day 90 to 180. For Post-14, Hymenoptera (e.g., ants) was the dominant group of soil arthropod on Day, Coleoptera dominant on Day 21, Hemiptera on Day 40 and Collembola dominated the soil on Day 90 and 180. This observation provides preliminary evidence that there could be succession by Order level among soil arthropod communities at the soil beside the carrion. The pattern of succession were almost similar between Treatments (Coleoptera

to Diptera, to Hemiptera, and lastly Collembola), hence, there was no significant difference found between Treatments (p = 0.842) (Figure 4.56).

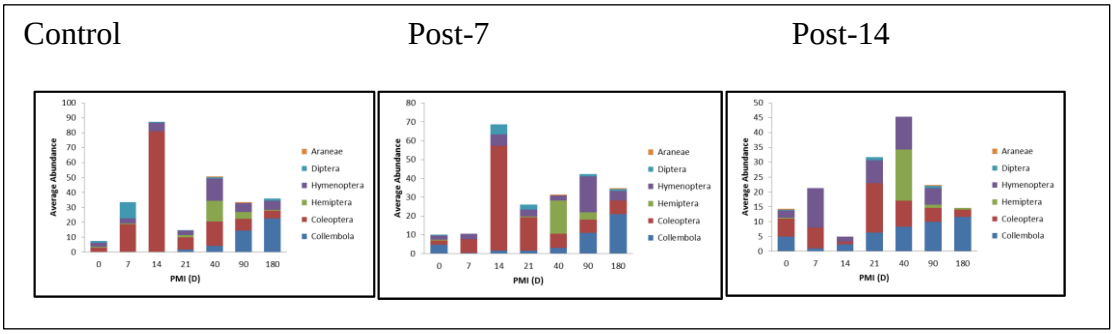

Figure 4.56. Soil arthropod community abundance (by Order) beside the carrion according to Treatments in summer 2014 at Snook, Texas.

Soil 5 m

Soil arthropod community at the soil 5 m away (serve as control soil) from all carrion regardless of Treatments mainly consisted of Collembola, Coleoptera, and Hymenoptera. This observation indicates soil arthropod community structure at 5 m away was different from those in soil beneath (where Diptera larvae were much more abundance) and more similar to the soil beside the carcasses (Figure 4.57). There was no significant difference found between Treatments at soil 5 m (p = 0.439).

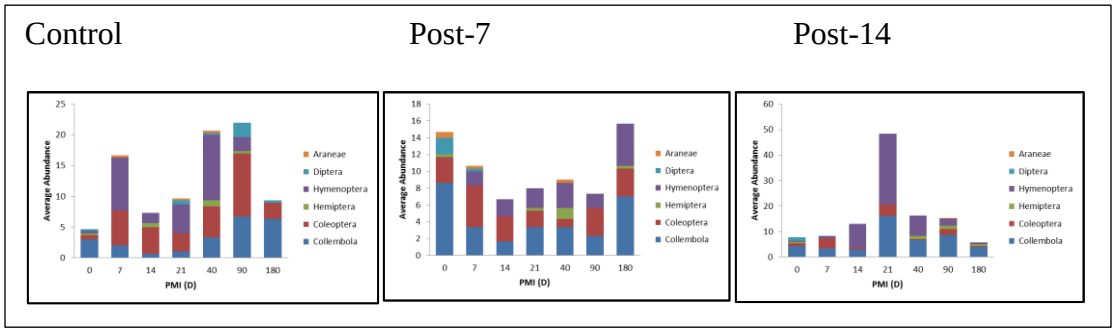

Figure 4.57. Soil arthropod community abundance (by Order) at soil 5 m away from the carrion according to Treatments in summer 2014 at Snook, Texas.

Abundance

The full model showed a significant difference in Day ($p = 0.0003$) and Region ($p < 0.0113$) and an interaction between Day x Region with $p = 0.0192$. No significant difference was detected for Treatments ($p = 0.5195$). However, there were significant differences (divergence) found in abundance between treatments on Day 7 at soil beneath (Control x Post-14, $p = 0.0071$ and Control x Post-7, $p = 0.0149$) and Day 14 (Control x Post-14, $p = 0.0024$, and Control x Post-7, $p = 0.0217$). For soil lateral, significant difference was found on Day 14 (Control x Post-14, $p = 0.0024$, and "Control x Post-7, $p = 0.0355$). As for soil 5 m, significant difference was detected on Day 21 (Control x Post-14, $p = 0.0001$ and Post-7 x Post-14, $p = 0.0002$) (Figure 4.58). Resilience was tested only for soil beneath for all treatments and all treatments showed a stable soil arthropod community by abundance (Table 4.50).

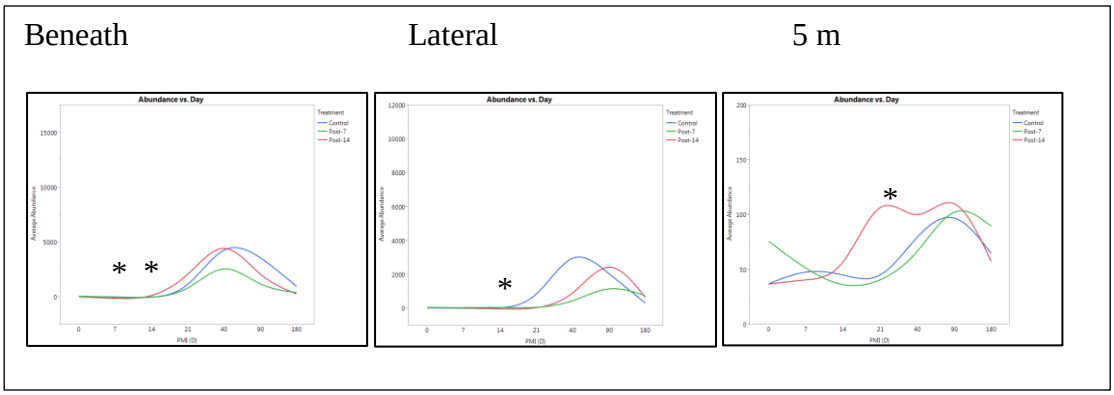

Figure 4.58. Soil arthropod community abundance (by Order) across Treatments over time at different soil regions in summer 2014 at Snook, Texas.

Table 4.50. Resilience of soil arthropod community abundance (by Order) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.5836	Resistance
Post-7	None	0.0872	Resistance
Post-14	None	0.1718	Resistance

Richness

The full model showed a significant difference in Day ($p < 0.0001$), Treatment ($p = 0.0028$) and Region ($p = 0.0142$) without significant interaction. Statistical difference (divergence) was detected on Day 7 at soil beneath ($p = 0.0496$) and Day 14 (Post-7 x Post-14, $p = 0.0310$), and then convergence occurred on Day 21. In general, richness at soil beneath of Control carcasses showed higher species richness compared to delayed carcasses. However, richness at soil lateral showed similar trend among all treatments (Figure 4.59). Resilience was tested only for soil beneath for all treatments and all treatments showed a stable soil arthropod community by richness (Table 4.51).

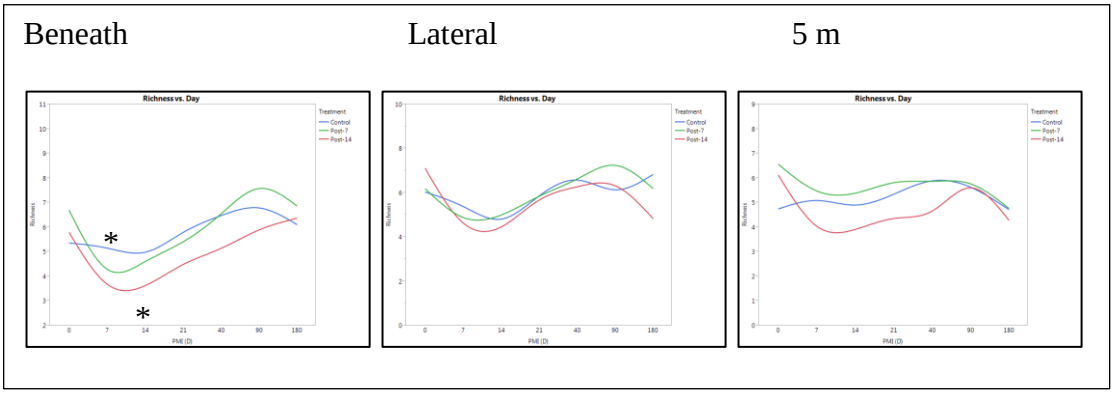

Figure 4.59. Soil arthropod community richness (by Order) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference).

Table 4.51. Resilience of soil arthropod community richness (by Order) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1980	Resistance
Post-7	None	0.0319	Resistance[#]
Post-14	None	0.0239*	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Simpson's diversity index

The full model showed a significant difference in Day ($p < 0.0001$), and Region ($p < 0.0001$) with a significant interaction Day x Region ($p = 0.0116$). Treatment had no significant difference ($p = 0.2669$). Simpson diversity index varied across Treatments over time although there was no significant difference detected at soil lateral and soil 5 m. At soil beneath, the Post-14 group had the highest Simpson's index (means lower diversity) from Day 21 to Day 90, followed by Post-7 group. Significant difference (divergence) was detected at soil beneath on Day 14, where ANOVA showed a significant difference between Control and Post-7 ($p = 0.0351$), however, convergence

occurred on Day 21 (Figure 4.60). Resilience was tested only for soil beneath for all treatments and only Control carcasses showed a stable soil arthropod community by Simpson's diversity, while Post-14 had resilience on Day 90. Post-7 did not have resilience even on Day 180 (Table 4.52).

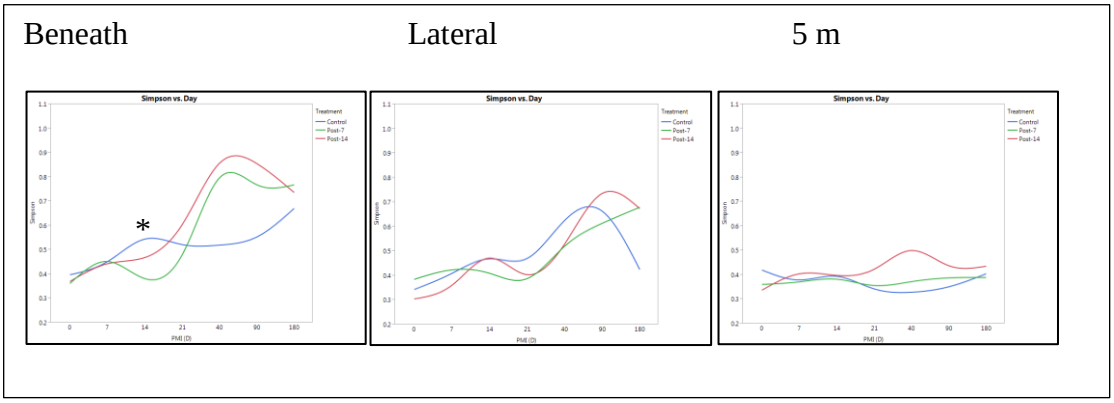

Figure 4.60. Simpson's diversity index of soil arthropods (by Order) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference).

Table 4.52. Resilience of soil arthropod community Simpson's diversity (by Order) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.8852	Resistance
Post-7	0 x 40	0.0002*	No resilience on Day
	0 x 90	0.0263*	180
	0 x 180	0.0041*	
Post-14	0 x 40	0.0169*	90

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p < 0.0001$), and Region ($p < 0.0001$) with a significant interaction Day x Region ($p = 0.0478$). Treatment had no significant difference ($p = 0.1308$). There was no statistical difference of Shannon-Wiener's diversity index found between Treatments over every sampling day in all soil regions, indicating a stable soil arthropod community. At soil beneath, diversity was decreasing for Post-7 and Post-14 carcasses from Day 0 to Day 40, while for Control carcasses, diversity decreased from Day 7 to Day 14, and then increased on Day 21 and Day 40, and decreased again on Day 90 and 180. Note that on Day 14, Post-7 group had the highest Shannon diversity and had a marginal p value ($p = 0.0541$) compared to other groups. Similar trend was observed between Treatments at soil lateral and soil 5 m (Figure 4.61). Resilience was tested only for soil beneath for all treatments and only Control carcasses showed a stable soil arthropod community by Shannon's diversity, while Post-14 had resilience on Day 180. Post-7 did not have resilience even on Day 180 (Table 4.53).

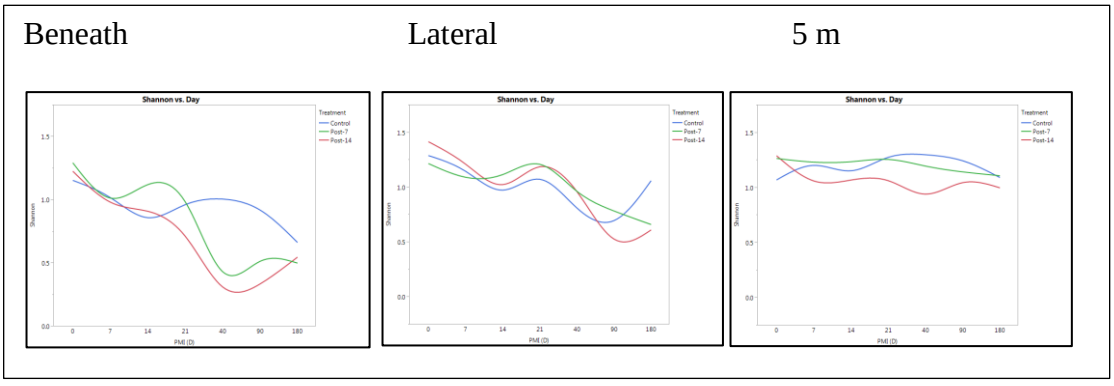

Figure 4.61. Shannon-Wiener's diversity index of soil arthropods (by Order) across Treatments over time at different soil region in summer 2014 at Snook, Texas.

Table 4.53. Resilience of soil arthropod community (by Order) Shannon-Wiener's diversity for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.9178	Resistance
Post-7	0 x 40	0.0002*	No resilience on Day
	0 x 90	0.0296*	180
	0 x 180	0.0037*	
Post-14	0 x 40	0.0108*	180
	0 x 90	0.0429*	

Evenness

The full model showed a significant difference in Day ($p < 0.0001$), and Region ($p < 0.0001$) with a significant interaction Day x Region ($p = 0.037$). Treatment had no significant difference ($p = 0.6423$). There was statistical difference (divergence) of evenness found at soil beneath on Day 14 for Control x Post-7 and Control x Post-14 ($p = 0.0205$ and 0.0213, respectively), followed by a convergence on Day 21. In general, evenness for all groups was decreasing over time. Evenness in Post-7 and Post-14 groups decreased from Day 14 to Day 40 at soil beneath. At soil lateral, evenness decreased from Day 0 to Day 90 for all groups, and then increased on Day 180. Evenness at soil 5 m was quite stable, except on Day 90 where Post-14 group had the lowest evenness than other groups (Figure 4.62). Resilience was tested only for soil beneath for all treatments and only Control carcasses showed a stable soil arthropod community by evenness, while Post-14 had resilience on Day 90. Post-7 did not have resilience even on Day 180 (Table 4.54).

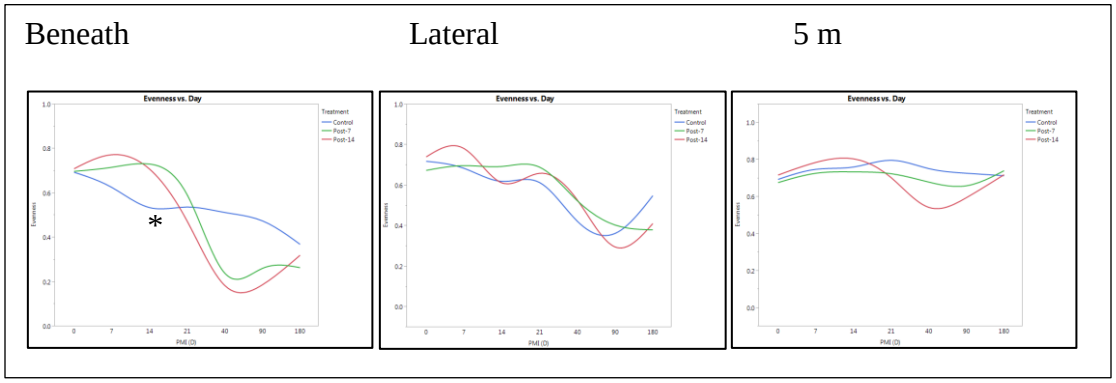

Figure 4.62. Evenness of soil arthropods (by Order) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference).

Table 4.54. Resilience of soil arthropod community (by Order) evenness for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.8314	Resistance
Post-7	0 x 40	0.0002*	No resilience on Day
	0 x 90	0.0261*	180
	0 x 180	0.0042*	
Post-14	0 x 40	0.0198*	90

Effective number of species

The full model showed a significant difference in Day ($p < 0.0001$), and Region ($p < 0.0001$) without any significant interaction. Treatment had no significant difference ($p = 0.1279$). Statistical difference (divergence) of effective number of species (Order) was found between Control x Post-7 at soil beneath on Day 14 ($p = 0.0379$) followed by convergence on Day 21. In general, effective number of species (Order) decreased from Day 0 to Day 90 for Post-7 and Post-14 groups, although there was slight increased on Day 14. Control carcasses showed a decreased ENS from Day 0 to Day 14, and then increased on Day 21 to day 40, and then decreased after that. The trends of ENS in soil 5

m were similar among treatments (Figure 4.63). Resilience was tested only for soil beneath for all treatments and only Control carcasses showed a stable soil arthropod community by ENS, while Post-14 had resilience on Day 180. Post-7 did not have resilience even on Day 180 (Table 4.55).

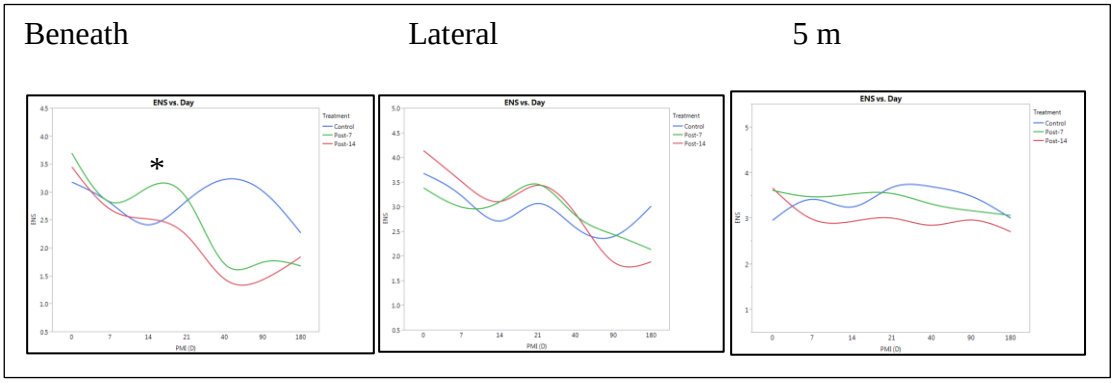

Figure 4.63. Effective number of species (by Order) of soil arthropods across Treatments over time at different soil regions in summer 2014 at Snook, Texas.

Table 4.55. Resilience of soil arthropod community ENS (by Order) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.8931	Resistance
Post-7	0 x 40	0.0002*	No resilience on Day
	0 x 90	0.0092*	180
	0 x 180	0.0016*	
Post-14	0 x 40	0.0072*	180
	0 x 90	0.0195*	

Family in 2014

PERMANOVA was performed on soil arthropod data by Family level. Note that at this taxonomical level, replicate showed a marginal effect ($p = 0.074$). Results showed that there was Day, Treatment and Region effects. Moreover, there were interactions between Day x Treatment, and Day x Region (Table 4.56).

Table 4.56. Analysis of the soil arthropod community structure (by Family) in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	11.3059	0.001*
Treatment	2	2.8316	0.002*
Region	2	6.1330	0.001*
Day x Treatment	2	1.5984	0.050*
Day x Region	2	2.7196	0.001*
Treatment x Region	4	1.2416	0.161
Day x Treatment x Region	4	0.9106	0.627

Since there was significant effect in Day, Treatment and Region, further analyses were conducted. All soil regions were significantly different from each other ($p < 0.05$), indicating soil community structure changes according to location (Table 4.57). For Treatment, significant differences were found on the pairs of Control x Post-7 and Control x Post-14, with p value 0.008 and 0.001, respectively. For Post-7 x Post-14 comparison, a marginal significant value was obtained ($p = 0.065$) (Table 4.58). This result indicates delayed Diptera colonization on carrion did significantly impact the soil arthropod community structure at the Family level, thus highlighting the importance of taxonomical scale in ecological studies. As for day of decomposition, all day to day comparisons were significantly different (Table 4.59). The NMDS plot of stress for soil

arthropod community structure (Figure 4.64) and NMDS ordinations for Day, Treatment and Region were provided for visualization of data distribution (Figure 4.65, 4.66 and 4.67, respectively). Minimum stress for given dimensionality was 0.2065 with r^2 = 0.7179. The MRPP analysis for soil region showed a significant difference (A value = 0.0268; Significant of Delta = 0.001 based on 999 permutations). The MRPP analysis for soil treatment showed a significant difference (A value = 0.0100; Significant of Delta = 0.001 based on 999 permutations) while the MRPP for day also showed a significant difference with A value 0.0875 and Significant of Delta 0.001.

Table 4.57. Pairwise comparisons between Regions on soil arthropod community structure by Family in summer 2014 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R^2	P value
Beneath x Lateral	Region	1	0.739	0.7932	2.7628	0.0218	0.008*
	Residual	124	35.601	0.2871		0.9782	
	Total	125	36.394			1.0000	
Beneath x 5 m	Region	1	2.608	2.6076	8.6704	0.0654	0.001*
	Residual	124	37.293	0.3007		0.9346	
	Total	125	39.900			1.0000	
Lateral x 5 m	Region	1	1.390	1.3904	5.0731	0.0393	0.001*
	Residual	124	33.986	0.2740		0.9607	
	Total	125	35.377			1.0000	

Table 4.58. Pairwise comparisons between Treatments on soil arthropod community structure by Family in summer 2014 at Snook, Texas after Bonferroni's correction.

Treatment		df	SS	MS	F model	R^2	P value
Control x Post-7	Treatment	1	0.532	0.5324	1.8561	0.0147	0.036*
	Residual	124	35.567	0.2868		0.9853	
	Total	125	36.100			1.0000	
Control x Post-14	Treatment	1	1.186	1.1859	3.909	0.0305	0.001*
	Residual	124	37.621	0.3033		0.9695	
	Total	125	38.807			1.0000	
Post-7 x Post-14	Treatment	1	0.494	0.4938	1.6491	0.0131	0.065•
	Residual	124	37.131	0.2994		0.9869	
	Total	125	37.625			1.0000	

•= Marginal significant difference.

Table 4.59. Pairwise comparisons of soil arthropod community structure by Family between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
7	0.001*	-	0.001*	0.002*	0.001*	0.001*	0.001*
14	0.001*	0.001*	-	0.049*	0.001*	0.001*	0.001*
21	0.001*	0.002*	0.049*	-	0.007*	0.001*	0.001*
40	0.001*	0.001*	0.001*	0.007*	-	0.001*	0.001*
90	0.001*	0.001*	0.001*	0.001*	0.001*	-	0.004*
180	0.001*	0.001*	0.001*	0.001*	0.001*	0.004*	-

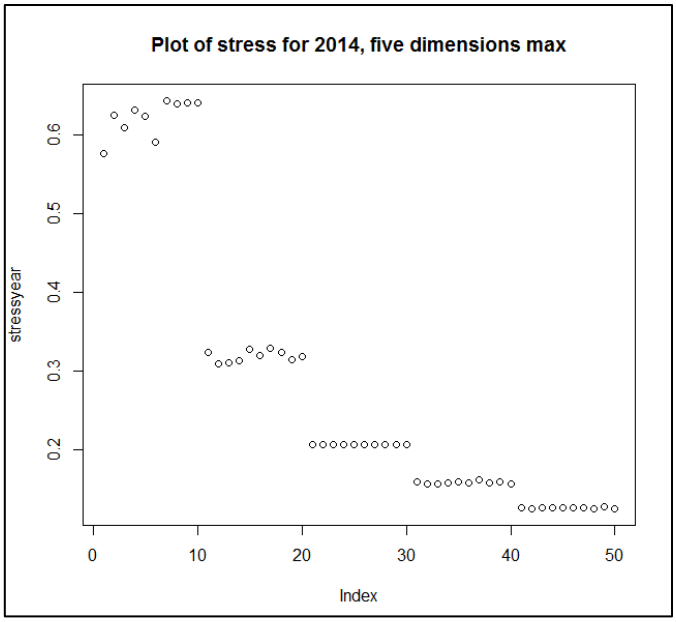

Figure 4.64. NMDS plot of stress for soil arthropod community structure (by Family) in summer 2014 at Snook, Texas (Stress test 0.2065; $r^2 = 0.7179$).

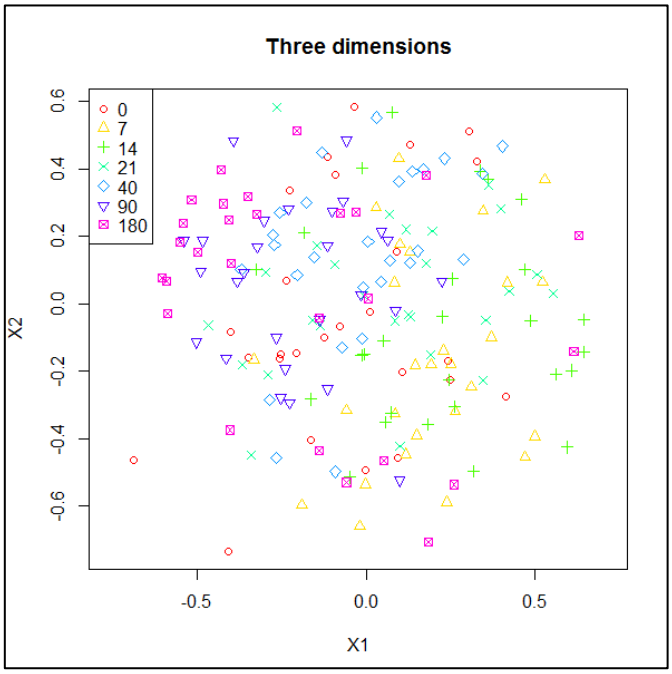

Figure 4.65. NMDS ordinations for soil arthropod community structure (by Family) according to days of carrion decomposition in summer 2014 at Snook, Texas.

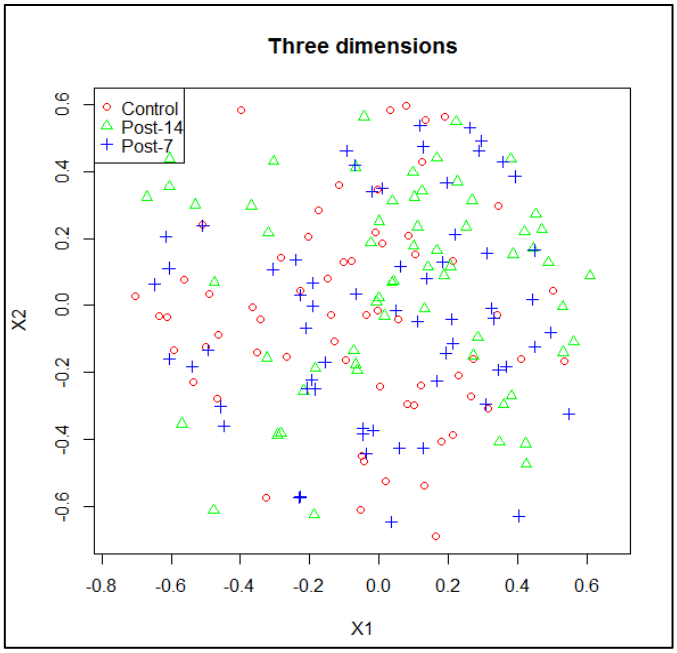

Figure 4.66. NMDS ordinations for soil arthropod community structure (by Family) according to Treatments in summer 2014 at Snook, Texas.

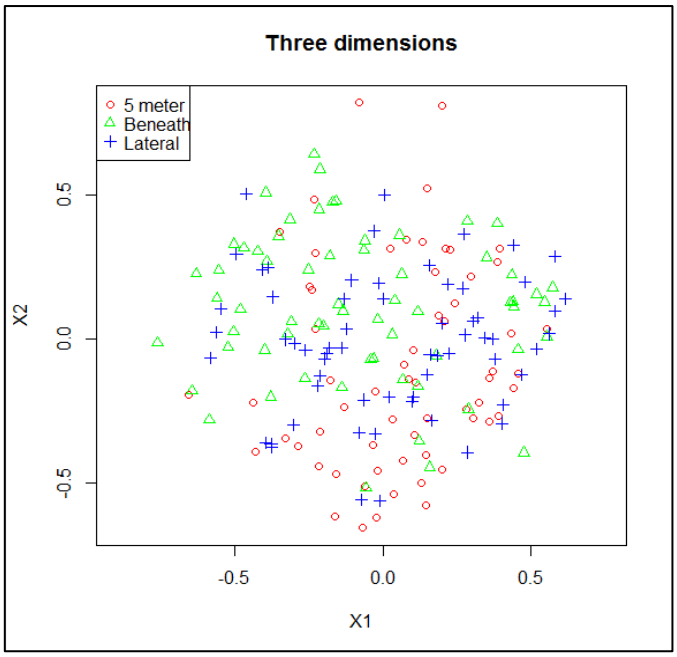

Figure 4.67. NMDS ordinations for soil arthropod community structure (by Family) according to soil regions in summer 2014 at Snook, Texas.

The ISA results showed 11 family indicators among soil arthropods in 2014 trial. They were Japygidae, Tenebrionidae, Muscidae, Staphylinidae, Nitidulidae, Carabidae, Stratiomyidae, Aphididae, Liposcelidae, Entomobryidae and Ptilidae (Table 4.60).

Table 4.60. Indicator species analysis by Family for soil arthropods in summer 2014 at Snook, Texas.

Type	Family	Indicator value	P value
All soils	Japygidae	0.1772	0.017*
	Tenebrionidae	0.1880	0.003*
	Muscidae	0.2552	0.004*
	Staphylinidae	0.2154	0.001*
	Nitidulidae	0.2000	0.014*
	Carabidae	0.4444	0.028*
	Stratiomyidae	0.3092	0.027*
	Aphididae	0.2222	0.016*
	Liposcelidae	0.4444	0.022*
	Entomobryidae	0.1012	0.019*
	Ptilidae	0.4815	0.047*

Abundance of soil arthropod community structure (by Family) according to soil region (excluding mites) in 2014

Soil beneath

There was significant difference between treatments at soil beneath ($p = 0.044$) where Control x Post-14 had a significant difference in terms of soil arthropod community structure ($p = 0.01$). Soil arthropod community beneath the Control pigs showed higher abundance of predatory rove beetles, Staphylinidae, on Day 7. Muscid larvae can be seen on Day 7, 14 and 40. The collembolans, Entomobryidae, increased in abundance from Day 21 to Day 180 (dominant family). For the Post-7 carcasses, muscid

larvae became dominant on Day 14 while staphylinid beetles dominated the soil on Day 21. On Day 180, Formicidae and Entomobryidae were the most abundant families. In Post-14 group, after a week exposure to the environment following the insect-exclusion cage removal, the abundance of Staphylinidae and Muscidae increased on Day 21. Likewise, entomobryid collembolans increased on Day 90 and 180 (Figure 4.68). Specific attention was given to the necrophagous families at soil beneath, hence the dynamics of the following families were plotted to determine differences between treatments over day of decomposition. For Muscidae, significant difference was found on Day 14 (Control x Post-7 and Control x Post-14, with p values 0.0334 and 0.0264, respectively) and Day 40 (Control x Post-14 and Post-7 x Post-14; $p = 0.0202$ and 0.0077, respectively). For Sarcophagidae, there was significant difference on Day 7 (Control x Post-7 and Control x Post-14, both p values were 0.0435) and only marginally significant difference on Day 21 ($p = 0.0623$). Similarly, for Staphylinidae, significant difference was found on Day 7 (Control x Post-7 and Control x Post-14, with p values 0.0050 and 0.0028, respectively) and Day 21 ($p = 0.0453$).

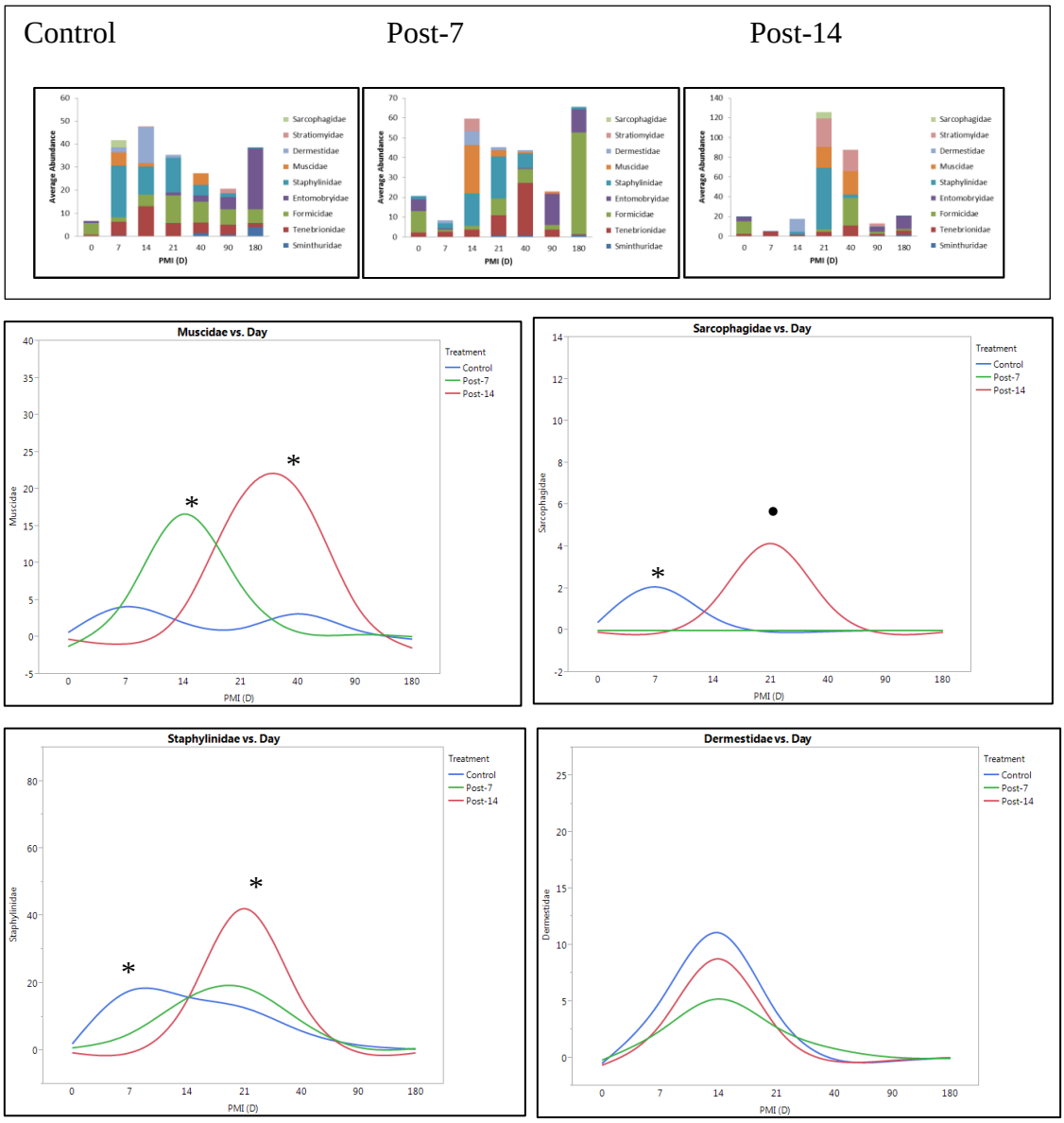

Figure 4.68. Above. Soil arthropod community abundance (by Family) beneath the carrion according to Treatments in summer 2014 at Snook, Texas. Middle Left. Abundance of Muscidae (larvae) at soil beneath the carrion across Treatments over day. Middle Right. Abundance of Sarcophagidae (larvae) at soil beneath the carrion across Treatments over day. Bottom Left. Abundance of Staphylinidae (adults) at soil beneath the carrion across Treatments over day. Bottom Right. Abundance of Dermestidae (adults and larvae) at soil beneath the carrion across Treatments over time (* indicates significant difference, • denotes marginal significant difference).

Soil lateral

There was no significant difference between treatments at soil lateral (p = 0.115). Soil arthropod community beside the Control pigs showed higher abundance of Staphylinidae and Sarcophagidae larvae on Day 7 while Tenebrionidae was abundant on Day 14. Formicidae and Entomobryidae were dominant on Day 90 and Day 180. However, for Post-7, Staphylinidae and Dermestidae were dominant on Day 14 and 21, and ants and entomobryid collembolans were the dominant group from Day 90 to 180. For Post-14, Tenebrionidae, Formicidae, Entomobryidae and some Staphylinidae were prevalent from Day 0 to Day 180, although entomobryid collembolans were the most abundant family on Day 90 and 180 (Figure 4.69).

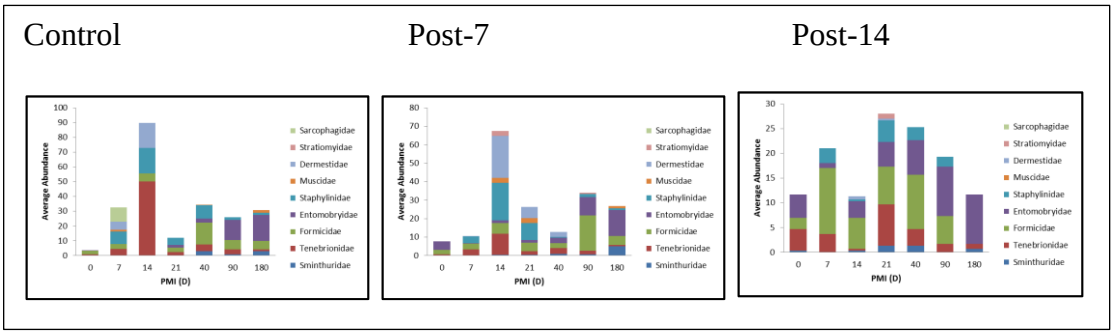

Figure 4.69. Soil arthropod community abundance (by Family) beside the carrion according to Treatments in summer 2014 at Snook, Texas.

Soil 5 m

There was significant difference between treatments at soil 5 m (p = 0.011) where Control x Post-14 was significant different by soil arthropod community structure (p = 0.033). Soil arthropod community at the soil 5 m away (serve as control soil) from all carrion regardless of Treatments mainly consisted of Formicidae, and Entomobryidae. In Control carcasses, abundance of Staphylinidae increased on Day 7, 14 and 21. Note that there was one Stratiomyidae larva observed on Day 90 at soil 5 m away from carrion, this indicates the dispersal range demonstrated by this family. For

Post-7 carcasses, the abundance of Sminthuridae (globular springtails) decreased from Day 0 to Day 21, and then recovered gradually from Day 21 to Day 40, and on Day 180, Sminthuridae and Formicidae were the dominant families. For Post-14 group, Entomobryidae were present in all sampling days. On Day 21, Formicidae was the most abundant family at soil 5m (Figure 4.70).

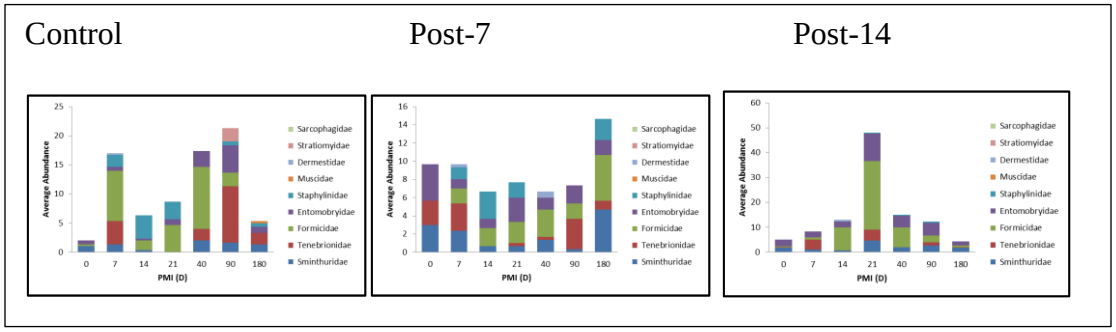

Figure 4.70. Soil arthropod community abundance (by Family) at soil 5 m away from the carrion according to Treatments in summer 2014 at Snook, Texas.

Abundance

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) and three interactions include Day x Treatment with $p < 0.0001$, Day x Region with $p < 0.0001$, and Day x Treatment x Region, with $p = 0.0357$. No significant difference was detected for Treatments ($p = 0.9845$). Significant difference was found in abundance at soil beneath on Day 7 (Control x Post-14, $p = 0.0006$ and Control x Post-7, $p = 0.0009$), on Day 14 ($p = 0.0491$), Day 21 (Control x Post-14, $p = 0.0083$ and Post-7 x Post-14, $p = 0.0152$) and Day 90 (Post-7 x Post-14, $p = 0.0306$). For soil beneath, convergence happened on Day 40 and Day 180. For soil lateral, divergence occurred on Day 0 (Control x Post-14, $p = 0.0227$) and followed by convergence on Day 7. For soil 5 m, a marginal significant difference was detected on Day 21 ($p = 0.0572$) (Figure 4.71). Resilience was tested only for soil beneath for all treatments and resilience was observed

on Day 90 for Post-14 carcasses while Control and Post-7 carcasses demonstrated a stable abundance over time (Table 4.61).

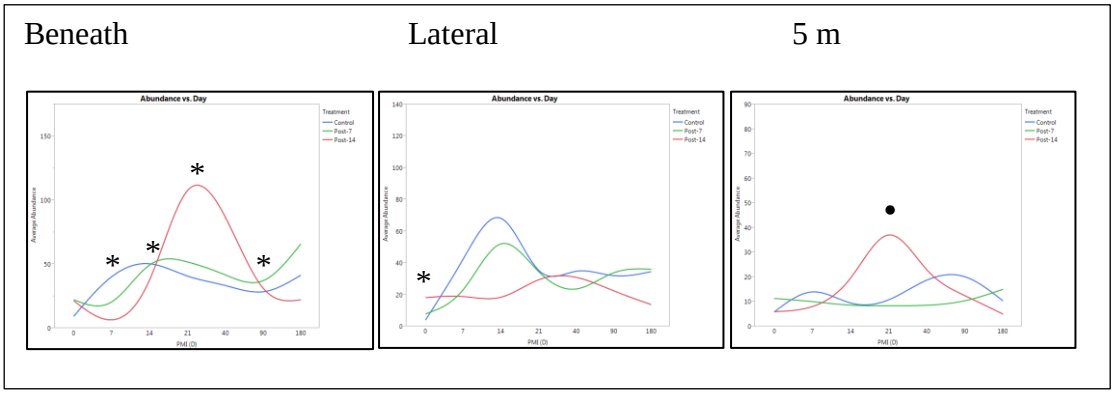

Figure 4.71. Soil arthropod community abundance (by Family) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference; • denotes marginally significant difference).

Table 4.61. Resilience for soil arthropod community abundance (by Family) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0666	Resistance
Post-7	None	0.4193	Resistance
Post-14	0 x 21	0.0002*	90
	0 x 40	0.0201*	

Richness

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) and an interaction Day x Treatment with $p < 0.0001$. No significant difference was detected for Treatments ($p = 0.0893$). Statistical difference (divergence) was detected on Day 7 at soil beneath (Control x Post-14, $p = 0.0225$) and Day 21 (Control x Post-14 and Post-7 x Post-14, both p values 0.0234). It was marginally

significant on Day 14 (Post-7 x Post-14, p = 0.0539), however, this was considered convergence. For soil lateral, Control x Post-14 was significantly different on Day 0. As for soil 5 m, significant difference (p = 0.0456) was detected on Day 7. In general, Post-14 group had the lowest richness from Day 7 to Day 14, followed by Post-7 group (Figure 4.72). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable richness for all treatments over time (Table 4.62).

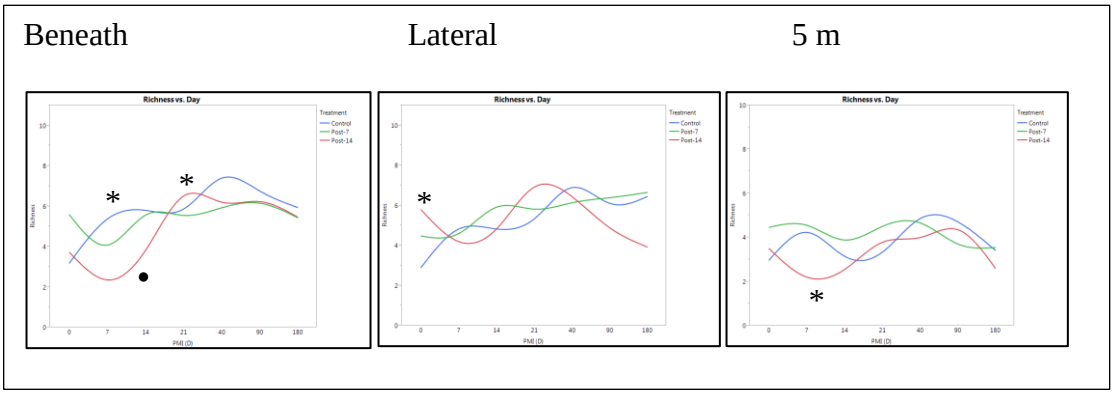

Figure 4.72. Soil arthropod community richness (by Family) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference; • denotes marginally significant difference).

Table 4.62. Resilience for soil arthropod community richness (by Family) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0909	Resistance
Post-7	None	0.3724	Resistance
Post-14	None	0.0074*	Resistance[#]

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Simpson's diversity index

The full model showed a significant difference in Day (p = 0.0288), Treatment (p = 0.0040), Region (p = 0.0060) and an interaction Day x Treatment with p = 0.0347. At soil beneath, the Post-14 group had the highest Simpson's index (means lower diversity) from Day 7 to Day 21, followed by Post-7 group. Significant difference (divergence) was detected at soil beneath on Day 21 between Control x Post-14 and Post-7 x Post-14 (p = 0.0032 and 0.0014), followed by convergence on Day 40. There was no significant difference of Simpson's Diversity detected at soil lateral. At soil 5 m, marginally significant difference was detected on Day 7 (Figure 4.73). Resilience was tested only for soil beneath for all treatments and Control carcasses demonstrated resilience on Day 90 while Post-7 and Post-14 carcasses were stable in diversity over time (Table 4.63).

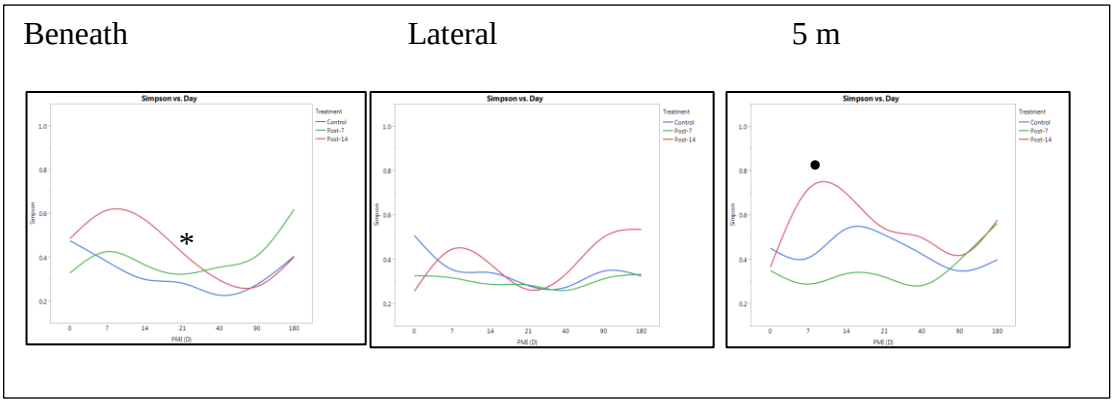

Figure 4.73. Simpson's diversity index of soil arthropods (by Family) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference, • denotes marginally significant difference).

Table 4.63. Resilience for soil arthropod Simpson's diversity (by Family) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 40	0.0319	90
Post-7	None	0.2425	Resistance
Post-14	None	0.1268*	Resistance

Shannon-Wiener's diversity index

The full model showed a significant difference in Day (p = 0.0020), Treatment (p = 0.0065), Region (p = 0.0001) and an interaction Day x Treatment with p = 0.0087. There was statistical difference (divergence) of Shannon-Wiener's diversity index found on Day 14 at soil beneath between Control x Post-14 (p = 0.0495) while Day 21 was marginally significant between Treatments, hence a convergence. On Day 40, significant difference was found between Control x Post-7 with p value 0.0186. At soil 5 m, significant difference was found on Day 7 between Post-7 x Post-14 (p = 0.0415) (Figure 4.74). Resilience was tested only for soil beneath for all treatments and Control carcasses demonstrated resilience on Day 90 while Post-7 and Post-14 carcasses were stable in diversity over time (Table 4.64).

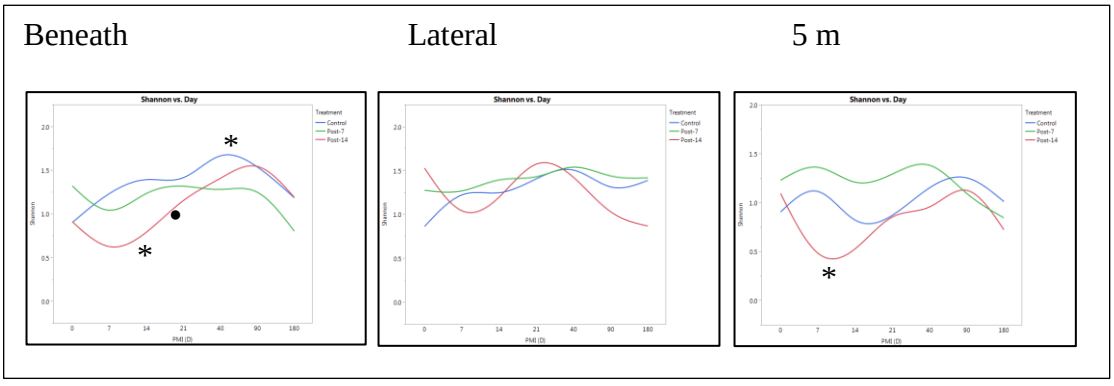

Figure 4.74. Shannon-Wiener's diversity index of soil arthropods (by Family) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* represents significant difference and • denotes marginally significant difference).

Table 4.64. Resilience for soil arthropod Shannon-Wiener's diversity (by Family) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 40	0.0047*	90
Post-7	None	0.3560	Resistance
Post-14	None	0.0194*	Resistance[#]

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Evenness

The full model showed a significant difference in Treatment ($p = 0.0067$) only, while Day and Region were not significantly difference ($p > 0.05$). There was statistical difference (divergence) of evenness found at soil beneath on Day 21 for Control x Post-14 and Post-7 x Post-14 ($p = 0.0001$ and < 0.0001, respectively), followed by a convergence on Day 40. At soil 5 m, marginally significant difference ($p = 0.0622$) was observed on Day 7. In general, Post-14 had the lowest evenness at soil beneath the carrion from Day 7- Day 21 (Figure 4.75). Resilience was tested only for soil beneath for all treatments and the results demonstrated that all treatments were in stable dynamics (by evenness) over time (Table 4.65).

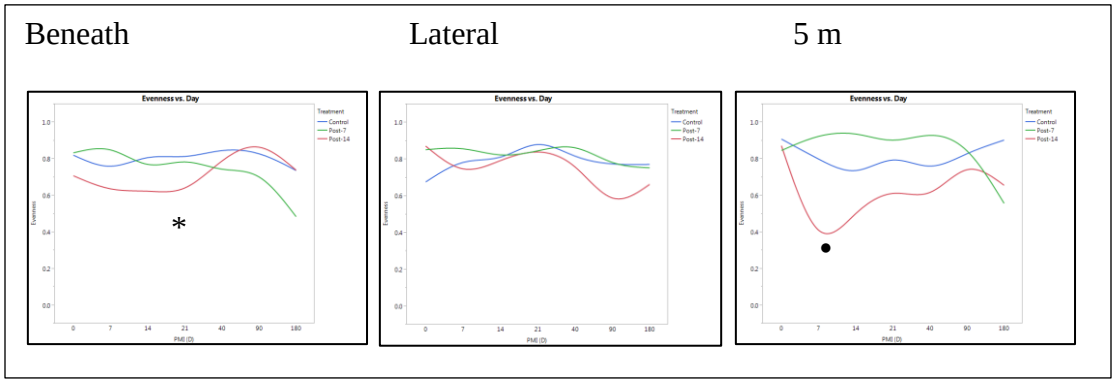

Figure 4.75. Evenness of soil arthropods across Treatments (by Family) over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference, • denotes marginally significant difference).

Table 4.65. Resilience for soil arthropod community evenness (by Family) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.5854	Resistance
Post-7	None	0.0637	Resistance
Post-14	None	0.7296	Resistance

Effective number of species

The full model showed a significant difference in Day ($p = 0.0002$), Treatment ($p = 0.0324$), Region ($p = 0.0003$) and a significant interaction Day x Treatment ($p = 0.0164$). At soil beneath, statistical difference (divergence) of effective number of species (Family) was found between Control x Post-14 on Day 14 ($p = 0.0245$). On Day 21, there was significant difference between Post-7 x Post-14 ($p = 0.0393$). Furthermore, Control x Post-7 and Control x Post-14 were significantly different on Day 40 ($p = 0.0201$ and 0.0422, respectively). Convergence then occurred on Day 90. At soil lateral, significant difference was found between Control x Post-14 on Day 7 ($p = 0.0434$). As for soil 5 m away from carrion, Post-7 x Post-14 was significantly different ($p = 0.0328$). In general, ENS was the highest at soil beneath for Control carcasses, followed by Post-

7, and then Post-14 group (Figure 4.76). Resilience was tested only for soil beneath for all treatments and Control carcasses demonstrated resilience on Day 90 while Post-7 and Post-14 carcasses were stable in ENS over time (Table 4.66).

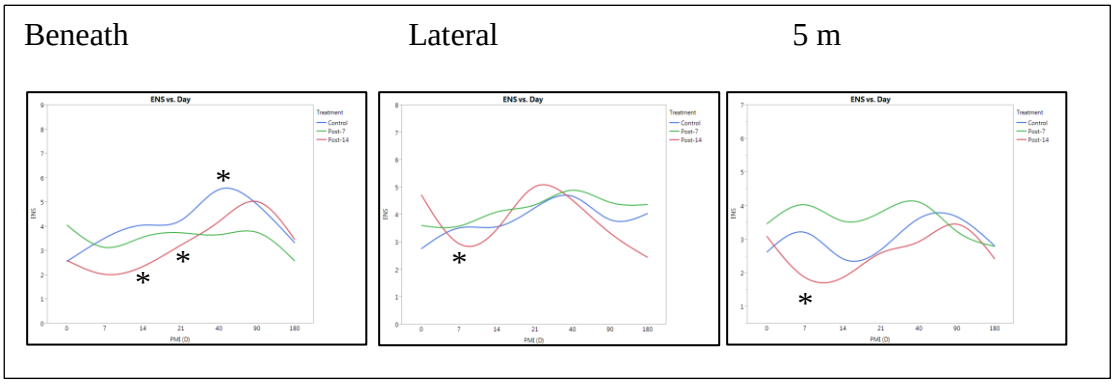

Figure 4.76. Effective number of species (by Family) of soil arthropods across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference, • denotes marginally significant difference).

Table 4.66. Resilience for soil arthropod community ENS (by Family) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 40	0.0077*	90
Post-7	None	0.5232	Resistance
Post-14	None	0.0275*	Resistance[#]

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Genus in 2014

PERMANOVA was performed on soil arthropod data by Genus level. Results showed that there was Day, Treatment and Region effects (p = 0.001, 0.015 and 0.001, respectively). There was no significant interaction was detected (Table 4.67).

Table 4.67. Analysis of the soil arthropod community structure (by Genus) in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	11.2532	0.001*
Treatment	2	2.4540	0.015*
Region	2	5.5230	0.001*
Day x Treatment	2	1.4222	0.142
Day x Region	2	1.1520	0.276
Treatment x Region	4	0.8007	0.721
Day x Treatment x Region	4	0.7005	0.831

There was a significant effect in Day, Treatment and Region, further analyses were carried out. For soil regions, all soil regions were significantly from each other ($p < 0.05$), indicating soil community structure changes according to region, although soil beneath and soil lateral was just 30 cm away (Table 4.68). For treatment, Control x Post-14 was significantly different (p = 0.003) in terms of soil arthropod community structure at the genus level (Table 4.69). This indicates the importance of taxonomical scale in ecological studies. As for day of decomposition, most of the pairwise comparisons between days of decomposition were significantly different, except five pairs namely Day 7 x Day 14, Day 7 x Day 21, Day 14 x Day 21, Day 90 x Day 180, and Day 0 x Day 180 where there were no significant difference detected (Table 4.70). The NMDS plot of stress for soil arthropod community structure (Figure 4.77) and NMDS ordinations for

Day, Treatment and Region were provided for visualization about data distribution (Figure 4.78, 4.79 and 4.80 respectively). Minimum stress for given dimensionality was 0.1520 with r^2 = 0.8736. The MRPP analysis for soil region showed a significant difference (A value = 0.0358; Significant of Delta = 0.001 based on 999 permutations), the MRPP for treatments showed A value 0.0088 and Significant of Delta 0.026 while the MRPP for day also showed a significant difference with A value 0.1075 and Significant of Delta 0.001.

Table 4.68. Pairwise comparisons between Regions on soil arthropod community structure by Genus in summer 2014 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	1.023	1.0234	2.8826	0.0227	0.013*
	Residual	124	44.025	0.3550		0.9773	
	Total	125	45.049			1.0000	
Beneath x 5 m	Region	1	2.121	2.1214	6.5963	0.0505	0.001*
	Residual	124	39.880	0.3216		0.9495	
	Total	125	42.001			1.0000	
Lateral x 5 m	Region	1	1.915	1.9149	6.38	0.0489	0.002*
	Residual	124	37.217	0.3001		0.9511	
	Total	125	39.132			1.0000	

Table 4.69. Pairwise comparisons between Treatments on soil arthropod community structure by Genus in summer 2014 at Snook, Texas after Bonferroni's correction.

Treatment		df	SS	MS	F model	R2	P value
Control x Post-7	Treatment	1	0.512	0.5124	1.472	0.0117	0.18
	Residual	124	43.167	0.3481		0.9883	
	Total	125	43.679			1.0000	
Control x Post-14	Treatment	1	1.339	1.3386	3.9849	0.0312	0.003*
	Residual	124	41.654	0.3359		0.9688	
	Total	125	42.993			1.0000	
Post-7 x Post-14	Treatment	1	0.397	0.3972	1.2297	0.0098	0.253
	Residual	124	40.050	0.3229		0.9902	
	Total	125	40.447			1.0000	

Table 4.70. Pairwise comparisons of soil arthropod community structure by Genus in summer 2014 at Snook, Texas between decomposition days after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.014*	0.002*	0.001*	0.001*	0.006*	0.061•
7	0.014*	-	0.472	0.091	0.001*	0.001*	0.001*
14	0.002*	0.472	-	0.298	0.001*	0.001*	0.001*
21	0.001*	0.091	0.298	-	0.001*	0.001*	0.001*
40	0.001*	0.001*	0.001*	0.001*	-	0.002*	0.004*
90	0.006*	0.001*	0.001*	0.001*	0.002*	-	0.071•
180	0.061•	0.001*	0.001*	0.001*	0.004*	0.071•	-

• Marginally significant difference.

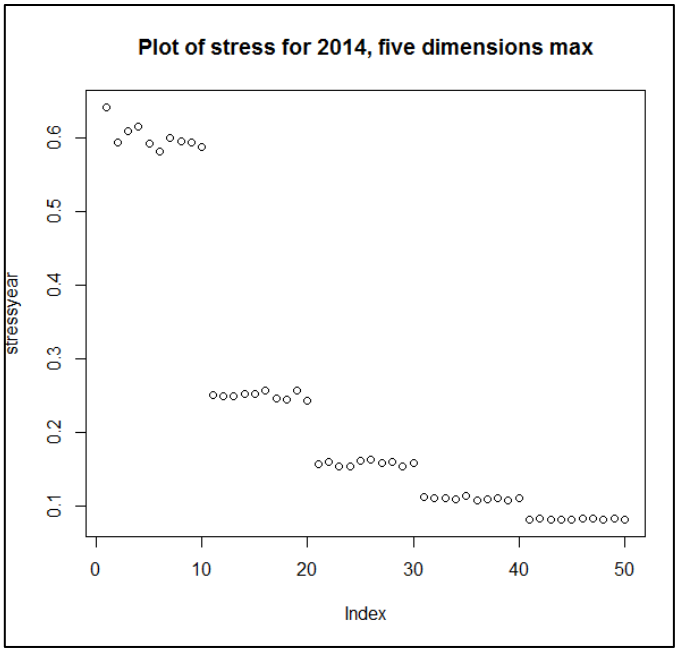

Figure 4.77. NMDS plot of stress for soil arthropod community structure (by Genus) in summer 2014 at Snook, Texas (Stress test 0.1520; $r^2 = 0.8736$).

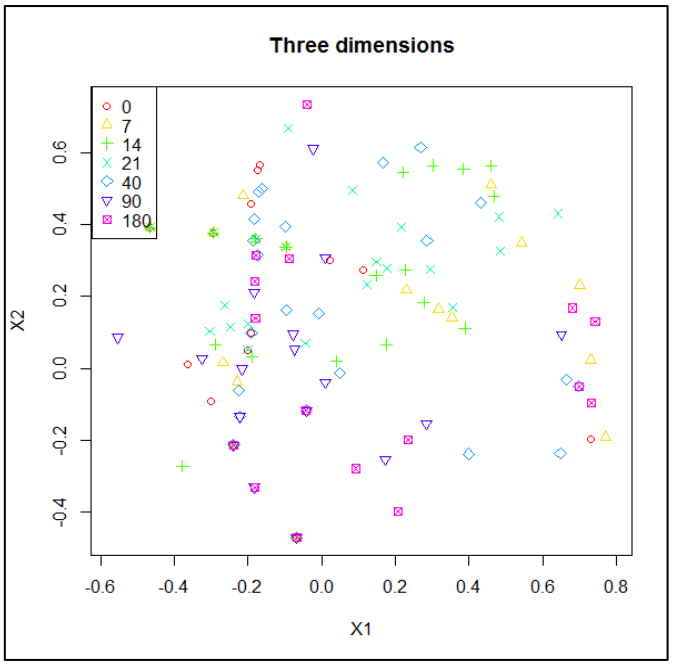

Figure 4.78. NMDS ordinations for soil arthropod community structure (by Genus) according to carrion decomposition days in summer 2014 at Snook, Texas.

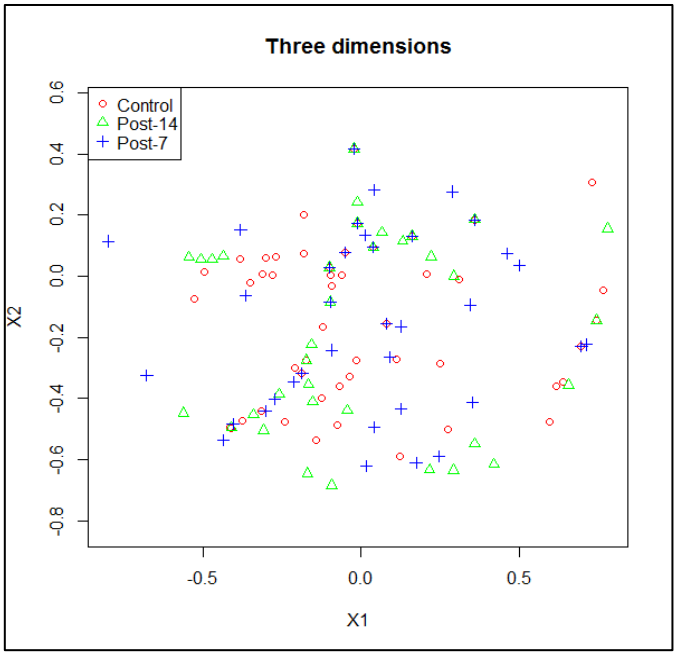

Figure 4.79. NMDS ordinations for soil arthropod community structure (by Genus) according to treatments in summer 2014 at Snook, Texas.

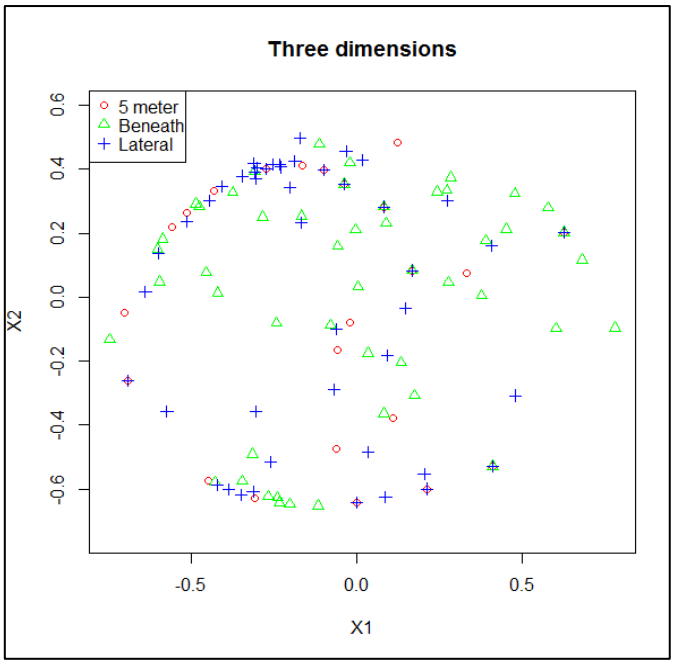

Figure 4.80. NMDS ordinations for soil arthropod community structure (by Genus) according to soil regions in summer 2014 at Snook, Texas.

The ISA results showed five genera of soil arthropods were the significant indicators in summer 2014. They were *Fannia* sp. (Fanniidae), *Baris* sp. (Curculionidae), *Hydrotaea* sp. (= *Hydroteae* sp.) (Muscidae), *Leptogenys* sp. (Formicidae), and *H. illucens* (Stratiomyidae) (Table 4.71). Note that three genera (*Fannia*, *Hydrotaea and Hermetia*) are in the Order Diptera, one genus in the Order Coleoptera (*Baris* sp.) and one genus (*Leptogenys*) in the Order Hymenoptera. All dipteran indicators are necrophagous, the ants (*Leptogenys* sp.) are predators while the curculionid beetles (*Baris* sp.) are herbivores.

Table 4.71. Indicator species analysis by Genus for soil arthropods in summer 2014 at Snook, Texas.

Type	Genus and species	Indicator value	P value
All soils	*Fannia sp.*	0.2857	0.035*
	Baris sp.	0.6667	0.008*
	Hydrotaea sp.	0.2570	0.008*
	Leptogenys sp.	0.1281	0.047*
	Hermetia illucens	0.3316	0.021*

Abundance of soil arthropod community structure (by Genus) according to soil regions (excluding mites) in 2014

Soil beneath

There was significant difference between Treatments at soil beneath the carrion ($p = 0.016$) where Control x Post-7 and Control x Post-14 were significantly different from each other ($p = 0.044$ and 0.006, respectively). Soil arthropod community beneath the Control pigs showed a very high abundance of *S. bullata* and *Hydrotaea* larvae on Day 7, suggesting these larvae were the dominant species underneath the swine carrion. There was a switch of larval species on Day 14 where *Hydrotaea* sp, *Fannia* sp. and *Hermetia illucens* Linnaeus larvae were dominated the soil beneath the carrion.

Hydrotaea larvae persisted in the soil until Day 40 while *H. illucens* larvae still can be seen on Day 90. Red imported fire ants, *S. invicta*, were the dominant genus from Day 40 to Day 180. For Post-7, *Hydrotaea* larvae and *H. illucens* larvae were the dominant groups of soil arthropods on Day 14. As for Post-14, *Hydrotaea* and *H. illucens* larvae were the dominant genera on Day 21 and Day 40. The shift in soil arthropod community (by Genus) beneath the carrion in different Treatments was obvious, and was statistically different between Treatments ($p = 0.016$) (Figure 4.81). The abundance of the *S. bullata*, *Fannia* sp., *H. illucens*, and *Hydrotaea* sp. at soil beneath the carrion were specifically highlighted as they are the major necrophagous genera in this study site (Figure 4.81).

For *S. bullata* larvae, abundance of *S. bullata* larvae were high for Control carcasses on Day 7, and it was statistically significant difference between Control x Post-7 and Control x Post-14 carcasses, p values were 0.0435 for both pairs of comparison. On Day 21, abundance of *S. bullata* larvae increased at Post-14 carcasses, however, it was not significant difference, although marginally, compared to other groups ($p = 0.0623$).

The larvae of *Fannia* sp. increased in abundance at the soil beneath the Control carcasses and were peaked on Day 14, although there was no significant difference between treatments in all sampling days. Likewise, *H. illucens* larvae were more abundant in Post-14 carcasses on Day 21 and Day 40. However, no statistically difference ($p > 0.05$) was detected between treatments at every sampling day.

Hydrotaea larvae increased in abundant on Day 14 (by Post-7 carcasses) and again on Day 21 (by Post-14 carcasses). For Day 14, Control x Post-7 and Post-7 x Post-14 were significantly different ($p = 0.0334$ and 0.0264). On Day 21, Control x Post-14 and Post-7 x Post-14 were also statistically different with p value 0.0202 and 0.0077, respectively.

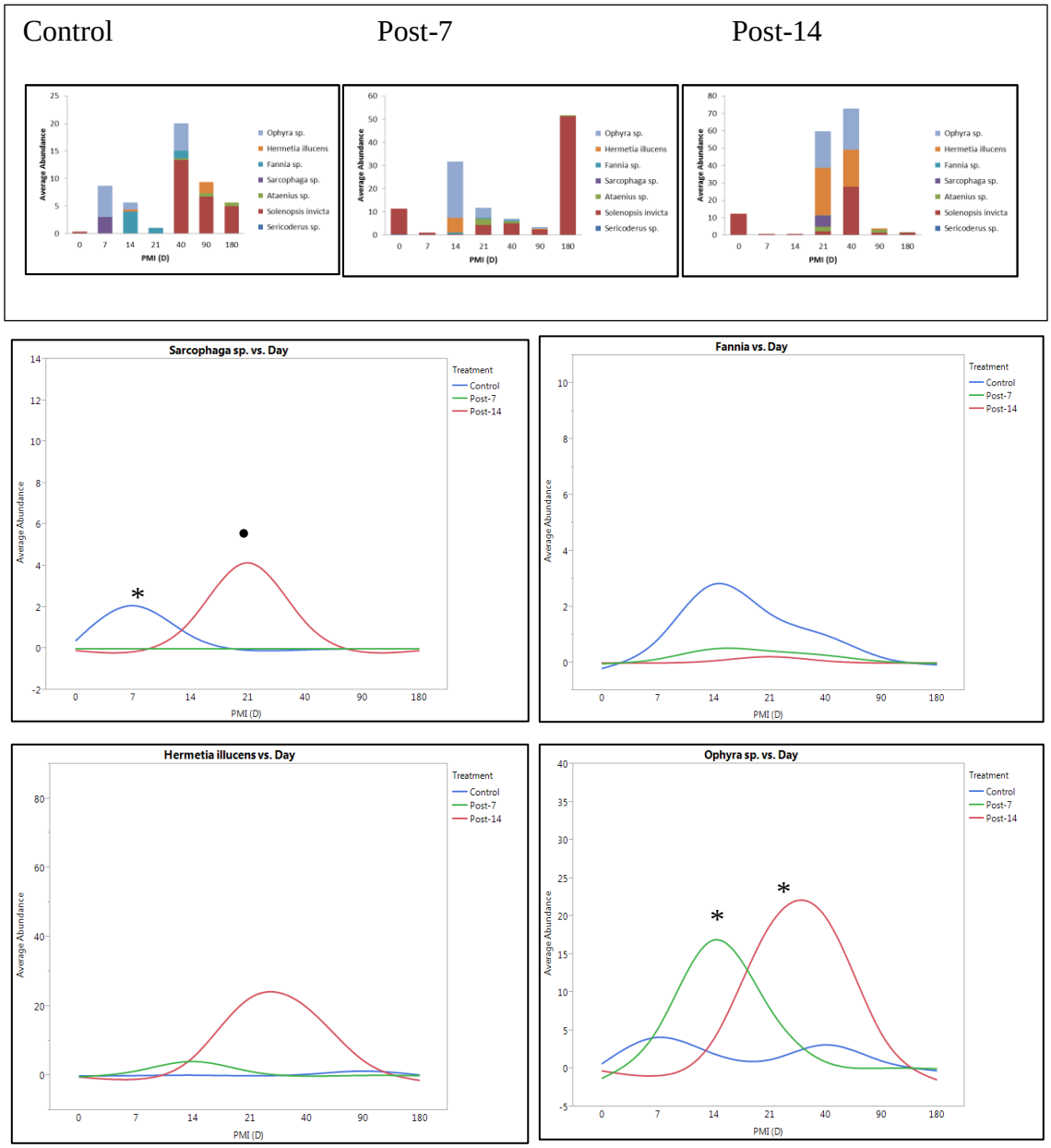

Figure 4.81. Above. Soil arthropod community abundance (by Genus) beneath the carrion according to Treatments over carrion decomposition days in summer 2014 at Snook, Texas. Middle Left. Abundance of *Sarcophaga bullata* (larvae) at soil beneath the carrion across Treatments over time. Middle Right. Abundance of *Fannia* sp. (larvae) at soil beneath the carrion across Treatments over time. Lower Left. Abundance of *Hermetia illucens* (larvae) at soil beneath the carrion across Treatments over time. Lower Right. Abundance of *Hydrotaea* sp. (larvae) at soil beneath the carrion across treatments over time (* represents significant difference. • denotes marginally significant difference).

Soil lateral

There was no significant difference between Treatments (p = 0.439) at soil beside the carrion. Soil arthropod community beside the Control pigs showed higher abundance of *S. bullata* and *Hydrotaea* larvae on Day 7, and *Fannia* larvae on Day 14. Note that the *Hydrotaea* larvae can still be collected on Day 180 underneath the Control pigs. Post-7 carcasses showed *H. illucens* larvae on Day 14 and *Hydrotaea* larvae on Day 14 and 21. For Post-14 group, *H. illucens* larvae were present on Day 21. In general, *S. invicta* were quite common on every treatment on every sampling day (Figure 4.82).

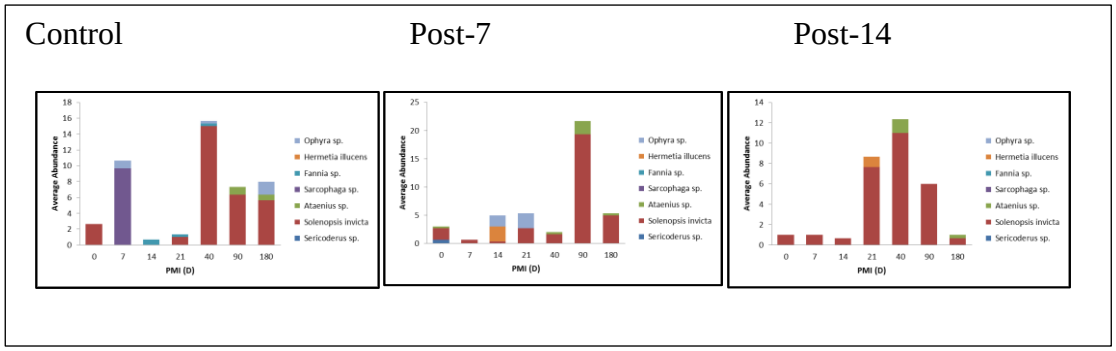

Figure 4.82. Soil arthropod community abundance (by Genus) beside the carrion according to Treatments over carrion decomposition days in summer 2014 at Snook, Texas.

Soil 5 m

There was no significant difference between Treatments (p = 0.667) at soil 5 m away from carrion. The soils at 5 m away from carrion served as the control to the soils collected from beneath and lateral of the carrion. Overall, *S. invicta* was the common and dominant arthropod in all treatments on every sampling day. Note that the larvae of *H. illucens* were collected on Day 90 from the Control swine (Figure 4.83).

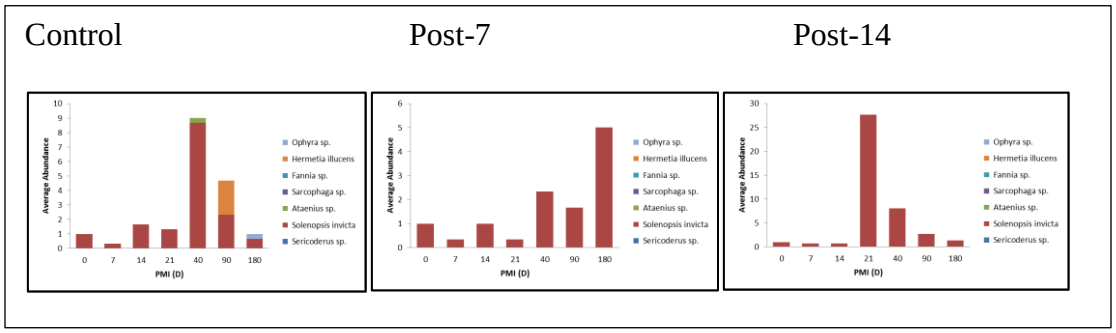

Figure 4.83. Soil arthropod community abundance (by Genus) at soil 5 m away from the carrion according to Treatments over carrion decomposition days in summer 2014 at Snook, Texas.

Abundance

The full model showed a significant difference in Day ($p = 0.0245$) and Region ($p < 0.0001$) and two interactions include Day x Treatment with $p = 0.0009$, and Day x Treatment x Region, with $p = 0.0399$. No significant difference was detected for Treatments ($p = 0.2644$). However, there was a significant difference found in abundance at soil beneath on Day 14 between Post-7 x Post-14 ($p = 0.0464$) and again on Day 21 (Post-7 x Post-14, $p = 0.0308$). Also, marginal significant difference was detected on Day 21 ($p = 0.0636$). There was no divergence occurred at soil lateral and soil 5 m (Figure 4.84). Resilience was observed on Day 90 for Post-14 carcasses while Control and Post-7 carcasses demonstrated a stable abundance over time (Table 4.72).

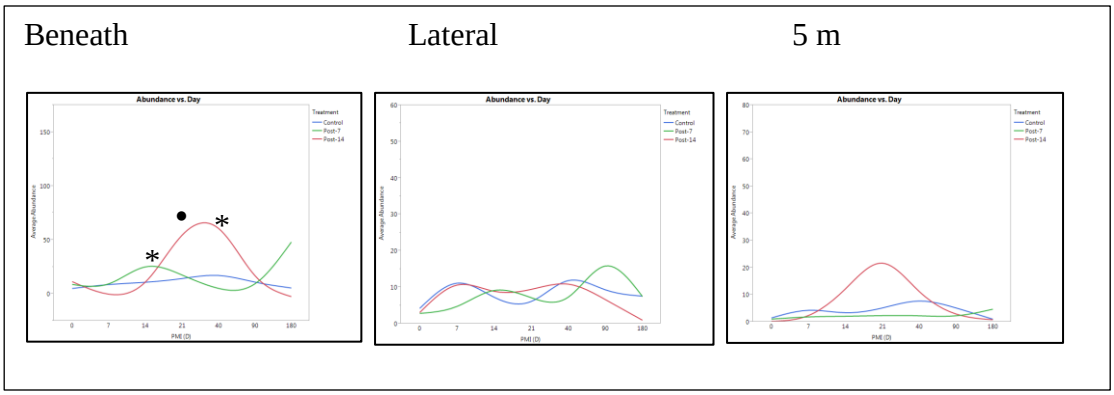

Figure 4.84. Soil arthropod community abundance (by Genus) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* denotes significant difference; • represents marginal significant difference).

Table 4.72. Resilience for soil arthropod community abundance (by Genus) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.3793	Resistance
Post-7	None	0.5406	Resistance
Post-14	0 x 40	0.0389*	90

Richness

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) and three interactions include Day x Treatment with $p = 0.0028$, Day x Region with $p = 0.0230$ and Day x Treatment x Region, with $p = 0.0052$. No significant difference was detected for Treatments ($p = 0.5473$). Statistical difference (divergence) was detected between Treatments at soil beneath and soil lateral. At soil beneath, Control and Post-14 was significantly different ($p = 0.0242$) on Day 21, followed by a convergence on Day 40. There was marginal significant ($p = 0.0593$) between treatments on Day 7. At soil lateral, Control x Post-14 had significant difference ($p = 0.0242$) on Day 90 and Day 180. No significant difference was detected between treatments for soil 5 m. In general, genus richness at soil beneath of Post-7 and Post-14 carcasses showed

higher richness compared to Control carcasses from Day 7 to Day 21. Similarly, at soil lateral, richness of Post-7 and Post-14 groups was higher on Day 14 and Day 21 compared to Control. However, the richness in Control carcasses increased from Day 40 to Day 180. As for soil at 5 m, richness of Control peaked on Day 21 and the second peak on Day 90, while Post-14 group showed a single peak on Day 14 (Figure 4.85). Resilience was tested only for soil beneath for all treatments and resilience was observed on Day 90 for Post-14 carcasses while Control and Post-7 carcasses demonstrated a stable richness over time (Table 4.73).

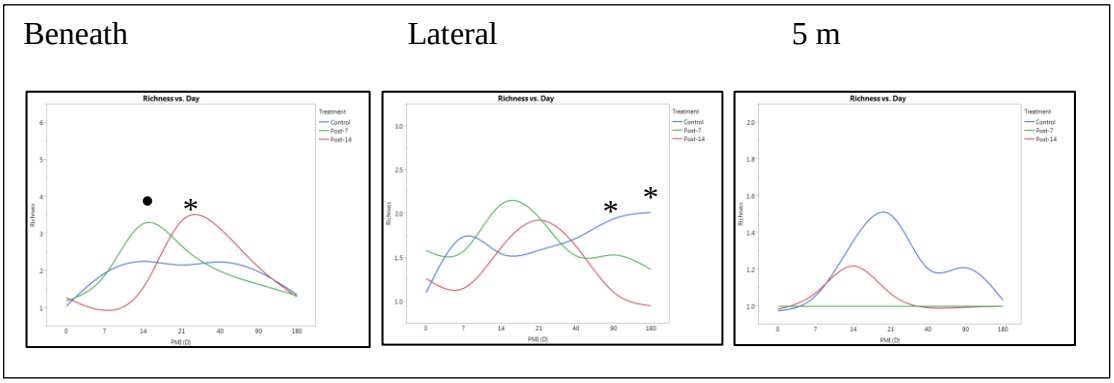

Figure 4.85. Soil arthropod community richness (by Genus) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* denotes significant difference; • represents marginal significant difference).

Table 4.73. Resilience for soil arthropod community richness (by Genus) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4267	Resistance
Post-7	None	0.0710	Resistance
Post-14	0 x 21	<0.0001*	90
	0 x 40	0.0002*	

Simpson's diversity index

The full model showed a significant difference in Day ($p < 0.0319$) and Region ($p < 0.0001$) without any significant interactions. Treatment was not significant as well ($p = 0.3763$). No significant difference in Simpson's diversity index across Treatments over every sampling time at all soil regions. In other words, no divergence occurred and the community was in stable dynamics. At soil beneath, Control carcasses showed increased in soil arthropod genus diversity from Day 0 to Day 7 (probably due to active decomposition process that attracts many different genera of arthropods), and the diversity decreased gradually on Day 21, and then increased again on Day 40. Post-14 groups had higher Simpson's index (means lower diversity in family) from Day 0 to Day 7, probably due to treatment effect, however, diversity increased sharply from Day 14 to Day 40. At soil lateral, Post-14 carcasses generally had lower diversity compared to Control carcasses on Day 7, 90 and 180. Post-7 had higher diversity compared to Post-14 and had a similar temporal dynamics as Post-14. At soil 5 m, there was very low diversity observed in all groups from Day 0 to Day 180 in general, except on Day 21 where Control had a higher diversity compared to other groups (Figure 4.86). Resilience was tested only for soil beneath for all treatments and resilience was observed on Day 180 for Post-14 carcasses while Control and Post-7 carcasses demonstrated a stable diversity over time (Table 4.74).

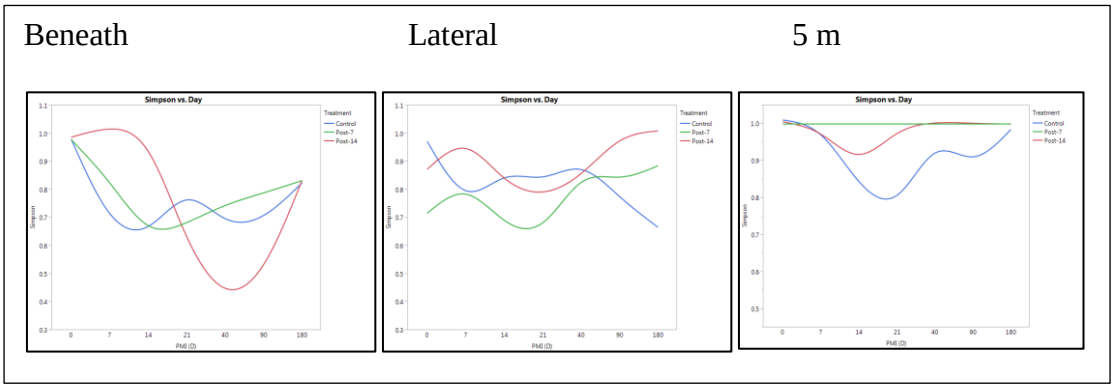

Figure 4.86. Simpson's diversity index of soil arthropod (by Genus) across Treatments over time at different soil regions in summer 2014 at Snook, Texas.

Table 4.74. Resilience for soil arthropod Simpson's diversity (by Genus) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.5460	Resistance
Post-7	None	0.8010	Resistance
Post-14	0 x 21	0.0206*	180
	0 x 40	0.0011*	
	0 x 90	0.0046*	

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p = 0.0053$) and Region ($p < 0.0001$) without any significant interactions. Treatment was not significant as well ($p = 0.3757$). There was no statistical difference of Shannon-Wiener's diversity index found between Treatments over every sampling day in all soil regions, although there were some marginal significant differences at soil beneath and soil lateral. At soil beneath, lower diversity in arthropod was noted on Post-14 carcasses on Day 7 and Day 14 (on Day 14, marginal significant difference between treatments was noted with $p = 0.0744$). This indicates delayed insect colonization on carrion decreased soil arthropod

diversity. While for Control carcasses, diversity increased from Day 0 to Day 14, and then deceased on Day 21, and increased again on Day 90, and then decreased on Day 180. Higher diversity was noted at soil lateral for Post-7 group from Day 7 to Day 21, possibly due to the impact of delayed Diptera colonization on this treatment group. On Day 180, marginal significant difference was detected between treatments ($p = 0.0749$) at soil lateral. At soil 5 m, low diversity was observed for all groups from Day 0 to Day 180. However, Control group had a higher diversity on Day 14 and 21, and Post-14 group had a smaller peak on Day 14 (Figure 4.87). Resilience was tested only for soil beneath for all treatments and resilience was observed on Day 180 for Post-14 carcasses while Control and Post-7 carcasses demonstrated a stable diversity over time (Table 4.75).

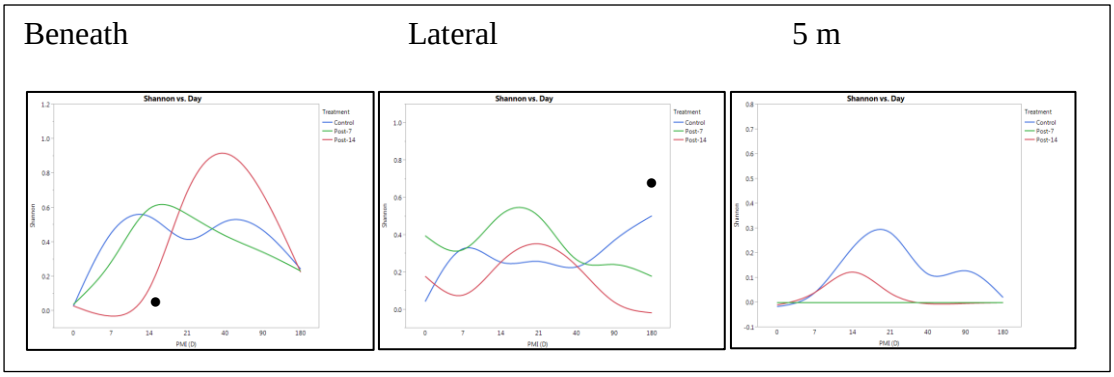

Figure 4.87. Shannon-Wiener's diversity index of soil arthropod (by Genus) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (• represents marginal significant difference).

Table 4.75. Resilience for soil arthropod Shannon-Wiener's diversity (by Genus) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.5006	Resistance
Post-7	None	0.8010	Resistance
Post-14	0 x 21	0.0028*	180
	0 x 40	0.0005*	
	0 x 90	0.0108*	

Evenness

The full model showed only one factor was significant difference, Region ($p < 0.0001$), while Day and Treatment were not significantly different ($p = 0.0741$ and 0.2530, respectively). Also, there was no significant interaction. There was no statistical difference of evenness (by Genus) found between Treatments on every sampling day in all soil regions, although there were marginal differences detected on Day 90 and 180 at soil lateral ($p = 0.0726$ and 0.0749, respectively). Again, treatments had no significant impact on evenness of soil arthropod community structure at the genus level. At soil beneath, evenness of Control and Post-7 carcasses increased from Day 0 to Day 14 while evenness decreased in Post-14 group from Day 0 to Day 14. Post-14 groups had very low evenness from Day 0 to Day 14 but increased gradually and peaked on Day 40. At soil lateral, Post-7 carcasses in general had the highest evenness at genus level on Day 0 to Day 40. At soil 5 m, Control carcasses in general had the highest evenness throughout 180 days of decomposition, followed by Post-14 carcasses (with a peak on Day 14) (Figure 4.88). Resilience was tested only for soil beneath for all treatments and resilience was observed on Day 180 for Post-14 carcasses while Control and Post-7 carcasses demonstrated a stable evenness over time (Table 4.76).

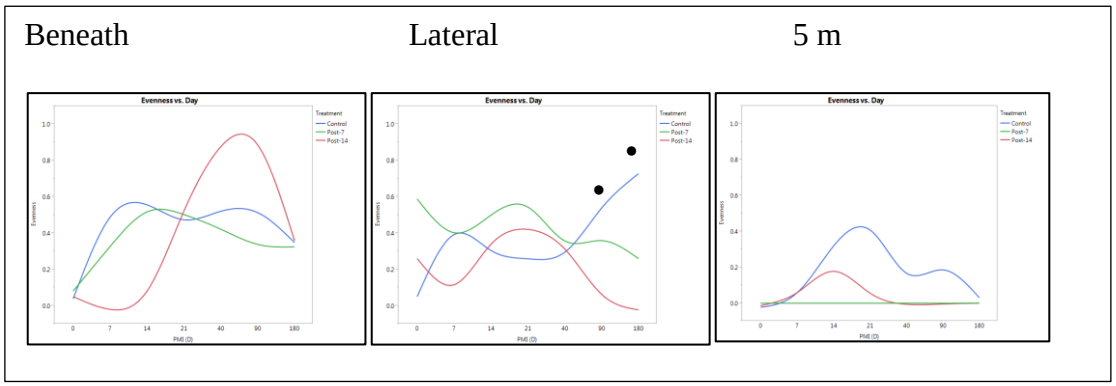

Figure 4.88. Evenness of soil arthropod (by Genus) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (• represents marginal significant difference).

Table 4.76. Resilience for soil arthropod community evenness (by Genus) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.6660	Resistance
Post-7	None	0.9022	Resistance
Post-14	0 x 40	0.0091*	180
	0 x 90	0.0027*	

Effective number of species

The full model showed a significant difference in Day ($p = 0.0053$) and Region ($p < 0.0001$) without any significant interactions. Treatment was not significant as well ($p = 0.4669$). There was no statistical difference of effective number of species (ENS) found between Treatments on every sampling day in all soil regions, hence the community was in stable dynamics. At soil beneath, ENS generally increased from Day 0 to Day 40 for both Control and Post-7 groups while the Post-14 carcasses, increased in effective number of genus from Day 21 to Day 40, and then decreased from Day 90 to Day 180. At soil lateral, ENS for Post-7 and Post-14 had a similar dynamic trend where they both increased from Day 7 to Day 21. As for Control carcasses at soil lateral, ENS

gradually decreased from Day 0 to Day 40, and then increased steadily from Day 90 to Day 180. On the other hand, Control carcasses had the highest ENS throughout the 180 days of decomposition (peaked on Day 14 and Day 21), followed by Post-14 group (with a smaller peak on Day 14) (Figure 4.89). Resilience was tested only for soil beneath for all treatments and resilience was observed on Day 180 for Post-14 carcasses while Control and Post-7 carcasses demonstrated a stable ENS over time (Table 4.77).

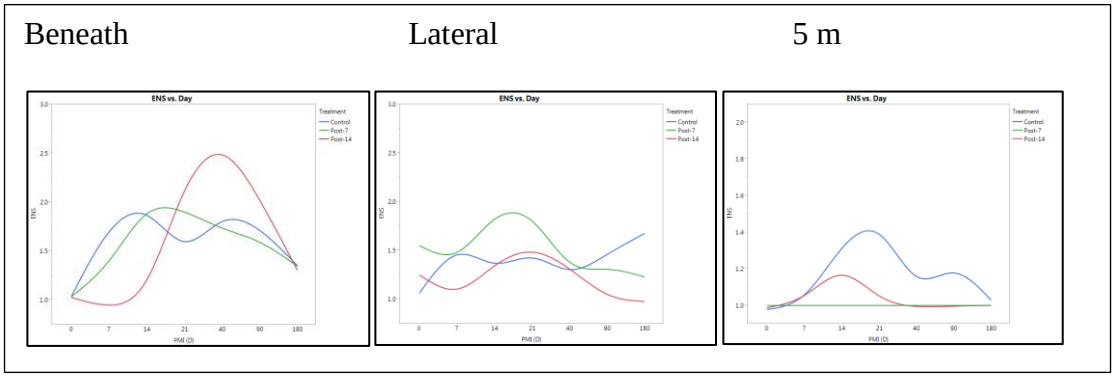

Figure 4.89. Effective number of genus of soil arthropods across Treatments over time at different soil regions in summer 2014 at Snook, Texas.

Table 4.77. Resilience for soil arthropod ENS (by Genus) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4444	Resistance
Post-7	None	0.6996	Resistance
Post-14	0 x 21	0.0054*	180
	0 x 40	0.0006*	
	0 x 90	0.0382*	

Function in 2014

PERMANOVA was performed on soil arthropod data by function. Results showed that there was Day effect and Region effect (both p value = 0.001) without any significant interaction (Table 4.78). Again, Treatment was not significantly difference by soil arthropod community function (p = 0.100).

Table 4.78. Analysis of the soil arthropod community function in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	11.3635	0.001*
Treatment	2	1.6082	0.100
Region	2	9.0023	0.001*
Day x Treatment	2	1.5176	0.116
Day x Region	2	1.3278	0.222
Treatment x Region	4	0.9359	0.516
Day x Treatment x Region	4	0.9060	0.600

There was a significant effect in Day and Region, therefore further analyses were conducted. For soil regions, all soil regions were significantly from each other ($p < 0.05$), indicating soil community structure changes according to region (Table 4.79). As for days of decomposition, most of the pairwise comparisons between days of decomposition were significantly different ($p < 0.05$), except Day 14 x Day 21 and Day 21 x Day 40 where there was no significant difference detected ($p = 0.098$) (Table 4.80). The NMDS plot of stress for soil arthropod community structure (Figure 4.90) and NMDS ordinations for Day and Region were provided for visualization about data distribution (Figure 4.91 and 4.92, respectively). Minimum stress for given dimensionality was 0.1218 with $r^2 = 0.9089$. The MRPP analysis for soil region showed a significant difference (A value = 0.0425; Significant of Delta: 0.001 based on 999

permutations) while the MRPP for day also showed a significant difference with A value 0.1004 and Significant of Delta 0.001.

Table 4.79. Pairwise comparisons between Regions on soil arthropod community functions in summer 2014 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	0.6222	0.6221	3.4907	0.0274	0.007*
	Residual	124	22.1018	0.1782		0.9726	
	Total	125	22.7240			1.0000	
Beneath x 5 m	Region	1	2.2435	2.2434	11.89	0.0875	0.001*
	Residual	124	23.3962	0.1886		0.9125	
	Total	125	25.6397			1.0000	
Lateral x 5 m	Region	1	1.6486	1.6485	9.7776	0.0731	0.001*
	Residual	124	20.9072	0.1686		0.9269	
	Total	125	22.5557			1.0000	

Table 4.80. Pairwise comparisons of soil arthropod community functions between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.03*	0.001*	0.001*	0.001*	0.001*	0.002*
7	0.03*	-	0.022*	0.006*	0.003*	0.001*	0.001*
14	0.001*	0.022*	-	0.098	0.018*	0.001*	0.001*
21	0.001*	0.006*	0.098	-	0.335	0.001*	0.001*
40	0.001*	0.003*	0.018*	0.335	-	0.001*	0.001*
90	0.001*	0.001*	0.001*	0.001*	0.001*	-	0.007*
180	0.002*	0.001*	0.001*	0.001*	0.001*	0.007*	-

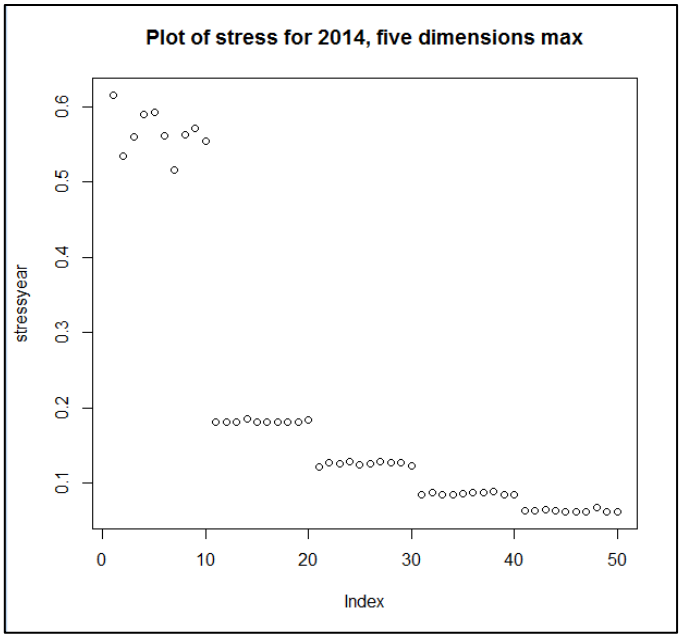

Figure 4.90. NMDS plot of stress for soil arthropod community function in summer 2014 at Snook, Texas (Stress test 0.1218; $r^2 = 0.9089$).

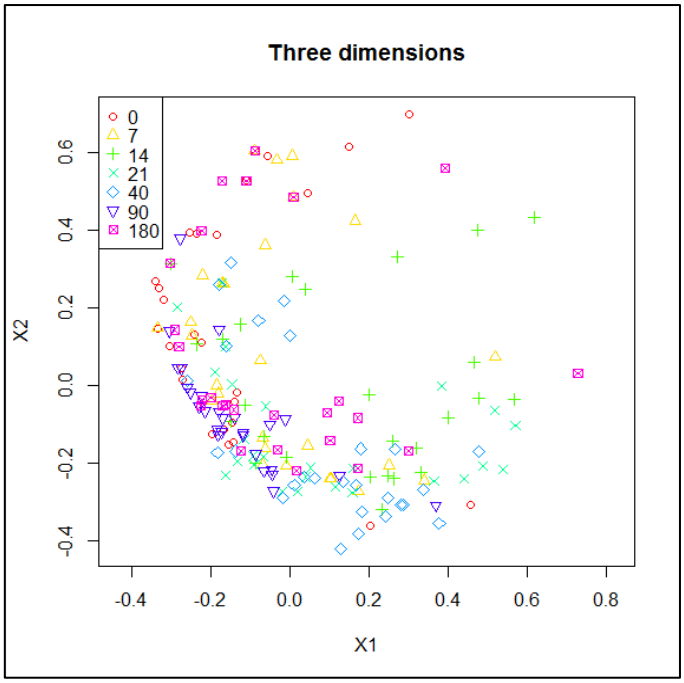

Figure 4.91. NMDS ordinations for soil arthropod community functions according to carrion decomposition days in summer 2014 at Snook, Texas.

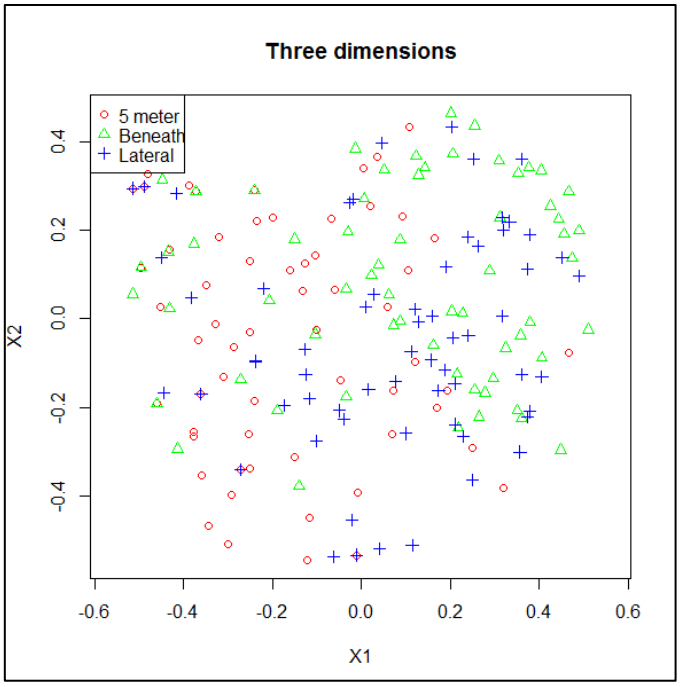

Figure 4.92. NMDS ordinations for soil arthropod community functions according to soil regions in summer 2014 at Snook, Texas.

The ISA results showed only one functional group of soil arthropod namely detritivore as the sole indicator for summer 2014 (Table 4.81), indicating that this guild is an important player during decomposition process.

Table 4.81. Indicator species analysis by Function for soil arthropods in summer 2014 at Snook, Texas.

Type	Functional group	Indicator value	P value
All soils	Detritivore	0.068	0.048*

Abundance of soil arthropod community structure (by Function) according to soil regions (excluding mites) in 2014

Soil beneath

The abundance of soil arthropod community by function was similar at soil beneath according to treatments (p = 0.176) (Figure 4.93). For the Control carcasses, generalist detritivores can be seen from Day 0 to Day 180, with an increasing trend over days. Necrophagous guild was present on Day 7, 14, 21 and 40. It is interesting to note that the predator abundance usually higher with the presence of necrophagous arthropods. Likewise, Post-7 had a similar composition of soil arthropod as in soil beneath. Post-14 had very low functional groups during the insect exclusion period (Day 0 to Day 14), and then increased in the abundance of detritivores and predators after the removal of insect-exclusion cages on Day 14.

Each functional group was highlighted individually (Figure 4.93). Interestingly, carcasses with delayed Diptera colonization (Post-7 and Post-14 groups) had higher abundance of detritivores than Control group from Day 14 to Day 90, There was a marginal statistical difference found between the treatments on Day 40 (p = 0.0513). On Day 90, statistical difference was found between Control x Post-7 and Post-7 x Post-14 for detritivores at soil beneath (p = 0.0398 and 0.0141, respectively). For predator / parasite guild, there was significant difference between Control x Post-7 and Control x Post-14 on Day 7 (p = 0.0071 and 0.0034, respectively) and Day 14 (Post-7 x Post-14, p = 0.0470). In general, predator abundance at soil beneath increased at the initial phase of experiment (for Control), and then decreased steadily. While for Post-7 and Post-14, they both had decreased in predator abundance at the initial phase of experiment and then increased after the insect-exclusion cages had been removed. Post-14 achieved the highest abundance of predators on Day 21.

For necrophagous guild, there was an increased for Control carcasses from Day 0 to Day 14 (the peak), and then decreased on Day 21 and all the way to Day 180. Significant difference was detected on Day 7 (Control x Post-14, with p value 0.0222). For Post-7 and Post-14, peaks of abundance were observed on Day 14 as well. However,

the peaks in treatment groups were lower compared to the one in Control. A marginal significant difference ($p = 0.0565$) was noted on Day 21 between treatments. On Day 40, a significant difference in Control x Post-14 was observed (p value 0.0389) at soil beneath for necrophagous communities. For herbivores in general, the abundance was low at the initial day of experiment regardless of treatments, remained low in numbers during the decomposition process (Day 7 to Day 21), and then gradually increased from Day 21 to Day 40, with Control carcasses had more abundance of herbivores than Post-7 and Post-14 groups. There was no significant difference ($p > 0.05$) between treatments for herbivores. For fungivore, the abundance was decreasing in all treatment groups at the initial phase of experiment (from Day 0 to Day 7). Since then, Post-7 and Post-14 groups did not have fungivore arthropods until Day 180 in the samples, while Control carcasses had an increase in abundance especially on Day 14 and Day 40, possibly due to the growth of fungi on the pig skeletons.

Resilience was tested only for all functional groups of soil arthropods at soil beneath for all treatments. The results showed fungivore and herbivore groups were stable over time (Table 4.82).

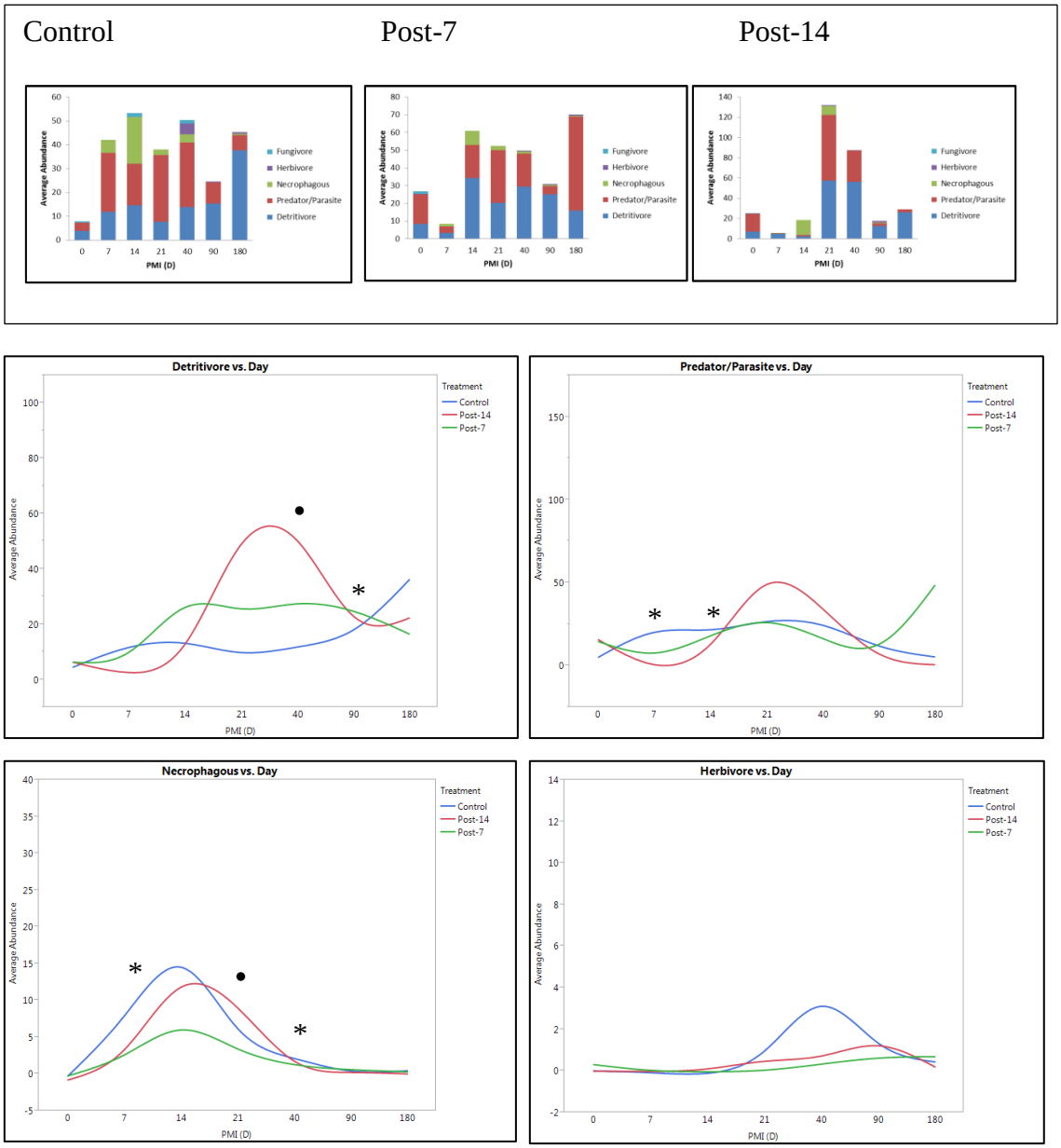

Figure 4.93. Above. Soil arthropod community abundance (by Function) beneath the carrion according to Treatments over carrion decomposition days in summer 2014 at Snook, Texas. Upper Left. Abundance of detritivores at soil beneath the carrion across treatments over time. Upper Right. Abundance of predator / parasite at soil beneath the carrion across treatments over time. Middle Left. Abundance of necrophagous at soil beneath the carrion across treatments over time. Middle Right. Abundance of herbivore at soil beneath the carrion across treatments over time. Lower Left. Abundance of fungivore at soil beneath the carrion across treatments over time.

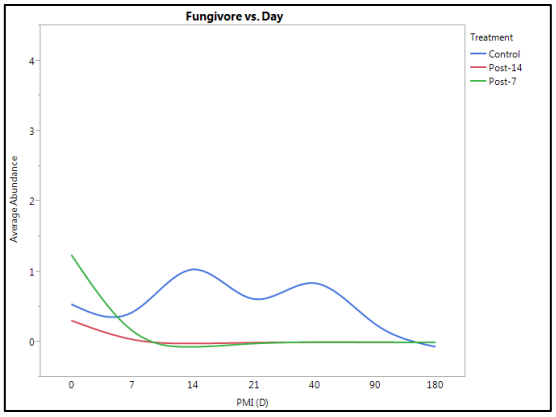

Figure 4.93 (Continued).

Table 4.82. Resilience of soil arthropod community functions for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Function	Treatment	Significant difference	P value	Resilience on Day
Necrophagous	Control	0 x 14	0.0084*	21
	Post-7	0 x 14	0.0184*	21
	Post-14	None	0.0254*	Resistance
Detritivore	Control	None	0.1219	Resistance
	Post-7	None	0.2219	Resistance
	Post-14	0 x 21	0.0477*	40
Predator	Control	None	0.0147*	Resistance
	Post-7	None	0.6673	Resistance
	Post-14	0 x 21	0.0245*	40
Fungivore	Control	None	0.3630	Resistance
	Post-7	None	0.0950	Resistance
	Post-14	None	0.4628	Resistance
Herbivores	Control	None	0.3850	Resistance
	Post-7	None	0.5794	Resistance
	Post-14	None	0.1294	Resistance

Soil lateral

Soil arthropod community function at soil lateral consisted of two majority functional groups namely predator and detritivore regardless of treatments (Figure 4.94), and therefore, statistically no significant different between the Treatments ($p = 0.731$). Necrophagous guild can be seen on Day 7 and Day 14 on the Control carcasses, possibly due to the dispersal of fly larvae. The abundant of necrophagous communities at soil lateral can be seen on Day 14, 21 and 40 for Post-7 carcasses. For Post-14 groups, necrophagous guilds were present on Day 21. Herbivore usually increased in abundance on Day 40 for all groups.

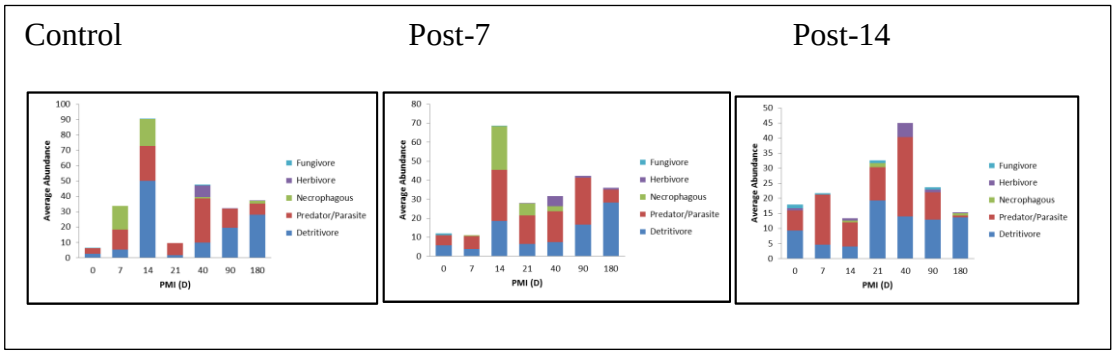

Figure 4.94. Soil arthropod community abundance (by Function) beside the carrion according to Treatments over carrion decomposition days in summer 2014 at Snook, Texas.

Soil 5 m

The soils at 5 m away from carrion served as the control to the soils collected from beneath and lateral of the carrion (Figure 4.95). Likewise, there was no significant difference in soil arthropod function between Treatments ($p = 0.4000$) at soil 5 m. The two major functional components found at soil 5 m were detritivores and predators, as similar to soil lateral. Several individuals of necrophagous insects (e.g., fly larvae) can be collected on Day 7 (for Control) and on Day 40 for Post-7 group. Again, this

demonstrates that the dispersal of fly larvae could reach the radius of 5 m away from carrion.

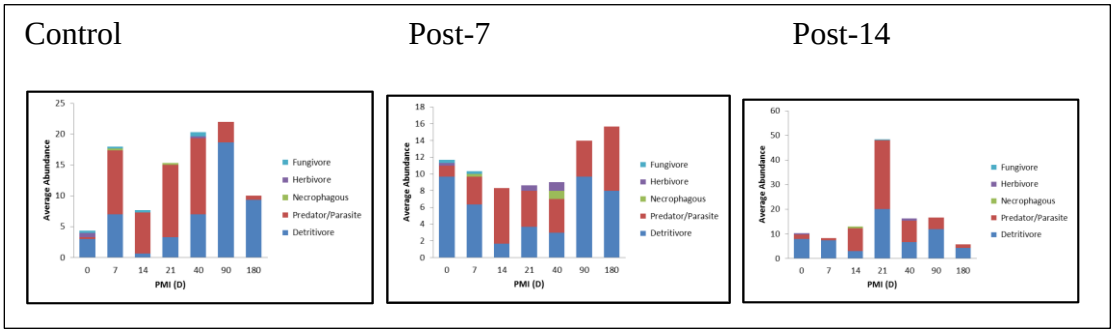

Figure 4.95. Soil arthropod community abundance (by Genus) at soil 5 m away from the carrion according to Treatments over carrion decomposition days in summer 2014 at Snook, Texas.

Mites associated with pig carrion in 2013

Year effect

There was a year effect (df = 1; F = 0.9326; p = 0.001) between two trials by morphospecies of mites (Figure 4.96 showed NMDS plot between years). Furthermore, when Function of mites was analyzed for Year effect, the results showed that there was significant difference between years (df = 1; F = 11.175; p = 0.001). Hence, data were analyzed separately.

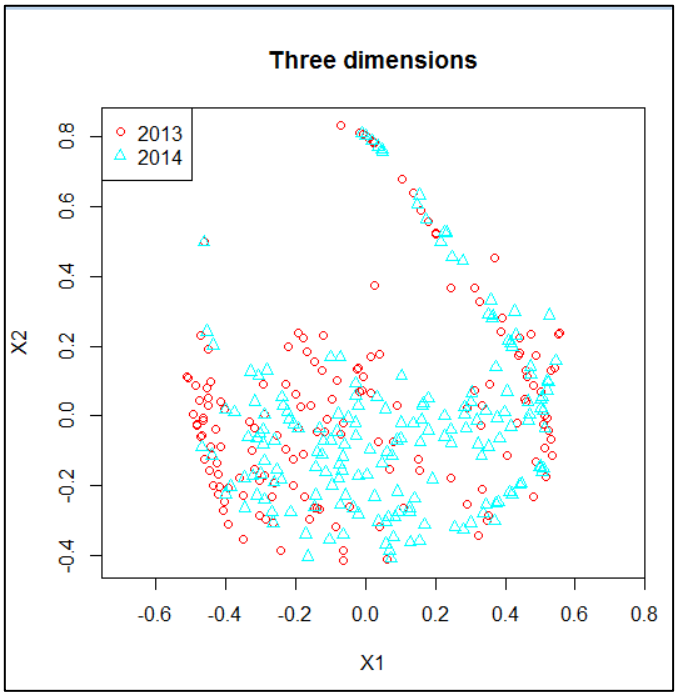

Figure 4.96. NMDS ordinations showing data distribution between years by acari Orders (minimum stress = 0.1048; r^2 = 0.9426).

Replicate effect

There was no replicate effect (df = 1; F = 0.9326; p = 0.455) among the replicates by morphospecies of mites. Also, when replicate effect was tested on Function of soil arthropods, result showed that there was no significant difference (df = 1, F = 1.7254; p = 0.138). Therefore, all data in the replicates were pooled together and analyzed.

Linear Regression

To determine whether mite specimens mounted on slides are able to represent the total abundance of mite collected from the soil samples in both 2013 and 2014 trials, linear regressions were conducted on the proportions of oribatid and non-oribatid mites to examine this relationship. In summer 2013, for Oribatida, the results showed a positive correlation coefficient (r^2 = 0.3888), indicating a moderate goodness-of-fit between proportion of oribatid mites mounted on slides and the proportion of oribatid mites collected from the soil. Likewise, proportions of non-oribatid in the soils and on the slides showed a positive correlation with r^2 = 0.3888 (a moderate strength of relationship) (Figure 4.97).

In summer 2014, the strength of relationship improved. For Oribatida mites, the results showed a positive correlation coefficient (r^2 = 0.4013), indicating a moderate relationship between the Oribatida mites mounted on slides and the Oribatida collected from the soils. On the other hand, for non-Oribatida mites, the correlation coefficient (r^2) was 0.4066, suggesting a moderate goodness-of-fit between the proportion of non-oribatid mounted on slides and the proportion of non-oribatid mites collected from soil (Figure 4.98).

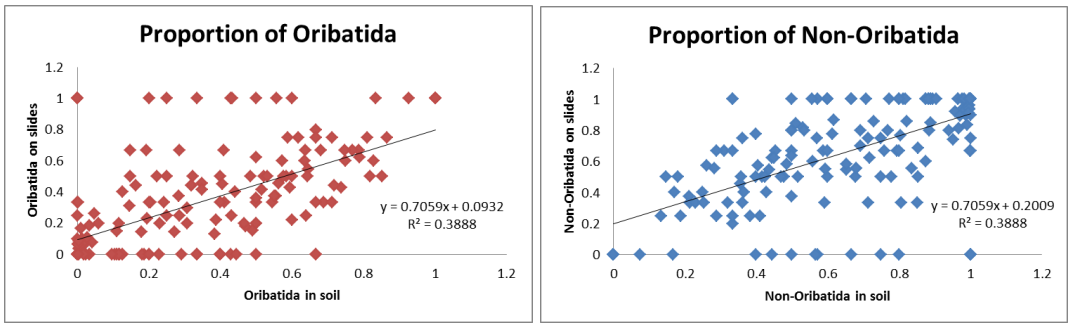

Figure 4.97. Linear regressions between proportions of mites collected in the soil samples with the mites mounted on slides in summer 2013 at Snook, Texas.

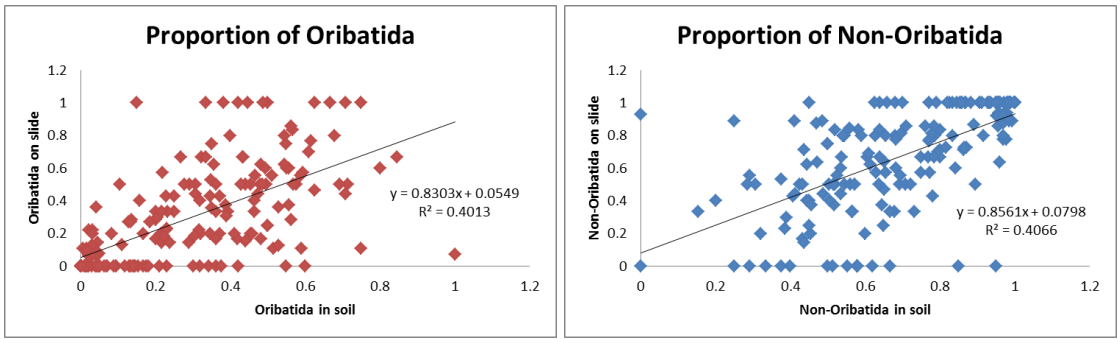

Figure 4.98. Linear regressions between proportions of mites collected in the soil samples with the mites mounted on slides in summer 2014 at Snook, Texas.

Community structure and function of soil mites in 2013

Total morphospecies in 2013

A total of two morphospecies were identified under the dissecting microscope, namely Oribatida mites and Non-Oribatida mites. The following figures demonstrated the total abundance of Oribatida and Non-Oribatida across Treatments over decomposition days according to soil regions (i.e., beneath, lateral and soil 5 m). In 2013 trial, full factorial model for Oribatida mites showed a significant difference in Day ($p < 0.0001$*) and Region ($p = 0.0187$). However, full factorial model for Non-Oribatida mites demonstrated Day ($p < 0.0001$), Region ($p < 0.0001$) and an interaction Day x

Region ($p < 0.0001$) were significantly different. Again, no significant treatment effect ($p > 0.05$) was observed for both groups of mites in summer 2013.

Soil beneath

For Oribatida, Day was significantly different at soil beneath ($p = 0.0217$) while treatments did not showed a significant difference ($p = 0.2288$). Similarly, Non-Oribatida abundance was also showing significance difference by days ($p < 0.0001$). Note that for both mite groups, there was no significant difference in mite abundance between treatments in every sampling day ($p > 0.05$). In other words, the mite abundance was in stable dynamics over time regardless of treatments, hence, no divergence or convergence in the mite abundance. In general, Oribatida decreased during decomposition process (between Day 7 and Day 40), and then abundance of Oribatida increased thereafter. On and after Day 40, the oribatid mites in Control carcasses increased in a faster rate than Post-7 and Post-14 groups. Although Control carcasses had a higher abundance on Day 180, but it was not significant difference from Post-7 and Post-14 ($p = 0.4476$).

For Non-Oribatida mites, mite abundance in all treatments increased sharply (the majority of the mites were *Sancassania* sp. in the Family Acaridae) on Day 40, and then decreased on Day 90. On Day 180, non-oribatid mites in Control carcasses remained low, but there were increased in abundance for Post-7 and Post-14 groups (Figure 4.99).

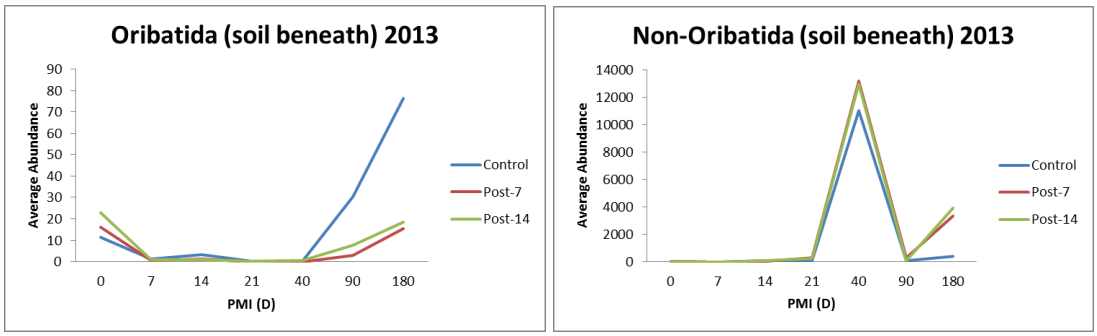

Figure 4.99. Total abundance of acari morphospecies across treatments over days of decomposition at soil beneath the carrion in summer 2013 at Snook, Texas. Right. Abundance of Oribatida. Left. Abundance of Non-Oribatida.

Soil lateral

For Oribatida, Day was significantly different at soil lateral ($p < 0.0001$) while treatments did not showed a significant difference ($p = 0.1750$). Similarly, Non-Oribatida abundance was also showing significant difference by days ($p < 0.0001$) but not treatments ($p = 0.3395$). Note that for both mite groups, there was no significant difference in mite abundance between treatments in every sampling day ($p > 0.05$). At the soil lateral, the oribatid decreased during the decomposition process, and then increased in a faster rate after Day 40 or Day 90. Likewise, the abundance of Non-Oribatida mites in all treatments increased sharply (the majority of the mites were *Sancassania* sp. in the Family Acaridae) on Day 40, although Control group showed a lower abundance, but there was no significant difference with each other ($p = 0.4673$). The mite abundance decreased on Day 90. On Day 180, a slight increase of non-oribatid mites was observed in Post-14 group (Figure 4.100).

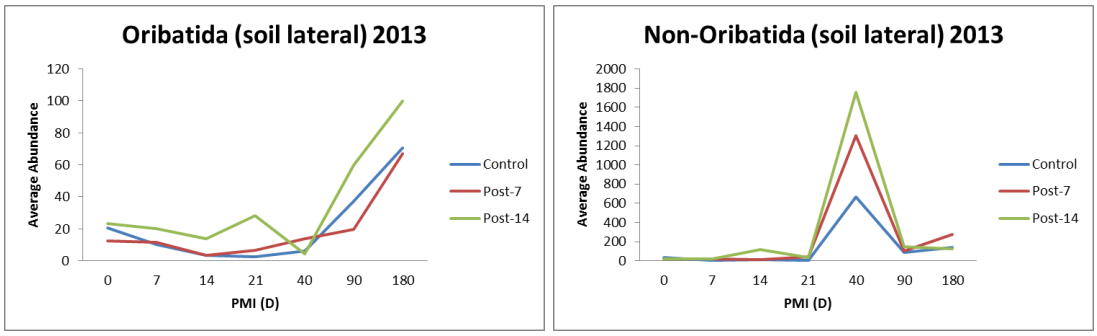

Figure 4.100. Total abundance of acari morphospecies across treatments over days of decomposition at soil lateral of the carrion in summer 2013 at Snook, Texas. Right. Abundance of Oribatida. Left. Abundance of Non-Oribatida.

Soil 5 m

For Oribatida, Day was significantly different at soil 5 m ($p = 0.0007$) while treatments did not showed a significant difference ($p = 0.5132$). However, Non-Oribatida abundance was showing no significant difference by days ($p = 0.2265$) and by treatments ($p = 0.4738$). Note that for both mite groups, there was no significant difference in mite abundance between treatments in every sampling day ($p > 0.05$). The mite abundance at soil 5 m was thus considered a stable community by having no effect in days and treatments. In general, oribatid mites in all treatment had increased in abundance from Day 21 to Day 180, except Post-14 group, which decreased in abundance on Day 180. However, no significant found was found ($p = 0.4033$). For Non-Oribatida mites, Post-7 had an exponential rate of increase from Day 90 to day 180, again, there was no significant difference ($p = 0.5091$) (Figure 4.101).

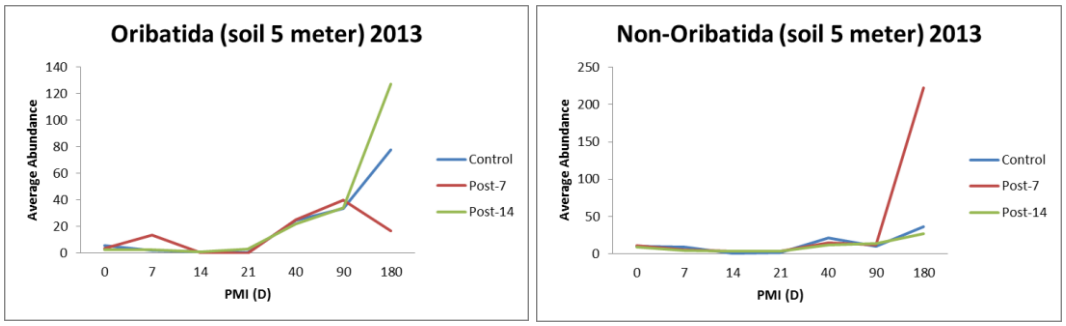

Figure 4.101. Total abundance of acari morphospecies across treatments over days of decomposition at soil 5 m away from the carrion in summer 2013 at Snook, Texas. Right. Abundance of Oribatida. Left. Abundance of Non-Oribatida.

Total Superorder / Order / Suborder / Cohort in 2013

Subsampling of mite specimens (from the total morphospecies in 2013 trial) for slide mounting was conducted as mentioned in Materials and Methods. A total of 1565 mite specimens were mounted on slides and identified to the lowest taxonomical rank as possible. Two Superorders have been identified namely Parasitiformes and Acariformes. Under the superorder Parasitiformes, only the Order Mesostigmata had been identified. While for Acariformes, two Orders have been identified from the samples in this study, namely Order Trombidiformes and Sarcoptiformes. Suborder Prostigmata (under Trombidiformes) was identified. Suborder Oribatida, Suborder Endeostigmata and Cohort Astigmatina have been identified as well (all belonged to Order Sarcoptiformes). Table 4.83 showed the mites' Orders and other taxonomic ranks identified in 2013 trial. The most dominant group mounted on slides was the Order Mesostigmata (36.67%) followed by the Cohort Astigmatina (36.54%).

Table 4.83. Total abundance and dominance of Orders and other lower taxonomic ranks of slide-mounted Acari identified from all soil samples in 2013 trials at Snook, Texas.

No.	Taxonomic rank		Total abundance	Dominance
1.	Order	Mesostigmata	574	36.67
2.	Cohort	Astigmatina	572	36.54
3.	Suborder	Oribatida	299	19.10
4.	Suborder	Prostigmata	95	6.07
5.	Suborder	Endeostigmata	25	1.59
		Total	1565	100

Total Family in 2013

For the suborder Oribatida, specimens were not identified to family level. The results presented below were the families identified from Non-Oribatida mites. A total of 26 families were identified from all the non-oribatid acari mounted on slides. One family was identified in the Suborder Endeostigmata, two families in the Cohort Astigmatina, ten families in the Order Mesostigmata, and 13 families in the Suborder Prostigmata. The most dominant family was Acaridae (46.47%), followed by Macrochelidae (11.08%) and Ascidae (8.95%) (Table 4.84). Note that Acaridae are the detritivores or necrophagous, Macrocehlidae and Ascidae are the predators in the soil. Only certain number of mite specimens has been identified to species. Hence, statistical analysis at the genus-species level was not performed. For the list of mite species recovered from soil samples, see Appendix H.

Table 4.84. Total abundance and dominance of Families of slide-mounted non-oribatid Acari identified from all soil samples in 2013 trial at Snook, Texas.

No.	Higher rank	Family	Total abundance	Dominance
1.	Astigmatina	Acaridae	566	46.47
2.	Mesostigmata	Macrochelidae	135	11.08
3.	Mesostigmata	Ascidae	109	8.95
4.	Mesostigmata	Uropodidae	93	7.64
5.	Mesostigmata	Parasitidae	92	7.55
6.	Mesostigmata	Laelapidae	46	3.78
7.	Prostigmata	Cunaxidae	29	2.38
8.	Endeostigmata	Nanorchestidae	26	2.13
9.	Mesostigmata	Phytoseiidae	21	1.72
10.	Prostigmata	Pygmephoridae	18	1.48
11.	Prostigmata	Anystidae	14	1.15
12.	Mesostigmata	Ameroseiidae	13	1.07
13.	Prostigmata	Teneriffiidae	9	0.74
14.	Prostigmata	Erythraeoidea	9	0.74
15.	Prostigmata	Bdellidae	5	0.41
16.	Prostigmata	Ereynetidae	5	0.41
17.	Mesostigmata	Melicharidae	5	0.41
18.	Mesostigmata	Digamasellidae	5	0.41
19.	Prostigmata	Scutacaridae	4	0.33
20.	Astigmatina	Histiostomatidae	3	0.25

Table 4.84 (Continued).

	Higher rank	Family	Total abundance	Dominance
21.	Prostigmata	Tetranychidae	3	0.25
22.	Mesostigmata	Eviphididae	3	0.25
23.	Prostigmata	Calligonellidae	2	0.16
24.	Prostigmata	Smaridiidae	1	0.08
25.	Prostigmata	Adamystidae	1	0.08
26.	Prostigmata	Raphignathidae	1	0.08
		Total	1218	100

Total function in 2013

Five major functional groups were identified from all the slide-mounted acari. They were herbivores, predators/parasites, detritivores, fungivores, and nectarivores / pollenivores. The most abundance functional group during carrion decomposition was the detritivores (62.44%), followed by the predators or parasites (33.13%) (Table 4.85). To see the list of mite families with its respective functional role, see Appendix I.

Table 4.85. Total abundance and dominance of Functions of slide-mounted acari identified from all soil samples in 2013 trials at Snook, Texas.

No.	Function	Total abundance	Dominance
	Detritivores	931	62.44
	Predators/Parasites	494	33.13
	Fungivores	60	4.02
	Herbivores	3	0.20
	Nectarivores/Pollenivores	3	0.20
	Total	1491	100

Superorder / Order / Suborder / Cohort in 2013

PERMAVONA was performed on the acari at this taxonomic level (Superorder / Order / Suborder / Cohort) to determine the effects of independent variables. The results showed Replicate had significant difference ($p = 0.045$). However, this significant result may be due to subsampling method for slide mounting. For the other factors, Day and Region were significantly different ($p = 0.001$) with no other significant interactions (Table 4.86).

Table 4.86. Analysis of the soil mite community structure (by Order and other ranks) in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	7.4483	0.001*
Treatment	2	0.8277	0.613
Region	2	9.9219	0.001*
Day x Treatment	2	1.2879	0.254
Day x Region	2	0.8374	0.607
Treatment x Region	4	0.8574	0.631
Day x Treatment x Region	4	0.4732	0.973

Since there was a significant effect in Day and Region, further analyses were carried out. For soil regions, all soil regions were significantly from each other ($p < 0.001$), indicating soil community structure changes according to region (Table 4.87). As for day of decomposition, most of the pairwise comparisons between days of decomposition were significantly different ($p < 0.05$), except Day 0 x Day 7 and Day 14 x Day 21 where there was no significant difference detected (Table 4.88). The NMDS plot of stress for soil mite community structure (Figure 4.102) and NMDS ordinations for Day and Region were provided for visualization about data distribution (Figure 4.103 and 4.104, respectively). Minimum stress for given dimensionality was 0.1598 with $r^2 = 0.8336$. The MRPP analysis for soil region showed a significant difference (A value = 0.0538; Significant of Delta = 0.001 based on 999 permutations) while the MRPP for day also showed a significant difference with A value 0.0786 and Significant of Delta 0.001.

Table 4.87. Pairwise comparisons between Regions on soil mite community structure by Order in summer 2013 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	1.0206	1.0206	4.4067	0.0343	0.001*
	Residual	124	28.7184	0.2316		0.9657	
	Total	125	29.7390			1.0000	
Beneath x 5 m	Region	1	3.725	3.7249	13.628	0.0990	0.001*
	Residual	124	33.891	0.2733		0.9010	
	Total	125	37.616			1.0000	
Lateral x 5 m	Region	1	2.3614	2.3614	10.471	0.0779	0.001*
	Residual	124	27.9654	0.2255		0.9221	
	Total	125	30.3268			1.0000	

Table 4.88. Pairwise comparisons of soil mite community structure by Order between decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.144	0.001*	0.001*	0.001*	0.003*	0.001*
7	0.144	-	0.001*	0.015*	0.001*	0.045*	0.001*
14	0.001*	0.001*	-	0.475	0.001*	0.001*	0.001*
21	0.001*	0.015*	0.475	-	0.001*	0.001*	0.002*
40	0.001*	0.001*	0.001*	0.001*	-	0.002*	0.01*
90	0.003*	0.045*	0.001*	0.001*	0.002*	-	0.035*
180	0.001*	0.001*	0.001*	0.002*	0.01*	0.035*	-

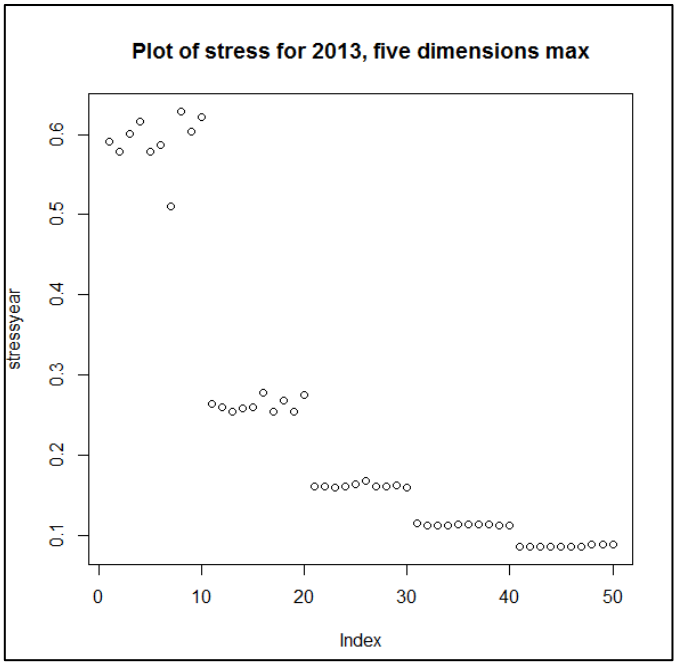

Figure 4.102. NMDS plot of stress for soil mite community structure (by Order) in summer 2013 at Snook, Texas (Stress test 0.1598; $r^2 = 0.8336$).

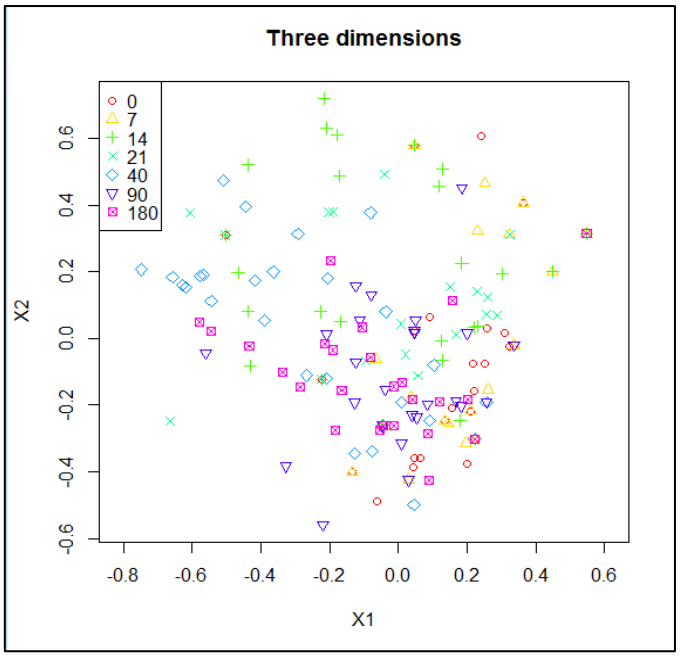

Figure 4.103. NMDS ordinations for soil mite community structure (by Order) according to carrion decomposition days in summer 2013 at Snook, Texas.

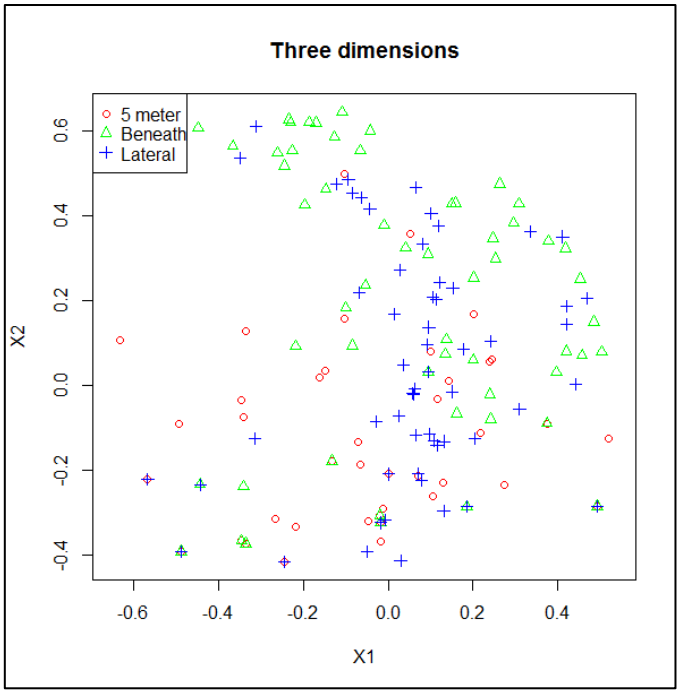

Figure 4.104. NMDS ordinations for soil mite community structure (by Order) according to soil regions in summer 2013 at Snook, Texas.

The ISA results showed two groups of soil mites namely Suborder Prostigmata and Cohort Astigmatina were the indicators for 2013 trial (Table 4.89).

Table 4.89. Indicator species analysis by Order/Suborder for soil mites in summer 2013 at Snook, Texas.

Type	Order/Suborder/Cohort	Indicator value	P value
All soils	Suborder Prostigmata	0.1579	0.001*
	Cohort Astigmatina	0.1521	0.005*

Abundance of soil mite community structure (by Order) according to soil region in 2013

Soil beneath

There was no significant difference in soil mite abundance between treatments at soil beneath ($p = 0.2475$). However, there is Day effect ($p = 0.0082$). Pairwise comparisons showed Day 0 x Day 40 and Day 7 x Day 40 were significantly different ($p = 0.0279$ and 0.0099, respectively). Four specific groups of mites at the soil beneath of Control carcasses namely Mesostigmata, Prostigmata, Astigmatina and Oribatida were plotted for their abundance over time (Figure 4.105). For Prostigmata, significant difference was found on Day 14 (Control x Post-14 and Control x Post-7, both with $p = 0.0044$), while a marginal significant difference ($p = 0.0609$) was found on Day 180 for Cohort Astigmatina, where the abundance of Post-7 carcasses was much higher than the other two groups.

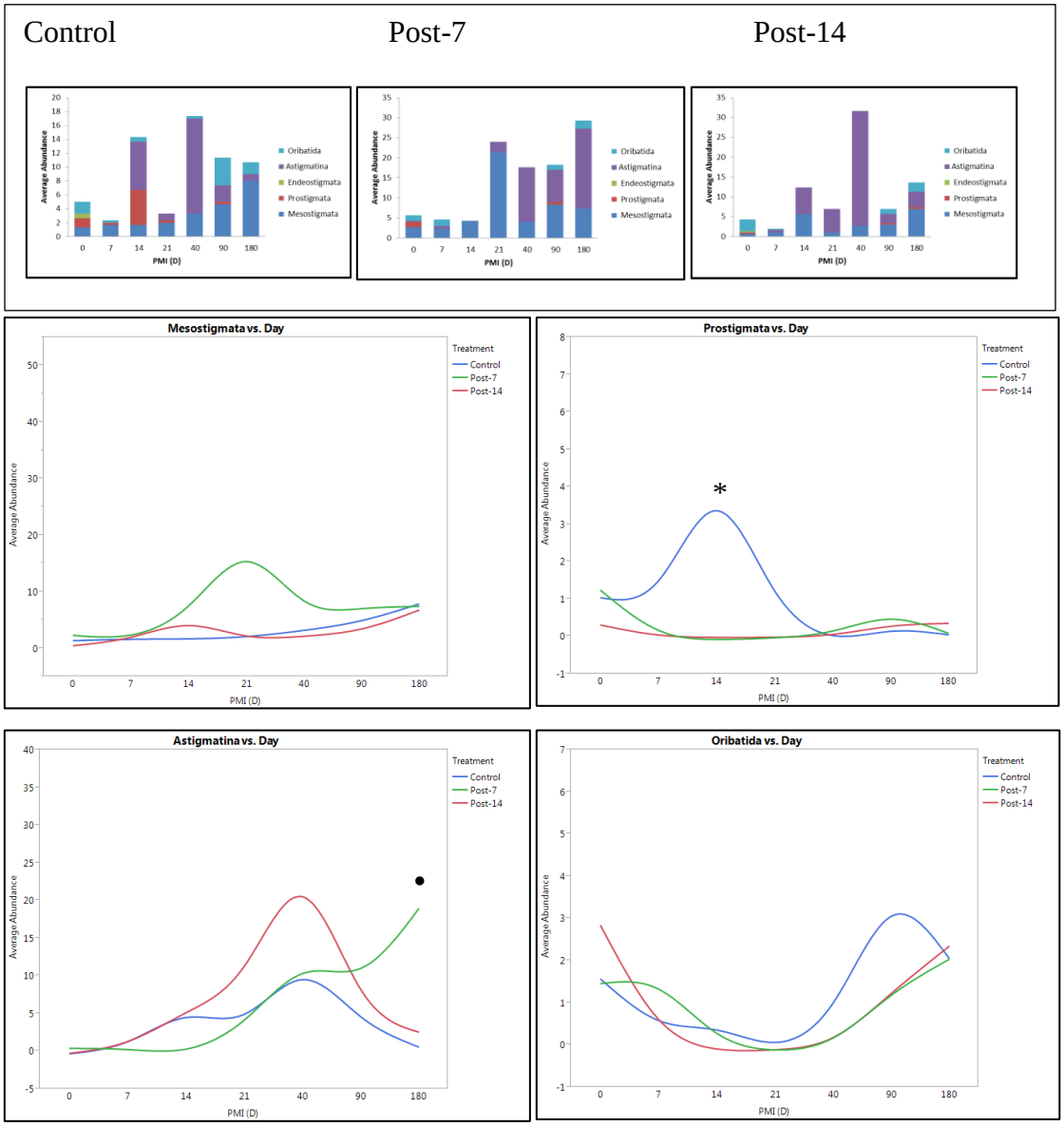

Figure 4.105. Above. Soil mite community abundance (by Order) beneath the carrion according to Treatments in summer 2013 at Snook, Texas. Middle Left. Abundance of Mesostigmata at soil beneath the carrion across treatments over time. Middle Right. Abundance of Prostigmata at soil beneath the carrion across treatments over time. Bottom Left. Abundance of Astigmatina at soil beneath the carrion across treatments over time. Bottom Right. Abundance of Oribatida at soil beneath the carrion across Treatments over time (* indicates significant difference; • indicate marginally significant difference).

Soil lateral

There was no significant difference in soil mite abundance between treatments at soil lateral (p = 0.4188). However, there was Day effect (p = 0.0002) among the soil mite abundance. In general, Astigmatina was the most abundance mites on Day 40 for all treatments. Mesostigmata was the second most abundance group, followed by Oribatida (Figure 4.106).

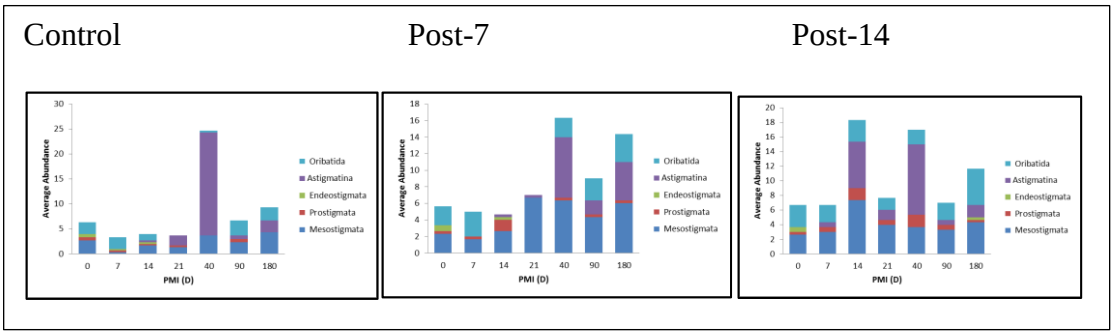

Figure 4.106. Soil mite community abundance (by Order) beside the carrion according to Treatments in summer 2013 at Snook, Texas.

Soil 5 m

There was no significant difference in soil mite abundance between treatments at soil 5 m (p = 0.5801), and there was significant difference in Day (p = 0.0085) (Figure 4.107). In general, Oribatida was the dominant group on the initial day of experiment, decreased during active decomposition stage, and then increased again on Day 40 onwards.

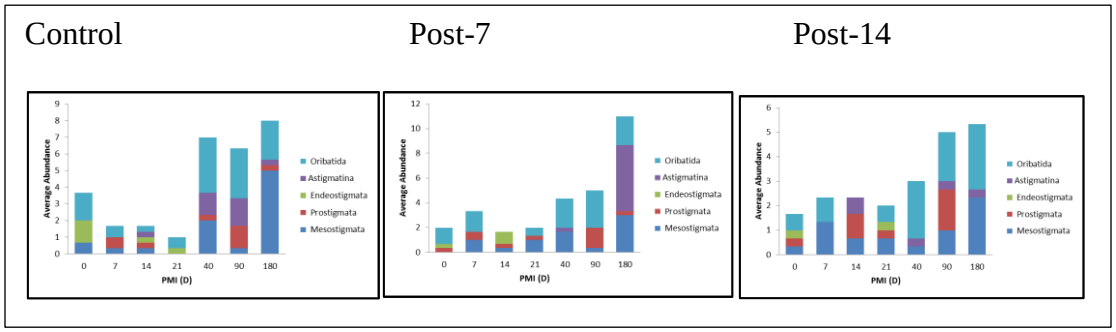

Figure 4.107. Soil mite community abundance (by Order) at soil 5 m away from the carrion according to Treatments in summer 2013 at Snook, Texas.

Abundance

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$). No significant difference was detected for Treatments ($p = 0.3118$) or any interactions. No significant difference was found in abundance between treatments in all sampling day at every soil region ($p > 0.05$) (Figure 4.108). Resilience was tested only for soil beneath for all treatments and resilience was observed on Day 90 for Post-14 carcasses while Control and Post-7 carcasses demonstrated a stable abundance over time (Table 4.90).

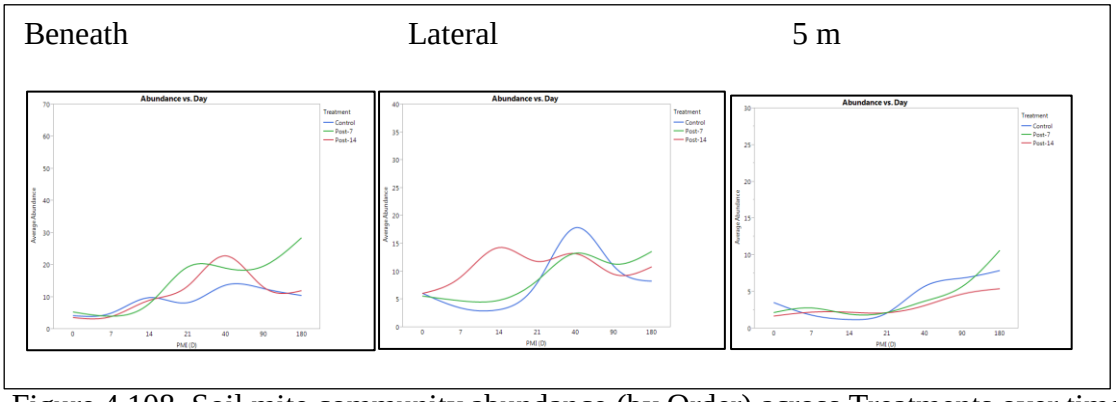

Figure 4.108. Soil mite community abundance (by Order) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.90. Resilience for soil mite community abundance (by Order) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2674	Resistance
Post-7	None	0.3635	Resistance
Post-14	0 x 40	0.0012*	90

Richness

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$). Treatment was not significantly difference ($p = 0.4049$) and no significant interaction was detected. There was no significant difference in richness between treatments on every sampling day at all soil regions ($p > 0.05$) (Figure 4.109). In general, mite community richness at soil beneath was decreasing during active decomposition and then increased again when the decomposition process was completed. Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable richness over time (Table 4.91).

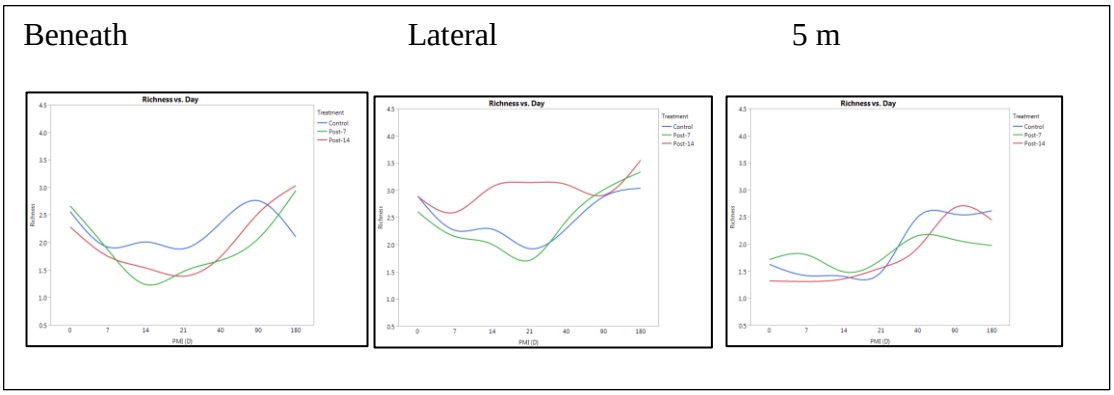

Figure 4.109. Soil mite community richness (by Order) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.91. Resilience for soil mite community richness (by Order) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.5969	Resistance
Post-7	None	0.1575	Resistance
Post-14	None	0.0587	Resistance

Simpson's diversity index

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$), without any significant interactions. Treatment was not significantly difference ($p = 0.3160$). Significant difference in Simpson's Diversity Index was found (divergence) only at soil lateral of carrion on Day 21 ($p = 0.0493$) and on again on Day 40 (Control x Post-14 with $p = 0.0130$). Convergence then occurred on Day 90 (Figure 4.110). No significant difference was detected between treatments in every sampling day at soil beneath as well as soil 5 m. Note that at soil beneath, diversity decreased over decomposition process, and increased after Day 21 for all treatments. Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable Simpson's diversity over time (Table 4.92).

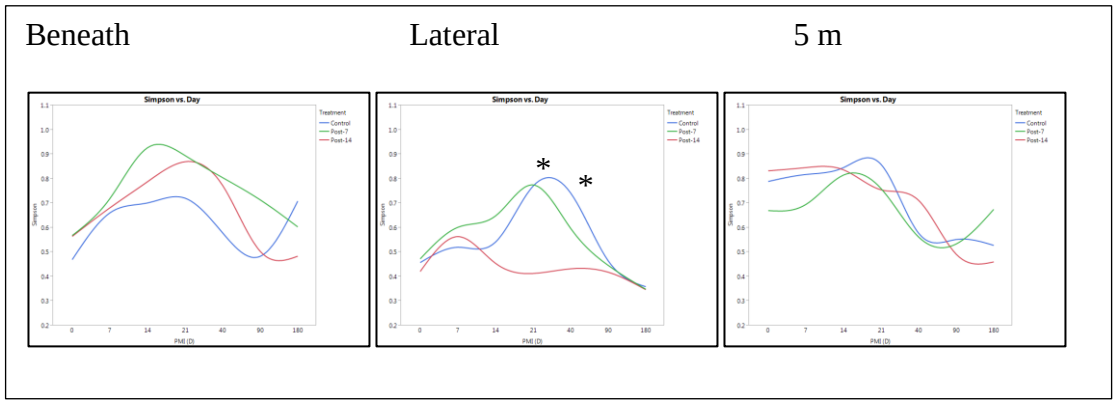

Figure 4.110. Simpson's diversity index (by Order) of soil mites across Treatments over time at different soil regions in summer 2013 at Snook, Texas (* indicates significant difference).

Table 4.92. Resilience for soil mite Simpson's diversity (by Order) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.3396	Resistance
Post-7	None	0.3077	Resistance
Post-14	None	0.0687	Resistance

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) without any significant interactions. Treatment was not significantly different ($p = 0.2821$). There was no statistical difference of Shannon-Wiener's diversity index found between Treatments over every sampling day in soil beneath and soil 5 m (the soil mite community diversity was in stable dynamics). However, significant difference (divergence) was found on soil lateral on Day 21 ($p = 0.0477$) and Day 40 (Control x Post-14 with $p = 0.0093$), convergence then occurred on Day 90. In general, soil beneath showed decreased in soil mite diversity in all treatments during active decomposition process and increased on Day 21 and onwards, although no significant

divergence was observed (Figure 4.111). Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable Shannon-Wiener's diversity over time (Table 4.93).

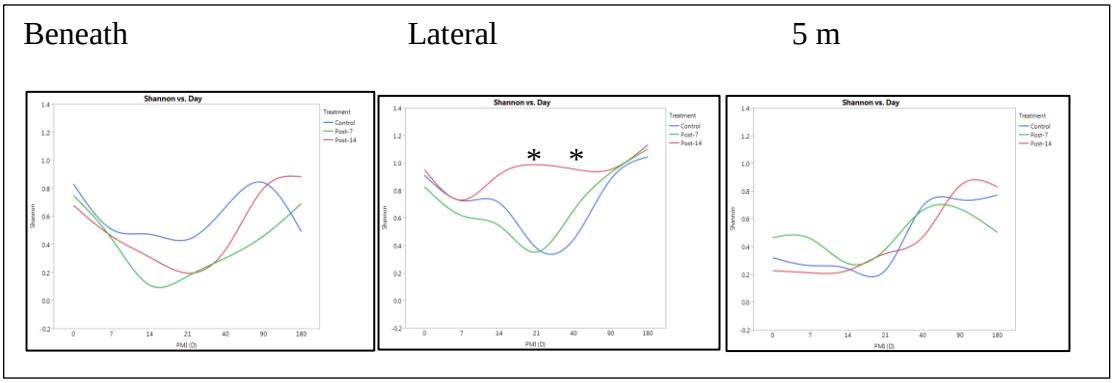

Figure 4.111. Shannon-Wiener's diversity index of soil mites across Treatments over time at different soil regions in summer 2013 at Snook, Texas (* indicates significant difference).

Table 4.93. Resilience for soil mite Shannon-Wiener's diversity (by Order) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.3976	Resistance
Post-7	None	0.0591	Resistance
Post-14	None	0.2526	Resistance

Evenness

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p = 0.0005$) without any significant interactions. Also, treatment had no significant difference ($p = 0.5385$). There was no statistical difference of evenness detected between treatments in every sampling day at all soil regions. In other words, the soil mite community evenness was in a stable dynamics. In general, soil mite evenness at soil

beneath decreased during active decomposition process and increased after the decomposition process was completed (Figure 4.112). Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable evenness over time (Table 4.94).

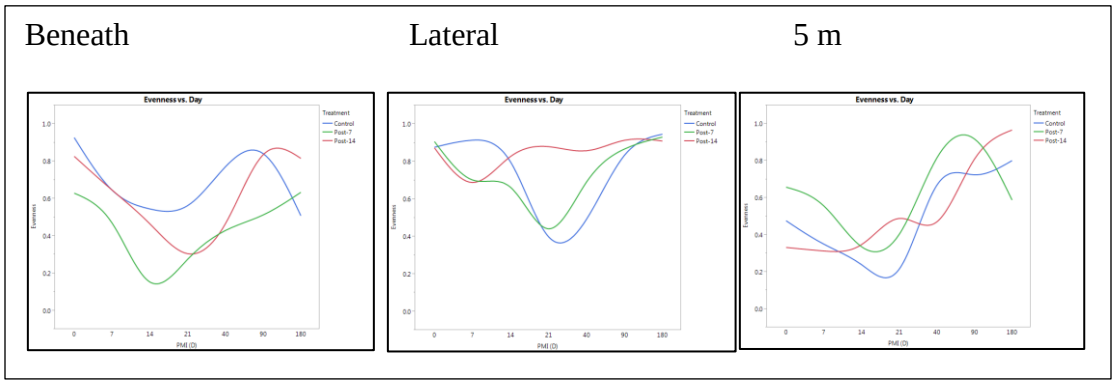

Figure 4.112. Evenness of soil mites (by Order) across Treatments over time at different soil regions in summer 2014 at Snook, Texas.

Table 4.94. Resilience for soil mite community evenness (by Order) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.5800	Resistance
Post-7	None	0.4590	Resistance
Post-14	None	0.2410	Resistance

Effective number of species

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) without any significant interactions. Treatment was not significant difference ($p = 0.1993$). Statistical difference of effective number of species (Order) or a significant divergence was found at soil lateral on Day 21 (Post-7 x Post-14, $p = 0.0298$)

and Day 40 (Control x Post-14, p = 0.0142), and convergence followed on Day 90. No significant difference was found at soil beneath and soil 5 m in terms of ENS. Likewise, ENS at soil beneath decreased during decomposition process and increased after Day 14 or 21 (Figure 4.113). Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable ENS over time, although there was a significant difference in the model for Post-14 group (Table 4.95).

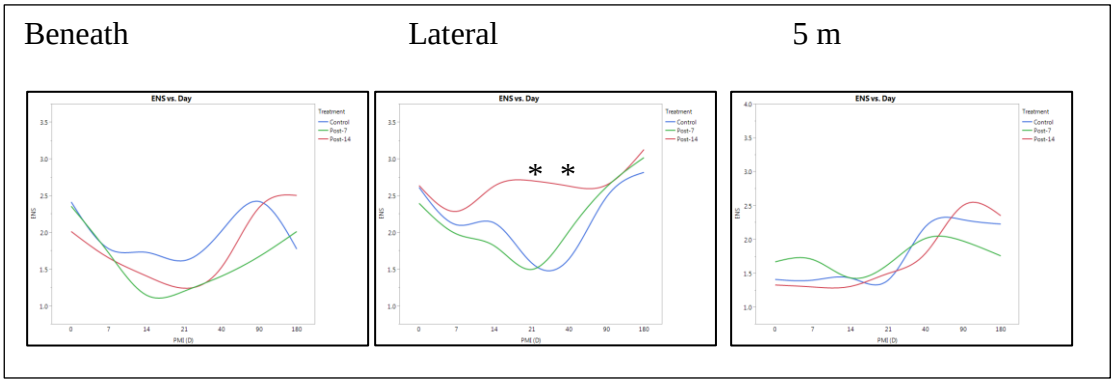

Figure 4.113. Effective number of species (by Order) of soil mites across Treatments over time at different soil regions in summer 2013 at Snook, Texas (* represents significant difference).

Table 4.95. Resilience for soil mite community ENS (by Order) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.3508	Resistance
Post-7	None	0.2161	Resistance
Post-14	None	0.0486*	Resistance

Family in 2013

PERMAVONA was performed on the acari at this taxonomic level to determine the effects of independent variables. The results showed Replicate had no significant

difference ($p = 0.414$). For the other factors, Day, Treatment and Region were significantly different ($p < 0.05$) with no other significant interactions detected (Table 4.96).

Table 4.96. Analysis of the soil mite community structure (by Family) in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	8.2682	0.001*
Treatment	2	1.7383	0.024*
Region	2	3.9044	0.001*
Day x Treatment	2	1.3355	0.136
Day x Region	2	1.2890	0.177
Treatment x Region	4	1.0307	0.437
Day x Treatment x Region	4	0.7766	0.846

Since there was a significant effect in Day, Treatment and Region, further analyses were conducted. All soil regions were significantly different from each other ($p < 0.001$), indicating soil community structure changes according to region (Table 4.97). Most of the pairwise comparisons between days of decomposition were significantly different ($p < 0.05$), except Day 7 x Day 21 (Table 4.98). Results showed treatments were significance with p value 0.035 for Control x Post-7 (Table 4.99). The NMDS plot of stress for soil mite community structure (Figure 4.114) and NMDS ordinations for Day, Region and Treatment were provided for visualization about data distribution (Figure 4.115, 4.116, and 4.117, respectively). Minimum stress for given dimensionality was 0.2311 with $r^2 = 0.6003$. The MRPP analysis for soil region showed a significant difference (A value = 0.0187; Significant of Delta = 0.001 based on 999 permutations), the MRPP for treatments showed A value 0.0039 and Significant of Delta 0.082 (note the hypothesis null was not rejected although PERMANOVA showed a significant

difference in treatments) while the MRPP for day also showed a significant difference with A value 0.0759 and Significant of Delta 0.001.

Table 4.97. Pairwise comparisons between Regions on soil mite community structure by Family in summer 2013 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	0.782	0.7815	2.1298	0.0169	0.026*
	Residual	124	45.503	0.3669		0.9831	
	Total	125	46.285			1.0000	
Beneath x 5 m	Region	1	2.468	2.4679	6.2335	0.0479	0.001*
	Residual	124	49.095	0.3959		0.9521	
	Total	125	51.563			1.0000	
Lateral x 5 m	Region	1	1.038	1.0376	2.6635	0.0210	0.004*
	Residual	124	48.310	0.3895		0.9790	
	Total	125	49.347			1.0000	

Table 4.98. Pairwise comparisons of soil mite community structure by Family between carrion decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.039*	0.001*	0.001*	0.001*	0.001*	0.001*
7	0.039*	-	0.002*	0.305	0.001*	0.001*	0.001*
14	0.001*	0.002*	-	0.031*	0.001*	0.002*	0.001*
21	0.001*	0.305	0.031*	-	0.001*	0.002*	0.002*
40	0.001*	0.001*	0.001*	0.001*	-	0.001*	0.001*
90	0.001*	0.001*	0.002*	0.002*	0.001*	-	0.002*
180	0.001*	0.001*	0.001*	0.002*	0.001*	0.002*	-

Table 4.99. Pairwise comparisons between Treatments on soil mite community structure by Family in summer 2013 at Snook, Texas after Bonferroni's correction.

Treatment			df	SS	MS	F model	R2	P value
Control	x	Treatment	1	0.791	0.7906	1.9702	0.0156	0.035*
Post-7		Residual	124	49.762	0.4013		0.9844	
		Total	125	50.552			1.0000	
Control	x	Treatment	1	0.528	0.5277	1.3739	0.0110	0.167
Post-14		Residual	124	47.633	0.3841		0.9890	
		Total	125	48.161			1.0000	
Post-7	x	Treatment	1	0.590	0.5903	1.5036	0.0120	0.134
Post-14		Residual	124	48.684	0.3926		0.9880	
		Total	125	49.274			1.0000	

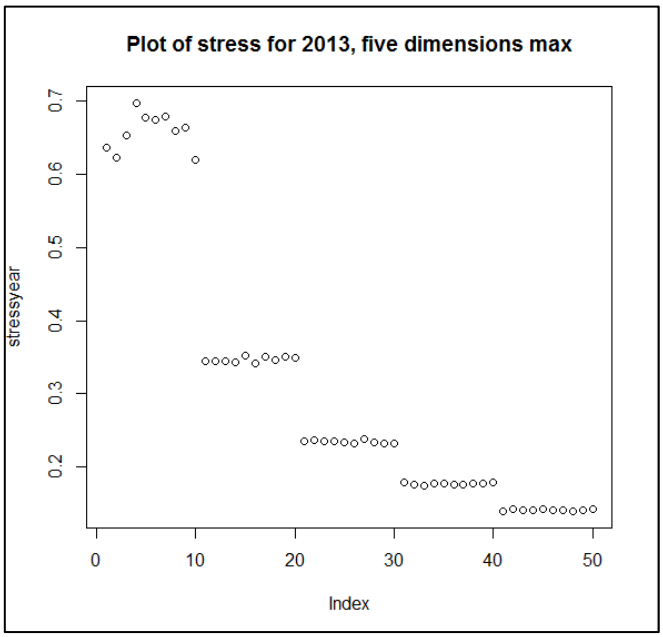

Figure 4.114. NMDS plot of stress for soil mite community structure (by Family) in summer 2013 at Snook, Texas (Stress test 0.2311; $r^2 = 0.6003$).

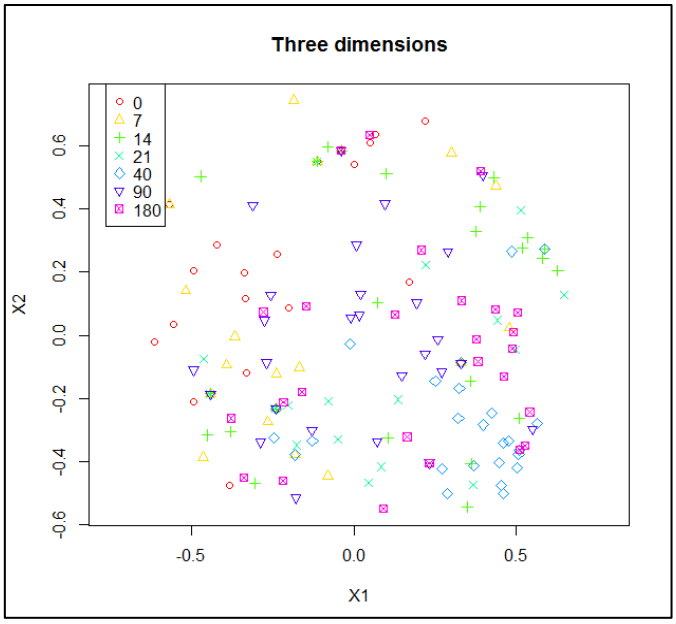

Figure 4.115. NMDS ordinations for soil mite community structure (by Family) according to carrion decomposition days in summer 2013 at Snook, Texas.

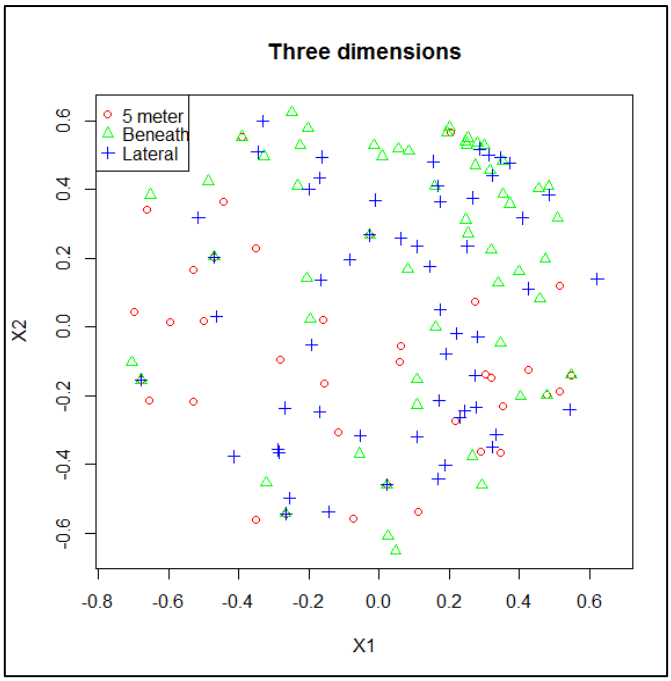

Figure 4.116. NMDS ordinations for soil mite community structure (by Family) according to soil regions in summer 2013 at Snook, Texas.

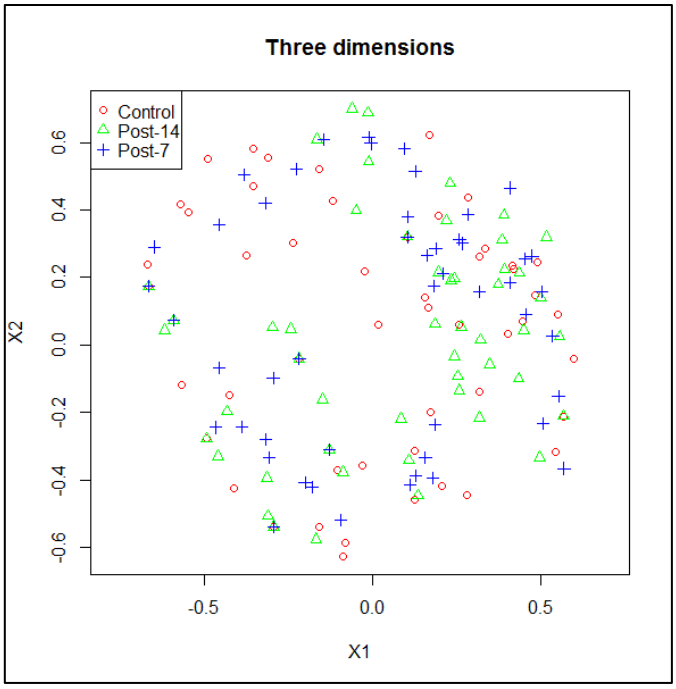

Figure 4.117. NMDS ordinations for soil mite community structure (by Family) according to treatments in summer 2013 at Snook, Texas.

The ISA results showed three families of soil mites namely Phytoseiidae, Pygmephoridae and Acaridae were the indicators for 2013 trial (Table 4.100).

Table 4.100. Indicator species analysis by Family for soil mite community in summer 2013 at Snook, Texas.

Type	Family	Indicator value	P value
All soils	Phytoseiidae	0.1364	0.042*
	Pygmephoridae	0.6667	0.001*
	Acaridae	0.1537	0.005*

Abundance of soil mite community structure (by Family) according to soil regions in 2013

Soil beneath

There was no significant difference in soil mite abundance between treatments at soil beneath ($p = 0.1552$). However, there is Day effect ($p = 0.0004$). Five acari families namely Ascidae, Acaridae, Macrochelidae, Parasitidae and Uropodidae were highlighted to demonstrate population dynamics in response to treatments over decomposition days at soil beneath the carrion. Only a marginal significant difference was detected in the family Acaridae on Day 180 ($p = 0.0609$). There was no significant difference determined ($p > 0.05$) for the abundance of other mite families over time. In general, Acaridae (*Sancassania* spp.) was the dominant family observed in all treatment groups. For Control carcasses, Acaridae was observed from Day 7, peaked on Day 40, and reduced in population on Day 180, and succeeded by Macrochelidae (*Macrocheles* spp.), who peaked in abundance on Day 180. Conversely, Acaridae was dominant for Post-7 group from Day 40 until Day 180 (Figure 4.118).

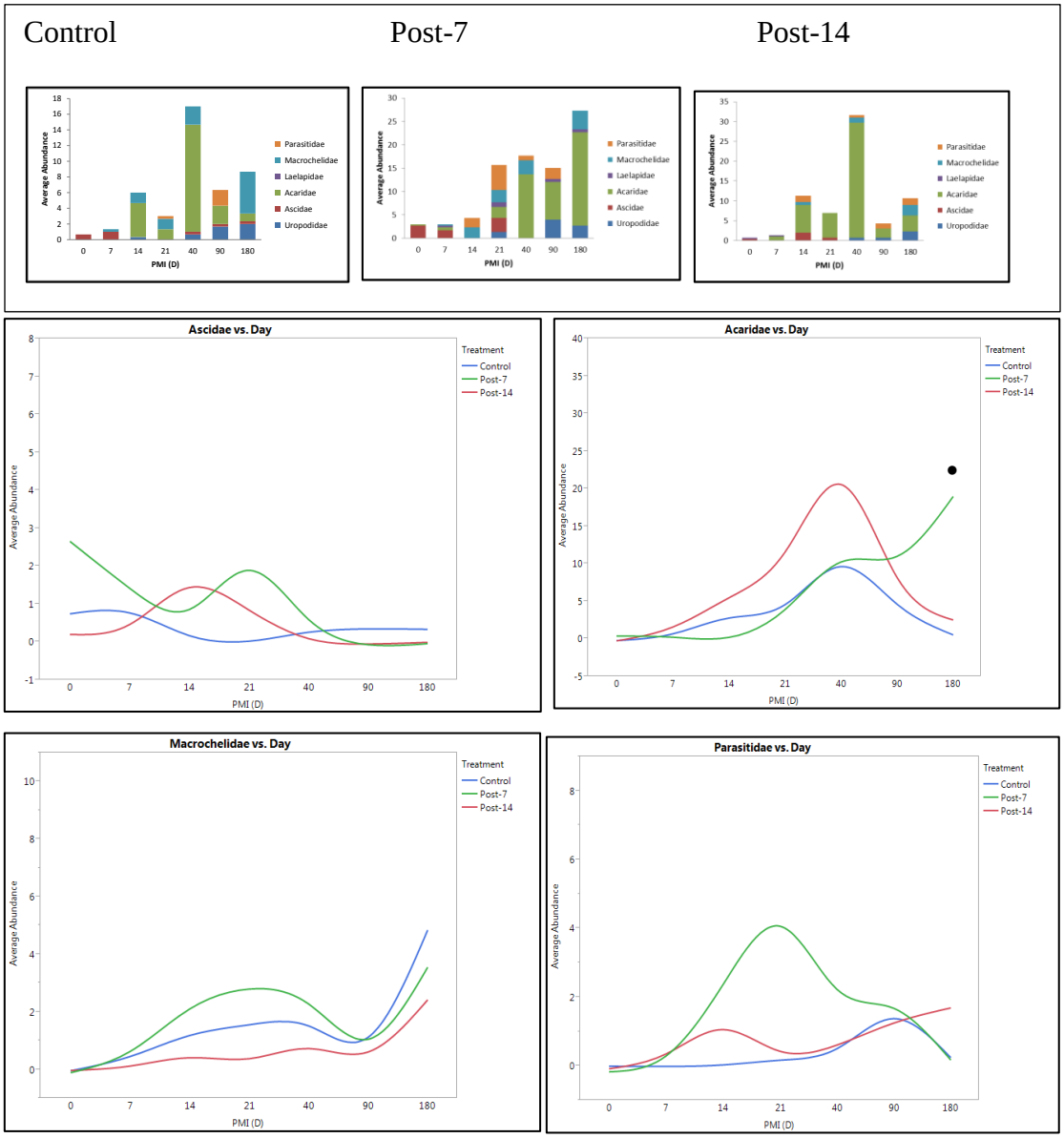

Figure 4.118. Above. Soil mite community abundance (by Family) beneath the carrion according to Treatments in summer 2013 at Snook, Texas. Upper Left. Abundance of Ascidae at soil beneath the carrion across treatments over time. Upper Right. Abundance of Acaridae at soil beneath the carrion across treatments over time. Middle Left. Abundance of Macrochelidae at soil beneath the carrion across treatments over time. Middle Right. Abundance of Parasitidae at soil beneath the carrion across treatments over time. Bottom Left. Abundance of Uropodidae at soil beneath the carrion across Treatments over time (• indicates marginally significant difference).

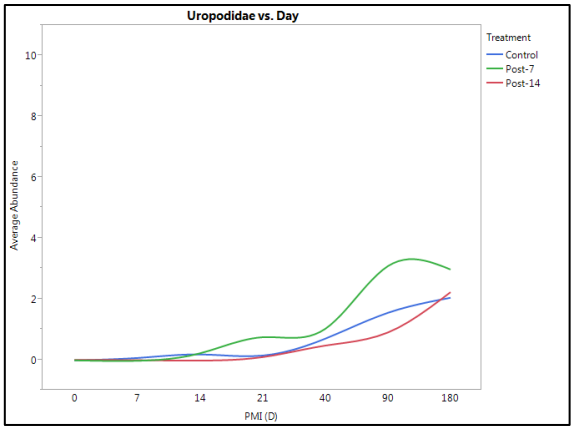

Figure 4.118 (Continued).

Soil lateral

There was no significant difference in soil mite abundance between treatments at soil lateral ($p = 0.7339$). However, there was Day effect ($p < 0.0001$) among the soil mite abundance. In general, Acaridae was the most abundance mites on Day 40 for all treatments. For the Control pigs, Ascidae and Macrochelidae were the other two major families that occurred at the soil lateral of the carrion. For Post-7 and Post-14 carcasses, families such as Parasitidae and Laelapidae were also collected from the soil from Day 14 to Day 180 (Figure 4.119).

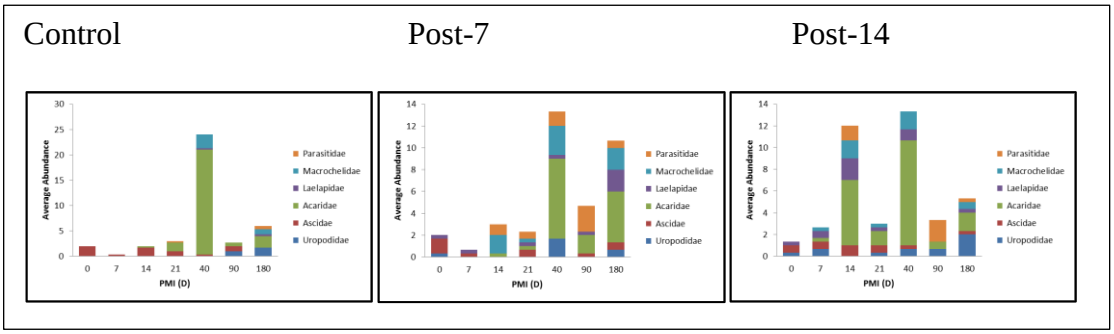

Figure 4.119. Soil mite community (by Family) abundance beside the carrion according to Treatments in summer 2013 at Snook, Texas.

Soil 5 m

There was no significant difference in soil mite abundance between treatments at soil 5 m ($p = 0.4713$) as well as no significant in Day ($p = 0.1247$) (Figure 4.120). In general, Acaridae was the dominant group on Day 40 for Control and Post-14 group, and Day 180 for Post-7 group (apparent delayed in abundance compared to other groups). It is noteworthy to mention that acarid mites can disperse to the soil 5 m away from the carrion. In reality, soils at 5 m away from carrion were dominated by Oribatida mite. However, the families of Oribatida were not identified and hence were not included. It is obvious that the predatory mites (e.g., Ascidae, Macrochelidae, Parasitidae) were present especially at the beginning of the experiment and between the Days of 90 and 180 (Figure 4.120).

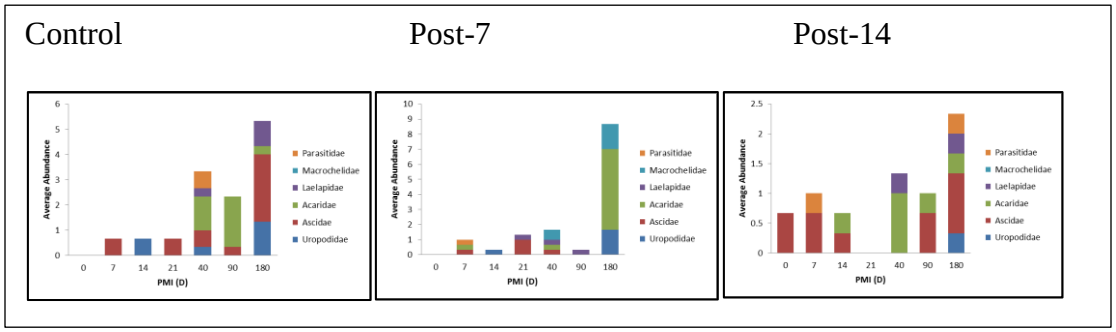

Figure 4.120. Soil mite community (by Family) abundance at soil 5 m away from the carrion according to Treatments in summer 2013 at Snook, Texas.

Abundance

The full model showed a significant difference in Day ($p < 0.0001$), Region ($p < 0.0001$), and an interaction between Day x Region ($p = 0.0020$). Treatment was not significantly different ($p = 0.3307$). However, there was no significant difference in soil mite abundance (by Family) between treatments on every sampling day at all soil regions in 2013 trial (Figure 4.121). In other words, the soil mite community was in a stable equilibrium regardless of treatments. Resilience was tested only for soil beneath for all treatments and there was resilience on Day 90 for Post-14 groups while Control and Post-7 groups demonstrated a stable abundance over time (Table 4.101).

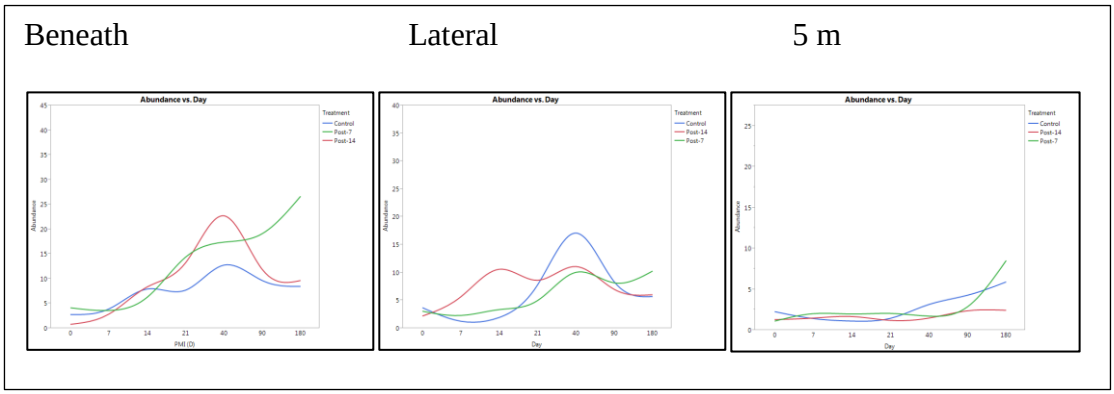

Figure 4.121. Soil mite family abundance across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.101. Resilience for soil mite community abundance (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2506	Resistance
Post-7	None	0.2288	Resistance
Post-14	0 x 40	0.0001*	90

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Richness

The full model showed a significant difference in Day ($p = 0.0174$) and Region ($p < 0.0001$) without any significant interactions. Furthermore, treatment was not significantly different ($p = 0.8156$). There was no significant difference in richness between treatments on every sampling day at all soil regions ($p > 0.05$) (Figure 4.122). In general, mite community family richness at soil beneath was lower in Post-14 compared to Post-7 and Control groups throughout the decomposition process. Contrary, Acari family richness was higher in Post-14 group at soil lateral. Perhaps this observation suggest a lateral movement of soil arthropods to the side of the carrion to avoid the highly concentrated nutrient island at the soil beneath, considering the treatment Post-14 (i.e., delayed blow fly colonization for 14 days) allowed the soil

arthropods to move away from the soil beneath in a sufficient time line. Another explanation would be the soil arthropods were attracted to the carrion resource, but were congregated at the soil lateral before entering the soil beneath. Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable richness over time (Table 4.102).

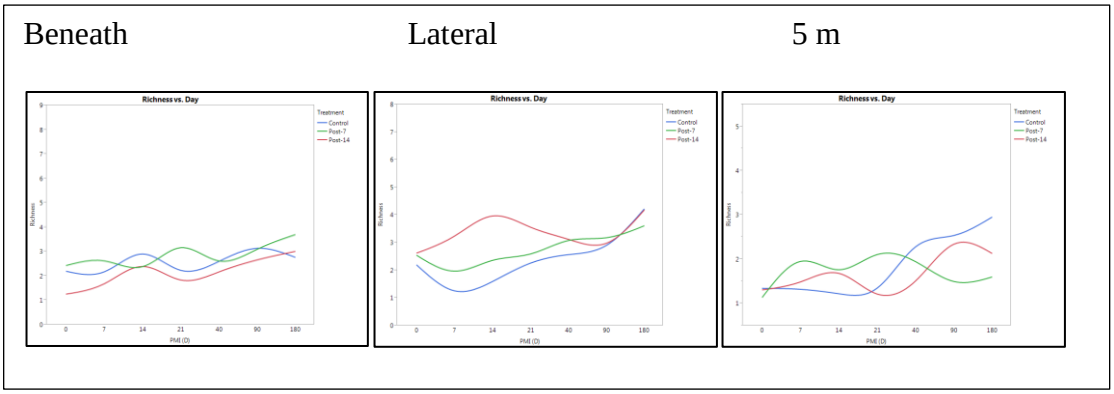

Figure 4.122. Soil mite family richness across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.102. Resilience for soil mite community richness (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.6208	Resistance
Post-7	None	0.7145	Resistance
Post-14	None	0.1393	Resistance

Simpson's diversity index

The full model showed a significant difference in Day ($p = 0.0051$) and Region ($p < 0.0001$) without any significant interactions. Also, treatment was not significant difference ($p = 0.6251$). No significant difference was detected between treatments in

every sampling day at all soil regions (Figure 4.123). This indicates that the soil mite diversity was in stable dynamics. Note that at soil beneath, diversity was lower for Post-14, and the diversity increased from Day 40 to Day 180. In contrast, Post-14 had the highest diversity at soil lateral. Perhaps this suggests the lateral movement or aggregation of soil arthropods to the soil beside the carcasses to avoid the center of nutrient toxicity (i.e., the soil beneath). Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable diversity over time (Table 4.103).

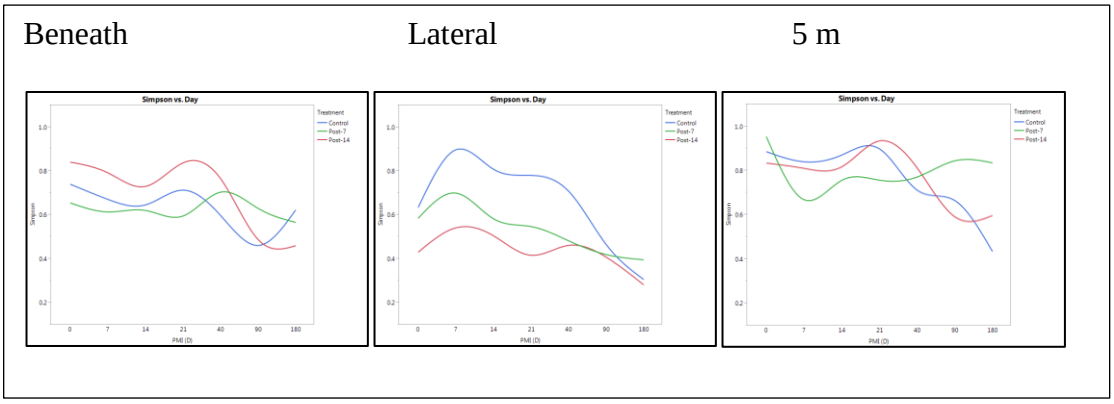

Figure 4.123. Simpson's diversity index of soil mite (by Family) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.103. Resilience for soil mite Simpson's diversity (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.7313	Resistance
Post-7	None	0.9399	Resistance
Post-14	None	0.1516	Resistance

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p = 0.0083$) and Region ($p < 0.0001$) without any significant interactions. Besides, treatment was not significant difference ($p = 0.6736$). There was no statistical difference of Shannon-Wiener's diversity index found between Treatments over every sampling day in all soil regions, indicating a stable soil mite community. Similarly, soil beneath showed decreased in soil mite diversity during active decomposition process and increased on Day 21 and onwards. Note that at soil lateral, Post-14 had the highest diversity while Control carcasses had the lowest diversity (Figure 4.124). Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable diversity over time (Table 4.104).

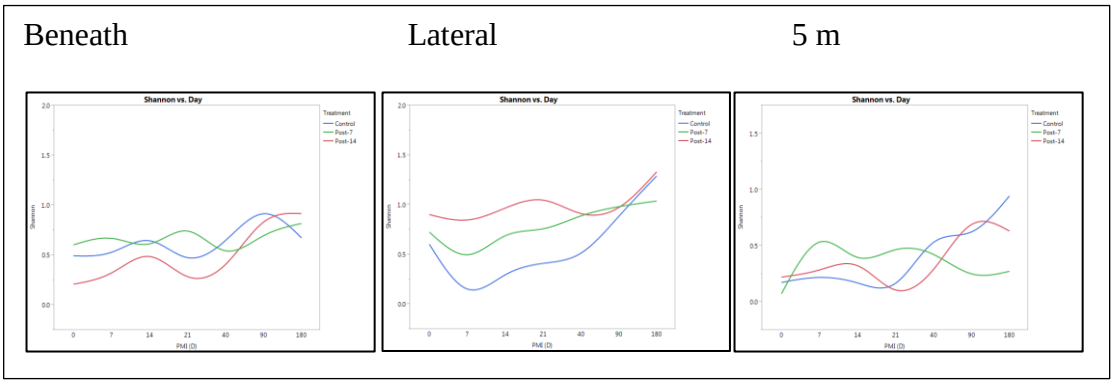

Figure 4.124. Shannon-Wiener's diversity index of soil mites (by Family) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.104. Resilience for soil mite Shannon-Wiener's diversity (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.7048	Resistance
Post-7	None	0.8783	Resistance
Post-14	None	0.1344	Resistance

Evenness

The full model showed a significant difference in Day ($p = 0.0196$) and Region ($p < 0.0001$) without any significant interactions. Moreover, treatment was not significantly different ($p = 0.7339$). There was no statistical difference of evenness detected between treatments in every sampling day at all soil regions, indicating the soil mite community was in stable dynamics. In general, soil mite evenness at soil beneath decreased during active decomposition process and increased over time (Figure 4.125). Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable evenness over time (Table 4.105).

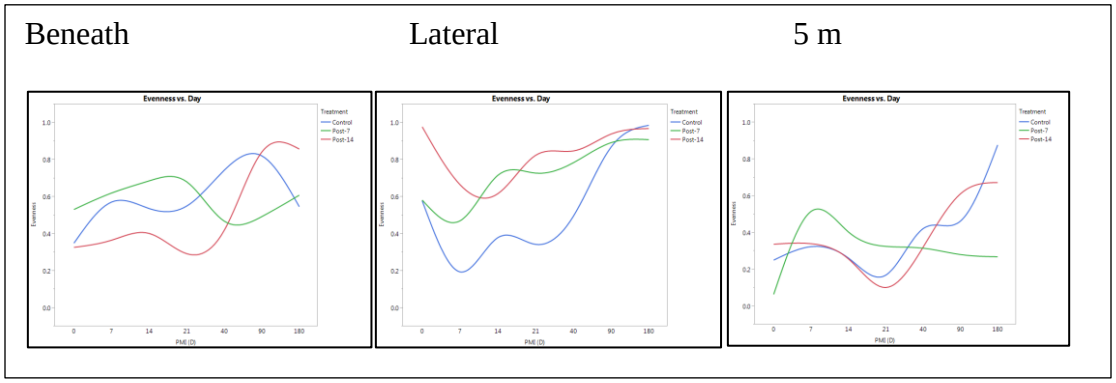

Figure 4.125. Evenness of soil mite (by Family) across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.105. Resilience for soil mite community evenness (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.7757	Resistance
Post-7	None	0.9482	Resistance
Post-14	None	0.2730	Resistance

Effective number of species

The full model showed a significant difference in Region (p = 0.0006). Day was marginally significantly difference (p = 0.0504) for ENS. No significant interaction was detected and treatment was not significantly different (p = 0.6942). No significant difference was found in ENS between treatments on every sampling day at all types of soil regions, suggesting a stable soil mite community. Likewise, ENS at soil beneath decreased during decomposition process and increased on and after Day 21. At soil lateral, Post-14 had the highest ENS compared to other treatment groups (Figure 4.126). Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable ENS over time (Table 4.106).

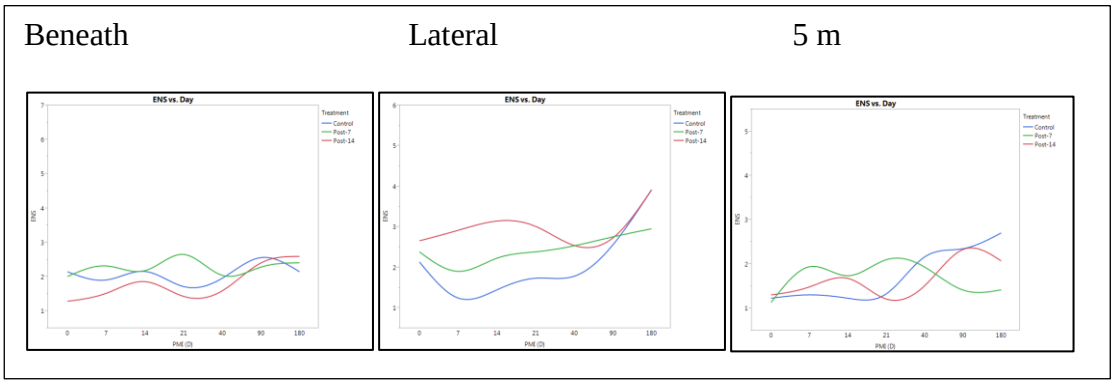

Figure 4.126. Effective number of species (by Family) of soil mites across Treatments over time at different soil regions in summer 2013 at Snook, Texas.

Table 4.106. Resilience for soil mite community ENS (by Family) for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.6757	Resistance
Post-7	None	0.8026	Resistance
Post-14	None	0.1357	Resistance

Soil mite function in 2013

PERMANOVA was performed on soil mite data by function. Results showed that there was significant Day effect and Region effect (both $p = 0.001$) without any significant interaction (Table 4.107). Again, Treatment was not significantly difference by soil mite community function ($p = 0.403$). Note that the Replicate had a significant difference by PERMANOVA ($p = 0.018$), suggesting functionally different among replicates. A separate PERMANOVA was performed to determine which pair of functional group was different. However, ANOVA test for individual functional group did not reveal significant effect in replicate ($p < 0.05$).

Table 4.107. Analysis of the soil mite community function in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	11.0829	0.001*
Treatment	2	1.0590	0.403
Region	2	6.5013	0.001*
Day x Treatment	2	0.8253	0.570
Day x Region	2	1.0267	0.424
Treatment x Region	4	0.7634	0.712
Day x Treatment x Region	4	0.4758	0.960

There was a significant effect in Day, Region and Replicate, further analyses were carried out. For soil regions, all soil regions were significantly from each other ($p < 0.05$), indicating soil community structure changes according to region (Table 4.108). As for days of decomposition, most of the pairwise comparisons between days of decomposition were significantly different ($p < 0.05$), except Day 0 x Day 7, Day 0 x Day 14, Day 0 x Day 21, Day 14 x Day 21, Day 40 x Day 180 and Day 90 x Day 180 where there were no significant difference detected ($p > 0.05$) (Table 4.109). For

replicate effect, replicate #1 and replicate #3 was found significant difference (p = 0.009) and there was marginal significant difference in Replicate #2 and #3 (p = 0.053) (Table 4.110). The NMDS plot of stress for soil arthropod community structure (Figure 4.127) and NMDS ordinations for Day, Region and Replicate were provided for visualization of data distribution (Figure 4.128, 4.129, and 4.130, respectively). Minimum stress for given dimensionality was 0.1209 with r^2 = 0.9144. The MRPP analysis for soil region showed a significant difference (A value = 0.0386; Significant of Delta = 0.001 based on 999 permutations) while the MRPP for day also showed a significant difference with A value 0.0784 and Significant of Delta 0.001. The MRPP for Replicate showed A value 0.0083 and Significant of Delta 0.025.

Table 4.108. Pairwise comparisons between Regions on soil mite community function in summer 2013 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	0.5082	0.5082	2.4479	0.0194	0.044*
	Residual	124	25.7434	0.2076		0.9806	
	Total	125	26.2519			1.0000	
Beneath x 5 m	Region	1	2.136	2.1356	8.5821	0.0647	0.001*
	Residual	124	30.857	0.2488		0.9353	
	Total	125	32.992			1.0000	
Lateral x 5 m	Region	1	1.4896	1.4896	7.3457	0.0559	0.001*
	Residual	124	25.1458	0.2027		0.9441	
	Total	125	26.6354			1.0000	

Table 4.109. Pairwise comparisons of soil mite community function between decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.062•	0.101	0.155	0.001*	0.003*	0.001*
7	0.062•	-	0.015*	0.639	0.001*	0.007*	0.001*
14	0.101	0.015*	-	0.243	0.001*	0.002*	0.001*
21	0.155	0.639	0.243	-	0.001*	0.007*	0.001*
40	0.001*	0.001*	0.001*	0.001*	-	0.005*	0.268
90	0.003*	0.007*	0.002*	0.007*	0.005*	-	0.106
180	0.001*	0.001*	0.001*	0.001*	0.268	0.106	-

Table 4.110. Pairwise comparisons between Replicates on soil mite community function in summer 2013 at Snook, Texas after Bonferroni's correction.

Replicate		df	SS	MS	F model	R2	P value
1 x 2	Replicate	1	0.1597	0.1596	0.7019	0.0056	0.617
	Residual	124	28.2076	0.2274		0.9944	
	Total	125	28.3673			1.0000	
1 x 3	Replicate	1	0.7001	0.7001	3.0579	0.0240	0.009*
	Residual	124	28.3900	0.2289		0.9760	
	Total	125	29.0901			1.0000	
2 x 3	Replicate	1	0.5881	0.5881	2.6384	0.0201	0.053•
	Residual	124	28.7292	0.2316		0.9799	
	Total	125	29.3173			1.0000	

• Marginal significant difference.

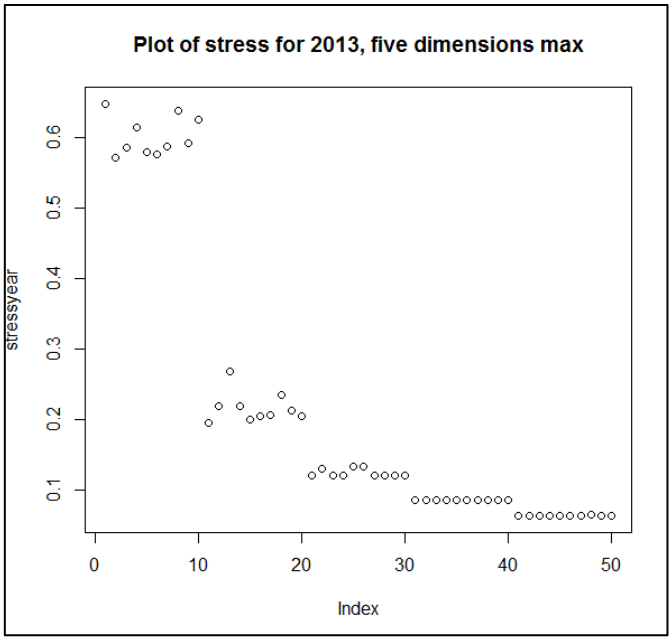

Figure 4.127. NMDS plot of stress for soil mite community function in summer 2013 at Snook, Texas (Stress test 0.1209; $r^2 = 0.9144$).

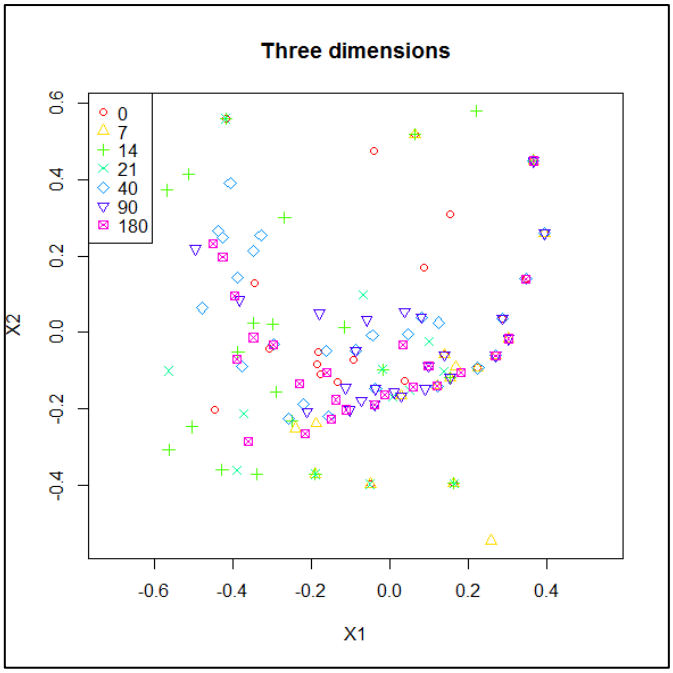

Figure 4.128. NMDS ordinations for soil mite community functions according to days of carrion decomposition in summer 2013 at Snook, Texas.

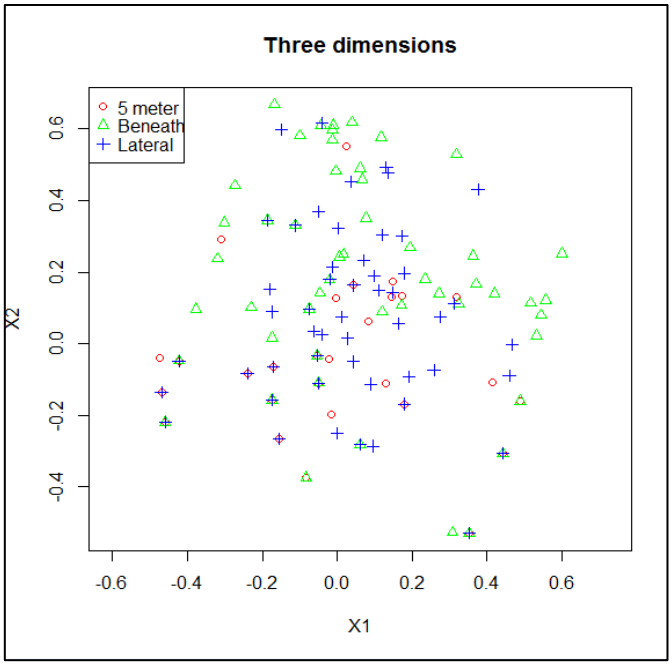

Figure 4.129. NMDS ordinations for soil mite community functions according to soil regions in summer 2013 at Snook, Texas.

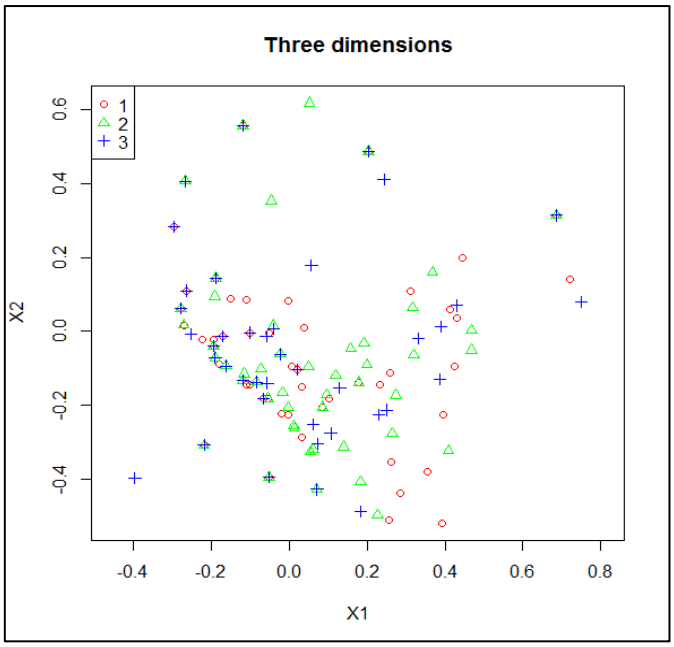

Figure 4.130. NMDS ordinations for soil mite community functions according to replicates in summer 2013 at Snook, Texas.

The ISA results demonstrated two functional groups of soil mites, detritivores and fungivores, as the indicators for summer 2013 (Table 4.111). These guilds are important players during decomposition process of pig carrion. The detritivore could be the generalists performing necrophagy while the fungivores feed on the fungi growth on the skin or skeletons.

Table 4.111. Indicator species analysis by Function for soil mites in summer 2013 at Snook, Texas.

Type	Functional group	Indicator value	P value
All soils	Fungivore	0.2333	0.002*
	Detritivore	0.0956	0.011*

Abundance of soil mite community structure (by Function) according to soil regions in 2013

Soil beneath

Soil mite community function was not significant difference between Treatments at soil beneath ($p = 0.418$). However, there was significant difference in Day ($p = 0.007$). In general, the major functional groups of mites for all treatments were the detritivores, and then followed by the predators. Detritivore became dominant on Day 14, 40 and 90 for Control carcasses; Day 40, 90 and 180 for Post-7 carcasses and almost every sampling day for Post-14 group. Although no significant difference was observed on the predator / parasite group, Post-7 had a higher abundance of predator guild at soil beneath on Day 21. Fungivores was present in a higher quantity under the Control carcasses on Day 14, and then re-appeared on Day 90. For fungivore, significant difference (divergence) was observed on Day 14 where Control carcasses had higher fungivore abundance than other groups (Control x Post-7 and Control x Post-14, both have p value 0.0180) followed by a convergence on Day 21 (Figure 4.131). For detritivore, a marginal significant difference was observed on Day 180 ($p = 0.0723$). Detritivores in Control and Post-14 group increased to its peak on Day 40 while Post-7 group continue to increase in detritivore abundance even to Day 180.

Resilience was tested only for all functional groups of soil mites at soil beneath for all treatments. The results showed predators were stable over time regardless of treatments (Table 4.112).

Figure 4.131. Above. Soil mite community abundance (by Function) beneath the carrion according to Treatments over decomposition days in summer 2013 at Snook, Texas. Upper Left. Abundance of predator / parasite at soil beneath the carrion across treatments over time. Upper Right. Abundance of detritivore at soil beneath the carrion across treatments over time. Lower Left. Abundance of fungivore at soil beneath the carrion across treatments over time (* represents significant difference; • denotes marginal significant difference).

Table 4.112. Resilience of soil mite community function for each treatment at soil beneath the carrion in summer 2013 at Snook, Texas.

Function	Treatment	Significant difference	P value	Resilience on Day
Detritivore	Control	None	0.2394	Resistance
	Post-7	None	0.0781	Resistance
	Post-14	0 x 40	0.0014*	90
Predator	Control	None	0.6992	Resistance
	Post-7	None	0.5089	Resistance
	Post-14	None	0.2209	Resistance
Fungivore	Control	0 x 14	0.0358*	21
	Post-7	None	0.4782	Resistance
	Post-14	None	0.5216	Resistance

Soil lateral

Soil mite community function was not significant difference between Treatments at soil lateral ($p = 0.447$). However, there was significant difference in Day ($p = 0.001$). Similarly, the major functional groups of mites for all treatments at soil lateral were the detritivores, and then followed by the predators. In general, detritivore increased its abundance over time and reached the peak on Day 40. Predators and parasites were also increased in abundance along the decomposition process but its population abundance usually lower compared to the numbers of detritivores (Figure 4.132).

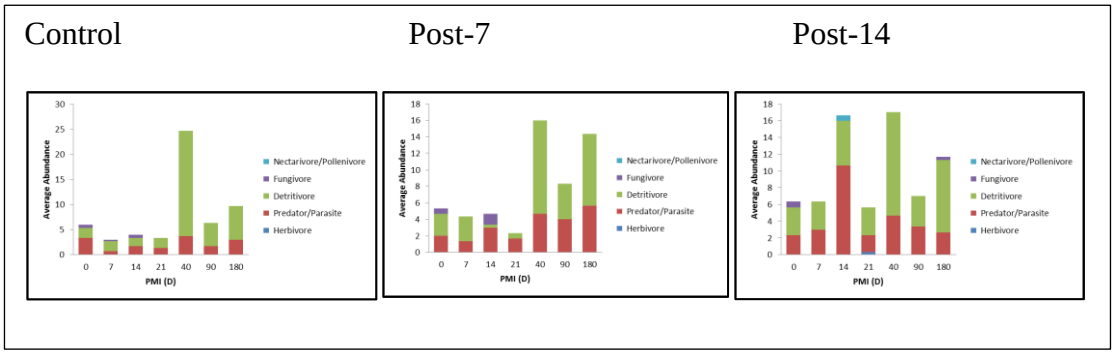

Figure 4.132. Soil mite community abundance (by Function) beside the carrion according to Treatments over carrion decomposition days in summer 2013 at Snook, Texas.

Soil 5 m

Soil mite community function was not significant difference between Treatments at soil 5 m ($p = 0.785$). However, there was significant difference in Day ($p = 0.006$). Similarly, the major functional groups of mites for all treatments at soil 5 m were the detritivores, and then followed by the predators. In general, detritivore increased its abundance over time and became dominant on Day 40, 90 and 180 (Figure 4.133). It is interesting to note that detritivores such as the family Acaridae was able to dispersal or migrate to the soil 5 m away from carrion. However, it is well known that *Sancassania* sp. (Acaridae) is able to perform phoresy on varieties of insects such as beetles. Predators and parasites were also increased in abundance along the decomposition process, usually at the beginning of decomposition and after the decomposition process, but the population abundance of predators are usually lower compared to the numbers of detritivores.

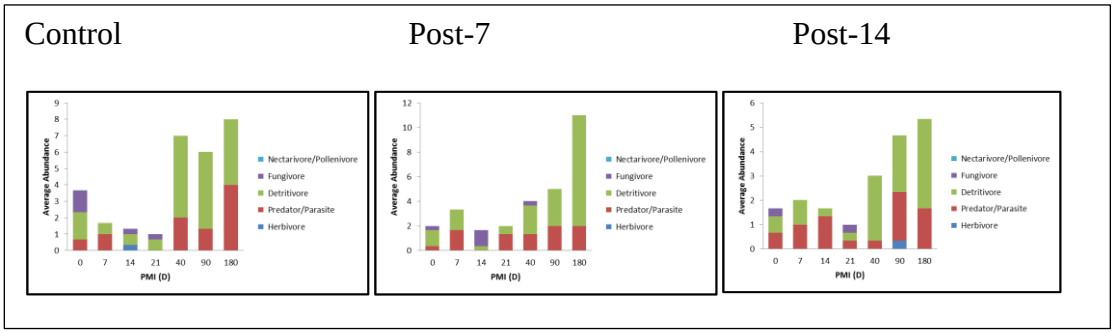

Figure 4.133. Soil mite community abundance (by Function) at soil 5 m away from the carrion according to Treatments over carrion decomposition days in summer 2013 at Snook, Texas.

Community structure and function of soil mites in 2014

Total morphospecies in 2014

A total of two morphospecies were identified under the dissecting microscope, namely Oribatida mites and Non-Oribatida mites. The following figures demonstrated the total abundance of Oribatida and Non-Oribatida across Treatments over decomposition days according to soil regions (i.e., beneath, lateral and soil 5 m). In 2014 trial, full factorial model for Oribatida mites showed a significant difference in Day ($p < 0.0001$) and Region ($p = 0.0002$). On the other hand, full factorial model for Non-Oribatida mites demonstrated Day ($p = 0.0004$), Region ($p = 0.0131$) and an interaction Day x Region ($p = 0.0204$) were significantly different. Again, no significant treatment effect ($p > 0.05$) was observed for both groups of mites in 2014 trial.

Soil beneath

For Oribatida, Day was significantly different at soil beneath ($p = 0.0060$) while treatments did not showed a significant difference ($p = 0.9349$). Similarly, Non-Oribatida abundance was also showing significance difference by days ($p = 0.0097$). In general, Oribatida decreased during decomposition process (between Day 7 and Day 21), and then abundance of Oribatida increased thereafter. On Day 21, the abundance of oribatid at Post-14 carcasses increased in a higher rate while Post-7 increased

exponentially on Day 40. However, there was no significant difference determined among these treatments ($p > 0.05$).

For Non-Oribatida mites, there was a significant difference on Day 7 between Control x Post-14 ($p = 0.0497$) and also on Day 14 between Control x Post-14 and Control x Post-7 with p value 0.0056 and 0.0112, respectively. No significant difference was detected on Day 40 between treatments ($p = 0.9249$) although Post-14 had a lower abundance of mites (the major composition of the mites on this day were *Sancassania* sp. in the Family Acaridae). Mite abundance was then decreased on Day 90 and 180 for all treatments (Figure 4.135).

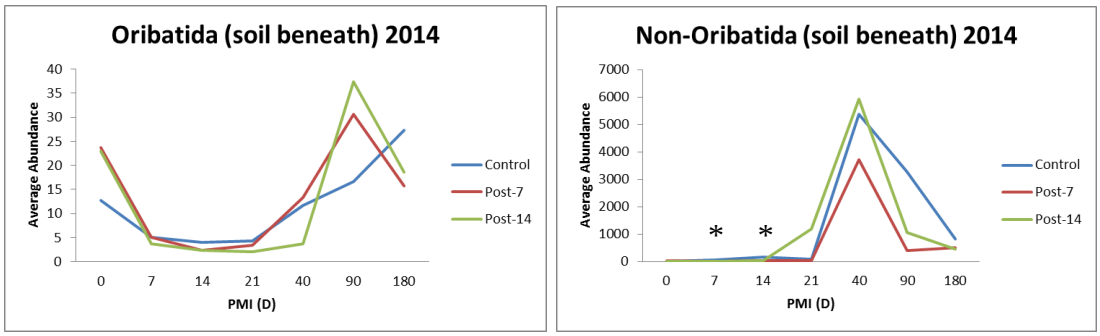

Figure 4.134. Abundance of Oribatida (Left) and Non-Oribatida (Right) mites across Treatments over carrion decomposition days at soil beneath the carrion in summer 2014 at Snook, Texas (* represent significant difference).

Soil lateral

For Oribatida, Day was significantly different at soil lateral ($p = 0.0006$) while treatments did not showed a significant difference ($p = 0.5718$). However, Non-Oribatida abundance did not have significant difference in days ($p = 0.1141$) and treatments ($p = 0.6723$). At the soil lateral, there were marginal significant differences detected on Day 7 ($p = 0.0506$) and Day 21 ($p = 0.0577$). The oribatid decreased during the decomposition process, and then increased gradually after Day 14 for all treatments.

It is interesting to note that oribatid mites for Control group continue to increase even on Day 180 while the Post-7 and Post-14 groups were decreased in oribatid abundance.

For Non-Oribatida mites, significant difference was found on Day 14 between Control x Post-14 ($p = 0.0075$) and Control x Post-7 ($p = 0.0308$). Likewise, the abundance of Non-Oribatida mites in Control group achieved the peak (the majority of the mites were *Sancassania* sp. in the Family Acaridae) on Day 40, although the Post-7 and Post-14 groups were still low in abundance ($p = 0.4323$) and delayed their peaks to Day 90 (however, no significant difference was found between treatments, $p = 0.7218$), probably this was due to the lateral movement or dispersal of acarid mites from soil beneath to the soil lateral of carrion. The mite abundance in all treatments was then subsequently decreased on Day 180 (Figure 4.135).

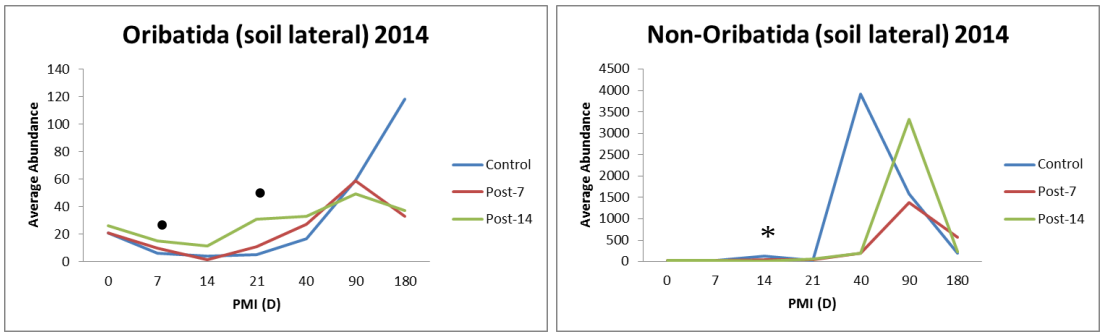

Figure 4.135. Abundance of Oribatida (Left) and Non-Oribatida (Right) mites across Treatments over carrion decomposition days at soil lateral the carrion in summer 2014 at Snook, Texas (• denotes marginal significant different; * represent significant difference).

Soil 5 m

For Oribatida, Day was significantly different at soil 5 m ($p < 0.0001$) while treatments did not showed a significant difference ($p = 0.4338$). Similarly, Non-Oribatida abundance was showing significant difference by days ($p = 0.0478$) and by treatments ($p = 0.7439$). The mite abundance at soil 5 m was thus considered a stable

community by having no effect in days and treatments. In general, oribatid mites in all treatments peaked on day 90, and eventually decreased in abundance on Day 180. For Non-Oribatida mites, significant difference was found on Day 21 between Control x Post-14 with p value 0.0244. The divergence at soil 5 m suggests natural stochastic events in the soil that led into the change of soil mite abundance (Figure 4.136).

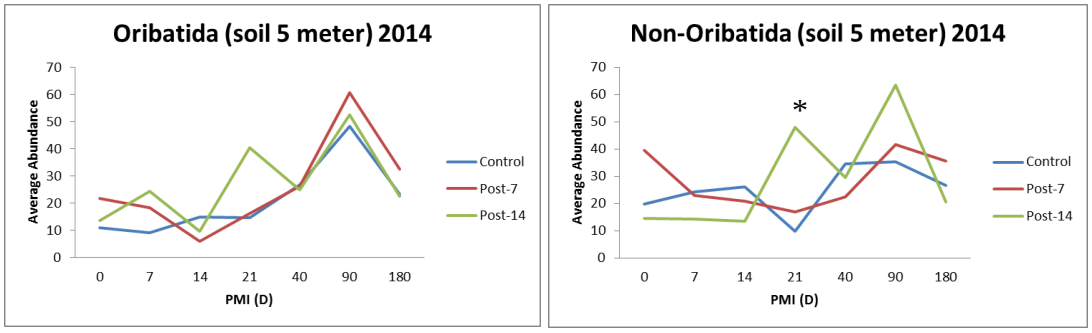

Figure 4.136. Abundance of Oribatida (Left) and Non-Oribatida (Right) mites across Treatments over carrion decomposition days at soil 5 m from the carrion in summer 2014 at Snook, Texas (* represent significant difference).

Total Superorder / Order / Suborder / Cohort in 2014

Subsampling of mite specimens (from the total morphospecies in 2014 trial) for slide mounting was conducted as mentioned in Materials and Methods. A total of 1740 mite specimens were mounted on slides and identified to the lowest taxonomical rank as possible. Two Superorders have been identified namely Parasitiformes and Acariformes. Under the superorder Parasitiformes, only the Order Mesostigmata had been identified. While for Acariformes, two Orders have been identified from the samples in this study, namely Order Trombidiformes and Sarcoptiformes. Suborder Prostigmata (under Trombidiformes) was identified. Suborder Oribatida and Cohort Astigmatina have been identified as well (all belonged to Order Sarcoptiformes). Note that none of the Suborder Endeostigmata was collected from the soil samples in summer 2014. This could be due to the absence of this mite during 2014 trial, or due to the chance that this mite was not

collected from the soil samples, or perhaps this mite was collected in the soil samples but was not fell into the randomly chosen square on the petri dish to be picked up for mounting during the subsampling process. Table 4.113 showed the mites' Orders and other taxonomic ranks identified in 2014 trial. The most dominant group mounted on slides was the Order Mesostigmata (54.02%) followed by the Suborder Oribatida (24.31%) and Cohort Astigmatina (18.51%). Note that the purpose of slide mounting was to identify the taxonomical group of the mite specimens, and the abundance as well as the dominance calculated on the slides may not represent the true abundance in the field.

Table 4.113. Total abundance and dominance of Orders and other lower taxonomic ranks of slide-mounted Acari identified from all soil samples in 2014 trials.

No.	Taxonomic rank		Total abundance	Dominance
1.	Order	Mesostigmata	940	54.02
2.	Suborder	Oribatida	423	24.31
3.	Cohort	Astigmatina	322	18.51
4.	Suborder	Prostigmata	55	3.16
		Total	1740	100

Total Family in 2014

For the suborder Oribatida, specimens were not identified to family level. The results presented below were the families identified from Non-Oribatida mites. A total of 17 families were identified from all the non-oribatid acari mounted on slides. One superfamily was identified as Eupodoidea, one family in the Cohort Astigmatina, nine families in the Order Mesostigmata, and 7 families in the Suborder Prostigmata. The most dominant family was Macrochelidae (31.50%), followed by Acaridae (25.08%) and

Ascidae (14.10%) (Table 4.114). Note that Acaridae are the detritivores or necrophagous whereas Macrocehlidae and Ascidae are the predators in the soil. Only relatively few numbers of mite specimens have been identified to Genus and species. Hence, statistical analysis at the Genus-species level was not performed. For the list of mite species recovered from soil samples, see Appendix H.

Table 4.114. Total abundance and dominance of Families of slide-mounted non-oribatid Acari identified from all soil samples in 2014 trial at Snook, Texas.

No.	Higher rank	Superfamily/Family	Total abundance	Dominance
1.	Mesostigmata	Macrochelidae	405	31.54
2.	Astigmatina	Acaridae	322	25.08
3.	Mesostigmata	Ascidae	181	14.10
4.	Mesostigmata	Laelapidae	124	9.66
5.	Mesostigmata	Parasitidae	96	7.48
6.	Mesostigmata	Uropodidae	50	3.89
7.	Prostigmata	Cunaxidae	35	2.73
8.	Mesostigmata	Phytoseiidae	20	1.56
9.	Mesostigmata	Ameroseiidae	16	1.25
10.	Mesostigmata	Melicharidae	11	0.86
11.	Prostigmata	Ereynetidae	5	0.39
12.	Prostigmata	Pygmephoridae	5	0.39
13.	Prostigmata	Scutacaridae	4	0.31
14.	Prostigmata	Rhagidiidae	4	0.31
15.	Prostigmata	Erythraeoidae	2	0.16

Table 4.114 (Continued).

	Higher rank	Superfamily/Family	Total abundance	Dominance
16.	Mesostigmata	Eviphididae	3	0.23
17.	Prostigmata	Eupodoidea*	1	0.08
		Total	1284	100

* = Superfamily

Total function in 2014

Four major functional groups were identified from all the slide-mounted acari in 2014 trial. They were herbivores, predators/parasites, detritivores, and fungivores. Note that none of the mites identified in 2014 trial fell into the category of nectarivores / pollenivores, and hence this group was not included in the analysis. The most abundance functional group during carrion decomposition was the predators / parasites guild (50.77%), followed by the detritivores (46.75%) (Table 4.115). The list of mite families with its respective functional role can be seen at Appendix I.

Table 4.115. Total abundance and dominance of Functions of slide-mounted acari identified from all soil samples in 2014 trials at Snook, Texas.

No.	Function	Total abundance	Dominance
1.	Predators/Parasites	859	50.77
2.	Detritivores	791	46.75
3.	Fungivores	24	1.42
4.	Herbivores	18	1.06
	Total	1692	100

Superorder / Order / Suborder / Cohort in 2014

PERMAVONA was performed on the acari at this taxonomic level to determine the effects of independent variables. The results showed Day and Region were significantly different ($p = 0.001$). Furthermore, there were interactions determined between Day x Treatment ($p = 0.016$), and Day x Region ($p = 0.008$). Treatment was marginally significant difference ($p = 0.058$), and Replicate was not significant difference ($p = 0.733$) (Table 4.116).

Table 4.116. Analysis of the soil mite community structure (by Order and other ranks) in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	23.0240	0.0001*
Treatment	2	2.0812	0.058•
Region	2	15.4155	0.001*
Day x Treatment	2	2.3085	0.016*
Day x Region	2	2.8110	0.008*
Treatment x Region	4	1.3753	0.149
Day x Treatment x Region	4	0.7342	0.729

• Marginal significant difference.

Since there was a significant effect in Day and Region, therefore further analyses were conducted. For soil regions, all soil regions were significantly different from each other ($p < 0.05$), indicating soil community structure changes according to regions (Table 4.117). As for day of decomposition, most of the pairwise comparisons between days of decomposition were significantly different ($p < 0.05$), except Day 0 x Day 7, Day 14 x Day 21, Day 40 x Day 90, Day 40 x Day 180 and Day 90 x Day 180 where there were no significant difference detected (Table 4.118). The NMDS plot of stress for

soil mite community structure (Figure 4.137) and NMDS ordinations for Day and Region were provided for visualization about data distribution (Figure 4.138 and 4.139, respectively). Minimum stress for given dimensionality was 0.1018 with r^2 = 0.9365. The MRPP analysis for soil region showed a significant difference (A value = 0.0703; Significant of Delta = 0.001 based on 999 permutations) while the MRPP for day also showed a significant difference with A value 0.11 and Significant of Delta 0.001.

Table 4.117. Pairwise comparisons between Regions on soil mite community structure by Order in summer 2014 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	0.6218	0.6217	3.1195	0.0245	0.028*
	Residual	124	24.7164	0.1993		0.9755	
	Total	125	25.3382			1.0000	
Beneath x 5 m	Region	1	4.615	4.6150	24.34	0.1641	0.001*
	Residual	124	23.512	0.1896		0.8359	
	Total	125	28.127			1.0000	
Lateral x 5 m	Region	1	2.0116	2.0115	12.629	0.0924	0.001*
	Residual	124	19.7516	0.1592		0.9076	
	Total	125	21.7631			1.0000	

Table 4.118. Pairwise comparisons of soil mite community structure by Order between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.971	0.001*	0.003*	0.001*	0.001*	0.001*
7	0.971	-	0.005*	0.017*	0.002*	0.001*	0.001*
14	0.001*	0.005*	-	0.499	0.001*	0.001*	0.001*
21	0.003*	0.017*	0.499	-	0.001*	0.001*	0.001*
40	0.001*	0.002*	0.001*	0.001*	-	0.243	0.239
90	0.001*	0.001*	0.001*	0.001*	0.243	-	0.418
180	0.001*	0.001*	0.001*	0.001*	0.239	0.418	-

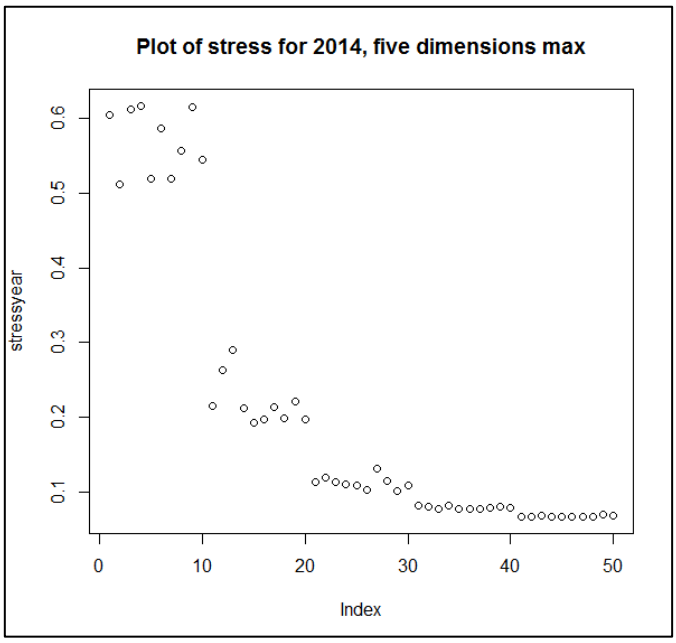

Figure 4.137. NMDS plot of stress for soil mite community structure (by Order) in summer 2014 at Snook, Texas (Stress test 0.1018; $r^2 = 0.9365$).

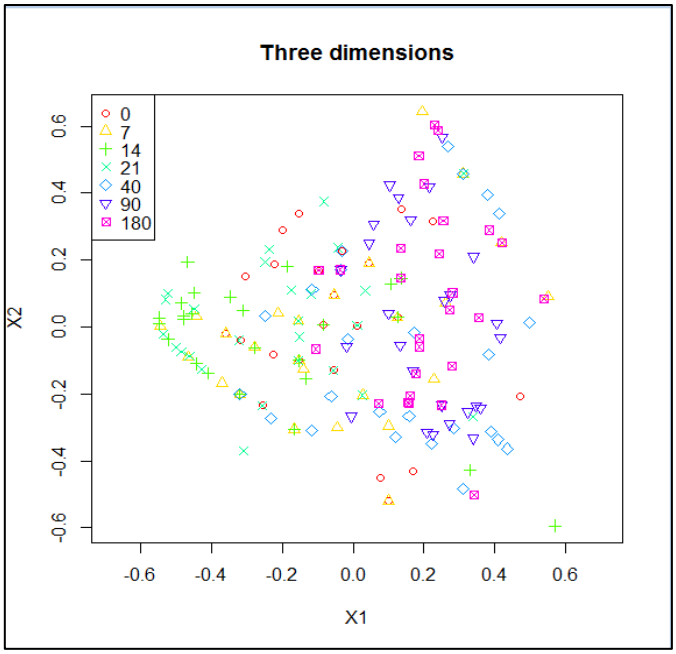

Figure 4.138. NMDS ordinations for soil mite community structure (by Order) according to days of carrion decomposition in summer 2014 at Snook, Texas.

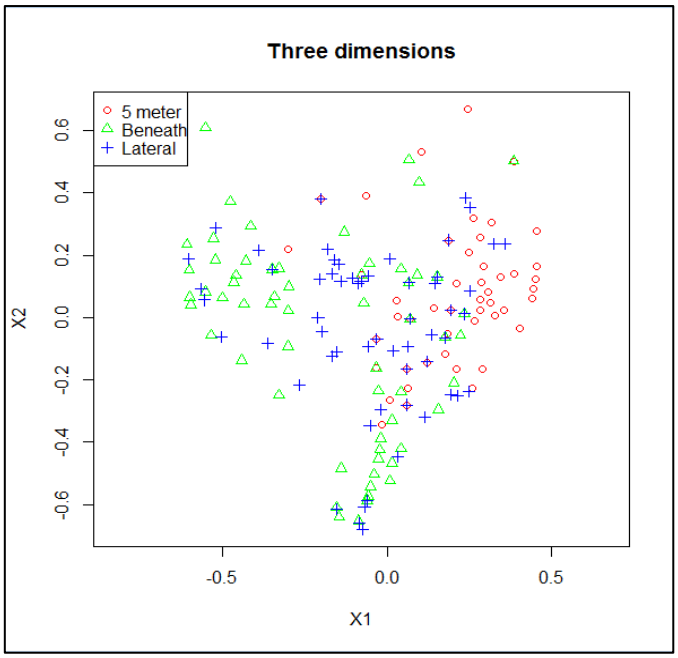

Figure 4.139. NMDS ordinations for soil mite community structure (by Order) according to soil regions in summer 2014 at Snook, Texas.

For ISA, results showed two groups of soil mites namely Suborder Prostigmata and Cohort Astigmatina were the indicators for 2014 trial (Table 4.119).

Table 4.119. Indicator species analysis by Order/Suborder for soil mites in summer 2014 at Snook, Texas.

Type	Order/Suborder/Cohort	Indicator value	P value
All soils	Prostigmata	0.1273	0.045*
	Astigmatina	0.0870	0.036*

Abundance of soil mite community structure (by Order) according to soil regions in 2014

Soil beneath

There was a marginal significant difference in soil mite abundance between treatments at soil beneath ($p = 0.061$). However, there was also Day effect ($p = 0.001$). Order Mesostigmata was the dominant Order for Control carcasses throughout the sampling days. While for Post-7 and Post-14 groups, Mesostigmata was the dominant Order from Day 0 to Day 21, but succeeded by the Cohort Astigmatina from Day 40 to Day 180. Four specific groups of mites at the soil beneath of Control carcasses namely Mesostigmata, Prostigmata, Astigmatina and Oribatida were plotted for their abundance over time (Figure 4.140). For Mesostigmata, significant differences between Treatments were found on Day 7 (Control x Post-7 and Control x Post-14, with p values 0.0186 and 0.0066, respectively) and Day 40 (Control x Post-7 and Control x Post-14, with p values 0.0139 and 0.0023). For Prostigmata, a significant difference ($p = 0.0467$) was found on Day 180, while a marginal significant difference ($p = 0.0569$) was found on Day 40 for Cohort Astigmatina. There was no significant difference detected among treatments in Oribatida.

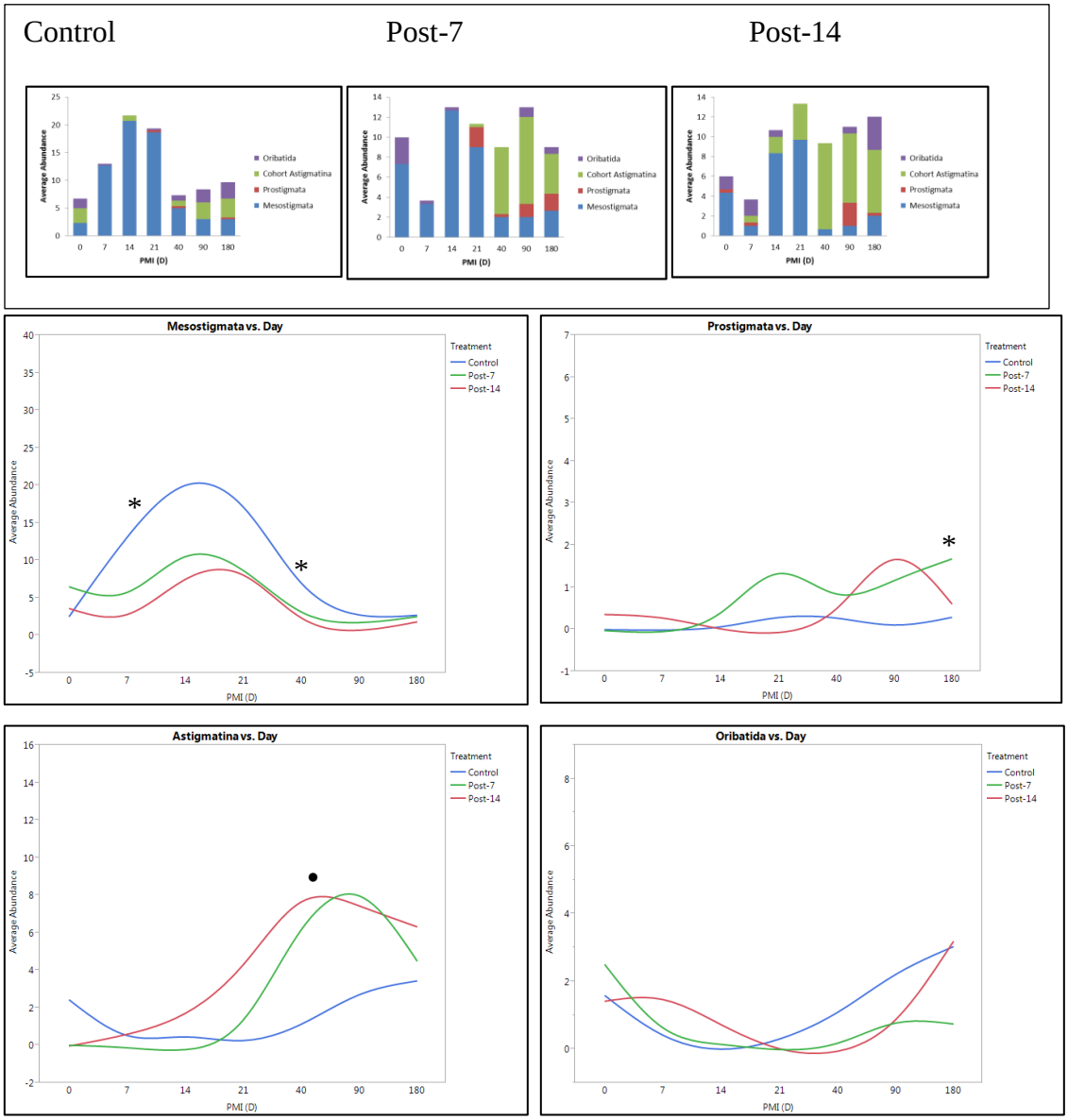

Figure 4.140. Above. Soil mite community abundance (by Order) beneath the carrion according to Treatments in summer 2014 at Snook, Texas. Middle Left. Abundance of Mesostigmata at soil beneath the carrion across treatments over time. Middle Right. Abundance of Prostigmata at soil beneath the carrion across treatments over time. Bottom Left. Abundance of Astigmatina at soil beneath the carrion across treatments over time. Bottom Right. Abundance of Oribatida at soil beneath the carrion across Treatments over time (* indicates significant difference; • indicate marginally significant difference.).

Soil lateral

There was no significant difference in soil mite abundance between treatments at soil lateral (p = 0.644). However, there was Day effect (p = 0.0001) among the soil mite abundance. In general, Mesostigmata was the dominant Order mounted on slides on the early phase of decomposition (Day 0 to Day 21). However, Astigmatina was the dominant Order from Day 40 to Day 180 (Figure 4.141).

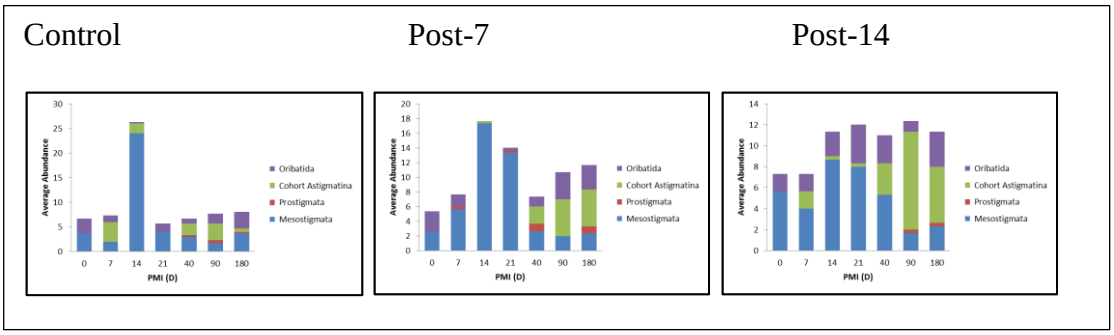

Figure 4.141. Soil mite community abundance (by Order) beside the carrion according to Treatments in summer 2014 at Snook, Texas.

Soil 5 m

There was no significant difference in soil mite abundance between treatments at soil 5 m (p = 0.286), and there was significant difference in Day (p = 0.001) (Figure 4.142). In general, Oribatida and Mesostigmata were the two dominant groups throughout all the decomposition stages and Oribatida was the dominant group on Day 40 and onwards (Figure 4.142).

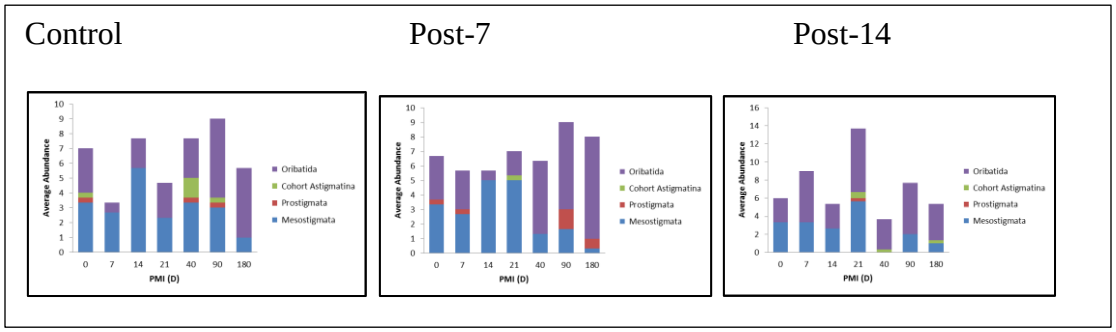

Figure 4.142. Soil mite community abundance (by Order) at soil 5 m away from the carrion according to Treatments in summer 2014 at Snook, Texas.

Abundance

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p < 0.0001$) with an interaction Day x Region ($p = 0.0494$). No significant difference was detected for Treatments ($p = 0.8543$). However, when comparing among treatments on every individual day, significant difference (divergence) was found on Day 7 at soil beneath between Control x Post-7 ($p = 0.0141$) and between Control x Post-14 ($p = 0.0141$), followed by convergence on Day 14. For soil 5 m, significant difference was found on Day 21 between Control x Post-14 ($p = 0.034$) and between Post-7 x Post-14 ($p = 0.0475$) (Figure 4.143). Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable abundance over time (Table 4.120).

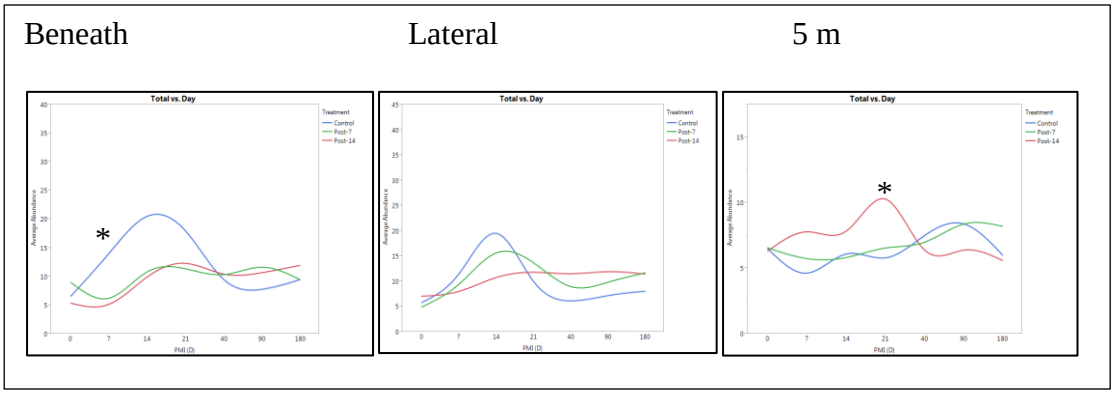

Figure 4.143. Soil mite community abundance (by Order) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference).

Table 4.120. Resilience for soil mite community abundance (by Order) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1678	Resistance
Post-7	None	0.1921	Resistance
Post-14	None	0.0542	Resistance

Richness

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p = 0.0158$), with a significant interaction Day x Region ($p = 0.0158$). Treatment was not significantly different ($p = 0.5950$). There was no significant difference between treatments at soil beneath in all sampling days, indicating a stable soil mite community. There was a significant difference detected on Day 90 at soil 5 m between Post-7 x Post-14 ($p = 0.0310$). There were marginal significant differences determined at soil lateral (on Day 90, $p = 0.0787$) and soil 5 m (on Day 40, $p = 0.0527$). In general, mite community richness at soil beneath in all treatments was decreasing during active decomposition and then increased again when the decomposition process was completed

(Figure 4.144). Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable richness over time (Table 4.121).

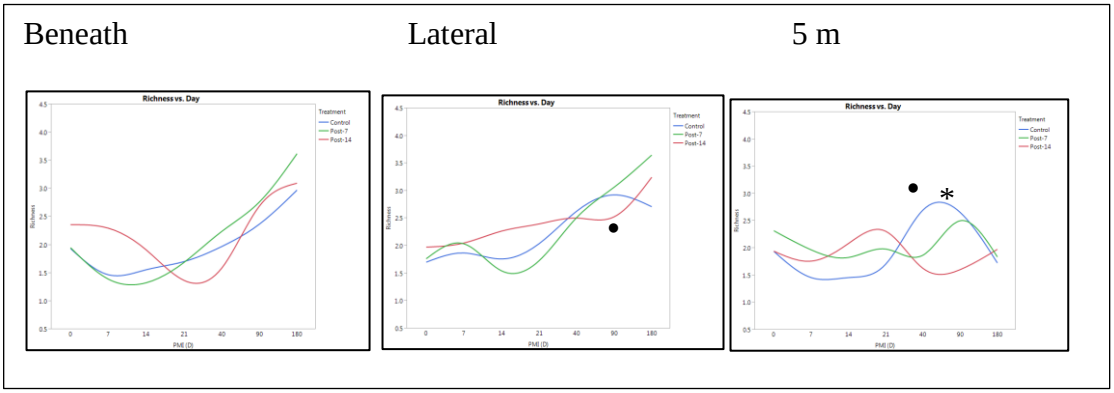

Figure 4.144. Soil mite community richness (by Order) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (• indicates marginal significant difference; * indicates significant difference).

Table 4.121. Resilience for soil mite community richness (by Order) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2693	Resistance
Post-7	None	0.0455*	Resistance
Post-14	None	0.0926	Resistance

Simpson's diversity index

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p = 0.0090$) with a significant interaction Day x Region ($p = 0.0002$). There was no significant difference in Treatments ($p = 0.5184$). Significant difference in Simpson's Diversity Index was found (divergence) only at soil lateral of carrion on Day 180 (Control x Post-7, $p = 0.0485$). Furthermore, there was a marginal significant difference detected on Day 21 ($p = 0.0716$) at soil lateral. No significant difference ($p > 0.05$) was

detected between treatments in every sampling day at soil beneath as well as soil 5 m. Note that at soil beneath, diversity decreased over decomposition process, and increased on Day 21 for Control and Post-7 group, while Post-14 increased in diversity on Day 40 (Figure 4.145). Resilience was tested only for soil beneath for all treatments and all results demonstrated a stable diversity over time (Table 4.122).

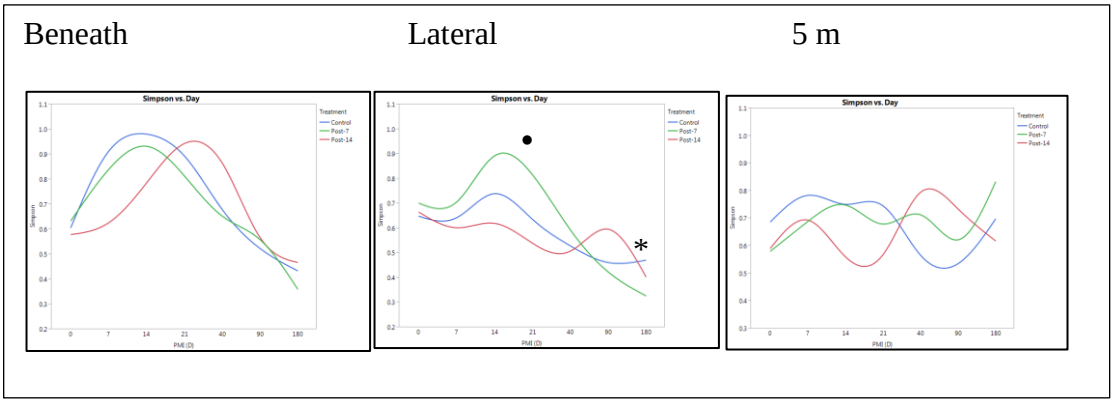

Figure 4.145. Simpson's diversity index (by Order) of soil mites across Treatments over time at different soil regions in summer 2014 at Snook, Texas (• indicates marginal significant difference; * indicates significant difference).

Table 4.122. Resilience for soil mite Simpson's diversity (by Order) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0025*	Resistance#
Post-7	None	0.0868	Resistance
Post-14	None	0.0110*	Resistance#

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p = 0.0153$), with an interaction Day x Region ($p = 0.0007$). No significant difference was detected on Treatment ($p = 0.2758$). There was no statistical difference of Shannon-Wiener's diversity index found between Treatments over every sampling day in all soil regions, suggesting the mite community was in stable dynamics. However, a marginal significant difference ($p = 0.0795$) was found on soil lateral on Day 21. In general, soil beneath showed decreased in soil mite diversity in all treatments during active decomposition process and increased after Day 14 (for Control and Post-7) or after Day 21(for Post-14) (Figure 4.146). Resilience was tested only for soil beneath for all treatments and resilience occurred on Day 21 for Control carcasses while Post-7 and Post-14 carcasses demonstrated a stable diversity over time (Table 4.123).

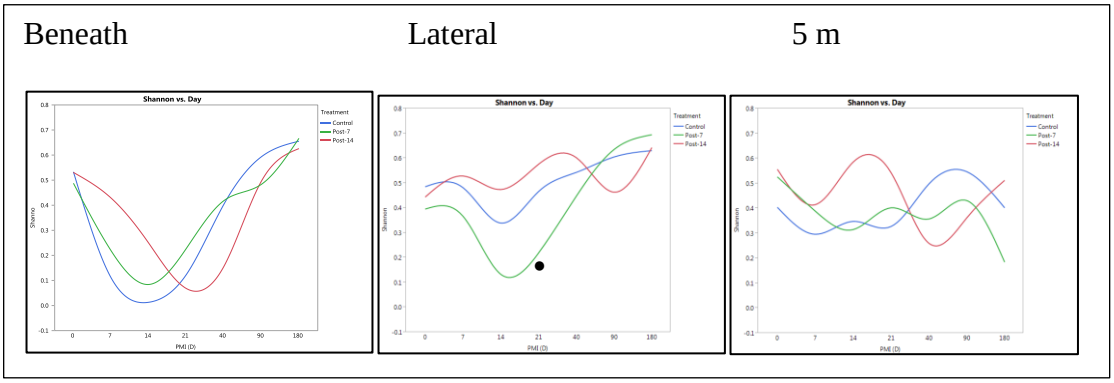

Figure 4.146. Shannon-Wiener's diversity index (by Order) of soil mites across Treatments over time at different soil regions in summer 2014 at Snook, Texas (• indicates marginal significant difference).

Table 4.123. Resilience for soil mite Shannon-Wiener's diversity (by Order) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 14	0.0471*	21
Post-7	None	0.1613	Resistance
Post-14	None	0.0102*	Resistance[#]

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Evenness

The full model showed a significant difference in Day ($p = 0.0056$) and Region ($p = 0.0166$) with an interaction Day x Region ($p = 0.0017$). Treatment had no significant difference ($p = 0.0600$), although it was marginal. There was no statistical difference of evenness detected between treatments in every sampling day at all soil regions. This indicates that the soil mites were in stable dynamics and were not sensitive to treatment effects. In general, soil mite evenness at soil beneath decreased during active decomposition process and increased after the decomposition process was completed (Figure 4.147). Resilience was tested only for soil beneath for all treatments and resilience occurred on Day 40 for Control carcasses while Post-7 and Post-14 carcasses demonstrated a stable evenness over time (Table 4.124).

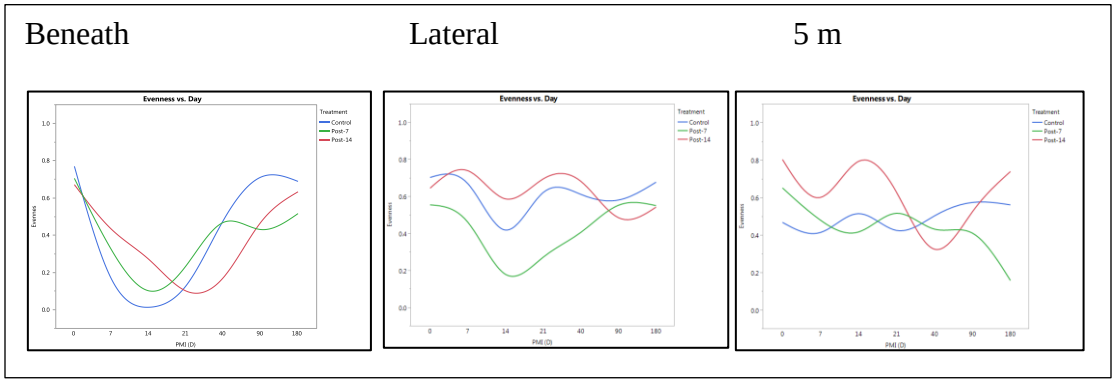

Figure 4.147. Evenness (by Order) of soil mites across Treatments over time at different soil regions in summer 2014 at Snook, Texas.

Table 4.124. Resilience for soil mite evenness (by Order) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 7	0.0413*	40
	0 x 14	0.0364*	
	0 x 21	0.0396*	
Post-7	None	0.2155	Resistance
Post-14	None	0.0540	Resistance

Effective number of species

The full model showed a significant difference in Day ($p < 0.0001$) and Region ($p = 0.0180$), with an interaction Day x Region ($p = 0.0011$). Treatment showed no significant difference ($p = 0.3143$). No statistical difference of effective number of species (Order) was found at all soil regions, although there was a marginal significant difference at soil lateral on Day 21 ($p = 0.0717$). In general, ENS at soil beneath decreased during decomposition process and increased after Day 14 or 21 (Figure 4.148). Resilience was tested only for soil beneath for all treatments demonstrated a stable ENS over time (Table 4.125).

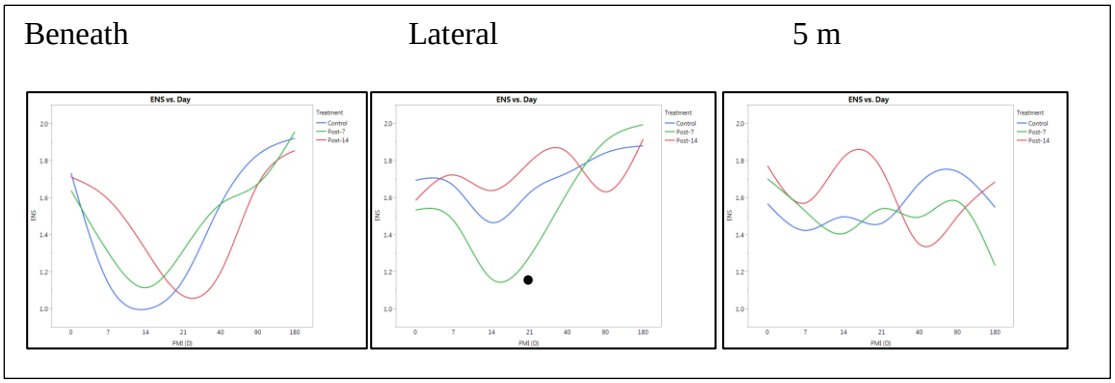

Figure 4.148. Effective number of species (by Order) of soil mites across Treatments over time at different soil regions in summer 2014 at Snook, Texas.

Table 4.125. Resilience for soil mite community ENS (by Order) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0021*	Resistance#
Post-7	None	0.0086*	Resistance#
Post-14	None	0.1815	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Family in 2014

PERMAVONA was performed on the acari at this taxonomic level to determine the effects of independent variables. The results showed Replicate had no significant difference ($p = 0.66$). For the other factors, Day, Treatment and Region were significantly different ($p < 0.05$). Besides, several significant interactions were also detected, such as Day x Treatment ($p = 0.011$), Day x Position ($p = 0.001$), and Treatment x Position ($p = 0.025$) (Table 4.126).

Table 4.126. Analysis of the soil mite community structure (by Family) in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	14.2435	0.001*
Treatment	2	2.2651	0.007*
Region	2	9.7355	0.001*
Day x Treatment	2	2.1302	0.011*
Day x Region	2	2.8505	0.001*
Treatment x Region	4	1.6717	0.025*
Day x Treatment x Region	4	0.8901	0.629

Since there was a significant effect in Day, Treatment and Region, further analyses were carried out. For soil regions, all soil regions were significantly from each other ($p < 0.05$), indicating soil community structure changes according to region (Table 4.127). As for day of decomposition, most of the pairwise comparisons between days of decomposition were significantly different ($p < 0.05$), except Day 0 x Day 7, Day 14 x Day 21 and Day 90 x Day 180 (Table 4.128). Results showed treatments were significance with p value 0.02 for Post-7 x Post-14 (Table 4.129). The NMDS plot of stress for soil mite community structure (Figure 4.149) and NMDS ordinations for Day, Region and Treatment were provided for visualization about data distribution (Figure 4.150, 4.151 and, 4.152 respectively). Minimum stress for given dimensionality was 0.1934 with $r^2 = 0.7281$. The MRPP analysis for soil region showed a significant difference (A value = 0.0496; Significant of Delta = 0.001 based on 999 permutations), the MRPP for treatments showed A value 0.0058 and Significant of Delta 0.004 while the MRPP for day also showed a significant difference with A value 0.0915 and Significant of Delta 0.001.

Table 4.127. Pairwise comparisons between Regions on soil mite community structure by Family in summer 2014 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	0.935	0.9350	2.8451	0.0224	0.016*
	Residual	124	40.753	0.3286		0.9776	
	Total	125	41.688			1.0000	
Beneath x 5 m	Region	1	5.133	5.1330	16.064	0.1147	0.001*
	Residual	124	39.623	0.3195		0.8853	
	Total	125	44.756			1.0000	
Lateral x 5 m	Region	1	2.275	2.2751	7.1256	0.0543	0.001*
	Residual	124	39.592	0.3192		0.9457	
	Total	125	41.868			1.0000	

Table 4.128. Pairwise comparisons of soil mite community structure by Family between decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.343	0.001*	0.001*	0.001*	0.001*	0.001*
7	0.343	-	0.003*	0.001*	0.001*	0.003*	0.001*
14	0.001*	0.003*	-	0.203	0.001*	0.001*	0.001*
21	0.001*	0.001*	0.203	-	0.001*	0.001*	0.001*
40	0.001*	0.001*	0.001*	0.001*	-	0.047*	0.005*
90	0.001*	0.003*	0.001*	0.001*	0.005*	-	0.349
180	0.001*	0.001*	0.001*	0.001*	0.001*	0.349	-

Table 4.129. Pairwise comparisons between Treatments on soil mite community structure by Family in summer 2014 at Snook, Texas after Bonferroni's correction.

Treatment			df	SS	MS	F model	R2	P value
Control	x	Treatment	1	0.504	0.5038	1.4488	0.0115	0.216
Post-7		Residual	124	43.124	0.3477		0.9885	
		Total	125	43.628			1.0000	
Control	x	Treatment	1	0.553	0.5533	1.6023	0.0128	0.115
Post-14		Residual	124	42.824	0.3453		0.9872	
		Total	125	43.377			1.0000	
Post-7	x	Treatment	1	0.884	0.8839	2.5757	0.0204	0.02*
Post-14		Residual	124	42.557	0.3432		0.9796	
		Total	125	43.441			1.0000	

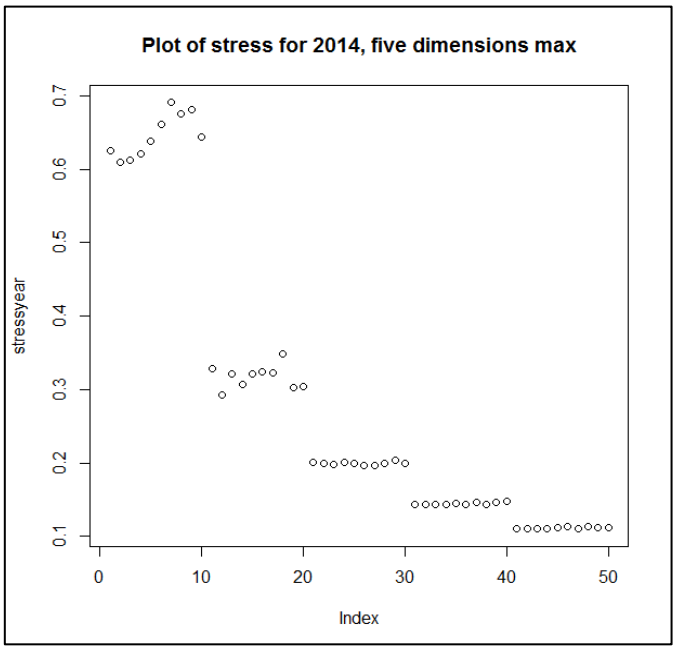

Figure 4.149. NMDS plot of stress for soil mite community structure (by Family) in summer 2014 at Snook, Texas (Stress test 0.1934; $r^2 = 0.7281$).

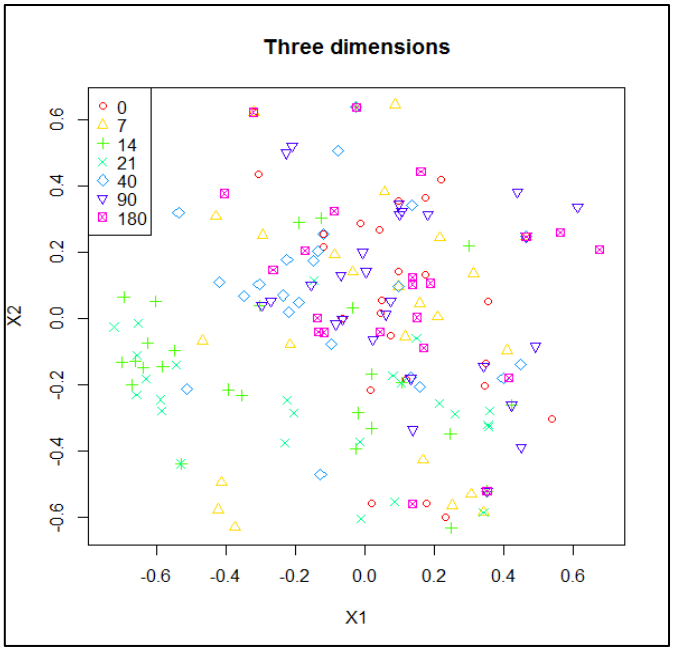

Figure 4.150. NMDS ordinations for soil mite community structure (by Family) according to days of carrion decomposition in summer 2014 at Snook, Texas.

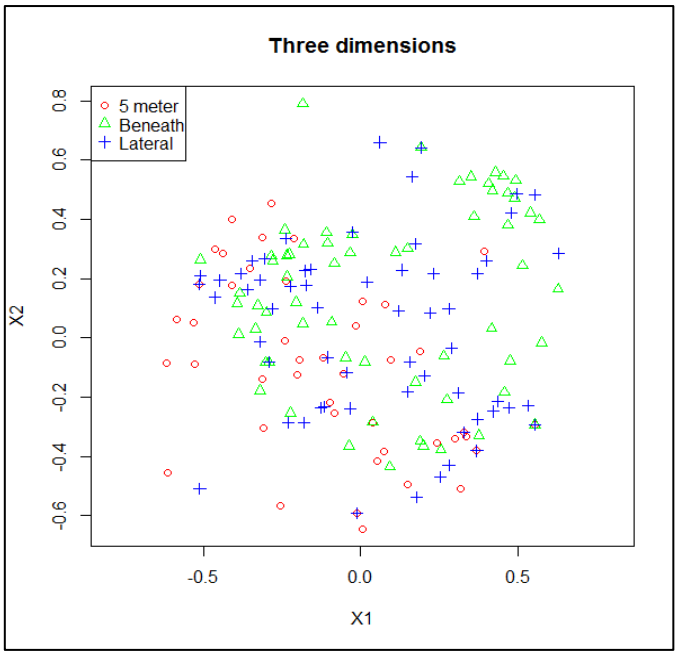

Figure 4.151. NMDS ordinations for soil mite community structure (by Family) according to soil regions in summer 2014 at Snook, Texas.

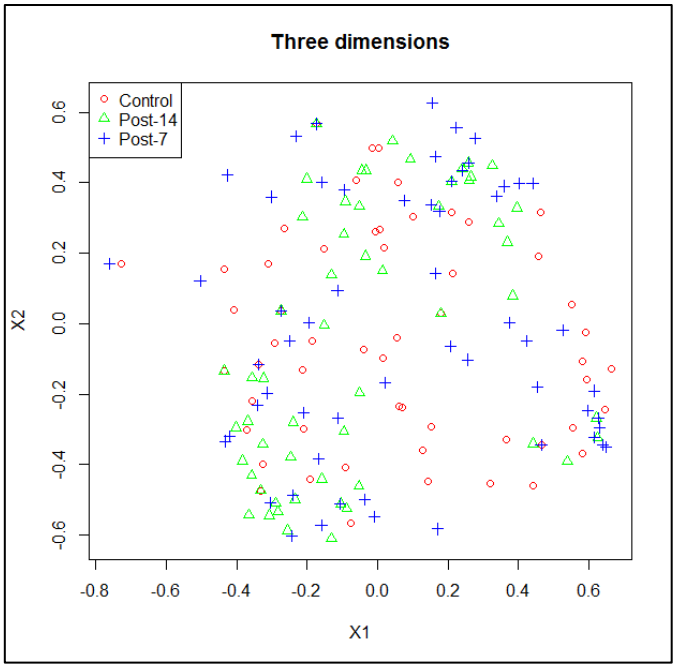

Figure 4.152. NMDS ordinations for soil mite community structure (by Family) according to treatments in summer 2014 at Snook, Texas.

The ISA results showed four families of soil mites namely Ascidae, Cunaxidae, Acaridae and Erythraeoidae were the indicators for 2014 trial (Table 4.130).

Table 4.130. Indicator species analysis by Family for soil mite community in summer 2014 at Snook, Texas.

Type	Family	Indicator value	P value
All soils	Ascidae	0.0884	0.044*
	Cunaxidae	0.2000	0.011*
	Acaridae	0.0870	0.039*
	Erythraeidae	0.6667	0.010*

Abundance of soil mite community structure (by Family) according to soil regions in 2014

Soil beneath

There was no significant difference in soil mite abundance between treatments at soil beneath ($p = 0.326$). However, there is Day effect ($p = 0.001$). In general, Ascidae and Parasitidae were observed on Day 0, Macrochelidae was then became dominant from Day 7 to Day 21, and succeeded by Acaridae from Day 40 to Day 180. It is important to note that on Day 0, several individuals of Acaridae (*Sancassania* sp.) were recovered in 2014 trial. This phenomenon suggests two possibilities that the acarid mites were present naturally in the soil or they were the remaining population of acarid mites from the previous trial. Six acari families namely Uropodidae, Ascidae, Acaridae, Laelapidae, Macrochelidae, and Parasitidae were highlighted herein to demonstrate population dynamics in response to treatments over decomposition days at soil beneath the carrion. Only a marginal significant difference was detected in the family Acaridae on Day 40 ($p = 0.0569$) and in the family of Macrochelidae on Day 40 ($p = 0.0527$). There was no significant difference determined ($p > 0.05$) for the abundance of other mite families over time. In general, abundance of Uropodidae decreased during the active decomposition stages, especially on Day 14, and then increased gradually after Day 21 onwards (Figure 4.153). The abundance of Ascidae decreased during the initial phase of decomposition, and then increased during the late phase of carrion decomposition. The abundance of Acaridae was following the sigmoidal curve, with the peaks on Day 40 for Post-7 and Post-14 groups. The free-living predators, Laelapidae, increased in abundance at the late phase of decomposition. The abundance of Macrochelidae was following the normal bell shape, with the peak on Day 14. Another group of free-living predator, Parasitidae, increased their abundance at the early phase of decomposition, and then decreased over time.

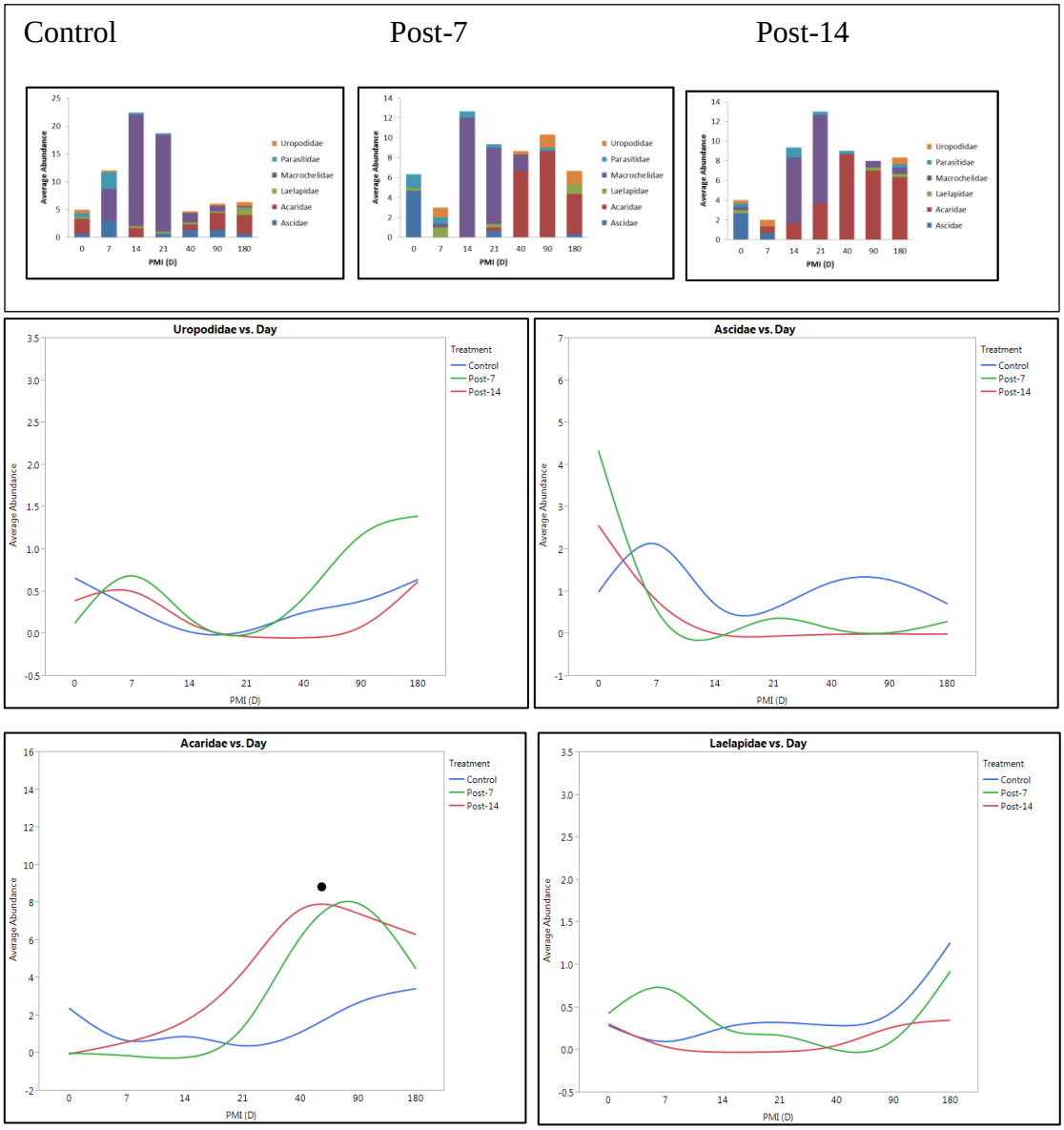

Figure 4.153. Above. Soil mite community abundance (by Family) beneath the carrion according to Treatments in summer 2014 at Snook, Texas. Upper Left. Abundance of Uropodidae at soil beneath the carrion across treatments over time. Upper Right. Abundance of Ascidae at soil beneath the carrion across treatments over time. Middle Left. Abundance of Acaridae at soil beneath the carrion across treatments over time. Middle Right. Abundance of Laelapidae at soil beneath the carrion across treatments over time. Bottom Left. Abundance of Macrochelidae at soil beneath the carrion across treatments over time. Bottom Right. Abundance of Parasitidae at soil beneath the carrion across Treatments over time (• indicates marginally significant difference).

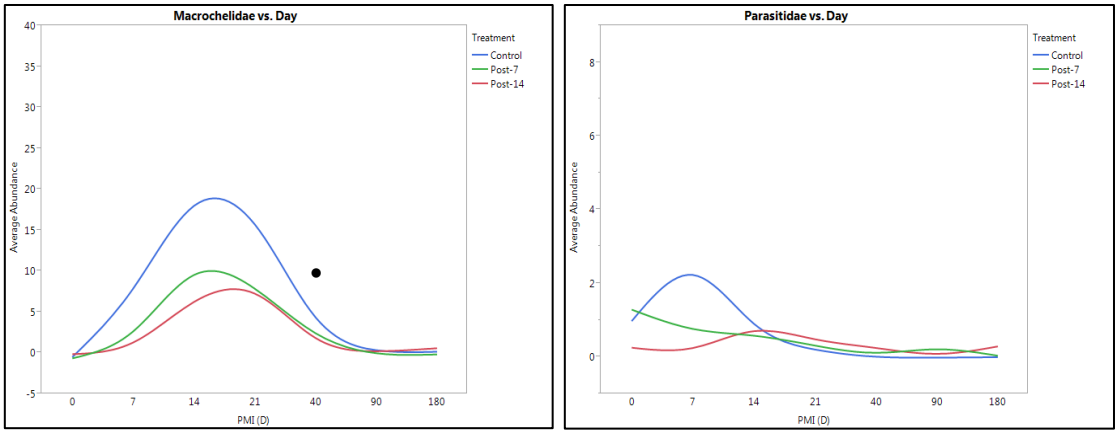

Figure 4.153 (Continued).

Soil lateral

There was no significant difference in soil mite abundance between treatments at soil lateral ($p = 0.341$). However, there was Day effect ($p = 0.0001$) among the soil mite abundance. In general, Acaridae was the most abundance mites during the late stages of decomposition. For the Control pigs, Ascidae and Macrochelidae were the other two major families that occurred at the soil lateral on Day 14. For Post-7 and Post-14 carcasses, Laelapidae mites were also collected from the soil from Day 21 to Day 180 (Figure 4.154).

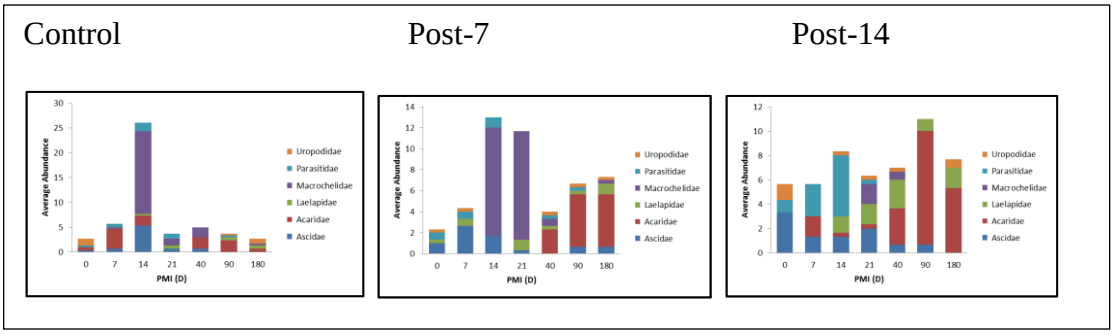

Figure 4.154. Soil mite community (by Family) abundance beside the carrion according to Treatments in summer 2014 at Snook, Texas.

Soil 5 m

There was a significant difference in soil mite abundance between treatments at soil 5 m ($p = 0.004$) as well as significant difference in Day ($p = 0.021$). For treatments, PERMAVONA showed Control x Post-7 was significantly different ($p = 0.003$) as well as Post-7 x Post-14 ($p = 0.001$). In general, Acaridae was quite abundant on Day 40 for Control and Post-14 groups and on Day 21 for Post-7 group. It is noteworthy to mention that acarid mites can disperse to the soil 5 m away from the carrion. In addition to that, acarid mites were recovered in the soil even on Day 0, suggesting the possibility of "contamination" from the remaining population from 2013 trial. In fact, soils at 5 m away from carrion were dominated by Oribatida mites. However, the families of Oribatida were not identified and hence were not included and presented in the figures below. It is obvious that the predatory mites (e.g., Ascidae, Macrochelidae, Parasitidae and Laelapidae) were present throughout the decomposition stages and were recovered up to Day 180 (Figure 4.155).

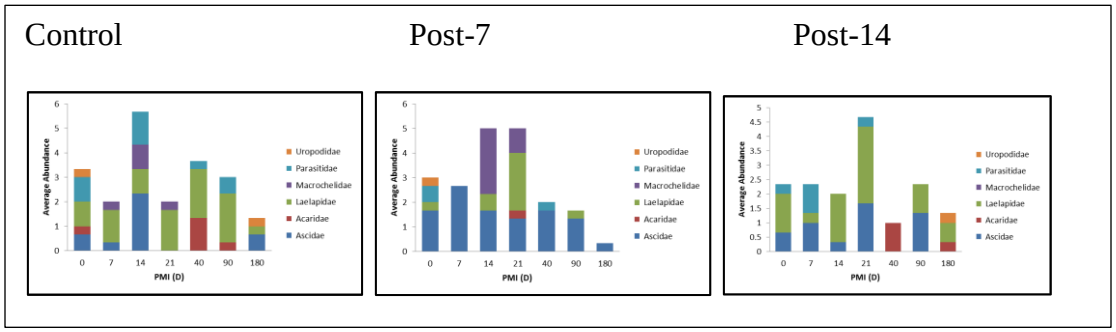

Figure 4.155. Soil mite community (by Family) abundance at soil 5 m away from the carrion according to Treatments in summer 2014 at Snook, Texas.

Abundance

The full model showed a significant difference in Day ($p < 0.0001$), Region ($p < 0.0001$), and interactions between Day x Treatment ($p = 0.0038$), Day x Region ($p = 0.0276$). Treatment had no significant difference ($p = 0.4536$). There was significant

difference (divergence) detected at soil beneath on Day 7 (Control x Post-7, p = 0.0282 and Control x Post-14, p = 0.0179) and at soil 5 m on Day 21 (Control x Post-7, p = 0.0237 and Control x Post-14, p = 0.0156). Convergence was then followed on Day 14 and Day 40 for soil beneath and soil 5 m, respectively (Figure 4.156). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable abundance over time (Table 4.131).

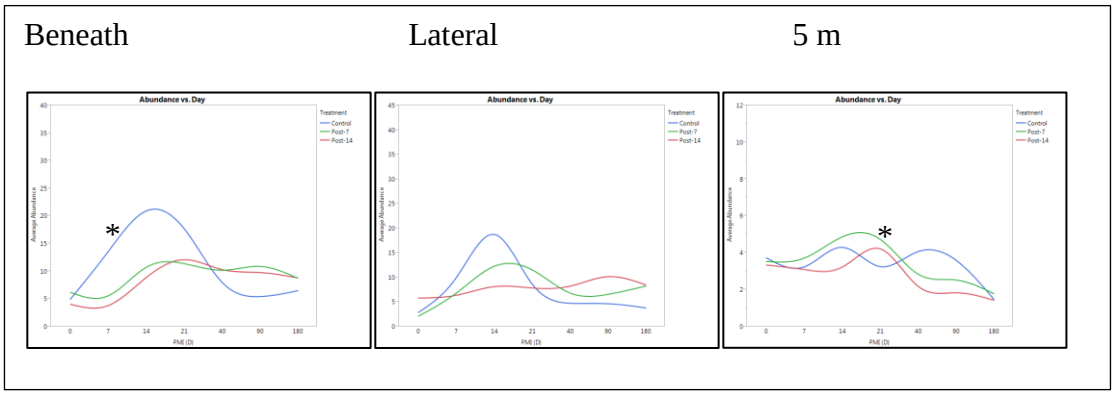

Figure 4.156. Soil mite family abundance across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* represents significant difference).

Table 4.131. Resilience for soil mite community abundance (by Family) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0838	Resistance
Post-7	None	0.1654	Resistance
Post-14	None	0.0323*	Resistance[#]

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Richness

The full model showed a significant difference in Region ($p = 0.0018$) while Day and Treatment were not significantly different ($p > 0.05$). No significant interaction was detected as well. There were significant differences (divergences) in richness on Day 40 at soil beneath (Control x Post-14, $p = 0.0297$), at soil lateral on Day 7 (Control x Post-7, $p = 0.0405$) and at soil 5 m on Day 21 (Control x Post-7, $p = 0.0128$ and Control x Post-14, $p = 0.0128$). All divergences were followed by convergences where there was no significant difference between treatments ($p > 0.05$). In general, mite community family richness at soil beneath was lower in Post-14 compared to Post-7 and Control groups throughout the decomposition process. In contrast, Acari family richness was higher in Post-14 group at soil lateral. Perhaps this observation suggest a lateral movement of soil arthropods to the side of the carrion to avoid the toxicity of the highly concentrated nutrient island at the soil beneath, considering the treatment Post-14 (i.e., delayed blow fly colonization for 14 days) allowed the soil arthropods to move away from the soil beneath. Another hypothesis would be the soil arthropods were attracted to the carrion resource, but were congregated at the soil lateral before entering the soil beneath, probably they were getting ready to penetrate the soil beneath the carrion when the environment becomes less toxic (Figure 4.157). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable richness over time (Table 4.132).

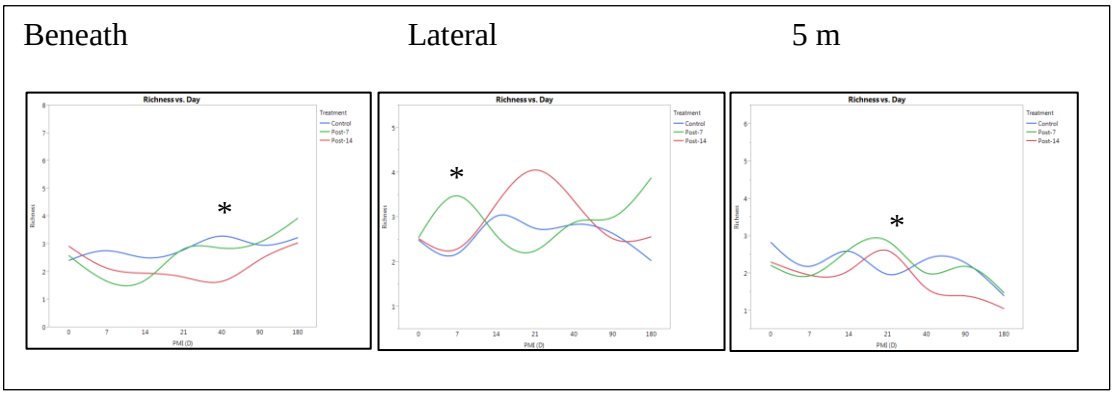

Figure 4.157. Soil mite richness (by Family) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* represents significant difference).

Table 4.132. Resilience for soil mite community richness (by Family) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.5285	Resistance
Post-7	None	0.4558	Resistance
Post-14	None	0.4999	Resistance

Simpson's diversity index

The full model showed a significant difference in Region ($p = 0.0365$) and an interaction between Day x Region ($p = 0.0003$). Treatment and Day were not significant difference ($p > 0.05$). Significant differences (divergences) were detected between treatments at soil beneath on Day 40 (Control x Post-14, $p = 0.025$ and Post-7 x Post-14, $p = 0.0339$), at soil lateral on Day 7 (Control x Post-14, $p = 0.0033$ and Post-7 x Post-14, $p = 0.0390$) and at soil 5 m on Day 21 (Control x Post-14, $p = 0.0305$ and Control x Post-7, $p = 0.0480$). All divergences were followed by convergences where there was no significant difference between treatments ($p > 0.05$). Note that at soil beneath, diversity was lower for Post-14 from Day 21 to Day 40, and the diversity increased from Day 90 to Day 180. In contrast, Post-14 had the highest diversity at soil lateral on Day 14 and

21. Perhaps this suggests the aggregation or lateral movement of soil arthropods to the soil beside the carcasses to avoid the center of nutrient toxicity (i.e., the soil beneath) (Figure 4.158). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable diversity over time (Table 4.133).

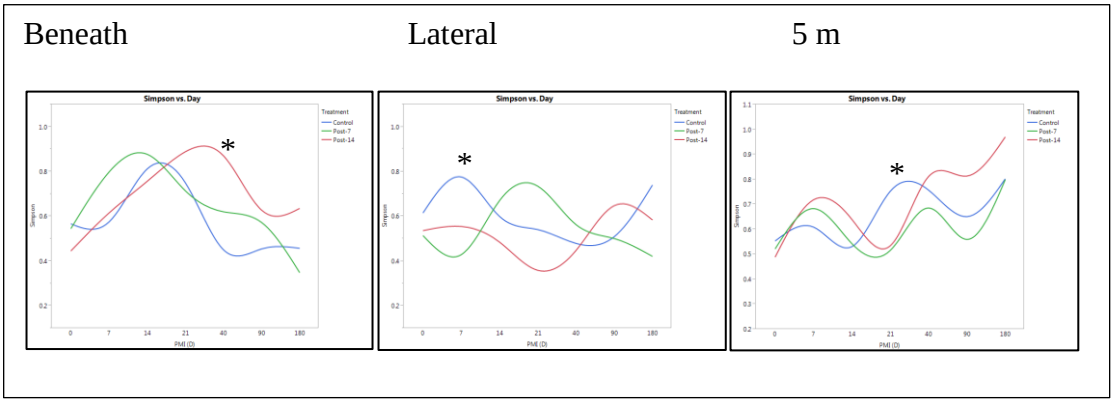

Figure 4.158. Simpson's diversity index of soil mites (by Family) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference).

Table 4.133. Resilience for soil mite Simpson's diversity (by Family) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0200*	Resistance[#]
Post-7	None	0.2132	Resistance
Post-14	None	0.0724	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Shannon-Wiener's diversity index

The full model showed a significant difference in Region ($p = 0.0151$) and an interaction between Day x Region ($p = 0.0013$). Treatment and Day were not significantly different ($p > 0.05$). There was statistical differences (divergence) of Shannon-Wiener's diversity index found between Treatments at soil beneath on Day 40 (Control x Post-14, $p = 0.0054$), at soil lateral on Day 7 (Control x Post-7, $p = 0.0050$ and Post-7 x Post-14, $p = 0.0491$) and at soil 5 m on Day 21 (Control x Post-14, $p = 0.0203$ and Control x Post-7, $p = 0.0272$). All divergences were followed by convergences where there was no significant difference between treatments ($p > 0.05$). Similarly, soil beneath showed decreased in soil mite diversity during active decomposition process and increased on Day 40 and onwards. Note that at soil lateral, Post-14 carcasses had the highest soil mite diversity (Figure 4.159). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable diversity over time (Table 4.134).

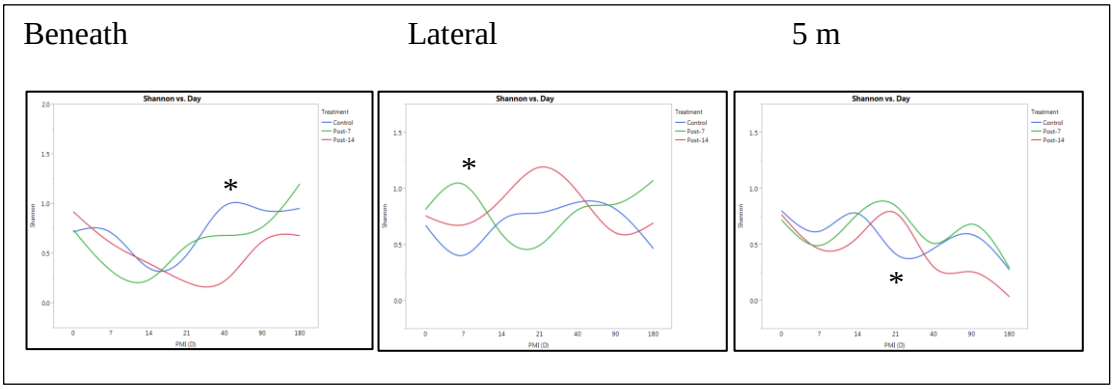

Figure 4.159. Shannon-Wiener's diversity index of soil mites (by Family) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference).

Table 4.134. Resilience for soil mite Shannon-Wiener's diversity (by Family) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0489*	Resistance[#]
Post-7	None	0.2728	Resistance
Post-14	None	0.1361	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Evenness

The full model showed a significant difference in Day ($p = 0.0233$) and no significant difference in Region ($p = 0.0990$) and Treatment ($p = 0.8687$). However, there was an interaction between Day x Region ($p = 0.0006$). There were statistical differences of evenness detected between treatments at soil beneath on Day 40 (Control x Post-7, $p = 0.0120$ and Control x Post-14, $p = 0.0019$) and at soil lateral on Day 7 (Control x Post-7, $p = 0.0098$ and Control x Post-14, $p = 0.0104$). All divergences were followed by convergences where there was no significant difference between treatments ($p > 0.05$). In general, soil mite evenness in all treatments at soil beneath decreased during active decomposition process and increased over time (Figure 4.160). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable evenness over time (Table 4.135).

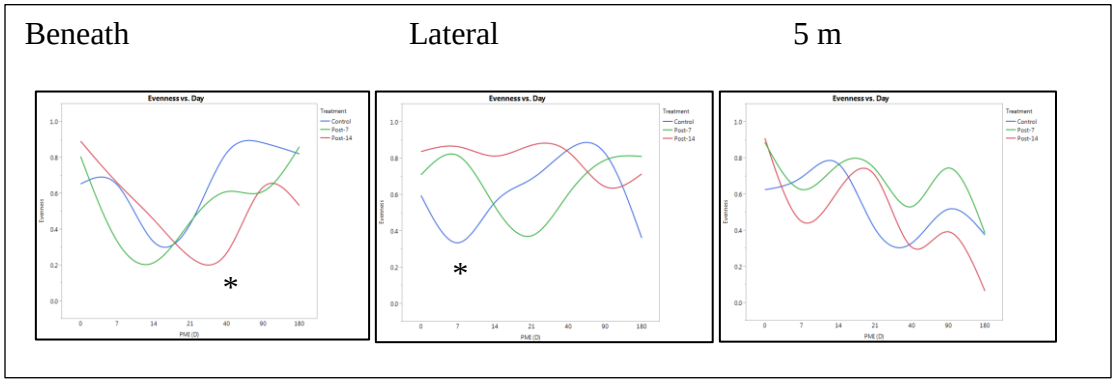

Figure 4.160. Evenness of soil mites (by Family) across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference).

Table 4.135. Resilience for soil mite community evenness (by Family) for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0168*	Resistance[#]
Post-7	None	0.1740	Resistance
Post-14	None	0.1118	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Effective number of species

The full model showed a significant difference in Region ($p = 0.0220$) and an interaction between Day x Position ($p = 0.0148$). There was no significant difference in Day and Treatment ($p > 0.05$). Significant differences were found in ENS between treatments at soil beneath on Day 40 (Control x Post-14, $p = 0.0201$), at soil lateral on Day 7 (Control x Post-7, $p = 0.0051$ and Post-7 x Post-14, $p = 0.0209$) and at soil 5 m on Day 21 (Control x Post-14, $p = 0.0411$). All divergences were followed by convergences where there was no significant difference between treatments ($p > 0.05$). Likewise, ENS at soil beneath decreased during active decomposition process and increased on and after

Day 14 (except Post-14 where it increased on Day 40) (Figure 4.161). Resilience was tested only for soil beneath for all treatments and the results demonstrated a stable ENS over time (Table 4.136).

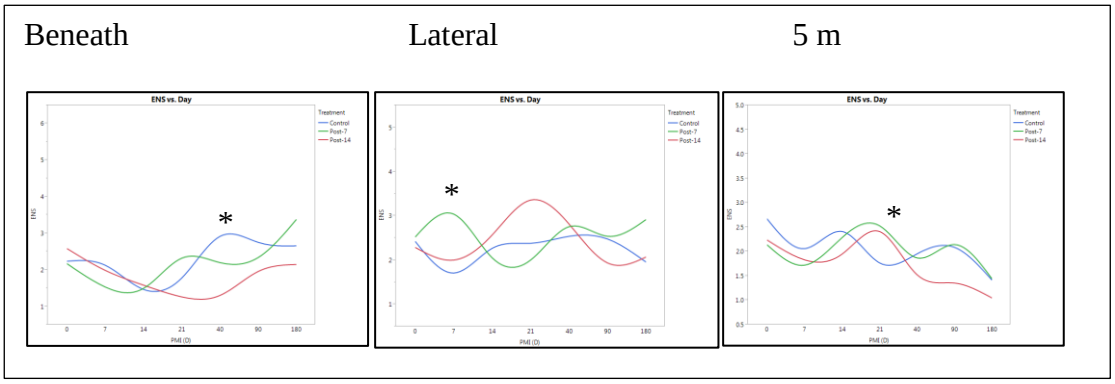

Figure 4.161. Effective number of species (by Family) of soil mites across Treatments over time at different soil regions in summer 2014 at Snook, Texas (* indicates significant difference).

Table 4.136. Resilience for soil mite community ENS (by Family) for each treatment at soil beneath the carrion the carrion in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0802	Resistance
Post-7	None	0.3905	Resistance
Post-14	None	0.1245	Resistance

Soil mite function in 2014

PERMANOVA was performed on soil mite data by function. Results showed that there was a significant Day, Treatment and Region effect ($p < 0.05$) without any significant interaction (Table 4.137). Furthermore, Replicate was not significant difference ($p = 0.624$).

Table 4.137. Analysis of the soil mite community function in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	31.1117	0.001*
Treatment	2	2.6718	0.032*
Region	2	3.7370	0.003*
Day x Treatment	2	0.8069	0.558
Day x Region	2	1.1300	0.316
Treatment x Region	4	0.9423	0.474
Day x Treatment x Region	4	0.5222	0.885

There was a significant effect in Day, Region and Replicate, further analyses were carried out. For soil regions, only soil beneath x soil 5 m were significantly ($p = 0.005$) (Table 4.138). As for days of decomposition, most of the pairwise comparisons between days of decomposition were significantly different ($p < 0.05$), except Day 0 x Day 7, Day 14 x Day 21, Day 40 x Day 180 and Day 90 x Day 180 where there were no significant difference detected ($p > 0.05$) (Table 4.139). For Treatment, Control x Post-14 and Post-7 x Post-14 were significantly different ($p < 0.05$) (Table 4.140).The NMDS plot of stress for soil arthropod community structure (Figure 4.162) and NMDS ordinations for Day, Region and Treatment were provided for visualization about data distribution (Figure 4.163, 4.164 and 4.165, respectively). Minimum stress for given dimensionality was 0.0845 with $r^2 = 0.9637$. The MRPP analysis for soil region showed a significant difference (A value = 0.0145; Significant of Delta = 0.007 based on 999 permutations) while the MRPP for day also showed a significant difference with A value 0.1678 and Significant of Delta 0.001. MRPP for Treatment showed A value 0.0050 and Significant of Delta 0.106 (not significantly different) based on 999 permutations.

Table 4.138. Pairwise comparisons between Regions on soil mite community function in summer 2014 at Snook, Texas after Bonferroni's correction.

Region		df	SS	MS	F model	R2	P value
Beneath x Lateral	Region	1	0.1433	0.1433	0.8814	0.0070	0.43
	Residual	124	20.1644	0.1626		0.9930	
	Total	125	20.3077			1.0000	
Beneath x 5 m	Region	1	0.9597	0.9597	6.0141	0.0463	0.005*
	Residual	124	19.7881	0.1598		0.9537	
	Total	125	20.7478			1.0000	
Lateral x 5 m	Region	1	0.3805	0.3804	2.6916	0.0212	0.068•
	Residual	124	17.5272	0.1413		0.9788	
	Total	125	17.9077			1.0000	

• Marginal significant difference.

Table 4.139. Pairwise comparisons of soil mite community function between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	7	14	21	40	90	180
0	-	0.589	0.001*	0.002*	0.008*	0.001*	0.001*
7	0.589	-	0.002*	0.005*	0.003*	0.001*	0.001*
14	0.001*	0.002*	-	0.571	0.001*	0.001*	0.001*
21	0.002*	0.005*	0.571	-	0.001*	0.001*	0.001*
40	0.008*	0.003*	0.001*	0.001*	-	0.035*	0.101
90	0.001*	0.001*	0.001*	0.001*	0.035*	-	0.251
180	0.001*	0.001*	0.001*	0.001*	0.101	0.251	-

Table 4.140. Pairwise comparisons between Treatments on soil mite community function in summer 2014 at Snook, Texas after Bonferroni's correction.

Treatment			df	SS	MS	F model	R2	P value
Control	x	Treatment	1	0.1363	0.1362	0.8083	0.0065	0.478
Post-7		Residual	124	20.9026	0.1685		0.9935	
		Total	125	21.0388			1.0000	
Control	x	Treatment	1	0.4332	0.4332	2.7932	0.0220	0.047*
Post-14		Residual	124	19.2325	0.1551		0.9780	
		Total	125	19.6657			1.0000	
Post-7	x	Treatment	1	0.4912	0.4911	3.4009	0.0267	0.022*
Post-14		Residual	124	17.9085	0.1444		0.9733	
		Total	125	18.3996			1.0000	

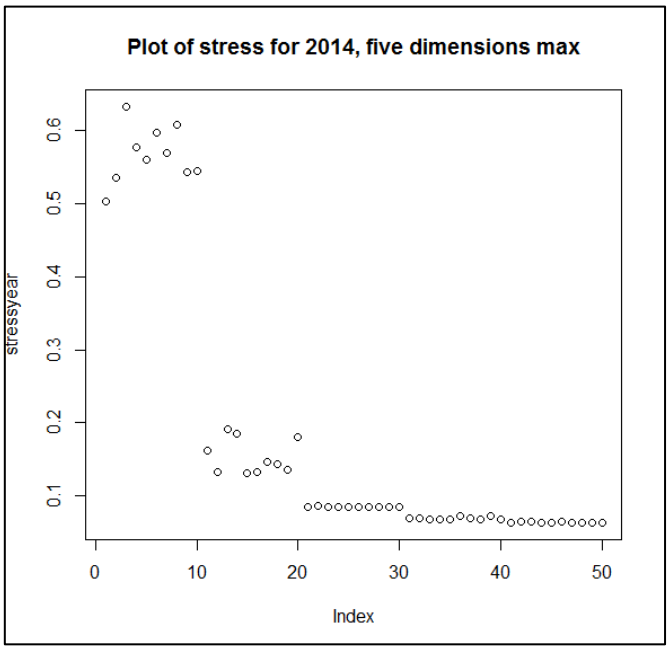

Figure 4.162. NMDS plot of stress for soil mite community function in summer 2014 at Snook, Texas (Stress test 0.0845; $r^2 = 0.9637$).

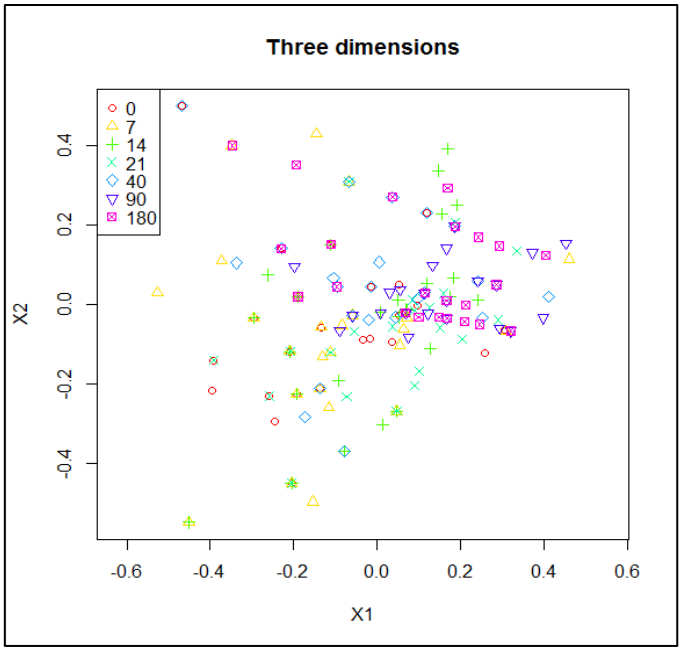

Figure 4.163. NMDS ordinations for soil mite community functions according to carrion decomposition days in summer 2014 at Snook, Texas.

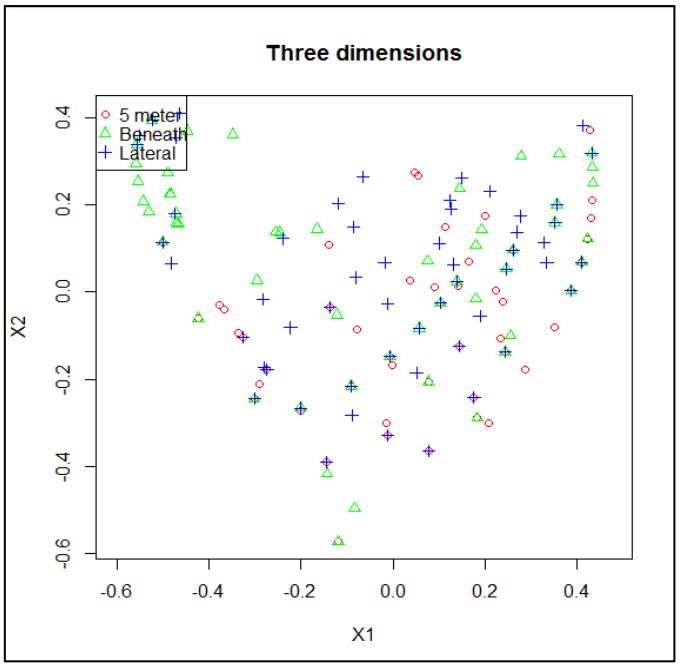

Figure 4.164. NMDS ordinations for soil mite community functions according to soil regions in summer 2014 at Snook, Texas.

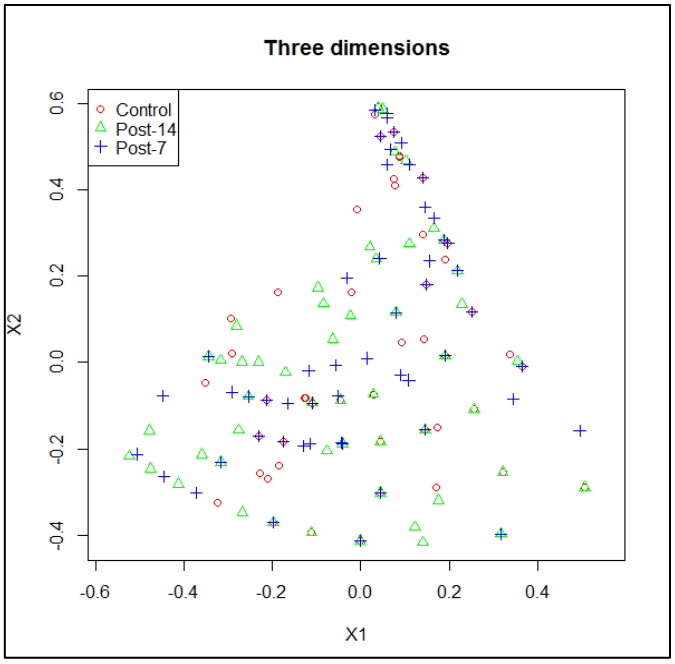

Figure 4.165. NMDS ordinations for soil mite community functions according to treatments in summer 2014 at Snook, Texas.

The ISA results showed none of the functional groups of soil mites was the indicator for 2014 trial.

Abundance of soil mite community structure (by Function) according to soil regions in 2014

Soil beneath

Soil mite community function was not significant difference between Treatments at soil beneath ($p = 0.19$). However, there was significant difference in Day ($p = 0.002$). In general, the major functional groups of mites for all treatments were the predators, and then followed by the detritivores. Predators became dominant at the early phase of decomposition and then it was succeeded by the detritivores. There were significant differences (divergences) in predator abundance between treatments at soil beneath on Day 7 (Control x Post-7, $p = 0.0029$ and Control x Post-14, $p = 0.0013$) and Day 40 (Control x Post-7, $p = 0.0202$ and Control x Post-14, $p = 0.0023$). Detritivores increased

their abundance on Day 14 or 21 and dominated the soil beneath even up to Day 180. There was a marginal significant in the abundance of detritivore on Day 0 ($p = 0.0604$). Fungivores usually increased in abundance during the late stage of carrion decomposition while herbivores decreased their abundance throughout the decomposition process (Figure 4.166).

Resilience was tested only for all functional groups of soil mites at soil beneath for all treatments. The results showed predators and fungivores were stable over time regardless of treatments (Table 4.141).

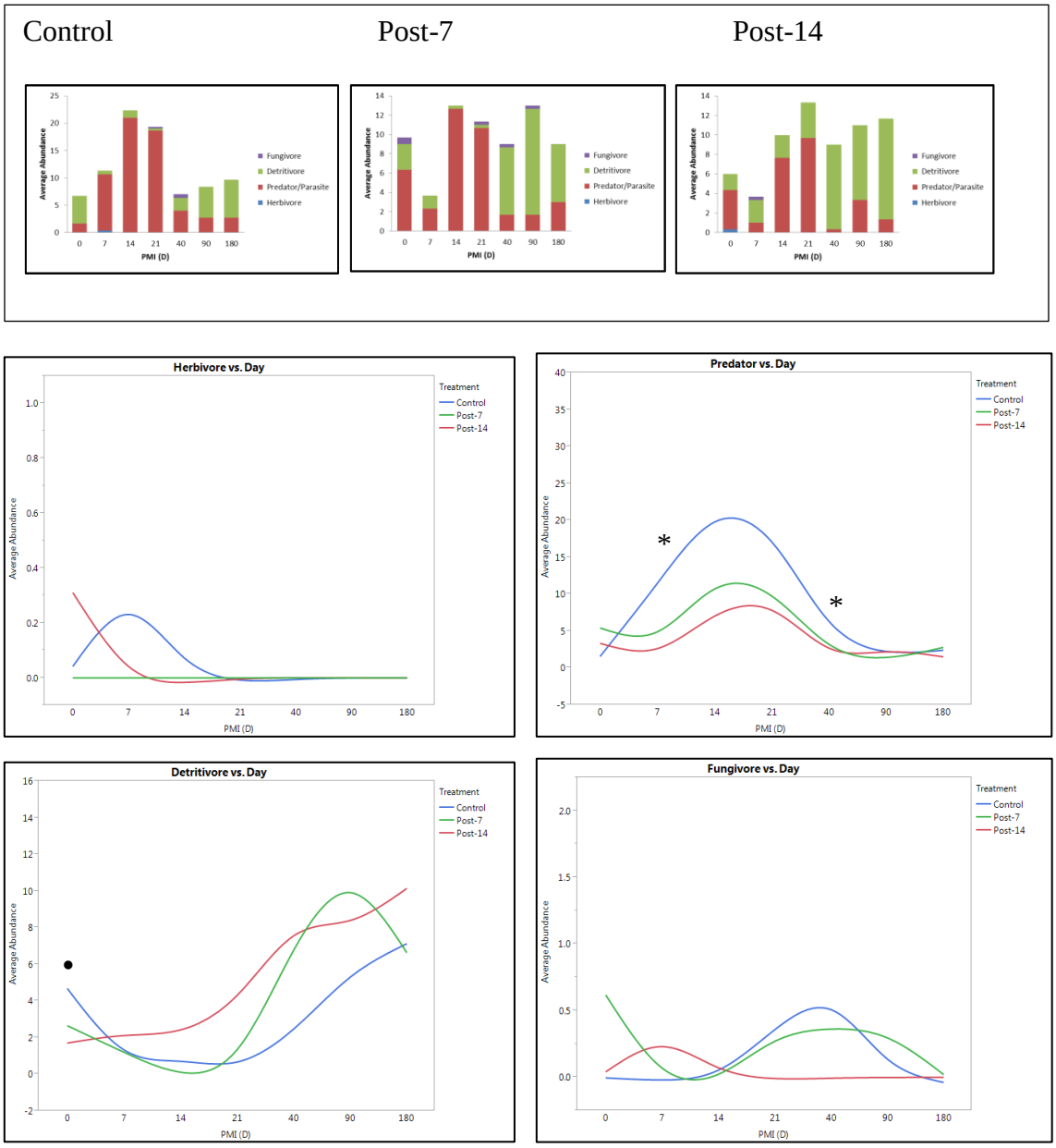

Figure 4.166. Above. Soil mite community abundance (by Function) beneath the carrion according to Treatments over decomposition days in summer 2014 at Snook, Texas. Upper Left. Abundance of herbivores at soil beneath the carrion across treatments over time. Upper Right. Abundance of predators at soil beneath the carrion across treatments over time. Lower Left. Abundance of detritivores at soil beneath the carrion across treatments over time. Lower Right. Abundance of fungivores at soil beneath the carrion across treatments over time (* represents significant difference while • denotes marginal significant difference).

Table 4.141. Resilience of soil mite community functions for each treatment at soil beneath the carrion in summer 2014 at Snook, Texas.

Function	Treatment	Significant difference	P value	Resilience on Day
Detritivore	Control	0 x 7	0.0394*	14
		0 x 21	0.0241*	40
	Post-7	0 x 90	0.0021*	180
	Post-14	None	0.0172*	Resistance
Predator	Control	None	0.0224*	Resistance
	Post-7	None	0.0053*	Resistance[#]
	Post-14	None	0.1065	Resistance
Fungivore	Control	None	0.5425	Resistance
	Post-7	None	0.7468	Resistance
	Post-14	None	0.4628	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Soil lateral

Soil mite community function was not significant difference between Treatments at soil lateral ($p = 0.221$). However, there was significant difference in Day ($p = 0.001$). Similarly, the major functional groups of mites for all treatments at soil lateral were the predators, and then followed by the detritivores. In general, predators increased its abundance over time and reached the peak on Day 14. Detritivores increased in abundance during the late stage of decomposition and dominated the soil lateral up to Day 180 (Figure 4.167).

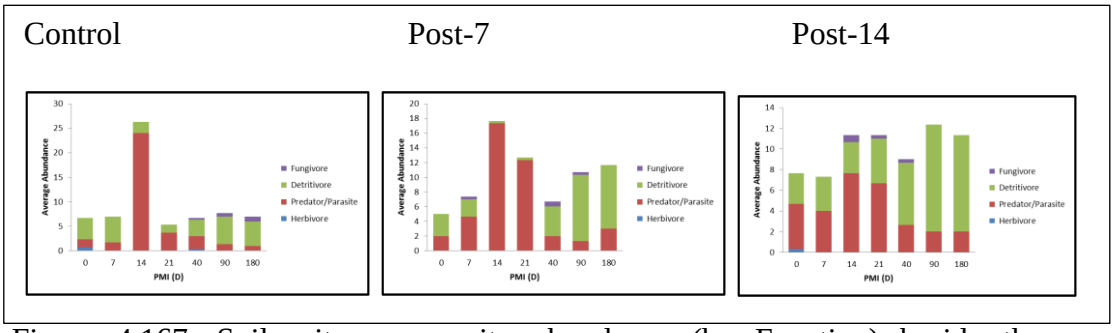

Figure 4.167. Soil mite community abundance (by Function) beside the carrion according to Treatments over carrion decomposition day in summer 2014 at Snook, Texas.

Soil 5 m

Soil mite community function was not significant difference between Treatments at soil 5 m ($p = 0.412$). However, there was significant difference in Day ($p = 0.001$). Similarly, the major functional groups of mites for all treatments at soil 5 m were the detritivores and predators. In general, predators dominated the early phase of decomposition and then succeeded by detritivores during the late stage of decomposition (Figure 4.168). It is interesting to note that detritivores such as the family Acaridae was able to dispersal or migrate to the soil 5 m away from carrion. However, it is well known that *Sancassania* sp. (Acaridae) is able to perform phoresy on varieties of insects such as beetles.

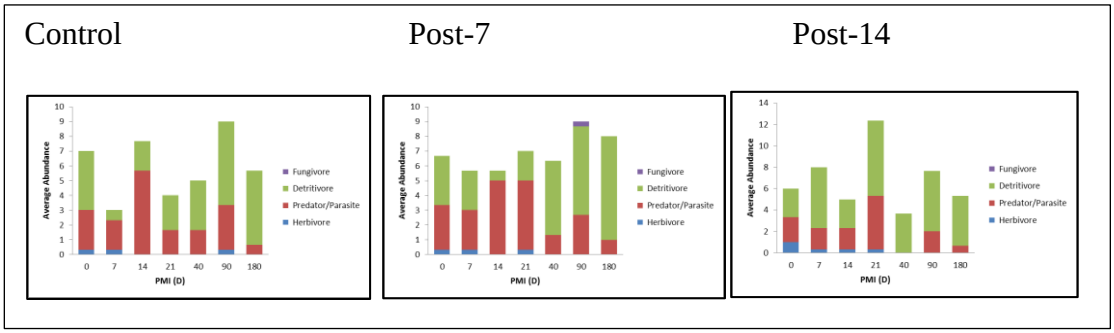

Figure 4.168. Soil mite community abundance (by Function) at soil 5 m away from the carrion according to Treatments over carrion decomposition days in summer 2014 at Snook, Texas.

Beetles association with phoretic mites in 2013 and 2014 trials

A total of six families and eight species of Coleoptera were found in association with phoretic mites in both 2013 and 2014 trials. These coleopterans families include Trogidae (one species), Dermestidae (three species), Cleridae (one species), Tenebrionidae (one species), Histeridae (one species) and Silphidae (one species). As for the phoretic mites, most of them were found underneath the elytra of the beetle species, and a few of them were found attached on the ventral surfaces or sternites of the beetle hosts (e.g., silphid beetles). A total of 10 families and 13 species of phoretic mites were identified from all beetle hosts collected in both trials. The phoretic mite families include Histiostomatidae (one species), Halolaelapidae (one species), Melicharidae (one species), Lardoglyphidae (two species), Acaridae (two species), Winterschmidtiidae (one species), Podapolipidae (one species), Ologamasidae (one species), Parasitidae (two species) and Macrochelidae (one species).

An unknown spiny Mesostigmata deutonymphs were also collected from *Omorgus suberosus* (Coleoptera: Trogidae) and *Hister* sp. (Coleoptera: Histeridae). Furthermore, *Hexanoetus* sp. (Histiostomatidae) collected underneath the elytra of *O. suberosus* could be another new species. Similarly, an unknown *Macrocheles* sp. (Natalie group) collected from *Nicrophorus marginatus* (Coleoptera: Silphidae) was morphologically distinctive from other *Macrocheles* identified from other carrion

beetles. Furthermore, there was a new mite in the Family Winterschmidtiidae (Parawinterschmidtia n.sp.) collected from *Necrobia rufipes*, and a mite species of the genus *Poecolochirus* (Family Parasitidae) collected from *N. marginatus* was a new species. And at least one of the *Sancassania* collected in the soil was possibly a new species (personal communication with Dr. O'Connor). Besides, an adult specimen of *Hydrotaea aenescens* (Diptera: Muscidae) was also collected in the field with an unknown *Macrocheles* sp. (Macrochelidae) attached on the sternite. There are possibilities that the phoretic mite species collected in this study are new to science and therefore follow-up studies on mite taxonomy should be pursued. However, note that not all mites attached on the arthropods are phoretic, some could be parasitic. In one occasion, an adult specimen of *Musca domestica* (Diptera: Muscidae) was collected along with two parasitic mites, Microtrombidiidae (Prostigmata), which attached themselves on both upper and lower squamae of the host insect.

A list of beetle hosts and their phoretic mites is provided in Table 4.142. Note that the presence of certain group mites may indicate the presence of the host insects and this information could provide information to forensic entomologists in determining the sequence of insect succession or perhaps further analyses on the determination of time of colonization (TOC). Nevertheless, the association of phoretic mites and their beetle hosts could be importance from the ecological perspectives such as resource utilization pattern, inter- and intra-competition, mutualism, dispersal, parasitism, and co-evolution between the host and the phoretic mites.

Table 4.142. List of beetle hosts and their phoretic mites recovered from the field site located at Snook, Texas in both summers 2013 and 2014.

No.	Beetle hosts		Phoretic mites
	Family	Genus and species	
1.	Trogidae	*Omorgus suberosus*	• Histiostomatidae: *Hexanoetus* sp. DN* • Halolaelapidae DN • Unknown "spiny" Mesostigmata DN*
2.	Dermestidae	*Dermestes caninus*	• Melicharidae: *Proctolaelaps* sp. • Lardoglyphidae: *Lardoglyphus anglolensis* • Lardoglyphidae: *Lardoglyphus zacheri* • Acaridae: *Tyrophagous putrescentiae* • Acaridae: *Sancassania* sp. DN
3.	Dermestidae	*Dermestes marmolatus*	• Melicharidae: *Proctolaelaps* sp. • Lardoglyphidae: *Lardoglyphus anglolensis* • Acaridae: *Sancassania* sp.
4.	Dermestidae	*Dermestes maculatus*	• Melicharidae: *Proctolaelaps* sp.
5.	Cleridae	*Necrobia rufipes*	• Winterschmidtiidae: *Parawinterschmidtia* sp. DN*
6.	Tenebrionidae	*Blapstinus* sp.	• Podapolipidae • Acaridae: *Sancassania* sp. DN

Table 4.142 (Continued).

	Beetle hosts		Phoretic mites
	Family	Genus and species	
7.	Histeridae	*Hister* sp.	• Ologamasidae: *Iphidosoma* sp. • Halolaelapidae • Unknown "spiny" Mesostigmata DN • Acaridae: *Sancassania* sp. DN
8.	Silphidae	*Necrophorus marginatus*	• Parasitidae: *Poecilochirus nr. monospinosus* • Parasitidae: *Poecilochirus "subterraneus"** • Macrochelidae: *Macrocheles* sp. (natalie group)*

DN= Deutonymphs (heteromorphic deutonymphs / hypopi)
* denotes possible a new species

Total number of Family, Genus and species identified from soil samples in both 2013 and 2014 trials

By taxonomic classification, a total of 10 families in the Order Mesostigmata, 15 families in the Suborder Prostigmata, two families in the Cohort Astigmatina, one family in the Suborder Endeostigmata and eight families of Suborder Oribatida were identified from soil samples in both trials, making a total of 36 acari families (Figure 4.169). The eight families of Oribatida were identified as Damaeidae, Lohmanniidae, Euphthiracaridae (*Rhysotritia* sp.), Cosmochthoniidae (*Cosmochthonius* sp.), Epilohmanniidae (*Epilohmannia* sp.), Hypochthoniidae (*Eohypochthonius* sp.), Galumnidae, and Oppiidae. All of them were considered as detritivores in the soil.

By functional group classification, a total of 11 families were identified as detritivores, 19 families were predators, four families as fungivores and two families as

herbivores (Figure 4.169). From the results, predators were more diverse than detritivores group.

A total of 30 species identified from 20 families of acari. Although species of acari were not subjected for community analysis and indices, the species names were provided in Appendix H.

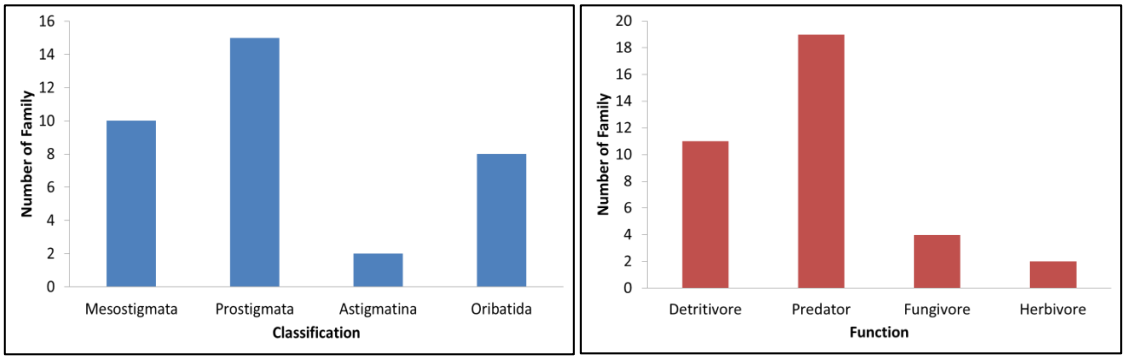

Figure 4.169. Number of acari families identified according to Order / Suborder / Cohort in both 2013 and 2014 trials at Snook, Texas (Left). Number of acari families identified according to acari functions in both 2013 and 2014 trials at Snook, Texas (Right).

Proposed hypothetical ecological framework for necrophilic acari

A hypothetical ecological framework for necrophilic acari was proposed (Figure 4.170). This framework serves as a purpose to illustrate the working mechanisms of how necrophilic acari arrive to ephemeral resources (e.g., carrion) and how do they disperse. When death occurred, the cell autolysis process started and released cell content. Microbial communities started to utilize these nutrients and started to release chemical signals in the forms of volatile organic molecules (VOCs) through quorum sensing. These molecules could serve as a means of interkingdom communication, for instance, as an attractant or repellent to certain groups of insects and arthropods. Blow flies and beetles could be attracted to these VOCs released by microbes on corpses (or necrobiome) and resulted in the colonization and consumption of the carrion. The

necrophilic acari could arrive to the body through phoresy by “hitchhiking” on the blow flies and beetles that are attracted to the bodies. At the same time, the decomposition activities caused by the microbes and insect communities started to change the physical and chemical state of the corpses and resulted in the formation of cadaver decomposition island (CDI). It is a pool of nutrient rich hotspot or highly fertilized island underneath the decomposing corpses due to the leakage of decomposition fluids, cadaveric materials and by-products into the soil. The presence of decomposition fluid could be highly toxic (nitrogen and phosphate toxicity) to the surrounding environments. As such, the formation, transformation and succession of CDI’s chemistry profiles could repel or attract certain acari communities underneath the soil of the corpses. For example, Oribatida mites, will be repel due to the overwhelming nutrients (e.g., high concentrations of ammonium) in the soil and as a consequence, the population of oribatid mites will decrease. On the other hands, the newly arrived phoretic mites (mostly predatory mites such as Macrochelidae or the detritivores such as Acaridae) from the insect hosts are most likely the mites that could be found in the soil during the active decomposition stage. During this period, the soil nutrients such as phosphates are highly concentrated and the macrochelids and acarids are probably able to tolerate or adapt in such environment.

The Astigmatina mites (e.g., Acaridae) that arrived through phoresy during the active decomposition stage will soon to reproduce, and it is known that acarid mites are detritivores and are of necrophagous. The acarid population will increase exponentially during the advanced or dry stage of carrion decomposition. However, due to the depleted carrion resource (as a consequence of the competition from both dipteran and coleopteran larvae), these acarid mite population started to metamorphosed into a dispersal stage called heteromorphic deutonymphs (also known as hypopi) characterized by the development of anal sucker plates and reduced mouthpart (it is also called calypstostases, which is the non-feeding stage). The formation of large numbers of heteromorphic deutonymphs in the soil resulted in the dispersal of mite population to other areas. These deutonymphs will attach themselves (using anal suckers in the case of

acarid mites) onto blow flies and beetles such as silphids, trogids, clerids and histerids for dispersal purposes in hoping to colonize new ephemeral resources.

Note that the body cooling phase during algor mortis will lead to the dispersal of ectoparasites that are living on human tissues such as lice and skin follicle mites, *Demodex folliculorum*. The time when ectoparasites are leaving the body has been proposed as an indicator in determining the time of death.

The mite communities associated with carrion decomposition processes are governed by several biotic factors such as inter-intra-competition, predation, parasitism, mutualism, birth rate, death rate, emigration rate and immigration rate (includes phoretic rate), as well as ecological effects such as priority effects, and non-consumption effects that could change and shape the acari successional sequence. Abiotic factors that can affect mite community dynamics include soil pH, temperature, moisture, precipitation, soil chemistry profiles, soil types, soil texture, soil porosity or even soil depth.

The goal of this ecological framework for necrophilic acari is to facilitate the holistic understanding of the systematic mechanism of mite community dynamics in the vicinity of CDIs and the role of acari during the decomposition of vertebrate carrion, and potentially, the exploration into the use of acari in forensic science applications.

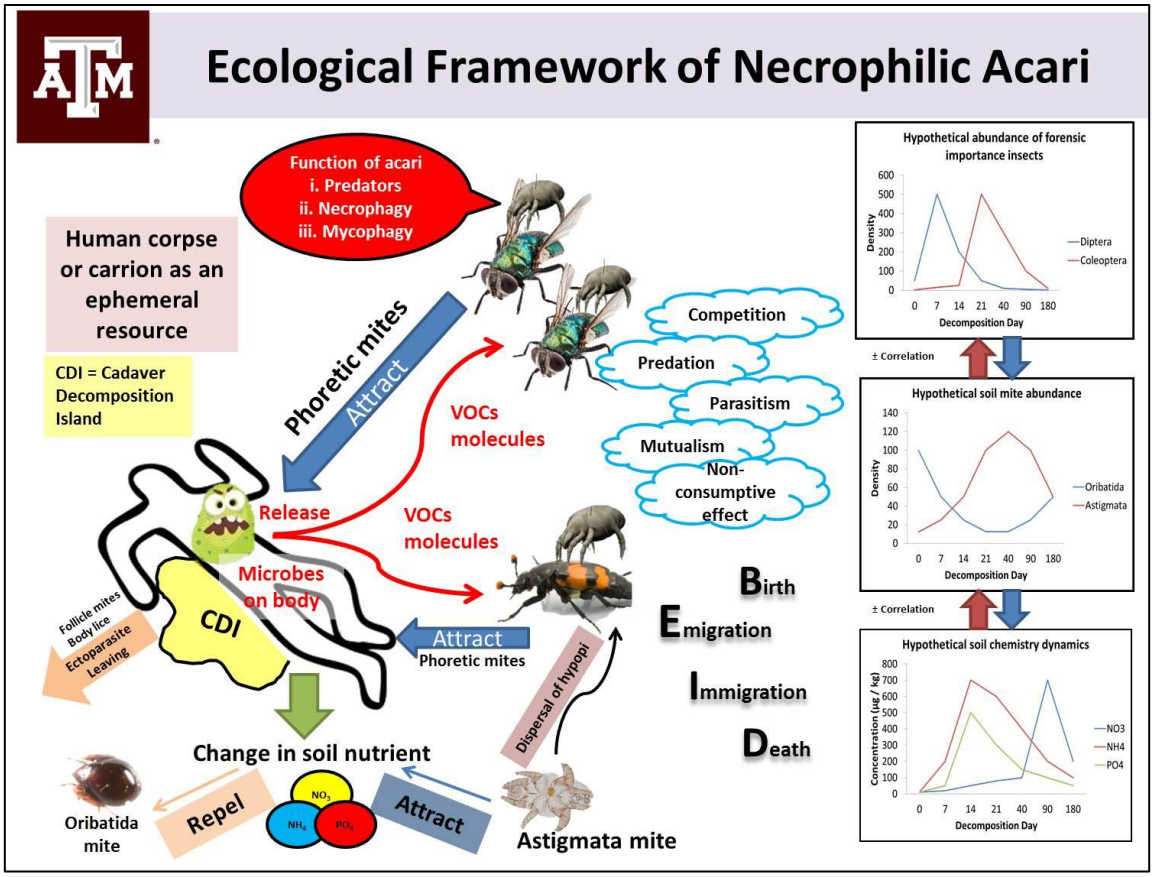

Figure 4.170. Proposed hypothetical ecological framework for necrophilic acari. Mites arrived to the corpse through phoresy by "hitchhiking" on the necrophagous flies and beetles, which are attracted to microbial volatile organic compounds released from the necrobiome. The decomposition fluid from the corpse change the soil nutrients underneath the body (the CDI) and subsequently change the community structure of soil dwelling mites, while the phoretic astigmatans thrived under such condition despite of high concentrations of soil ammonium and phosphate. However, the population abundance of necrophilic mites are substaintially depending on abiotic and biotic factors such as natality and mortality rates, emigration and immigration rates, as well as ecological relationships such as competition, predation and parasitism that may occur during the corpse decomposition process.

Comparison of soil arthropod results between 2013 and 2014 trials

Table 4.143 highlights the statistical results of soil arthropod communities between 2013 and 2014 trials at Snook, Texas (see Appendix N for ISA comparison).

Table 4.143. Significant results for soil arthropods collected in 2013 and 2014 trials at the field site located at Snook, Texas.

Factor	2013	2014
Temperature*	30.59±7.81°C	29.27±6.49°C
Precipitation	39.116 mm	171.45 mm
ADH (Base 10)*	29209.70	28080.67
Year effect		Yes
Replicate effect		No
Soil arthropods		
Soil arthropod Order	20 Orders	15 Orders
Soil arthropod Family (excluding mite)	50 Families	39 Families
Soil arthropod Genus (excluding mite)	26 Genera	14 Genera
Soil arthropod Function (excluding mite)	5 functional groups	5 functional groups
Significant results in community structure analyses:		
Order or Class	Day	Day
	Region	Region
	Day x Region	Day x Region
Family	Day	Day
	Treatment	Treatment
	Region	Region
	Day x Region	Day x Region
		Day x Treatment

Table 4.143 (Continued).

Factor	2013	2014
Genus	Day	Day
	Region	Treatment
		Region
Function	Day	Day
	Region	Region
	Day x Region	
	Day x Region x Treatment (marginal)	
Indicator species analysis (Order)	Thysanoptera	Thysanoptera
	Diptera	Diptera
	Collembola	Collembola
	Psocoptera	Psocoptera
	Megadrilacea	Diplura
		Oribatida
		Hemiptera
		Symphyla
Indicator species analysis (Family) (excluding mite)	Calliphoridae	Japygidae
	Dermestidae	Tenebrionidae
	Sarcophagidae	Muscidae
	Muscidae	Staphylinidae
	Erotylidae	Nitidulidae
	Sminthuridae	Carabidae
		Stratiomyidae
		Aphididae
		Liposcelidae
		Entomobryidae
		Ptilidae

Table 4.143 (Continued).

Factor	2013	2014
Indicator species analysis (Genus) (excluding mite)	*Ch. rufifacies* *Co. macellaria* *Hydrotaea* sp. *Omosita* sp. *Liposcelis* sp. *Dermestes* sp.	*Fannia* sp. *Baris* sp. *Hydrotaea* sp. *Leptogenys* sp. *H. illucens*
Indicator species analysis (Function) (excluding mite)	Detritivores Necrophagous	Detritivores
Soil mites		
Year effect		Yes
Replicate effect		Yes
Correlation coefficient	Oribatida (r^2= 0.3888) Non-Oribatida (r^2= 0.3888)	Oribatida (r^2= 0.4013) Non-Oribatida (r^2= 0.4066)
Soil mite Order / Suborder / Cohort	5 Orders	4 Orders
Soil mite Family	26 Families	17 Families
Soil mite Function	5 Functional groups	4 Functional groups
Significant results in community structure analyses:		
Order	Day Region	Day Treatment (marginal) Region Day x Treatment Day x Region

Table 4.143 (Continued).

Factor	2013	2014
Family	Day	Day
	Treatment	Treatment
	Region	Region
		Day x Treatment
		Day x Region
		Treatment x Region
Function	Day	Day
	Region	Treatment
	Replicate	Region
Indicator species analysis (Order)	Prostigmata	Prostigmata
	Astigmatina	Astigmatina
Indicator species analysis (Family)	Phytoseiidae	Ascidae
	Pygmephoridae	Cunaxidae
	Acaridae	Acaridae
		Erythraeidae
Indicator species analysis (Function)	Detritivores	None
	Fungivores	

* = Significant difference between 2013 and 2014 trials.

DISCUSSION

Delayed insect access to carrion shifted associated necrophagous insect community structure, turnover rates and insect assembly (Pechal et al. 2014b). When insects were allowed to colonize carrion previously excluded from insects there was an increased in necrophagous insect taxon richness and increased community turnover rates. Moreover, during the insect-exclusion period, insect-excluded carcasses remained

in bloat stage while those control carcasses (which were colonized by insects immediately) were in advanced decay stage of decomposition (Pechal et al. 2014b). Although there were some similar designs employed in the current study, there were indeed several distinctive differences between the current study and that of Pechal et al. (2014b). First, the duration of the current study was much longer (from Day 0 to Day 180), with more sampling frequency (a total of seven sampling points were conducted: Day 0, 7, 14, 21, 40, 90 and 180). Second, due to the longer period of study, the present study was able to examine the fundamental concepts of resilience, divergence and convergence in carrion ecology. Third, the current study examined the community structure and ecological indices on soil arthropods such as beetles, dipteran larvae, collembolans, ants as well as soil mites associated with carcasses with delayed-insect colonization, which had not been determined by the previous study. Fourth, the insect-exclusion period had been extended (as compared to Pechal (2012)), where carcasses had been excluded for seven days (i.e., Post-7) and 14 days (i.e., Post-14). Fifth, the current study employed more sticky traps to access community structure and abundance compared to Pechal et al. (2014b). The total of arthropod collection was much higher and much sharper in taxonomic resolution (same arthropod data had been sorted into different taxonomical ranks such as Order, Family and Genus, as well as arthropod function, and then analyze separately according to taxonomical rank). These analyses conveyed different meaning and interpretation to the carrion ecosystem, as well as highlighting the important of taxonomic resolutions and scales in carrion ecological studies (see Chapter 5). Results from this study demonstrated delayed blow fly colonization on carrion impacted soil arthropod community structure. This impact was partially due to the slower rate of biomass loss (see Figure 2.21 – 2.25) and slower rate of decomposition fluid release for both treatment groups (i.e., Post-7 and Post-14 groups) compared to the control carcasses, hence resulted in the different soil chemistry profiles (see Chapter 3) and eventually changed the soil arthropod community structure.

Year effect was significantly different for soil arthropod community structure (see Figure 4.8). This could be due to significant difference in ambient temperature as

well as ADH between summers 2013 and 2014 (see Figure 2.18 and 2.20). The soil arthropod community structure as well as associated food web shifted as a result of the abiotic environment (Whitford, 1989). Temperature has been known to affect the time of development and fecundity, as well as the appearance or dynamics of insect populations in the field (Ratte, 1984). A study demonstrated that the overall herbivory rates increase with temperature between 20 and 30°C, however, there is substantial variation in thermal responses among herbivore-plant pairs at the highest temperatures (Lemoine et al. 2014). Precipitation was much more abundant in 2014 trial compared to the previous year. This additional soil moisture could affect soil arthropod community structure as past study had demonstrated that water could affect collembolans and soil mites significantly in summer (Chikoski et al. 2006). In dry soils with water potential below -1.5 MPa, most bacteria, protozoan, and nematodes are not active (these taxa entered a state called anhydrobiosis, some fungivorous mites even entered a cryptobiosis state) (Whitford, 1989). For instance, supplemented summer rainfall resulted in an increase in vegetation cover, leading to an increase in the abundance of the insect herbivores, Auchenirrhyncha (Homoptera) (Masters et al. 1998).

Soil arthropod community structure was significantly different by days of carrion decomposition and also soil regions (see Table 4.143). Bornemissza (1957) found different stages of decomposition were correlated with the animal communities occupying them, and these communities were contrasted with the community dwelling in the leaf litter and soil. The various stages of decomposition influenced the underlying soil differently, with the greatest effect being observed during the “black putrefaction” and “butyric fermentation” stages (Bornemissza, 1957). The current study also determined similar results where Day 7 (in between active and advanced decomposition stage) of Control carcasses were significantly different in terms of soil arthropod community structures compared to the Post-7 and Post-14 carcasses. As for soil regions, the current study was in agreement with Bornemissza (1957) where soil arthropod community beneath the carrion were significantly different between soil beneath and soil lateral (soil at the distant of ~30 cm away from beneath the carrion), and between soil

beneath and soil 5 m away from carrion. Bornemissza (1957) defined soil beneath the carrion as "carrion zone" which was differed greatly from that of the control area. The soil fauna of the "intermediate zone", which defined as "the belt surrounding the carrion 10 cm wide", also showed substantial differences. Following these observations, I proposed that CDIs could be divided into several zones (i.e., beneath the carrion, or the side of carrion), due to the facts that each specific zone is inhabited by different soil arthropod community structure and function. The change in the soil arthropod community structure according to soil regions may be due to the different concentration of soil nutrients derived from the decomposition process, similar to the resource concentration hypothesis (Root, 1973) where the decomposers (i.e., microorganisms such as bacteria and fungi, mites, earthworms, collembolans) are attracted to resources and to recycle the organic matter or to perform carbon mineralization as part of their normal functions in the ecosystem (Brussaard, 1997). From a nutrient flow viewpoint, soil arthropods are envisioned as accelerating (or delaying) nutrient release from decomposing organic matter. They may do it directly, by feeding upon organic matter and associated microfloral, or indirectly, by channeling and mixing of the soil, improving the quality of substrate for microfloral and other consumers (Crossley, 1977). In another example, tillage system (may serve as a disturbance in this case) is known to affect soil physical and chemical environment, and thereby affecting soil organisms (Kladivko, 2001). No-tillage soils are usually physically and chemically stratified with more nutrients localized near the surface. However, plowed soils often demonstrated increased organic matter decomposition and nutrient mineralization (Hendrix et al. 1986).

Interpretation of soil arthropod community responses to delayed colonization of swine carrion was dependent on taxonomic resolution implemented. Based on the results obtained, Family level, unlike Order or Genus, assessment of the soil arthropod communities demonstrated more consistent results across parameters examined (see Table 4.141). Bowman & Bailey (1997) recorded similar phenomena with freshwater macro-invertebrate communities. They determined genus-level identification did not

provide a striking different description of community structure than higher levels (e.g., family, order) of taxonomic identification. Similarly, Olsgard et al. (1997) examined the relationship between taxonomic resolution in analyses of a microbenthic community along an established pollution gradient and they found that higher taxonomic levels are more likely to reflect a contamination gradient than are analyses based on species abundances. Another study conducted in Mid-Atlantic Highlands in U.S. on the effects of macroinvertebrate taxonomic resolution in large landscape bioassessment showed that the identification to the family level is sufficient for many bioassessment purposes (Waite et al. 2004).

Interpretation of results was dependent on the ecological indices employed. As previously mentioned, richness, Simpson's diversity, Shannon-Wiener's index, evenness and effective number of species (ENS) were studied. All indices (richness, diversity, evenness, ENS) decreased over time regardless of treatments, especially with carrion introduction and the ensuing decomposition process. Furthermore, all indices increased again (a sign of ecosystem recovery, or resilience) when the decomposition process was completed (for example, see Figure 4.145 – 4.148).

Based on the indices measured in this experiment, in general, carrion experiencing delayed colonization had less of an impact on soil arthropod communities (for example, see Figure 4.109 – 4.113). Barbercheck et al. (2009) determined similar results in their study. They examined soil mesofauna in disturbed ecosystems in North Carolina and found richness, evenness, abundance of mites and proportions of collembolans was dependent on the level of disturbance experienced. For example, mites, entomobryid and sminthurid collembolans, dipteran, and coleopteran larvae, and enchytraeids were associated with agricultural sites whereas onychiurid and isotomid collembolans, coleopteran and dipteran adults, and spiders were associated with undisturbed wetlands and high concentrations of soil organic matter. Hypogastrurid collembolans, diplurans, proturans, and symphylans were most closely associated with forest soils while ants and neanurine collembolans were associated with undisturbed wooded sites (Barbercheck et al. 2009). With the delay of insect colonization on carrion,

which subsequently slowed down the decomposition process will lead to the change of nutrient transformation and availability in the CDIs (Parmenter & MacMahon, 2009). Thus, reducing the quantity or rate of nutrient reintroduction into the environment may or may not impact soil arthropod communities that respond to nutrient pulses.

Delayed arthropod colonization of vertebrate carrion impacted soil communities in two ways. Firstly, a priority effect potentially was imposed on the carrion by the epinecrotic microbes from the external environment or from the microbiome of the carcass itself, which could ultimately play a significant role in changing the attractiveness of the carrion to arthropods through the release of microbial volatile organic compounds (Davis et al. 2013; Tomberlin et al. 2012; Janzen, 1977). Secondly, priority effect imposed to the carrion by the soil arthropod community in the soil, who dominated at the soil beneath the carrion, and consuming the side of carrion in contact with the soil surface. However, consumption rate by soil arthropods (e.g., collembolans, beetle larvae, ants or mites) might be slow and insignificant if compared to the consumption rate of fly larvae, and the soil arthropods abundance could be affected by the flow of decomposition fluid directly into the soil beneath, which could be highly toxic to certain group of soil detritivores (e.g., soil oribatid mites). Furthermore, there could be anaerobic condition at the soil beneath the carrion, which could deter the development of soil arthropods and soil microbes. However, with the absence of competition from Diptera larvae, it is hypothesized that certain soil arthropods that can adapt to such high levels of disturbance will flourish and dominate in the carrion environment, and subsequently reduce richness and diversity of soil fauna (Coleman et al. 1993).

Hawlena et al. (2012) demonstrated that grasshoppers stressed by spider predators have a higher body C:N ratio than other grasshoppers raised without spiders. This change in element content does not slow grasshopper decomposition but disturb belowground community function, decelerating the subsequent decomposition of plant litter. This legacy effect of predation on soil community function appears to be regulated by the amount of herbivore protein entering the soil. Similarly, the absent of

necrophagous dipteran community at the initial phase of decomposition in this study changes the cadaveric resource quality, which subsequently change the belowground community structure and function (as indicated by the change in ecological indices), and eventually change the soil nutrient dynamics (as highlighted in Chapter 3).

In general, soil arthropods are sensitive to days of decomposition and regions of soil (see Table 4.143), where Bornemissza (1957) noticed similar observations in their carrion decomposition studies. Note that "sensitivity" here is defined as how sensitive were the soil arthropods to the factors we measured (e.g., day, treatment, soil region etc.). However, the present study found that the soil arthropod sensitivity to treatment effect is an issue of taxonomic resolution. Soil arthropods were sensitive to treatment effects when the family (and genus level in 2014 trial) of taxonomic resolution was used in the community structure analyses, indicating changes in the family members within Orders were "sensitive" enough to the effects by delaying dipteran colonization on carrion. As previously mentioned, delayed Diptera colonization which lead to a slower rate of decomposition resulted in a change of carrion quality (i.e., the intactness of the carrion structure). The decomposition fluid that was transferred vertically into the soil changes the soil nutrient dynamics *in-situ* due to the absence of dipteran consumers and possibly due to the booming communities of soil microbes metabolizing the cadaveric nutrients. Under such situation, the change in nutrient availability eventually affects soil arthropods in terms of biomass, high-dominance of a few rapid-growing species, and reduction in species richness (Hägvar & Klanderud, 2009).

In general, soil arthropod communities exhibited divergence and convergence as related to succession trajectories experienced in relations to what was observed for those associated with the control (for example, see Figure 4.157). However, all divergences that occurred were eventually converged within the 180 days of study. Note that before divergence happened, there were dynamic equilibriums or stable states in all treatments, until a significant change was detected, hence, a divergence. Divergence is defined here as a significant difference in community abundance or indices across treatments at a particular time point (*sensu* Houseman et al. 2008), while community convergence

means there was recovery from community divergence where the abundance or indices were no longer significant difference across treatments at a time point after divergence occurred (*sensu* Houseman et al. 2008). Houseman et al. (2008) emphasized that perturbations can have differing effects on community dispersion, yielding either convergence or divergence. Based on the results, divergence of soil arthropod community at soil beneath could occur twice throughout the duration of study (for example, see Figure 4.72) or converged right after divergence (see Figure 4.73). Overall, the soil ecosystem is considered healthy by owning capability to maintain its structure (organization) and function (vigor) over time in the face of perturbation (Costanza & Mageau, 1999). Furthermore, soil arthropod communities exhibited different degrees of resilience dependent on the treatment experienced. Table 4.12 showed resilience of soil arthropod community beneath the Control and Post-14 carrion, while Table 4.13 demonstrated no resilience among soil arthropod community even on Day 180 on Post-7 carrion. Note that resilience is defined as the speed of recovery in which a system returns to equilibrium state following a perturbation (DeAngelis, 1980).

While this study was highly informative, weaknesses were determined. Identification of soil arthropods and mites was limited. As previously mentioned, identification to family level was most informative; however, with greater identification at the genus or species level, explanation of the impact of delayed arthropod colonization could have been enhanced. Furthermore, time was another limiting factor. Although the current study expand the observation duration up to 180 days, a longer period of observation time, for example, 365 days, could give a better picture about carrion decomposition and the impacts to soil arthropod communities as annual shifts in abiotic factors (e.g., temperature and precipitation) could have been considered. Also, the sample size in this study was three (n = 3), which reduce statistical power (Button et al. 2013). It would be ideal to increase the sample size to increase the power and confidence interval. However, considerations such as the study objectives, cost, time, and manpower should be taken into account before deciding on the desired sample size for statistical analyses. In the future, similar studies could be carried out on different types of soil,

different type of habitat or landscape, different ecoregions or climatic regions. More ecological indices (e.g., beta diversity) or network analyses can be investigated to better understand the ecological process between control and treatment carcasses. Furthermore, the current study was more directed to community ecology, it is recommended in the future that ecosystem ecology should be incorporated in the study design as it is imperative to know whether two ecosystems interact with each other, for example, the interactions of the aboveground arthropod community with the belowground arthropod community during the carrion decomposition process.

Note that the hypopi of acarid mites were recovered in the soil samples collected on the first day of field experiment in 2014 trial (see Figure 4.118), indicating certain degree of contamination at the study site by the remaining mite populations from the previous trial. It is well known that the soil mites, *Sancassania berlesi* (Michael) are general detritivores and are thought to live on local patches of organic matter and may persist for a few generations until the resource depleted entirely (Benton & Bowler, 2012). The early presence of acarid hypopi could change the soil arthropod community entirely due to the possible priority effect, as highlighted in Hanski & Kuusela (1977) who studied competition in carrion fly community and concluded that priority effect may decrease species diversity.

Based on the results generated in this study, we believe that the potential applications fall into three main categories namely forensic sciences (i.e., forensic entomology, forensic acarology), conservational sciences (biological conservation and sustainability, protection of endangered species, ecosystem health monitoring, bioindicators), and medicine (public health and disease ecology management). Most importantly, the current study enhances our understanding about nutrient recycling and contributes to the science of carrion ecology.

CONCLUSIONS

Soil arthropod community structures are sensitive to the years of trial (could be due to differences in abiotic factors), days of decomposition, and soil regions, but its

sensitivity to treatment effect depends on the taxonomic resolutions. Hence, based on these results, we reject the hypothesis null, that we had demonstrated that there was a shift in soil arthropod community structure and function in response to delayed vertebrate decomposition. We also discovered that soil arthropod community divergence, convergence and resilience are also taxonomic-scale dependent. We proposed that the family level of soil arthropod community is sufficient to detect significant differences for treatment effect. Furthermore, we suggest cadaver decomposition islands (CDIs) should be clearly defined as small distances away from the soil beneath the carrion could have significant difference in terms of soil arthropod community structure and function. There are many unexplored areas in soil acari diversity and function associated with carrion decomposition, and the future of pursuing carrion ecology and its applications (e.g., forensic acarology) is promising.

CHAPTER V

ABOVEGROUND ARTHROPOD COMMUNITY ASSOCIATED WITH DELAYED VERTEBRATE DECOMPOSITION

INTRODUCTION

Carrion is defined as dead and decaying vertebrate animal remains (Oxford Dictionary, 2016). However, carrion represents only a small part (approximately 1%) of the total detritus pool in ecosystems; a majority is phototrophically derived organic matter (Swift et al. 1979; Parmenter & MacMahon, 2009). Although much focus had been given to plant litter decomposition, understanding the heterotrophically derived component of the detrital pool- carrion is crucially important in the process of nutrient recycling (Benbow et al. 2015).

A great diversity of organisms are associated with carrion as it represents a valuable food source (i.e., narrow C:N ratio) (Carter et al. 2007). Resources like carrion are precious as they are ephemeral, unpredictable, and patchy; a number of organisms including microbial, invertebrates, plant, and vertebrate communities depend on it for survival (Benbow et al. 2015b). Soils are also affected by the introduction of vertebrate carrion as it alters associated chemical concentration (Bump et al. 2009). Carrion can therefore have direct and indirect effects on many parts of an ecological community, and contribute to the dynamics of species diversity and nutrient cycling (Barton et al. 2013a; Beasley et al. 2012; Hocking & Reynolds, 2011).

Invertebrates such as insects and other arthropods (e.g., mites) are important carrion consumers and drivers in the flow of chemical energy and carrion food web (Barton et al. 2013a). Arthropods are excellent agents in soft tissue removal. They recycle much of the biomass of carrion, making nutrients available for other organisms in the food chain (Forbes & Carter, 2015). In general, carrion becomes attractive to insects almost immediately after death (Anderson, 2001). However, successional waves of insects eventually modify the carrion resource either physically or biochemically

throughout the decomposition period (Anderson & Cervenka, 2002; Kreitlow, 2010). Ecological succession varies by region, climate, intra- and interspecies dynamics, and other random occurrences (Kreitlow, 2010; Gleason, 1917). Hence, ecological succession may not necessarily be deterministic or predictable as proposed by Clements (1916).

In general, carrion in terrestrial environments attracts a predictable assemblage of arthropods. Reed (1958) reported 240 taxa from dog (*Canis familiaris* L.) carcasses in Tennessee, USA. Payne (1965) documented 522 arthropod species collected from swine (*S. scrofa*) carcasses in South Carolina, USA. Goff et al. (1986) conducted necrophagous arthropod community study at Hawaiian Islands and reported 149 taxa from several kinds of carrion. At that same year, Braack (1986) reported 227 arthropods associated with impala (*Aepyceros melampus* (Lichtenstein)) carcasses in the northern Kruger National Park, South Africa. Since then, many studies have been conducted and different arthropod taxa associated with carrion decomposition process have been reported throughout the world (Tomberlin et al. 2015).

Taxonomic classification, however, will not provide a complete picture about what these arthropods do to the carrion. Hence, to understand the carrion community better, examining the arthropods from the perspective of ecological function is necessary. These ecological guilds include necrophagous, predators, parasites / parasitoids, and omnivores (Villet, 2011; VanLaerhoven, 2010; Catts & Haskell, 1990). Note that the term "grazer" was introduced to reflect those organism that appear to feed on the microbiological biofilms associated with carrion rather than the carrion itself or other macroorganisms, a phenomenon which has recently been documented to occur (Pechal et al. 2013, 2014a). Arthropods that fit into the "grazer" group are the larvae of Sphaeroceridae (lesser dung flies) which are microbial grazers when they are found in association with decomposing organic materials such as decaying plants, fungi, dung, and carrion (Roháček, 2001) and as well as fungivorous acari. Perhaps the most important functional group in this study is the necrophagous guild, which consist primarily the larvae of Calliphoridae and Sarcophagidae, and adults and larvae of

Silphidae, Dermestidae and Trogidae (Merritt & De Jong, 2015). Besides, predators are commonly found among the carrion frequenting arthropods such as the adults and larvae of Staphylinidae, Histeridae and adults of some Silphidae (Byrd & Castner, 2009). Specialized parasites and parasitoids of the carrion fauna generally come from the Order Hymenoptera, especially the families Braconidae and Pteromalidae (Frederickx et al. 2013). Interestingly, the staphylinid beetle *Aleochara* sp. is also a parasitoid of dipteran larvae (Prins, 1984). As for omnivores, this group feed on carrion and the other carrion-attendant fauna opportunistically. The omnivore members include ants, termites, cockroaches, wasps and many other beetles (Merritt & De Jong, 2015). Incidental taxa can be abundant on the traps placed at or around decomposing carrion. Basically there are three groups of incidental taxa: (i) those that actively live on the carcass, but do not feed on carrion but use it as shelter (ii) a staging location for predaceous arthropods to attack on the sudden increase in prey population (iii) those taxa that stochastically occur (i.e., passed by the carrion by chance) (Merritt & De Jong, 2015; Byrd & Castner, 2009; Catts & Haskell, 1990).

Immediate insect access to carrion is not always the case (Pechal et al. 2012; 2013; Bourel et al. 1999). In the absence of arthropods, such as when arthropods are experimentally excluded, the decomposition of carrion can be significantly delayed (Payne et al. 1968; Parmenter & MacMahon, 2009). Pechal et al. (2014b) demonstrated that delayed insect access to carrion (where insects excluded for five days) caused a marked shift in necrophagous insect community structure, turnover rates and assembly with overall effects on carrion decomposition. Although they found similarities between taxon (two orders and 11 families) arrival patterns, once insects were allowed to colonize carrion previously excluded from insects there was an increased necrophagous insect taxon richness and increased community turnover rates. Pechal et al. (2014b) found *Lucilia coeruleiviridis* (Macquart) and *P. regina* (Diptera: Calliphoridae) were the dominant taxa for insect access carcasses as well as insect exclusion carcasses. In 2010, there were significant difference of decomposition time, insect exclusion and their interaction on taxon richness. No significant difference was observed on evenness and

Simpson's diversity. However, in 2011 trial, they found no significant effects of decomposition time, marginal significant effects of insect exclusion ($p = 0.058$) and no interaction on taxon richness. Furthermore, evenness was nearly significant ($p = 0.057$) between control and delayed carcasses, and Simpson's diversity increased significantly over decomposition between control and delayed carcasses. These results suggest a year effect, possible due to differences in resource size or priority effects of initial colonizers altering subsequent community structure. Pechal et al. (2014b) also demonstrated that delayed insect access altered subsequent insect community assembly and was associated with a slower decomposition process. Changes in decomposition patterns are known to modify the rate of nutrient transformation and availability in the local habitat, these affect the ecosystem function and ultimately impact other members of the community that respond to nutrient pulses (Parmenter & MacMahon, 2009).

Many abiotic factors could contribute to the delay of insect access to carrion and consequently delay decomposition process (Campobasso et al. 2001). Abiotic factors that could cause delay in insect colonization on carrion include weather and location origins such as seasons, temperatures, rainfalls, snows, thunderstorms, tornados, beside a busy highway and high altitudes (e.g., highland or high-rise building etc.) which could deter immediate insect access and oviposition activities (Campobasso et al. 2001; Syamsa et al. 2012; Mahat et al. 2009). Another abiotic factors include burial activities or being hidden, and these could happen either naturally by animal behaviors (e.g., dogs burying bones or carcass hid by scavengers), or artificially (e.g., in criminal cases where cadavers have been wrapped, buried or hidden in concealed container, or in some rare cases where insecticide was applied on human corpse to prevent insect colonization) (Anderson, 2001). Furthermore, time could be another abiotic factor, for example, animals that die during night time where blow flies are not active and oviposition is likely to occur the next morning (Introna et al. 1998; Reibe & Madea, 2010).

A number of biotic factors can impact arthropod colonization of vertebrate carrion. It is well documented that predation of blow fly eggs and larvae by other organisms (e.g., ants, mites, beetles, or other species of flies) could affect the

colonization of blow fly on carrion (Norris, 1965). Bacterial quorum sensing on vertebrate carrion that results in the release of MVOCs could repel certain insects, which eventually delay the insect arrival time (Ma et al. 2012; Tomberlin et al. 2012; Davis et al. 2013). Ecological interactions (e.g., competition) and effects (priority effects, facilitation effects, inhibition effects) may play a role in deterring insect arrival and colonization or altering insect succession sequence although empirical studies are needed to confirm these observations (Connell & Slatyer, 1977). Changes in insect community structure and function following ecosystem perturbation are another hypothesis that deters initial insect colonization on carrion. For example, during mass mortality events (MMEs), where hundreds or thousands of vertebrate animals die during the same period of time (e.g., salmon runs, locust outbreak, large scale population die-off such as livestock population due to disease or draught), may cause large amount of nutrients flux into the ecosystems (Richey et al. 1975; Barton, 2015). Not only the introduction of large volume of carrion materials into the soil ecosystem, which could cause devastating effects such as nitrogen toxicity that kill most of the soil flora and fauna in that particular landscape (Bornemissza, 1957; Goyal & Huffaker, 1984), but the MMEs may also cause disturbance to the ecosystem function by reducing efficiency of decomposition process. The number or abundance of necrophagous arthropods, such as blow flies, is assumed to be predetermined based on the current spatiotemporal equilibrium dynamics in a given habitat. However, when an unexpected ecological disaster occurs, such as MMEs, there could be a "dilution effect" (*sensu* Ostfeld & Keesing, 2000a; Schmidt & Ostfeld, 2001), where population of necrophagous guild has been "diluted" or "divided" per unit carcass, this ultimately causes shortage in the number of necrophagous workers to decompose all the carrion simultaneously, eventually leads to the delay in carrion decomposition. It is possible that large-magnitude perturbation (i.e., MMEs) could change the equilibrium state of the disturbed habitat either temporarily or permanently, depending on the degree of resilience and the quality of its internal properties (e.g., network connectivity, community richness, diversity and functions etc.) that are inherited in the ecosystem (Gunderson, 2000).

Delayed carrion decomposition could have beneficial and deleterious effects. The benefits of delayed carrion decomposition are carrion could serve as a unique resource pool and alternative habitat for vast variety of organisms for an extended period of time. Furthermore, availability of carrion for a longer period could result in species diversity (especially necrophagous guilds) being maintained while enriching the soil ecosystem (Barton et al. 2013). The negative impacts of delayed carrion decomposition are the contamination of the environment with pathogenic bacteria (Houston & Cooper, 1975). Furthermore, carrion serves as a breeding ground for vast variety of insect vectors as well as provides food for scavenger animals, which could serve as pathogen reservoirs (Busvine, 2012; Jennelle et al. 2009; Jones & Pybus, 2001).

No previous studies have examined the terrestrial arthropod community associated with carrion experiencing insect-exclusion for more than five days. The objectives of this study were to examine the impact of delayed primary arthropod colonization of carrion on the successional trajectories of terrestrial arthropod community structure and function. Furthermore, this study aimed to determine how the aboveground arthropod community trajectories behaved (e.g., divergence, convergence, and resilience).

The ecological concepts in carrion ecosystem are demonstrated in Figure 5.1. Several ecological terminologies were introduced to depict the phenomena observed in the present study namely divergence, convergence, resilience and resistance. Axis Y in the figure represents measurable parameters such as the quantity of microbial function (e.g., average OD reading), soil nutrient concentration (e.g., NO_3^- (μg/kg)), or arthropod abundance (e.g., count number of Coleoptera) while Axis X represents the decomposition day (e.g., from Day 0 to Day 40). The Control carcasses are immediately colonized by insects and are represented by blue line, increased from Day 0 to Day 40. A disturbance (or treatment effect) was introduced for seven days (represented by pink area). The green line represents the quantity of response (by the microbes or soil nutrients or abundant of Coleoptera) to delayed insect colonization. Statistical analysis such as ANOVA can then be performed between Control and Treatment on every

sampling day to determine differences. If there was a significant difference between Control and Treatment (as seen on Day 14), then this situation is considered as "divergence" (*sensu* Weber & Legge, 2009). However, if there was no significant difference in response between Control and Treatment, for example, on Day 21, then this situation is called "convergence" (*sensu* Wassenaar et al. 2005). Convergence must come before Divergence. If there was no significant difference between Control and Treatment (brown line) throughout all sampling days (from Day 0 to Day 40) or there was no difference before a divergence occur, then this situation is considered "resistance", which is defined as staying essentially unchanged despite the presence of disturbance (Grimm & Wissel, 1997). Lastly, resilience is defined as the speed with which a system returns to initial state following a perturbation (DeAngelis, 1980), or speed of return to the equilibrium state (Pimm, 1984), this resilience may also be termed as engineering resilience (Holling, 1996b). Although Holling (1973) defined resilience in a different way (i.e, ecological resilience) which emphasizes condition far from any equilibrium steady state, where instabilities can flip a system into another regime of behavior (i.e., to another stability domain). Note that in this study, we defined resilience similar to DeAngelis (1980) and Pimm (1984), where we aimed to measure differences in response within a system, by comparing initial response on Day 0 with other sampling day. The two blue circles depicted in the Figure 5.1 showed the initial response of Day 0 and the last response of Day 40, and both of them were actually at similar level (or concentration), hence resilience occurred. Note that the term "resilience" was used after a significant loss of resistance. For example, the response level on Day 14 was much higher than the initial response on Day 0, with a significant difference (hence a loss of resistance). The time of first recovery, or resilience, could happen as earlier as Day 21 or Day 40 when there was no significant difference with Day 0. Note that the black line represents a hypothetical response where divergence (significant difference with Control carcass) and no resilience (significant difference between the initial respond on Day 0 with the respond on Day 40 within the Treatment).

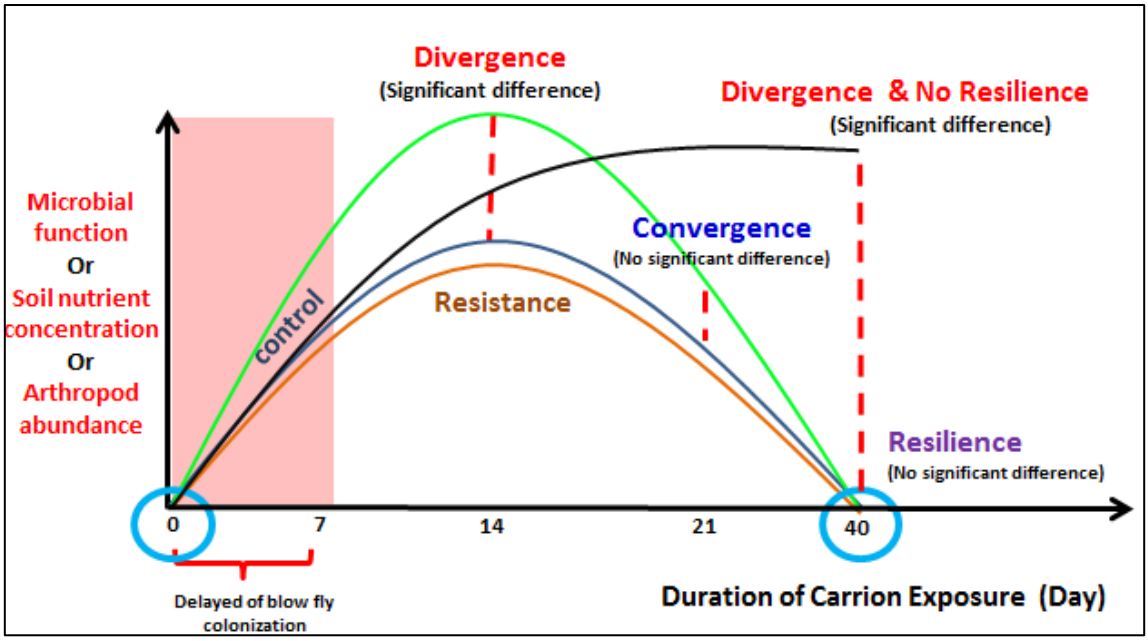

Figure 5.1. Ecological concepts in carrion ecosystem emphasized in the present study: community divergence, convergence, resilience, and resistance associated with carrion experiencing delay in dipteran colonization.

METHODS

Site description and experimental design

Swine carcass (*S. scrofa* L.) decomposition were studied at a site belonging to the Field Laboratory, Texas A&M University, College Station, Texas, USA (30°33' 18.54'' N 96°25'38.71'' W, 68 m a.s.l.). The perimeter of the study area was approximately 371 m and the area was approximately 7,943 m^2 (Figure 2.1 and 2.2). The soil at the study site was characterized as clay soil (Personal Communication- J.A. Peterson). There was a stream located at the north of the study site. The east and south edges were steep cliffs (~6 m) above the stream. Vegetation at the study site is considered part of the blackland prairie ecoregion (http://www.texasalmanac.com). Common vegetation found at the study site included Johnsongrass (*Sorgum halepense* L.) (dominant cover plant, covered approximately 75% at the study site approximately 90% of vegetation), oak (*Quercus* spp.), annual sunflower (*Helianthus annuus* L.), thistles (*Cirsium* spp. Mill.), Western

horse nettle (*Solanum dimidiatum* Raf.), Camphorweed (*Heterotheca subaxillaris* (Lam.)), muskmelon (*Cucumis melo* L.), jujube (*Zizyphus jujube* Miller),wild purple morning glory (*Ipomoea cordatotriloba* Dennst. tievine), pink evening primrose (*Oenothera speciose* Nutt.), poison ivy (*Toxicodendron radicans* (L.) Kuntze) and arrow-wood (*Viburnum dentatum* L.)

Studies were conducted in two consecutive summers during June 2013 and 2014. A total of nine pig carcasses purchased from a local pig farmer in Anderson, Texas were obtained for each year replicate. Sex and weight of each pig carcass was determined prior to placement in the field. The animals were deceased at the time of acquisition; therefore, the Texas A&M University Institutional Animal Care and Use Committee required no animal use protocol. The carcasses were double bagged and transported within one hour after death to the study site. Carcasses were placed in the field (approximately 1700 hr) and carcasses were randomly placed minimally 20 m apart along three transects. All carcasses were oriented with heads to cardinal north and dorsal side towards the east. The placement of pig carcasses in the field was calculated by using a Latin Square design and the arrangement of treatments groups were different between years (Figure 2.3 and 2.4). Each location was only used once. Subsequent locations were never less than five meters from a previous site used. During each field seasons, three random carcasses were enclosed in an individual 1.8 m^3 Lumite® screen (18 x 14 mesh size) portable field cages (BioQuip Products, Rancho Dominquez, CA, USA) for seven days, this treatment was designated as Post-7 group. Another three random carcasses were enclosed with similar manner as above but it was enclosed for 14 days, thus were designated as Post-14 group (Figure 2.5), while all insects were allowed access the remaining three carcasses, which were served as control (Figure 2.6). All carcasses were covered with hand-made anti-scavenging cages (0.6 m height x 0.9 m width x 1.2 m length) constructed of steel frames enclosed with poultry netting. Each anti-scavenging cage was topped with a layer of woven green fabric (Figure 2.6) to prevent direct sunlight and heat on the carcass. All cages were then properly labeled according to their designation. Stones were placed on top of each cage to increase weight in order to

prevent the movement of cage by extreme wind of scavenger activities. Furthermore, observations for vertebrate scavenging were made daily at approximately 2200 hours.

Observation on arthropod activities on pig carrion was recorded three times a day (morning, evening and night) until 40 days. The observations on the carrion decomposition process and major arthropod activities were documented (not included in the current Chapter, see Appendix K).

Climatological data such as temperatures and rainfall were recorded. NexSens DS1923 micro-T temperatures loggers (Fondriest Environmental, Inc., Alpha, OH, USA) (Figure 2.7) were placed at the study site 0.3 m above the ground on the anti-scavenging cage to measure local ambient temperature every 60 min for 40 days continuously. Temperature data were converted into accumulated degree hours (ADH) based on the following formula:

$$\mathrm{ADH} = \sum_{i=1}^{n} (\emptyset - \emptyset_0)$$

where $\emptyset$ is the ambient temperature (in °C), and the minimum threshold temperature $\emptyset_0$ (Higley & Haskell, 2009). The minimum development temperature threshold was set as 10°C in this study as that is the minimum used for blow flies common on vertebrate carrion during the summer months in Texas, USA. To obtain the value of accumulated degree days (ADD), the ADH was divided by 24 (i.e., ADD = ADH / 24). Precipitation during the study period was recorded daily with a rain gauge attached to a wooden stake approximately 1.3 m above the ground, and 1 m north from one of the carcasses in the field (Figure 2.8).

Hand collection

Arthropods, such as adult beetles and larvae, as well as maggots were collected using forceps. Specimens were preserved in labeled vials (23 mm (D) x 85 mm (L)) containing 70% ethanol (see Appendix L for the list of arthropods collected). Arthropods

were identified to lowest taxonomic level whenever possible. A dissecting microscope (Meiji, Japan) was used to examine specimens. Triplehorn & Johnson (2005) was used to identify to Family level, while taxonomical keys, such as Whitworth (2006) or taxonomists (e.g., Mr. Edward Riley from Texas A&M University and Dr. Gregory Dahlem from Northern Kentucky University) were consulted for greater taxonomic resolution in the arthropod collected (see Appendix J for the identification key of adult and larval Sarcophagidae associated with pig carrion in the current study). Adults and larval community structure and succession on pig carrion, as well as circadian rhythms of necrophagous insects were also recorded and analyzed (not included in the current Chapter, see Appendix M).

Sticky traps

Scent-free sticky traps (Trapper® Max Free Glue Trap, Bell Laboratory, Wisconsin, USA) (13.97 cm width x 19.05 cm length) were used. Four sticky traps were used for each pig carcass on every sampling day, with two sticky traps at the anterior of the pig carcass (~1 m north from the head) and two sticky traps at the posterior of the pig carcass (~1 m south from the tail). The overall distant between the anterior and posterior traps was approximately 3.12 m. At each location (anterior or posterior), two sticky traps were stapled at 0.3 m (bottom) and 1.0 m (above) on a 1.21 m wooden garden stake (Figure 5.2) facing away from the carcasses. Sticky traps remained in the field for 24 hours prior to removal. Sticky traps removed from the wooden stakes were placed in plastic bags (Hefty®) and stored in a freezer (-20°C) until further processing.

Pitfall traps

Pitfall traps (diameter 10.16 cm; volume 532 ml, buried into the ground with depth approximately 12 cm) were used in each trial. Four pitfall traps were placed on each side of the carcasses (Figure 5.3). Samples were taken at set time points (Day -5, 0, 3, 7, 10, 14, 21, and 40) during the study. No preservative fluid was added to the bottom of pitfall trap as arthropods were removed from the traps 24 hours of placement.

Arthropod specimens collected in a given pitfall trap were placed in Ziploc® storage bags, labeled, and stored in the freezer (-20°C) until further process. Identification was accomplished using methods previously described.

Sweep netting

Flying arthropods were collected using a standard hand sweep net (handle 0.6 m; net diameter 0.38 m). Three continuous sweeps (with uniform swings from right to left to right, with an angle of ~120°) were performed on each pig carcass (approximately 10 cm on top from the body) (Figure 5.4) on every sampling day (Day 0, 3, 7, 10, 14, 21 and 40). Collected arthropods were transferred to a killing jar (473 ml) charged with ethyl acetate. Euthanized insects were transferred to a labeled Ziploc® storage bag, and subsequently stored in the freezer (-20°C) until further process. Identification was accomplished using methods previously described. Several identified arthropods collected by sweep nets, pitfall traps, and hand picks were vouchered at TAMU Insect Collection with accession number #722.

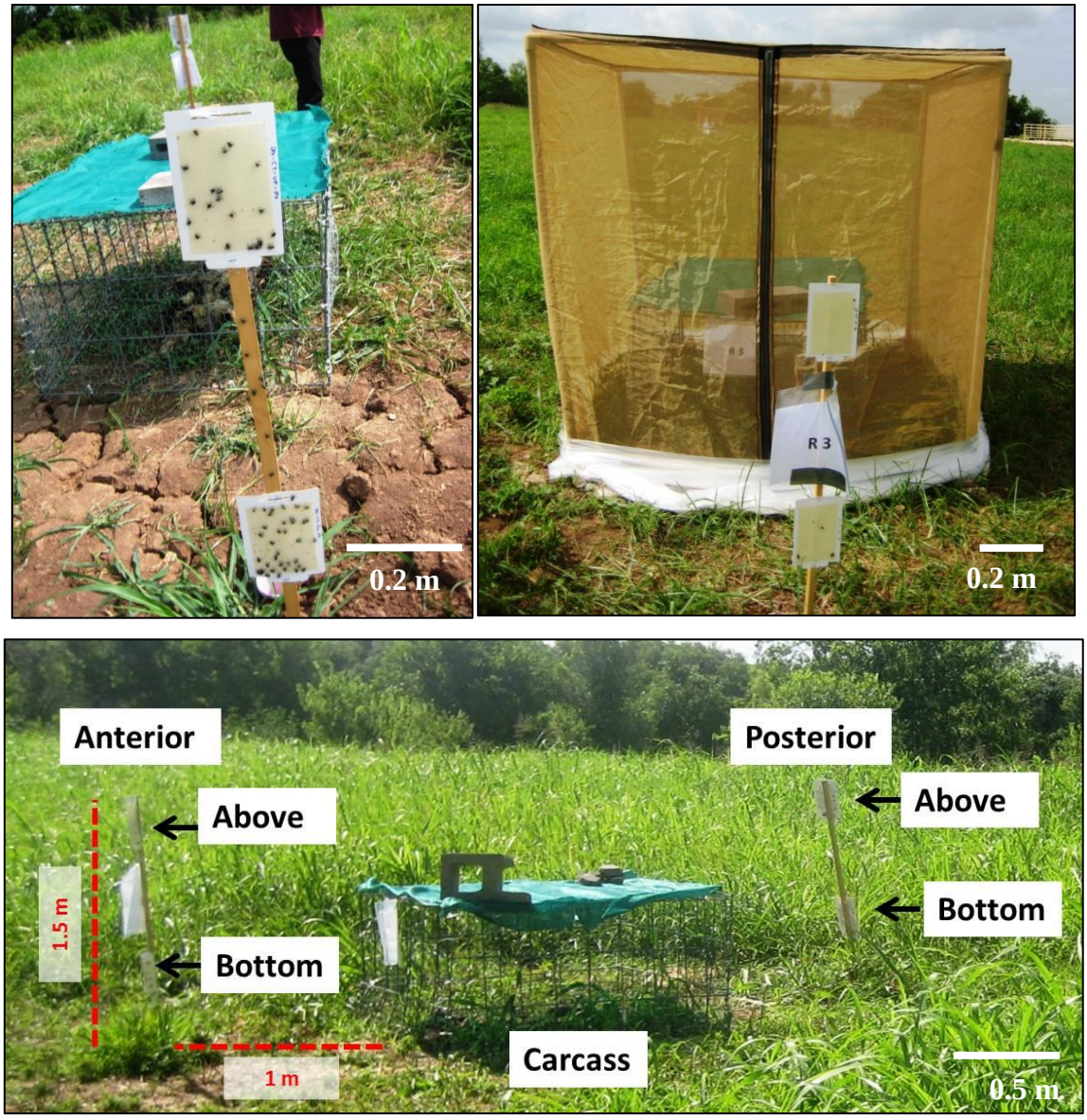

Figure 5.2. Sticky traps served as tools for trapping aboveground arthropods around the pig carrion (approximately 1 m away from the carrion) during summers 2013 and 2014 at the field site at Snook, Texas. Upper left. Sticky trap locations at the Control carcass. Upper right. Sticky trap locations at the carcass with delayed insect colonization (Post-14). Bottom. Overview of sticky trap locations for each pig carcass. Note that all sticky surfaces were facing outwards from the swine carcass.

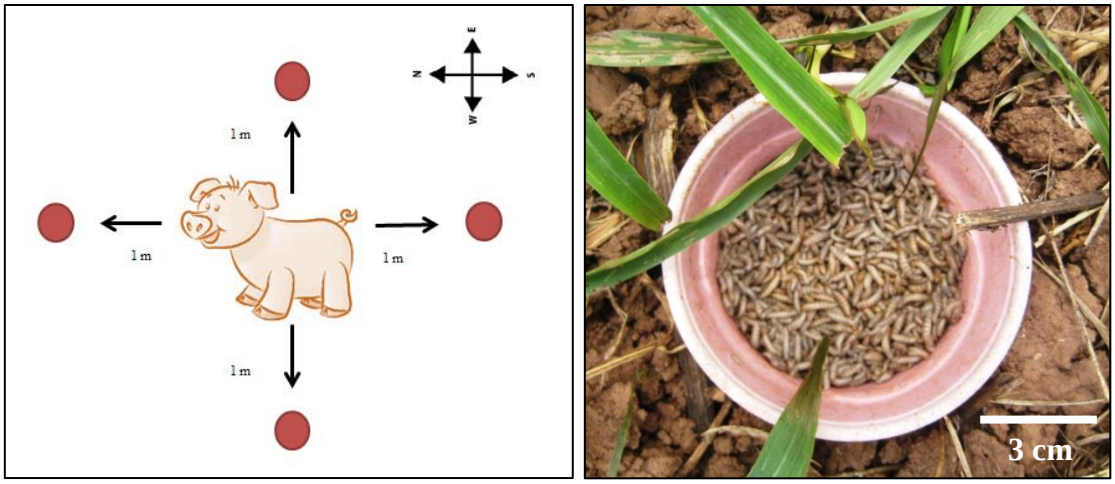

Figure 5.3. Left. Location of pitfall traps around each pig carcass in both summers 2013 and 2014 at Snook, Texas (image not to scale). Red circles represent the location of pitfall traps, which was 1 m away from the pig carrion. Right. Example of a pitfall trap filled with *Co. macellaria* (Diptera: Calliphoridae) 3rd instar on Day 7 after carcass placement in the field during summer 2014.

Figure 5.4. Sweep net was used to collect flying arthropods on and around the pig carcasses in both summers 2013 and 2014 at the field site of Snook, Texas.

Statistical analyses

Arthropod community data (separated by Order, Family, Genus and species, and Function) were analyzed using statistical program JMP® Pro version 11.0.0 (SAS Institute Inc., NC, USA) for analysis of variance (ANOVA) and Tukey-Kramer HSD post-hoc test. Arthropod was assigned to a certain functional group according to the Family level (see Appendix G). Aboveground arthropod community abundant data were also calculated for ecological indices such as species richness (S), Dominance (D_i), Simpson's diversity index (D), Shannon-Wiener's Diversity Index (H'), and Evenness (E). A diversity index is a quantitative measure that reflects how many different species there are in a dataset, and at the same time, taking into account how evenly the basic entities are distributed among those species.

Species Richness, S, simply quantifies how many different species of the dataset contained while Dominance (D_i) was calculated according to the equation below:

$$D_i = \frac{n_i}{N} \times 100$$

where n_i is the number of individual species collected, and N is the total number of specimen collected. Species dominant is classified according to Tischler's scale: eudominant $10\% \leq D_i \leq 100\%$, dominant $5\% \leq D_i \leq 10\%$, subdominant $2\% \leq D_i \leq 5\%$, recedent $1\% \leq D_i \leq 2\%$ and subrecedent $0\% \leq D_i \leq 1\%$ (Tischler, 1949).

Simpson's Index (D) measured both richness and proportion of each species and is calculated using this formula:

$$D = \sum_{i=1}^{S} P_i^2$$

where Pi is the proportion of species i. In brief, Simpson's index is the sum of proportion of each species in the community and represented the probability of two randomly selected individuals in the community belong to the same species. Shannon-Wiener

Index (H') is similar with Simpson's Index where the measurement takes species richness and proportion of species into account, and is calculated based on the following formula:

$$H' = -\sum_{i=0}^{n} P_i(\ln P_i)$$

In general, Shannon-Wiener Index is the negative sum of multiply products between species proportion (P_i) and natural log of species proportion ($\ln P_i$).

Evenness (E) is an indicator of similarity in abundance of different species. Evenness is measured on the scale from 0 to 1 where zero represents more variations in communities whereas one represents complete evenness. Evenness is defined as:

$$E = \frac{H'}{\ln S}$$

Evenness is the number obtained via dividing the value of Shannon-Wiener Index by natural log of species richness (S).

When Shannon-Wiener's Index was converted to Effective Number of Species (ENS), which is EXP (H').

$$ENS = EXP\ (H')$$

If the ENS value is close to 1, this indicates that the arthropod community has an equivalent diversity as a community with 1 equally-common species.

In addition, R project for statistical computing (R 3.0.2) was employed to analyze soil arthropod community data using vegan package (Oksanen et al. 2013). Vegan contains the methods of multivariate analysis, such as Permutational Analysis of Variance (PERMANOVA), needed in analyzing ecological communities, and tools for

diversity analysis. Bonferroni corrections were used to test for significance of pair-wise comparisons without an increased probability of rejecting the null when it was actually true (Type I error) (Cabin & Mitchell, 2000).

Non-metric multidimensional scaling (NMDS) was used to evaluate aboveground arthropod community structure and function between treatments over days in package Vegan function Adonis in R. It is an analysis of variance using distance matrices; for partitioning distances matrices among sources of variation and fitting linear models to distance matrices. It uses a permutation test with pseudo-F ratio. Generally, NMDS is a nonparametric ordination technique that avoids assuming linearity among community variables (McCune et al. 2002).

Multi-response permutation procedures (MRPP) was used for testing statistical differences between overlay groups of aboveground arthropod communities within the ordination using methods described elsewhere (Biodini et al. 1985). Indicator species analysis (ISA) completed MRPP by assigning significant indicator values to arthropod species that were indicative of community structural separation among treatments and over decomposition day (*sensu* McCune & Grace, 2002). The indicator value described which arthropod Order/Family/Genus or species was the best indicator among arthropod community based on the abundance data, with 0 representing no indication and 100 being a perfect indication for each grouping. All statistical results with value $p < 0.05$ were considered significant difference.

RESULTS

Weather data in summer 2013

The mean temperature was 30.59 ±7.81 °C, with maximum 47.67±4.48°C and minimum 15.50 ± 0.00 °C. Total accumulated degree hour (ADH) for 2013 trial was 29209.70 (base temperature of 10 °C). According to the nearest National Weather Station (KCLL) at Easterwood Field Airport, College Station, Texas (data downloaded from www.wunderground.com), there were 14 rain events recorded during the study period. Total precipitation recorded from rain gauge throughout the study was 39.12 mm.

Weather data in summer 2014

The mean temperature was 29.27 ±6.49 °C, with maximum 43.00±1.80°C and minimum 19.00±0 °C. Total accumulated degree hour (ADH) for 2014 trial was 28080.67 (base temperature of 10 °C). There were 24 rain events recorded during the study period. Total precipitation recorded from rain gauge throughout the study was 171.45 mm.

Weather comparison between summers 2013 and 2014

Generally, combined data showed mean temperature in summer 2013 is higher than mean temperature in summer 2014. Two-tailed T test was employed to compare two years temperature data and the results showed a significant difference ($p = 0.0004$). Table 2.1 showed the T-test result on weather comparison between summers 2013 and 2014. Figure 2.18 showed the mean temperatures data of both 2013 and 2014 trials and Figure 2.19 showed the amount of precipitation for both summers. Regarding precipitation, although summer 2014 received higher amount of precipitation (171.45 mm) compared to summer 2013 (39.12 mm), however, two-tailed T-test demonstrated that there was no significant difference between years ($p = 0.2725$). Table 2.2 showed the comparison of precipitation of both years.

Accumulated degree hours (ADH) and accumulated degree days (ADD)

Accumulated Degree Hours (base temperature 10 °C) for summer 2013 and 2014 was demonstrated in Figure 4.7, where the ADH in summer 2013 was significantly greater than summer 2014. T-test also demonstrated that there was significant difference in ADH between the two trials (t (1964.141) = -2. 1944, p = 0.0283). Based on the readings obtained from micro-T data logger, ADH and ADD was calculated up to 40 days of experiment. Table 4.1 demonstrated the ADH and ADD during sampling day in the field for 2013 and 2014 trials.

Community structure and function of aboveground arthropods collected by sticky traps in 2013

Year effect

There was a year effect (df =1; F = 7.2399; p = 0.001) between two trials by Order of arthropods (Figure 5.5 showed NMDS plot between years). Furthermore, when Function of soil arthropods was analyzed for Year effect, the results showed that there was significant difference between years (df = 1; F = 12.902; p = 0.001). Hence, data were analyzed separately.

Replicate effect

There was no replicate effect (df = 1; F = 1.3311; p = 0.225) among the replicates by Order of aboveground arthropods collected by sticky traps. Also, when replicate effect was tested on Function of aboveground arthropods, result showed that there was no significant difference (df = 1, F = 1.1867; p = 0.281). Therefore, all data in the replicates were pooled and analyzed.

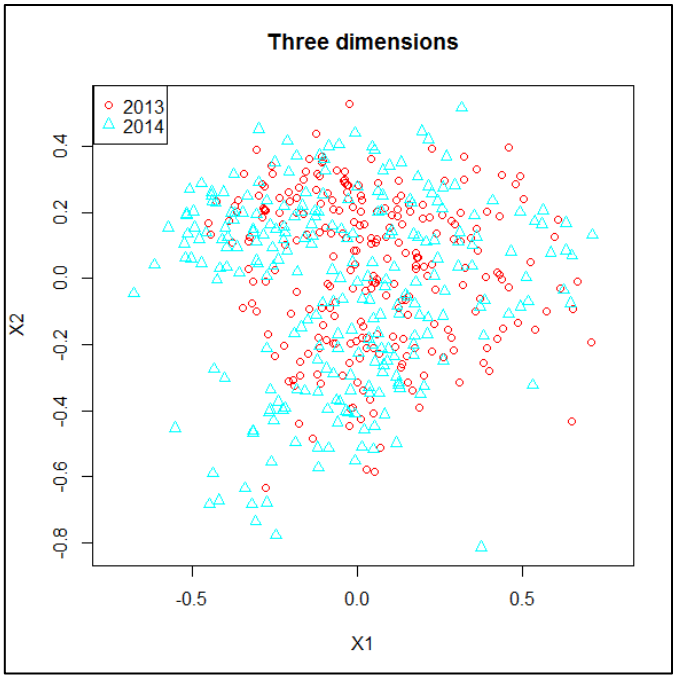

Figure 5.5. NMDS ordinations of aboveground arthropod data (by Order) between 2013 and 2014 trials. Minimum stress for given dimensionality 0.1631 and r^2 for minimum stress configuration was 0.8444.

Total Order in 2013

A total of 13 Orders of Insecta and one Order Araneae (Arachnida) have been recovered from all sticky traps in 2013 trial. Figure 5.6 showed the Orders identified in summer 2013 and the most dominant group was the Thysanoptera (29.65%), followed by Diptera (22.73%), Hemiptera (15.85%), Hymenoptera (14.93%), Coleoptera (9.04%) and others (all less than 3 %).

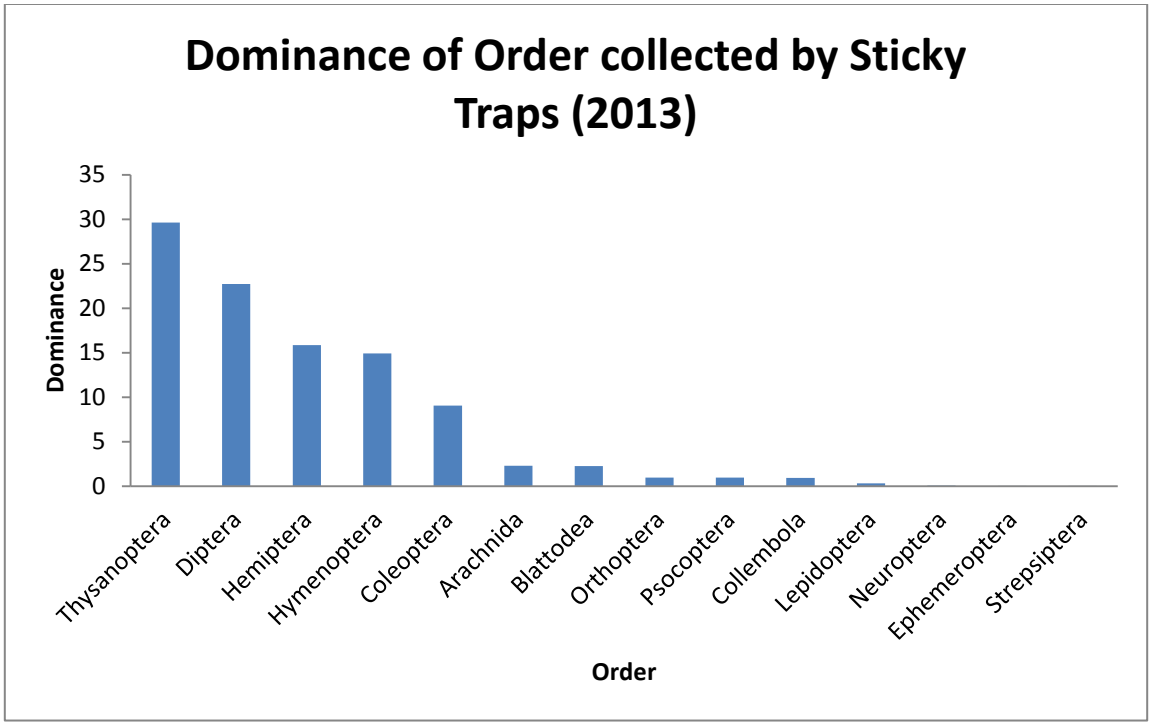

Figure 5.6. Dominance by Order of the aboveground arthropods collected using sticky traps in summer 2013 at the field site located at Snook, Texas.

Total collection of arthropod (by Order) by height and position in 2013

Out of 8105 arthropods collected in 2013 trial, 4557 arthropods (56.22%) were trapped by the sticky traps at the bottom (~0.3 m from ground surface) compared to 3548 arthropods (43.78%) by the sticky trap placed at above (~1 m from ground surface). For the location of sticky traps, anterior position of the pig carcasses collected 51.76% of total arthropods while posterior position caught 48.24%. In detail, sticky traps with position and height of "Anterior Bottom" collected 32.36% of the total arthropods, sticky trap with "Anterior Above" collected 19.12%, and sticky traps at "Posterior Bottom" collected 23.60%, while sticky traps with "Posterior Above" collected 24.65% of total arthropods (Figure 5.7).

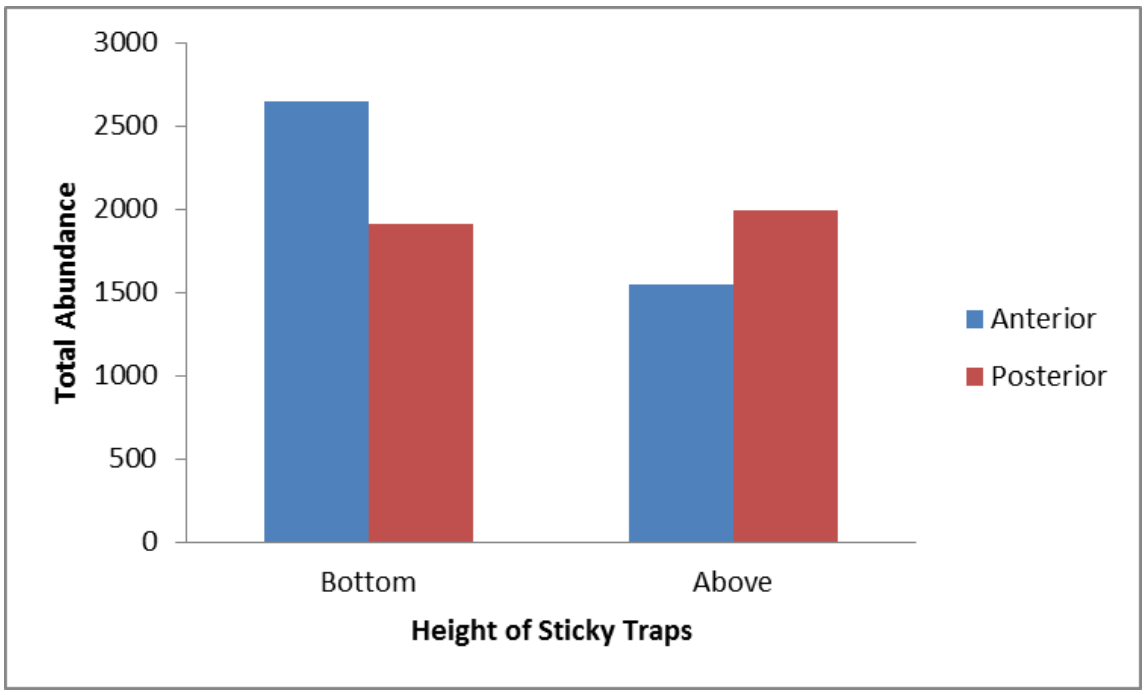

Figure 5.7. Total aboveground arthropods collected according to locations (anterior or posterior) and heights of sticky traps (above or bottom) in summer 2013 at the field site located Snook, Texas.

Total Family in 2013

A total of 117 families of arthropods (including eight families from the Class Arachnida) were identified from all sticky traps in summer 2013. Total abundance of all arthropods identified to Family level was 7412 individuals. The dominant family was Thripidae (32.49%), followed by Aphididae (11.43%), Dolichopodidae (9.92%), Formicidae (4.64%), Calliphoridae (3.47%), Staphylinidae (2.98%), Chloropidae (2.93%), Ectobiidae (2.46%), Trichogrammatidae (2.33%), Cicadellidae (2.23%), Muscidae (2.15%) and Ceraphronidae (2.05%). The other families were not shown as their dominance was less than 2%. Figure 5.8 showed the dominance of the some families collected in 2013.

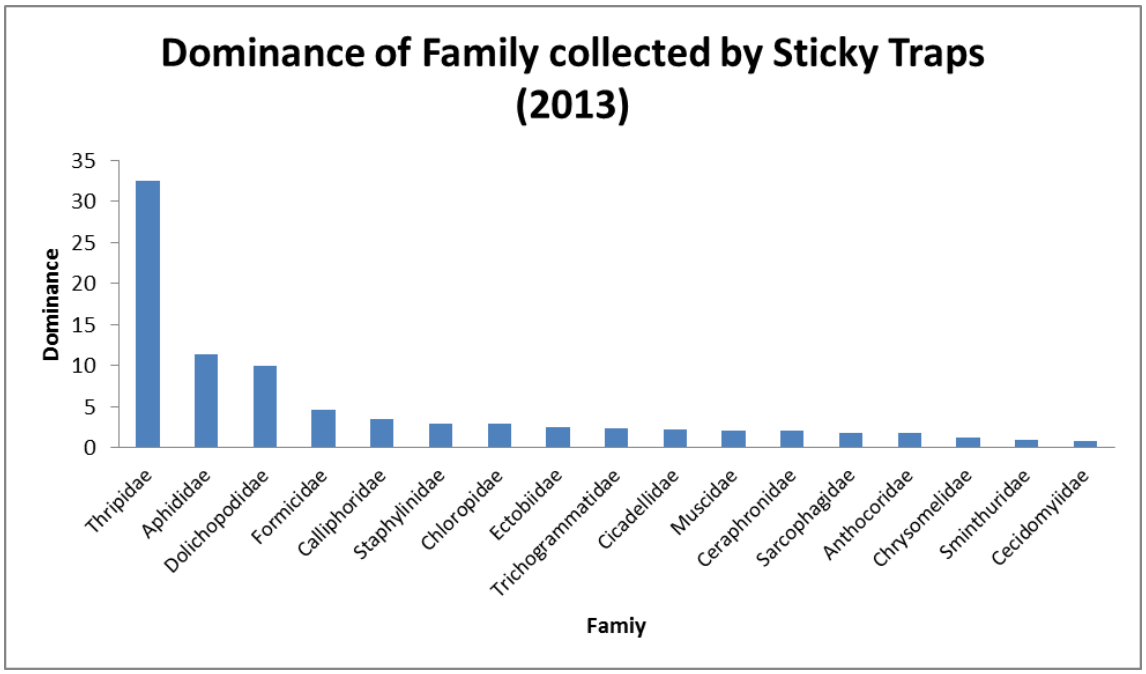

Figure 5.8. Dominance by Family of the aboveground arthropods collected using sticky traps in summer 2013 at the field site located at Snook, Texas.

Total Genus and species in 2013

A total of 48 genera and species of aboveground arthropods have been identified in 2013 trial (Figure 5.9). The most dominant genus and species collected was *Oligosita* sp. (Hymenoptera: Trichogrammatidae) (18.67%), followed by *Parcoblatta fulvescens* (Saussure & Zehntner, 1893) (Blattodea: Ectobiidae) (14.02%), *S. invicta* (Hymenoptera: Formicidae) (13.78%), *Ch. rufifacies* (10.54%), *Co. macellaria* (10.46%), *M. domestica* (7.30%), *Hydrotaea aenescens* (formerly known as *Ophyra aenescens*) (Wiedemann, 1830) (5.81%), *Ataenius* sp. (Coleoptera: Scarabaeidae) (4.23%) and others (all less than 3%).

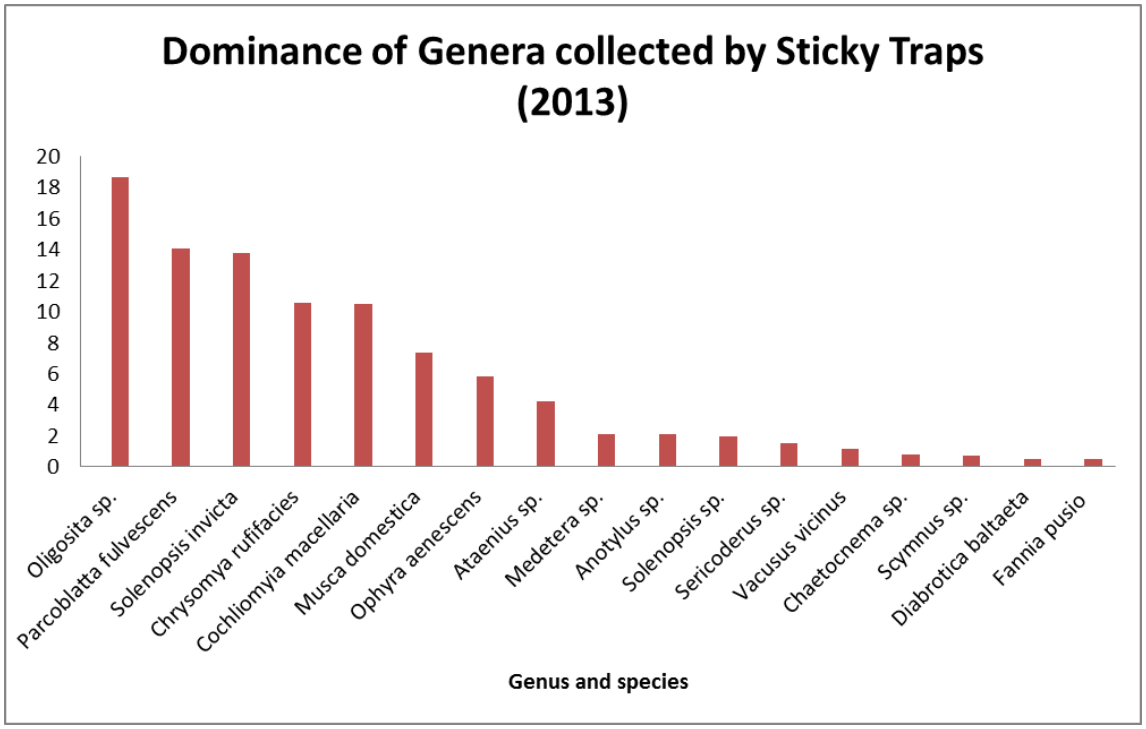

Figure 5.9. Dominance by Genera and species of the aboveground arthropods collected using sticky traps in summer 2013 at the field site located at Snook, Texas.

Total function in 2013

Eight functional groups were identified from aboveground arthropods collected on sticky traps in 2013. The most dominant group was herbivores (50.23%), followed by predators / parasites (28.31%), detritivores (7.27%), necrophagous (6.54%), nectarivores (4.68%), fungivore (2.46%), haematophagous (0.41%), and non-feeding group (0.12%) (Figure 5.10).

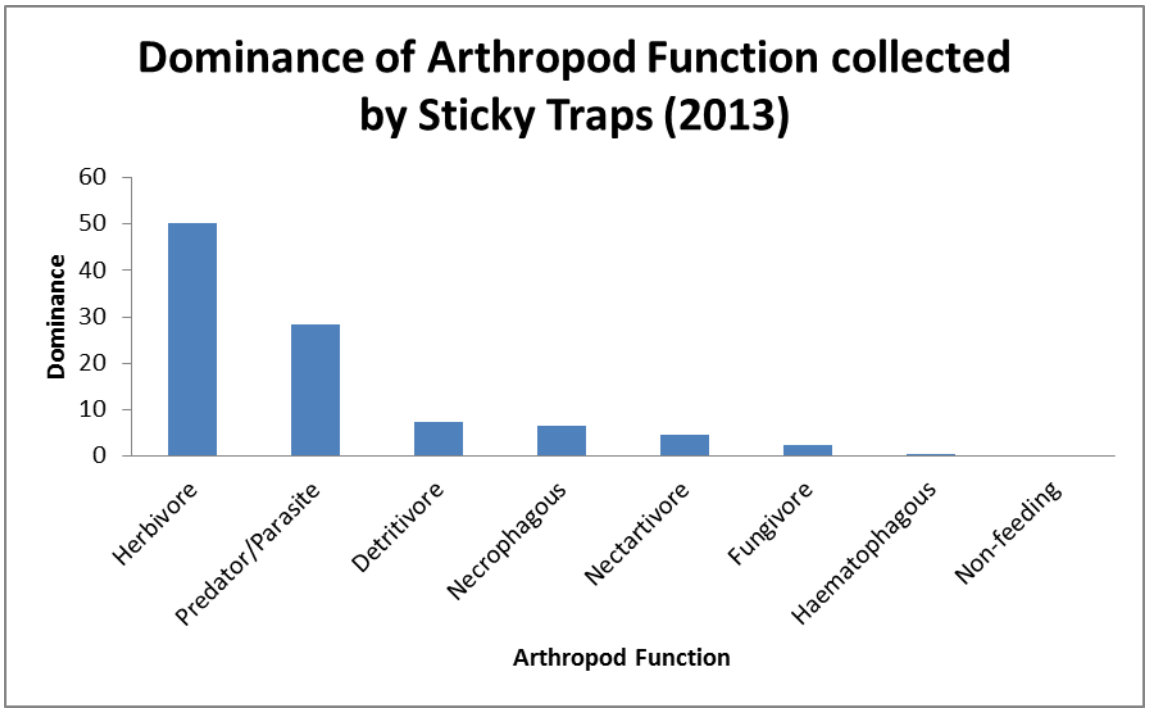

Figure 5.10. Dominance by functional groups of the aboveground arthropods collected using sticky traps in summer 2013 at the field site located at Snook, Texas.

Order in 2013

PERMANOVA was performed on aboveground arthropod data by Order level. Results showed that there was Day, Height, and Position effects ($p < 0.05$).There were interactions between Day x Height, Day x Position and Height x Position (Table 5.1).

Table 5.1. Analysis of the aboveground arthropod community structure (by Order) collected via sticky traps in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	35.413	0.001*
Treatment	2	1.456	0.134
Height	1	15.911	0.001*
Position	1	25.905	0.001*
Day x Treatment	2	1.081	0.355
Day x Height	1	3.223	0.009*
Treatment x Height	2	0.994	0.454
Day x Position	1	4.195	0.002*
Treatment x Position	2	0.745	0.742
Height x Position	1	5.965	0.001*
Day x Treatment x Height	2	0.607	0.860
Day x Treatment x Position	2	0.501	0.922
Day x Height x Position	1	1.193	0.314
Treatment x Height x Position	2	0.421	0.956
Day x Treatment x Height x Position	2	0.342	0.985

Since there was significant effect in Day, further analyses were carried out. For day of decomposition, all day to day comparisons were significantly different, except Day 7 x Day 10 where there was no significant difference (Table 5.2). The NMDS plot of stress for aboveground arthropod community structure (Figure 5.11) and NMDS ordinations for Day, Height, and Position were provided for visualization about data distribution (Figure 5.12, 5.13 and 5.14, respectively). Minimum stress for given dimensionality was 0.1598 with r^2 = 0.8439. The MRPP analysis for day showed a significant difference (A value = 0.1319; Significant of Delta = 0.001 based on 999 permutations), The MRPP for height also showed a significant difference with A value

0.0263 and Significant of Delta 0.001 while The MRPP for position was significantly different with A value 0.0446 and Significant of Delta 0.001.

Table 5.2. Pairwise comparisons of aboveground arthropod community structure (by Order) collected via sticky traps between carrion decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.001*	0.021*	0.026*	0.001*	0.001*	0.001*
3	0.001*	-	0.017*	0.001*	0.016*	0.001*	0.001*
7	0.021*	0.017*	-	0.114	0.001*	0.001*	0.001*
10	0.026*	0.001*	0.114	-	0.001*	0.001*	0.001*
14	0.001*	0.016*	0.001*	0.001*	-	0.001*	0.001*
21	0.001*	0.001*	0.001*	0.001*	0.001*	-	0.001*
40	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	-

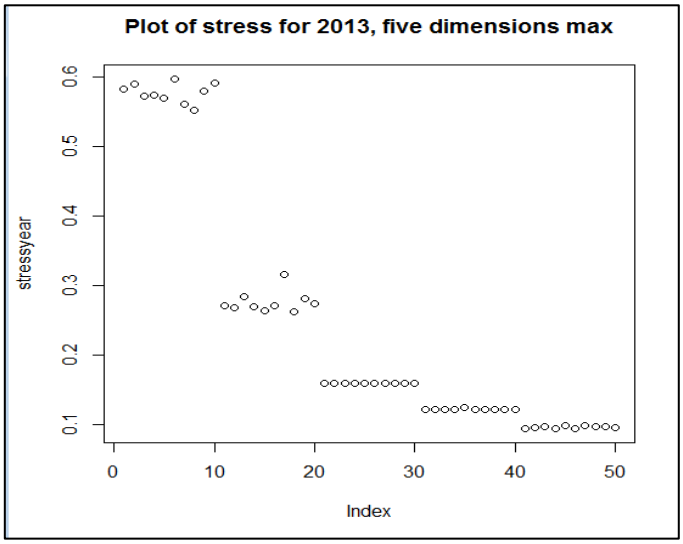

Figure 5.11. NMDS plot of stress for aboveground arthropod community structure (by Order) collected via sticky traps in summer 2013 at Snook, Texas (stress test 0.1598; r^2 = 0.8439).

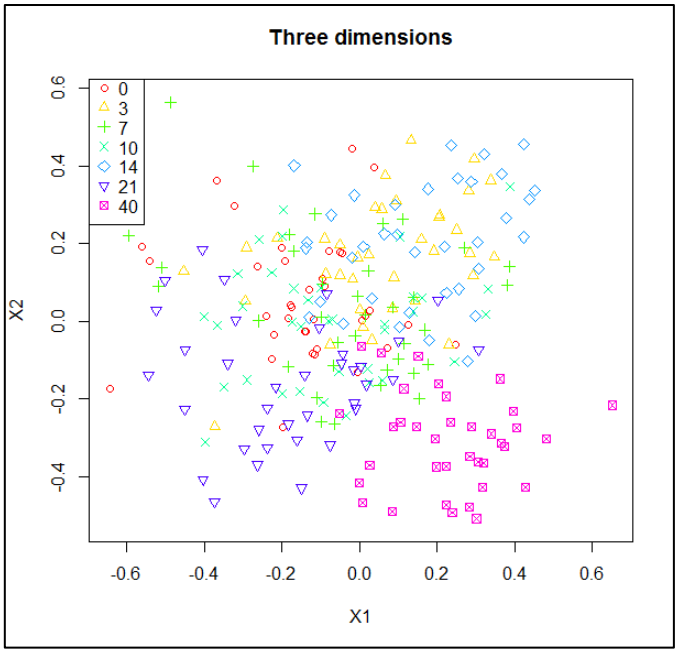

Figure 5.12. NMDS ordinations for aboveground arthropod community structure (by Order) by carrion decomposition days collected via sticky traps in summer 2013 at Snook, Texas.

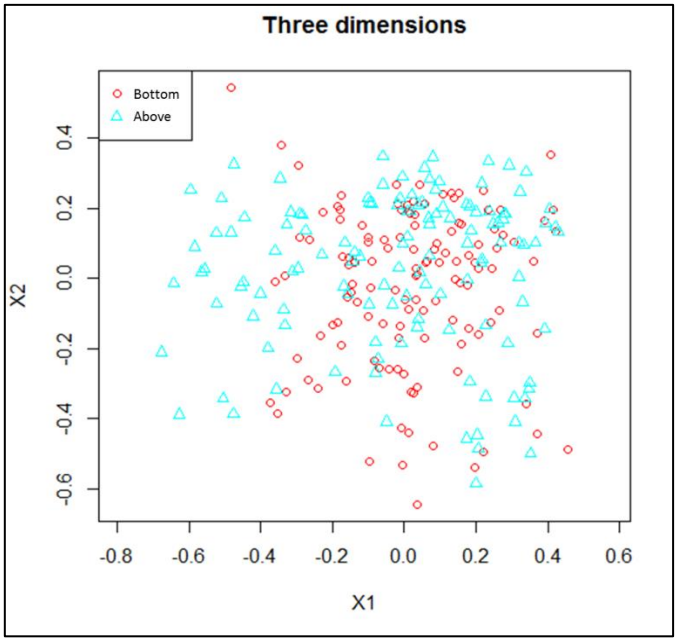

Figure 5.13. NMDS ordinations for aboveground arthropod community structure (by Order) by heights of sticky traps in summer 2013 at Snook, Texas.

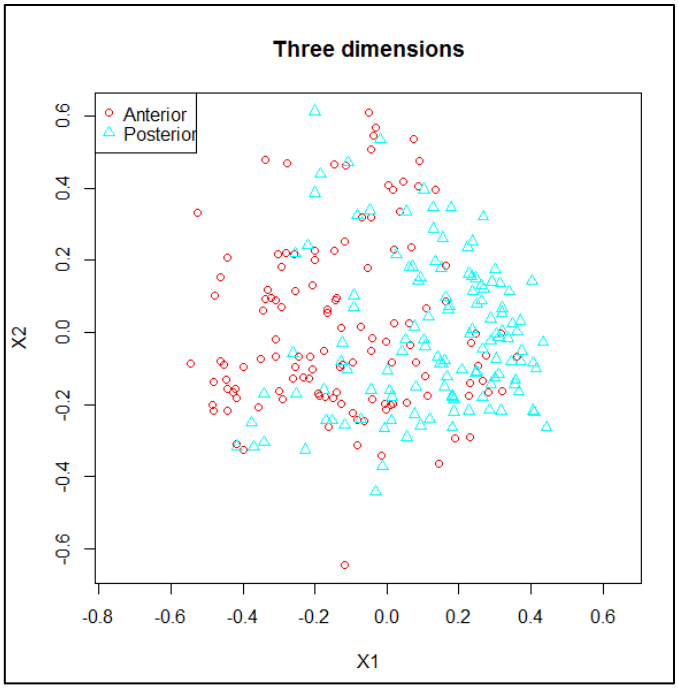

Figure 5.14. NMDS ordinations for aboveground arthropod community structure (by Order) by positions of sticky traps in summer 2013 at Snook, Texas.

The ISA results showed seven indicator Orders among aboveground arthropods in summer 2013. They were Psocoptera, Thysanoptera, Coleoptera, Diptera, Blattodea, Collembola, and Hemiptera (Table 5.3).

Table 5.3. Indicator species analysis by Order for aboveground arthropods collected via sticky traps in summer 2013 at Snook, Texas.

Type	Order	Indicator value	P value
Sticky traps	Psocoptera	0.0909	0.033
	Thysanoptera	0.0391	0.022
	Coleoptera	0.0628	0.018
	Diptera	0.0586	0.041
	Blattodea	0.1522	0.004
	Collembola	0.3947	0.002
	Hemiptera	0.1074	0.011

Abundance

The full model showed a significant difference in Day ($p < 0.0001$) and Height ($p = 0.0002$) and interactions between Day x Height ($p = 0.0493$), Height x Position ($p < 0.0001$), Day x Height x Position ($p = 0.0097$). There was a significant difference on Day 0 between Control x Post-7 with $p = 0.0005$ and there was no significant difference was found in abundance between treatments in all other sampling days ($p > 0.05$). Resilience was tested for all treatments and resilience was observed on Day 21 for Control carcasses while there was loss of resistance on Day 40 for Post-7 and Post-14 carcasses (Table 5.4). Average abundance of arthropods according to Orders collected at sticky trap in 2013 trial was demonstrated in Figure 5.15. For Diptera, there was a significant difference on Day 3 between Control x Post-7 ($p = 0.0367$). Thysanoptera was difference significantly on Day 0 between Control x Post-7 ($p = 0.0082$). For Hemiptera, there was significant difference on Day 10 between Post-7 x Post-14 ($p = 0.0300$). The abundance of Hymenoptera was significantly different on Day 0 between Control x Post-7 ($p = 0.0082$) and Control x Post-14 ($p = 0.0253$), and also on Day 10 (Control x Post-14, $p = 0.0419$) (Figure 5.16).

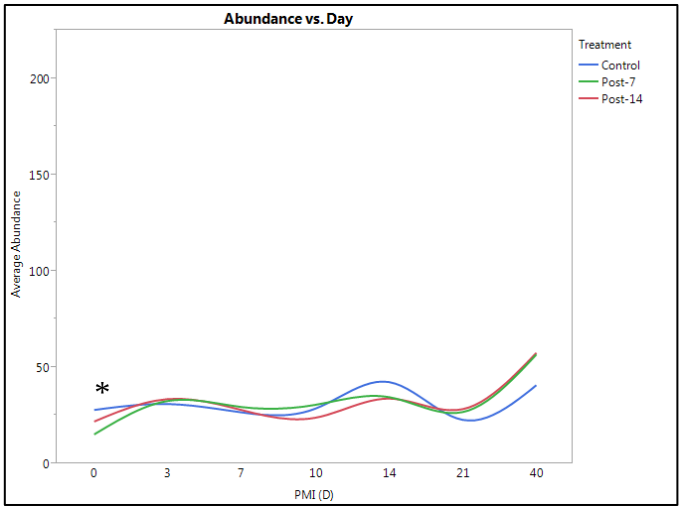

Figure 5.15. Aboveground arthropod community abundance (by Order) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (* denotes significant difference).

Table 5.4. Resilience for aboveground arthropod community abundance (by Order) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 14	0.0109	21
Post-7	0 x 40	0.0010	Loss of resistance on day 40
Post-14	0 x 40	<0.0001	Loss of resistance on day 40

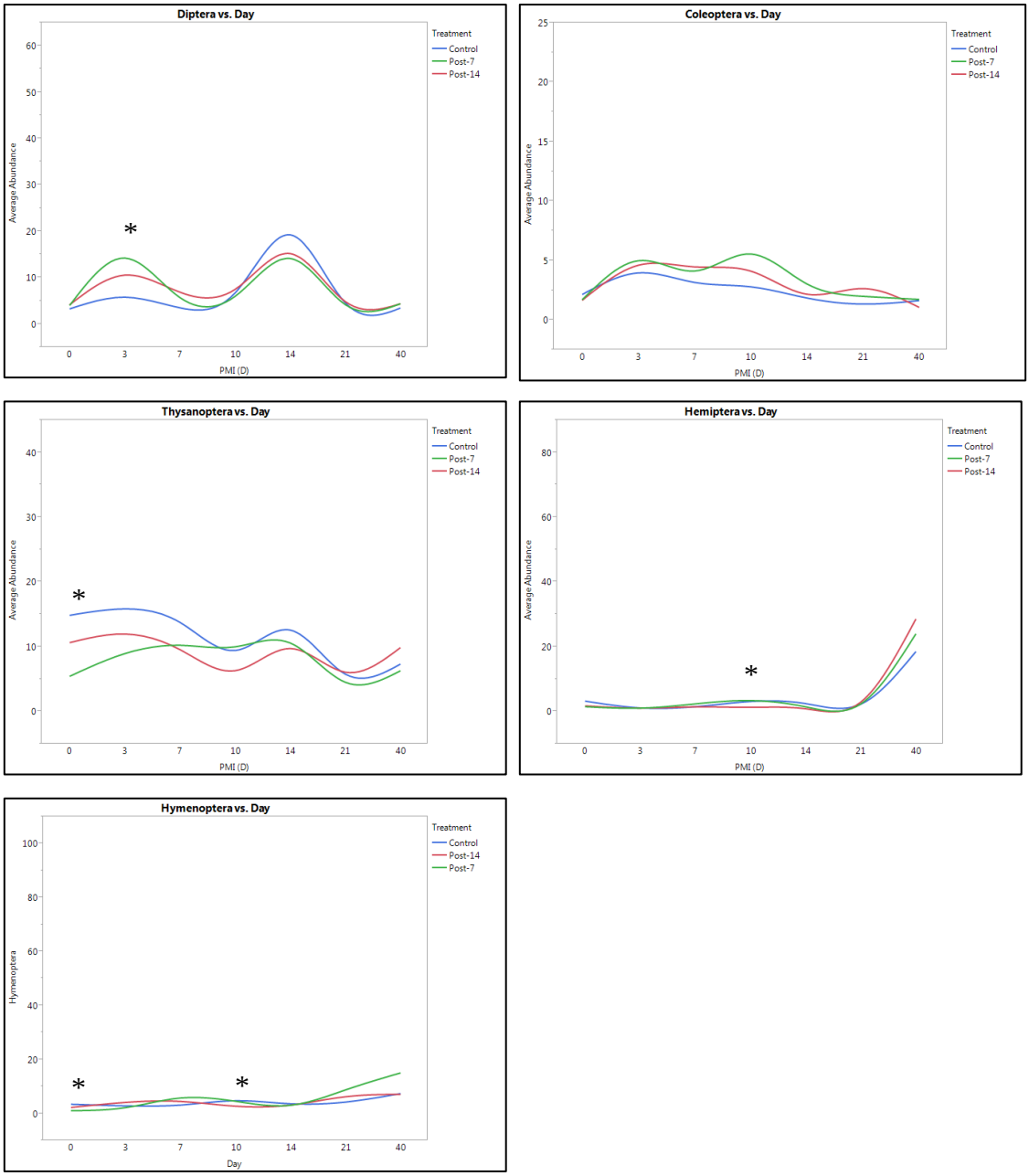

Figure 5.16. Average abundance of aboveground arthropods according to Orders collected via sticky traps in summer 2013 at Snook, Texas. Upper Left. Abundance of Diptera across Treatments over time. Upper Right. Abundance of Coleoptera across Treatments over time. Middle Left. Abundance of Thysanoptera across Treatments over time. Middle Right. Abundance of Hemiptera across Treatments over time. Lower Left. Abundance of Hymenoptera across Treatments over time (* represent significantly different).

Richness

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p < 0.0001$) and Position ($p = 0.0035$) and interactions between Day x Height ($p = 0.0055$), Day x Position ($p = 0.0004$), Height x Position ($p = 0.0480$) and Treatment x Height x Position ($p = 0.0417$). There was no significant difference was found in richness between treatments in all sampling days ($p > 0.05$) (Figure 5.17). In other words, the system was stable that no divergence or convergence was observed. Resilience was tested for all treatments and Control and Post-7 carcasses demonstrated resistance throughout the days while there was loss of resistance on Day 40 for Post-14 carcasses (Table 5.5).

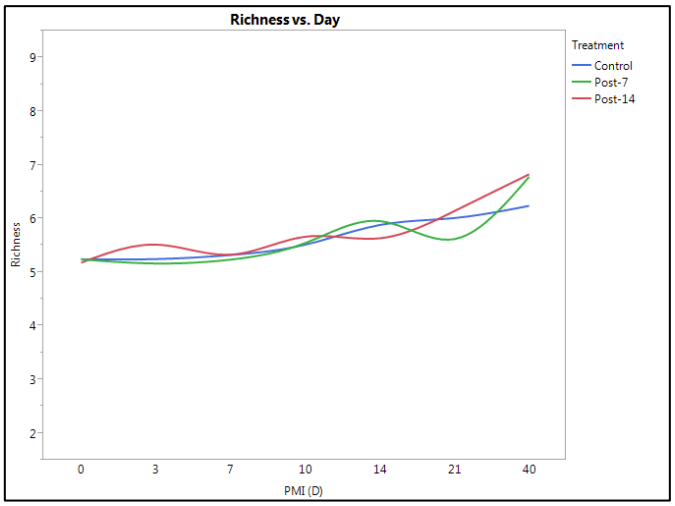

Figure 5.17. Aboveground arthropod community richness (by Order) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.5. Resilience for aboveground arthropod community richness (by Order) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.3511	Resistance
Post-7	None	0.0407	Resistance
Post-14	0 x 40	0.0196	Loss of resistance on Day 40

Simpson's diversity index

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p < 0.0001$) and Position ($p = 0.0003$) and interactions between Day x Height ($p = 0.0125$), Day x Position ($p < 0.0001$), Treatment x Position ($p = 0.0117$) and Height x Position ($p = 0.0133$). There was no significant difference was found in Simpson's Diversity between treatments in all sampling days ($p > 0.05$), although there was a marginal significant difference on Day 21 ($p = 0.0604$) (Figure 5.18). Resilience was tested for all treatments and Control and Post-7 carcasses demonstrated resistance throughout the days while there was loss of resistance on Day 40 for Post-14 carcasses (Table 5.6).

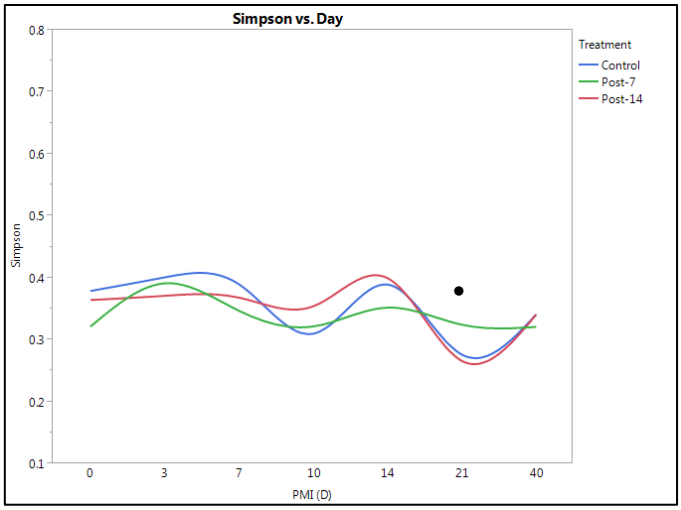

Figure 5.18. Simpson's diversity of the aboveground arthropod community (by Order) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (• denotes marginal significant difference).

Table 5.6. Resilience for Simpson's Diversity of the aboveground arthropod community (by Order) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0279*	Resistance
Post-7	None	0.4707	Resistance
Post-14	None	0.0614	Resistance

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Height ($p = 0.0210$), Day x Position ($p < 0.0001$), and Treatment x Position ($p = 0.0170$). There was no significant difference was found in Shannon-Wiener's Diversity between treatments in all sampling days ($p > 0.05$) (Figure 5.19). In other words, the system was resistance. Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.7).

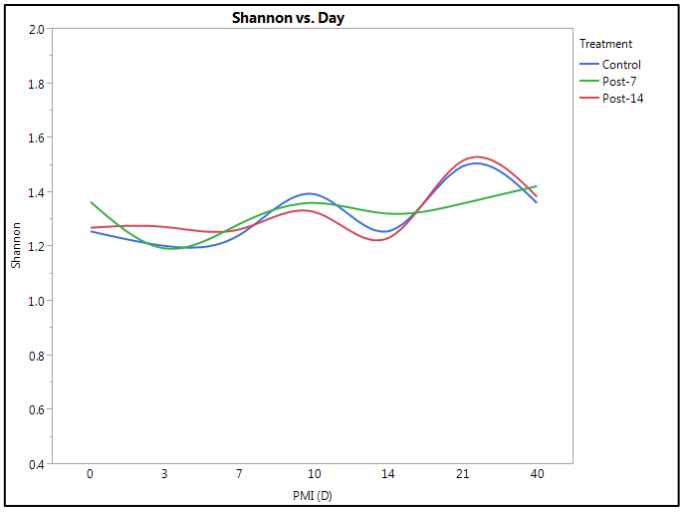

Figure 5.19. Shannon-Wiener's diversity of the aboveground arthropod community (by Order) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.7. Resilience for Shannon-Wiener's Diversity of the aboveground arthropod community (by Order) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0690	Resistance
Post-7	None	0.4292	Resistance
Post-14	None	0.0498*	Resistance[#]

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Evenness

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p = 0.0048$) and Position ($p = 0.0009$) and interactions between Day x Height ($p < 0.0001$), Day x Position ($p < 0.0001$), Treatment x Position ($p = 0.0251$), Height x Position ($p < 0.0001$) and Day x Height x Position ($p = 0.0369$). There was no significant difference was found in evenness between treatments in all sampling days ($p > 0.05$) (Figure 5.20).

In other words, the system was resistance. Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.8).

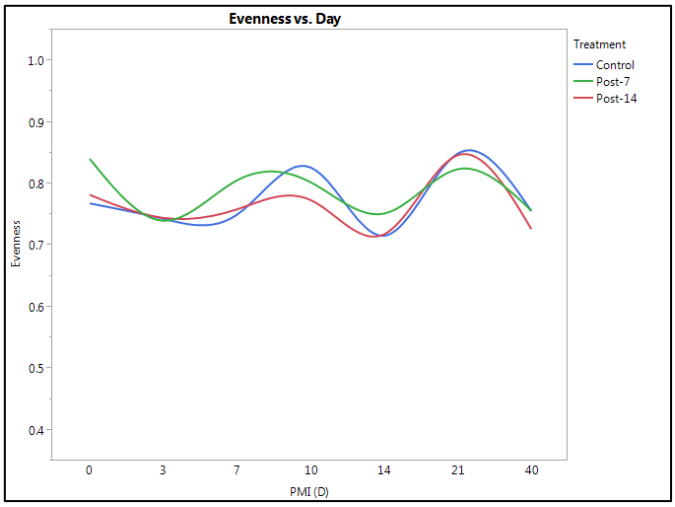

Figure 5.20. Evenness of the aboveground arthropod community (by Order) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.8. Resilience for evenness of the aboveground arthropod community (by Order) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0115*	Resistance#
Post-7	None	0.0502•	Resistance
Post-14	None	0.0273*	Resistance#

• Marginal significant difference.
= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Effective number of species

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Height ($p = 0.0056$), Day x Position ($p < 0.0001$), and Treatment x Position ($p = 0.0200$). There was no significant difference was found in ENS between treatments in all sampling days ($p > 0.05$) (Figure 5.21). In other words, the system was resistance. Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.9).

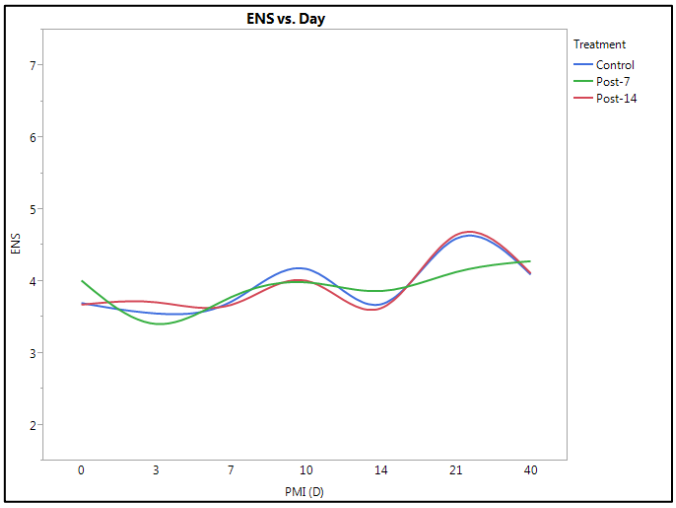

Figure 5.21. Effective Number of Species of the aboveground arthropod community (by Order) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.9. Resilience for ENS of the aboveground arthropod community (by Order) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1055	Resistance
Post-7	None	0.4649	Resistance
Post-14	None	0.0295	Resistance[#]

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Family in 2013

PERMANOVA was performed on aboveground arthropod data by Family level. Results showed that there was Day, Treatment, Height, and Position effects ($p < 0.05$).There were interactions ($p < 0.05$) between Day x Height, Day x Position and Height x Position (Table 5.10).

Table 5.10. Analysis of the aboveground arthropod community structure (by Family) collected via sticky traps in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	33.624	0.001*
Treatment	2	1.807	0.018*
Height	1	11.039	0.001*
Position	1	19.121	0.001*
Day x Treatment	2	1.051	0.373
Day x Height	1	2.921	0.002*
Treatment x Height	2	0.882	0.618
Day x Position	1	2.912	0.001*
Treatment x Position	2	0.784	0.756
Height x Position	1	3.033	0.002*
Day x Treatment x Height	2	0.455	0.992
Day x Treatment x Position	2	0.456	0.995
Day x Height x Position	1	0.686	0.773
Treatment x Height x Position	2	0.539	0.984
Day x Treatment x Height x Position	2	0.426	0.997

Since there was significant effect in Day and Treatment, further analyses were carried out. For day of decomposition, all day to day comparisons were significantly different, except Day 7 x Day 10 where there was no significant difference (Table 5.11). As for Treatment effect, significant difference was found between Control x Post-7 ($p = 0.034$) (Table 5.12). The NMDS plot of stress for aboveground arthropod community structure (Figure 5.22) and NMDS ordinations for Day, Treatment, Height, and Position were provided for visualization about data distribution (Figure 5.23, 5.24, 5.25 and 5.26, respectively). Minimum stress for given dimensionality was 0.2205 with $r^2 = 0.7023$. The MRPP analysis for day showed a significant difference (A value = 0.1358; Significant of Delta = 0.001 based on 999 permutations). The MRPP for treatment demonstrated significant difference with A value 0.0020 and Significant of Delta 0.084. The MRPP for height also showed a significant difference with A value 0.0189 and Significant of Delta 0.001 while the MRPP for position was significantly different with A value 0.0344 and Significant of Delta 0.001.

Table 5.11. Pairwise comparisons of aboveground arthropod community structure (by Family) collected via sticky traps between carrion decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.001*	0.002*	0.001*	0.001*	0.001*	0.001*
3	0.001*	-	0.003*	0.001*	0.001*	0.001*	0.001*
7	0.002*	0.003*	-	0.043*	0.001*	0.001*	0.001*
10	0.001*	0.001*	0.043*	-	0.001*	0.001*	0.001*
14	0.001*	0.001*	0.001*	0.001*	-	0.001*	0.001*
21	0.001*	0.001*	0.001*	0.001*	0.001*	-	0.001*
40	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	-

Table 5.12. Pairwise comparisons of aboveground arthropod community structure (by Family) collected via sticky traps between treatments in summer 2013 at Snook, Texas after Bonferroni's correction.

Treatment	df	SS	MS	F model	R2	P value
Control x Post-7	1	0.501	0.5008	1.7617	0.0105	0.034*
Residual	166	47.188	0.2846		0.9895	
Total	167	47.689			1.0000	
Control x Post-14	1	0.378	0.3777	1.3914	0.0083	0.146
Residual	166	45.074	0.2715		0.9917	
Total	167	45.452			1.0000	
Post-7 x Post-14	1	0.329	0.3288	1.1843	0.0070	0.251
Residual	166	46.091	0.2776		0.9930	
Total	167	46.420			1.0000	

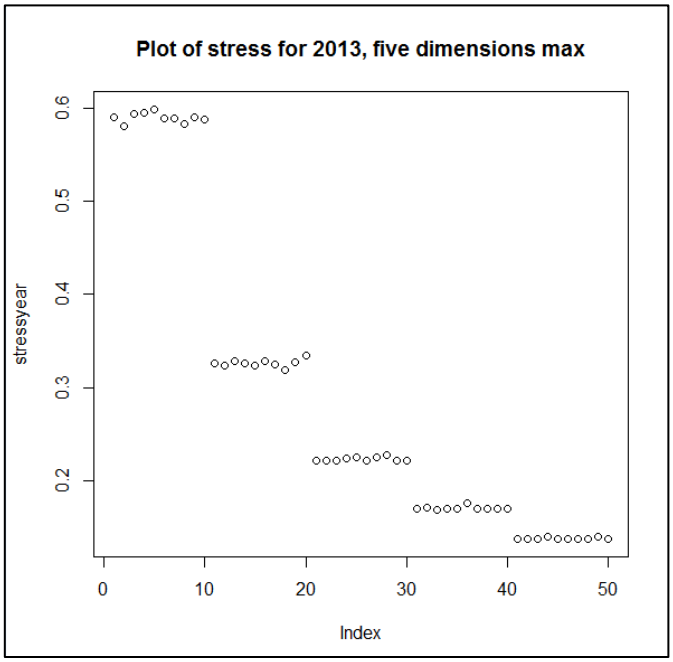

Figure 5.22. NMDS plot of stress for aboveground arthropod community structure (by Family) collected via sticky traps in summer 2013 at Snook, Texas (Stress test 0.2205; r^2 = 0.7023).

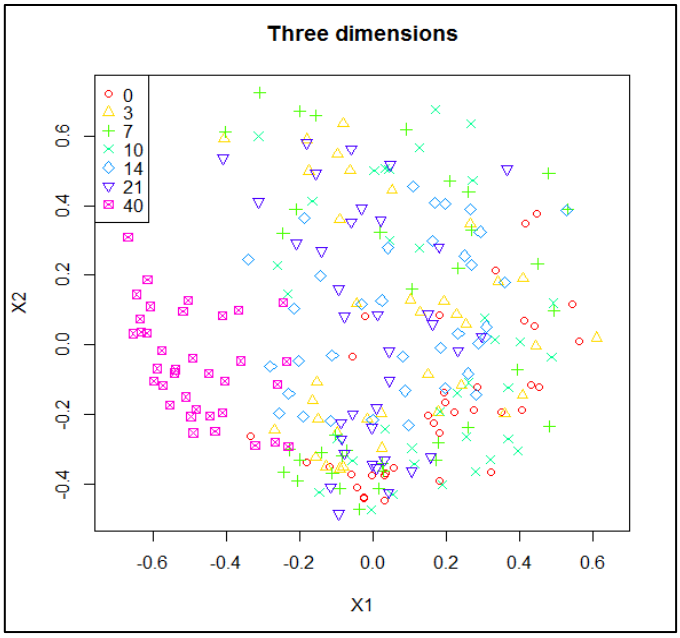

Figure 5.23. NMDS ordinations for aboveground arthropod community structure by carrion decomposition days (by Family) collected via sticky traps in summer 2013 at Snook, Texas.

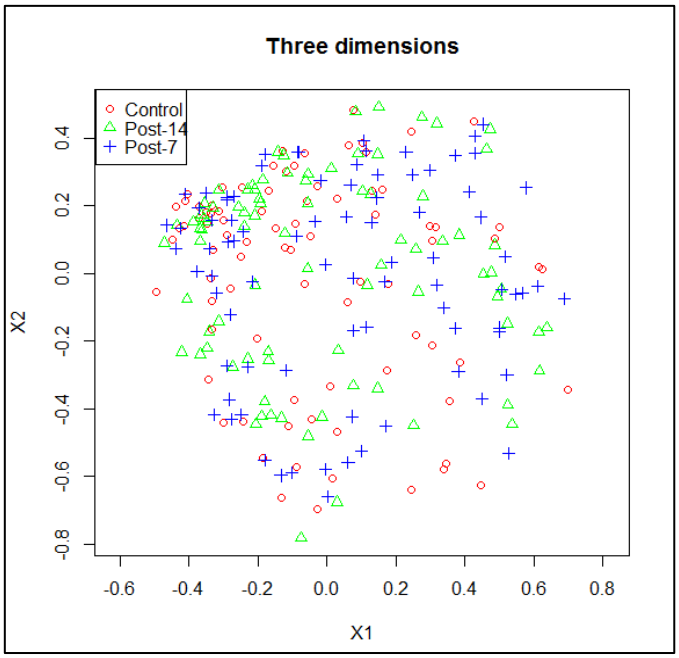

Figure 5.24. NMDS ordinations for aboveground arthropod community structure by Treatments (by Family) collected via sticky traps in summer 2013 at Snook, Texas.

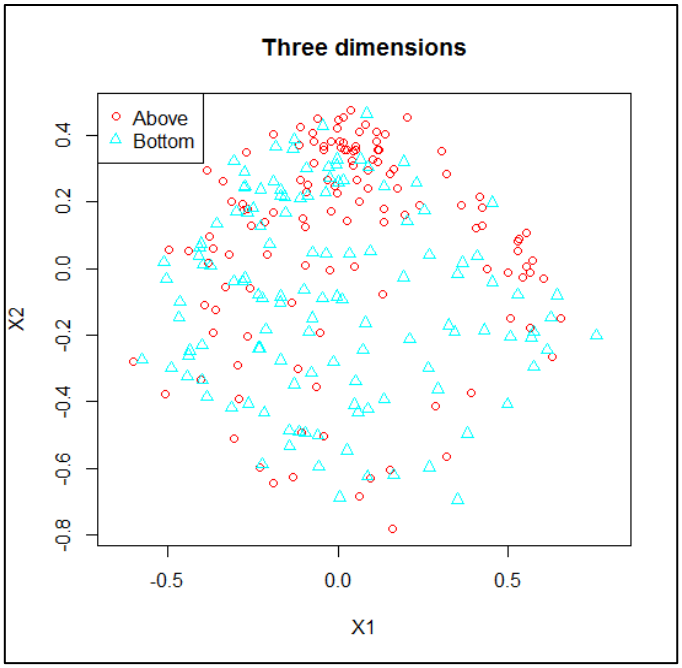

Figure 5.25. NMDS ordinations for aboveground arthropod community structure by heights (by Family) of sticky traps in summer 2013 at Snook, Texas.

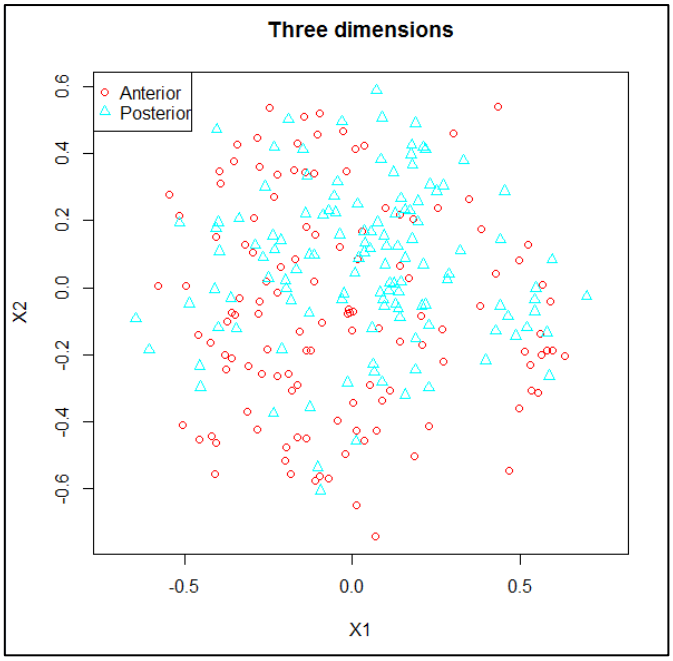

Figure 5.26. NMDS ordinations for aboveground arthropod community structure by positions (by Family) of sticky traps in summer 2013 at Snook, Texas.

For ISA, results demonstrated 23 indicator families among aboveground arthropods collected via sticky traps in summer 2013 at the field site (Table 5.13).

Table 5.13. Indicator species analysis by Family for aboveground arthropods collected via sticky traps in summer 2013 at Snook, Texas.

Type	Family	Indicator value	P value
Sticky traps	Rhyparochromidae	0.3000	0.004*
	Lachesilidae	0.7000	0.002*
	Latridiidae	0.1111	0.028*
	Thripidae	0.0390	0.030*
	Chrysomelidae	0.1461	0.018*
	Muscidae	0.1824	0.001*
	Anthocoridae	0.0752	0.030*
	Fanniidae	0.4000	0.019*
	Scarabaeidae	0.2041	0.005*
	Dolichopodidae	0.1252	0.003*
	Syrphidae	0.4444	0.001*
	Sarcophagidae	0.1087	0.014*
	Scelionidae	0.1304	0.013*
	Mymaridae	0.1020	0.030*
	Psyllidae	0.1333	0.037*
	Diapriidae	0.1667	0.015*
	Ectobiidae	0.1538	0.006*
	Pipuncluidae	0.6667	0.011*
	Tetranychidae	0.3478	0.004*
	Aphididae	0.1558	0.009*
	Trichogrammatidae	0.1040	0.027*
	Sminthuridae	0.4412	0.002*
	Culicidae	0.2857	0.036*

Abundance

The full model showed a significant difference in Day ($p < 0.0001$) and Height ($p = 0.0002$) and interactions between Height x Position ($p < 0.0001$), Day x Height x Position ($p = 0.0238$). There was a significant difference on Day 0 between Control x Post-7 with $p = 0.0019$ and there was no significant difference was found in abundance between treatments in all other sampling days ($p > 0.05$) (Figure 5.27). Resilience was tested for all treatments and resilience was observed on Day 21 for Control carcasses while there was loss of resistance on Day 40 for Post-7 and Post-14 carcasses (Table 5.14). Average abundance of arthropods according to Families collected at sticky trap in 2013 trial were demonstrated in Figure 5.28. For Thripidae was difference significantly on Day 0 between Control x Post-7 ($p = 0.0081$). For Calliphoridae, there was a significant difference on Day 3 between Control x Post-7 ($p = 0.0353$), Control x Post-14 ($p < 0.0001$), and Post-7 x Post-14 ($p = 0.0271$) and a significant difference on Day 14 ($p = 0.0496$). Muscidae also demonstrated a significant difference on Day 3 between Control x Post-7 ($p = 0.0249$).

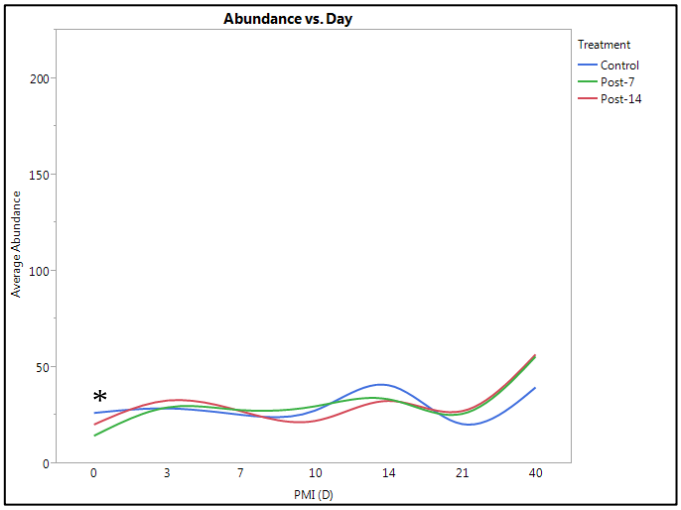

Figure 5.27. Aboveground arthropod community abundance (by Family) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (* denotes significant difference).

Table 5.14. Resilience for aboveground arthropod community (by Family) abundance collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 14	0.0144	21
Post-7	0 x 40	0.0010	Loss of resistance on Day 40
Post-14	0 x 40	<0.0001	Loss of resistance on Day 40

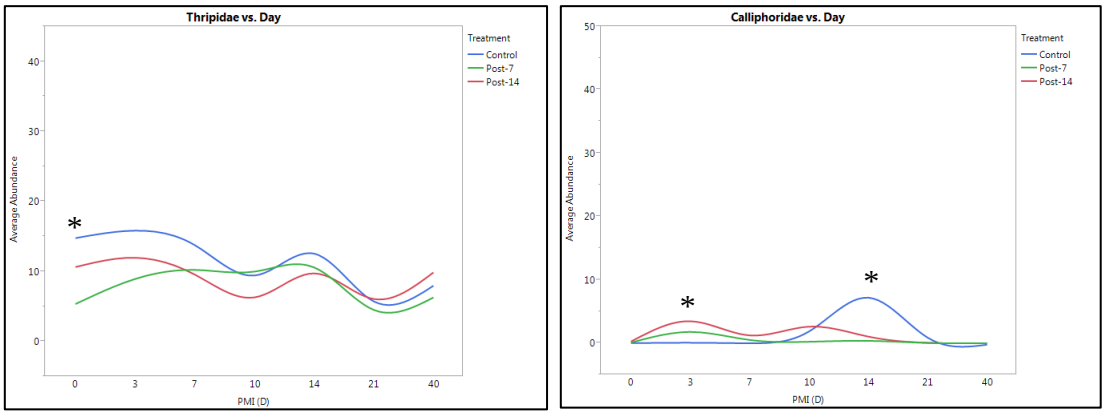

Figure 5.28. Average abundance of aboveground arthropods according to Families collected via sticky traps in summer 2013 at Snook, Texas. Upper Left. Abundance of Thripidae across Treatments over time. Upper Right. Abundance of Calliphoridae across Treatments over time. Middle Left. Abundance of Sarcophagidae across Treatments over time. Middle Right. Abundance of Muscidae across Treatments over time. Lower Left. Abundance of Dolichopodidae across Treatments over time. Lower Right. Abundance of Aphididae (* represents significantly different).

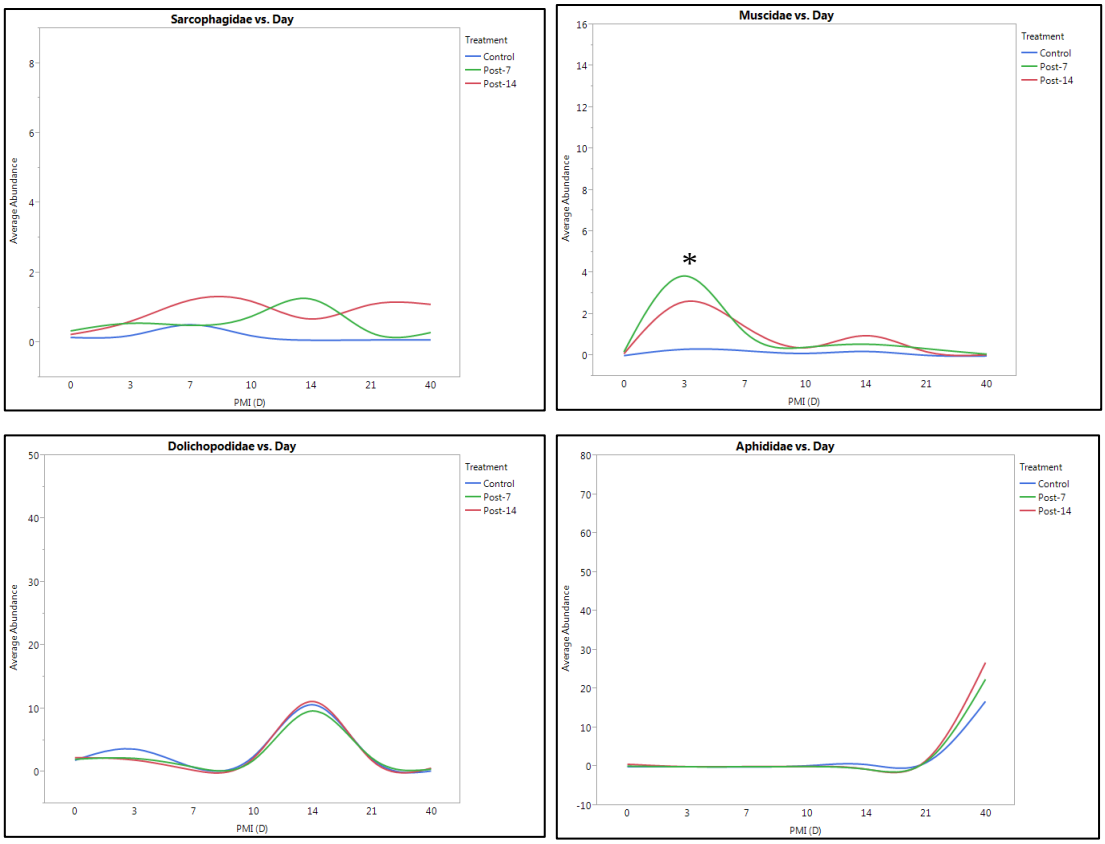

Figure 5.28 (Continued).

Richness

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Position ($p < 0.0001$), Height x Position ($p < 0.0001$) and Day x Height x Position ($p < 0.0001$). There was a significant difference (divergence) in richness between Control x Post-14 on Day 3 ($p = 0.0191$) (Figure 5.29). Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.15).

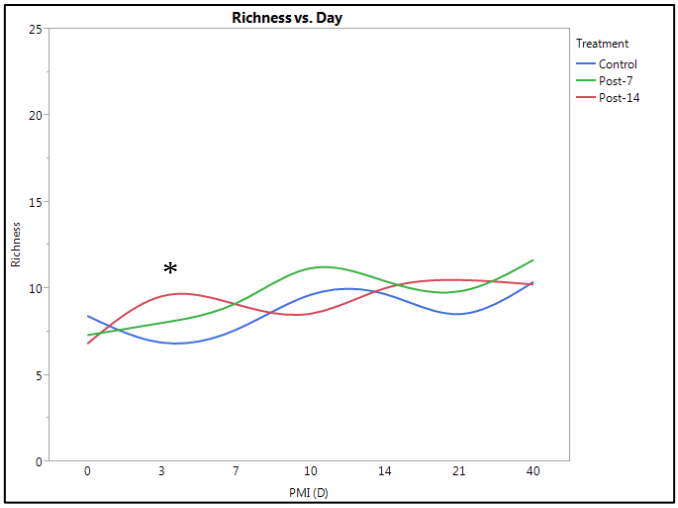

Figure 5.29. Aboveground arthropod community richness (by Family) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.15. Resilience for aboveground arthropod community (by Family) richness collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0936	Resistance
Post-7	None	0.0639•	Resistance
Post-14	None	0.1079	Resistance

• Marginal significant difference.

Simpson's diversity index

The full model showed a significant difference in Day ($p < 0.0001$), Treatment ($p = 0.0090$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Height ($p = 0.0025$), Day x Position ($p < 0.0001$), and Height x Position ($p = 0.0275$). There was no significant difference was found in Simpson's Diversity between treatments in all sampling days ($p > 0.05$) (Figure 5.30). In other words, the system was

resistance. Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.16).

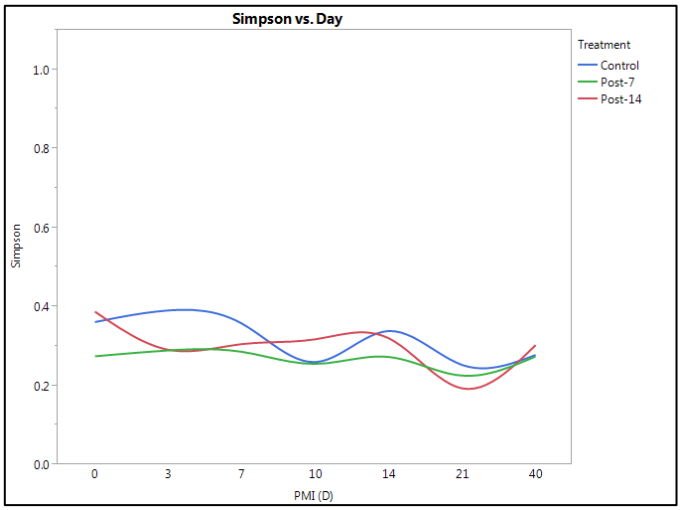

Figure 5.30. Simpson's diversity of the aboveground arthropod community (by Family) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.16. Resilience for Simpson's Diversity of the aboveground arthropod community (by Family) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0767	Resistance
Post-7	None	0.7602	Resistance
Post-14	None	0.1261	Resistance

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p < 0.001$), Treatment ($p = 0.0240$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Position ($p < 0.0001$), and Day x Height x Position ($p = 0.0264$). There was no

significant difference was found in Shannon-Wiener's Diversity between treatments in all sampling days ($p > 0.05$) (Figure 5.31). In other words, the system was resistance. Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.17).

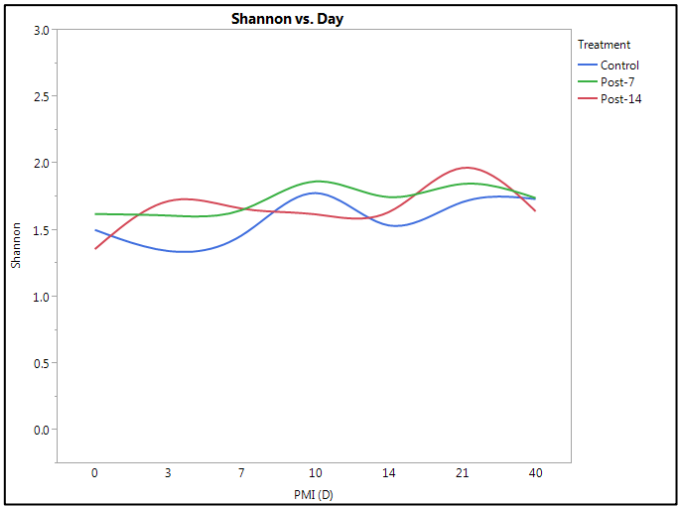

Figure 5.31. Shannon-Wiener's diversity of the aboveground arthropod community (by Family) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.17. Resilience for Shannon-Wiener's Diversity of the aboveground arthropod community (by Family) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1109	Resistance
Post-7	None	0.5044	Resistance
Post-14	None	0.0889	Resistance

Evenness

The full model showed a significant difference in Day ($p < 0.0001$), Treatment ($p = 0.0218$), Height ($p < 0.0001$) and Position ($p = 0.0009$) and interactions between Day x Height ($p = 0.0003$), Day x Position ($p < 0.0001$), Treatment x Position ($p = 0.0453$), and Height x Position ($p < 0.0001$). There was a significant difference in evenness on Day 0 ($p = 0.0441$) (Figure 5.32). Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.18).

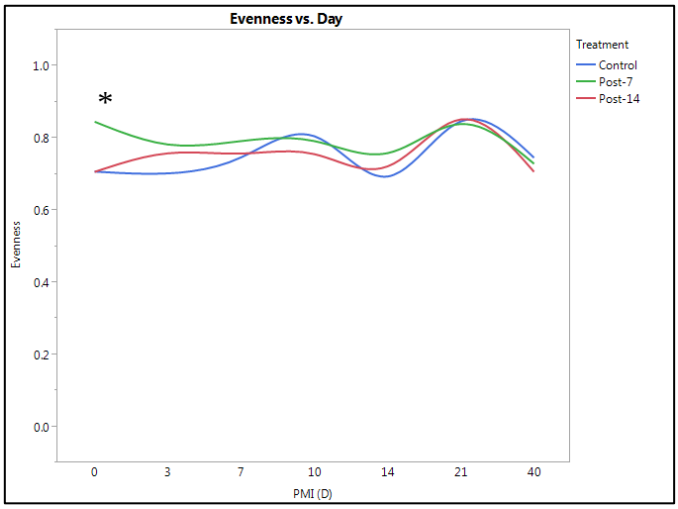

Figure 5.32. Evenness of the aboveground arthropod community (by Family) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.18. Resilience for evenness of the aboveground arthropod community (by Family) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0152*	Resistance[#]
Post-7	None	0.1082	Resistance
Post-14	None	0.1072	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Effective number of species

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Position ($p < 0.0001$), Height x Position ($p = 0.0230$) and Day x Height x Position ($p = 0.0101$). There was no significant difference was found in ENS between treatments in all sampling days ($p > 0.05$) (Figure). In other words, the system was resistance. Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.19).

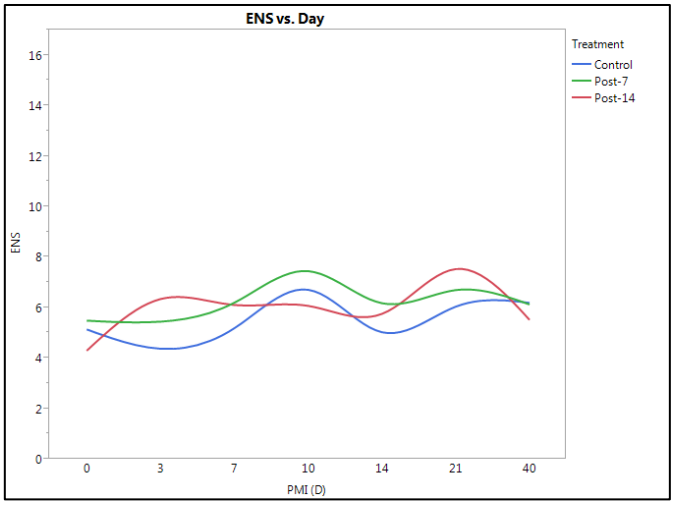

Figure 5.33. Effective Number of Species of the aboveground arthropod community (by Family) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.19. Resilience for ENS of the aboveground arthropod community (by Family) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2122	Resistance
Post-7	None	0.4960	Resistance
Post-14	None	0.0863	Resistance

Genus and species in 2013

PERMANOVA was performed on aboveground arthropod data by Genus and species level. Results showed that there was Day, Treatment, Height, and Position effects ($p < 0.05$).There was an interaction between Day x Treatment ($p = 0.002$) (Table 5.20).

Table 5.20. Analysis of the aboveground arthropod community structure (by Genus and species) collected via sticky traps in summer 2013 using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	8.6051	0.001*
Treatment	2	4.7516	0.001*
Height	1	6.5113	0.001*
Position	1	5.4548	0.001*
Day x Treatment	2	2.6019	0.002*
Day x Height	1	1.3557	0.198
Treatment x Height	2	1.4240	0.142
Day x Position	1	1.7580	0.104
Treatment x Position	2	0.5890	0.897
Height x Position	1	1.0766	0.331
Day x Treatment x Height	2	0.7905	0.679
Day x Treatment x Position	2	0.5392	0.944
Day x Height x Position	1	0.5659	0.824
Treatment x Height x Position	2	0.3922	0.994
Day x Treatment x Height x Position	2	0.5911	0.909

Since there was significant effect in Day and Treatment, further analyses were carried out. For day of decomposition, all day to day comparisons were significantly different, except Day 7 x Day 10 and Day 7 x Day 14 where there were no significant difference (Table 5.21). As for Treatment effect, significant difference was found between Control x Post-7 ($p = 0.001$), Control x Post-14 ($p = 0.007$) and Post-7 x Post-14 ($p = 0.014$) (Table 5.22). The NMDS plot of stress for aboveground arthropod community structure (Figure 5.34) and NMDS ordinations for Day, Treatment, Height, and Position were provided for visualization about data distribution (Figure 5.35, 5.36, 5.37 and 5.38, respectively). Minimum stress for given dimensionality was 0.1937 with

r^2 = 0.8066. The MRPP analysis for day showed a significant difference (A value = 0.0719; Significant of Delta = 0.001 based on 999 permutations). The MRPP for treatment demonstrated significant difference with A value 0.0214 and Significant of Delta 0.001. The MRPP for height also showed a significant difference with A value 0.0175 and Significant of Delta 0.001 while the MRPP for position was significantly different with A value 0.0106 and Significant of Delta 0.001.

Table 5.21. Pairwise comparisons of aboveground arthropod community structure (by Genus and species) collected via sticky traps between carrion decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
3	0.001*	-	0.005*	0.002*	0.008*	0.001*	0.001*
7	0.001*	0.005*	-	0.366	0.124	0.002*	0.002*
10	0.001*	0.002*	0.366	-	0.023*	0.002*	0.003*
14	0.001*	0.008*	0.124	0.023*	-	0.001*	0.001*
21	0.001*	0.001*	0.002*	0.002*	0.001*	-	0.001*
40	0.001*	0.001*	0.002*	0.003*	0.001*	0.001*	-

Table 5.22. Pairwise comparisons of aboveground arthropod community structure (by Genus and species) collected via sticky traps between treatments in summer 2013 at Snook, Texas after Bonferroni's correction.

Treatment	df	SS	MS	F model	R2	P value
Control x Post-7	1	2.320	2.3204	6.7673	0.0392	0.001*
Residual	166	56.920	0.3428		0.9608	
Total	167	59.241			1.0000	
Control x Post-14	1	1.235	1.2346	3.8518	0.0227	0.007*
Residual	166	53.208	0.3205		0.9773	
Total	167	54.443			1.0000	
Post-7 x Post-14	1	1.023	1.0232	2.7758	0.0165	0.014*
Residual	166	61.195	0.3686		0.9835	
Total	167	62.218			1.0000	

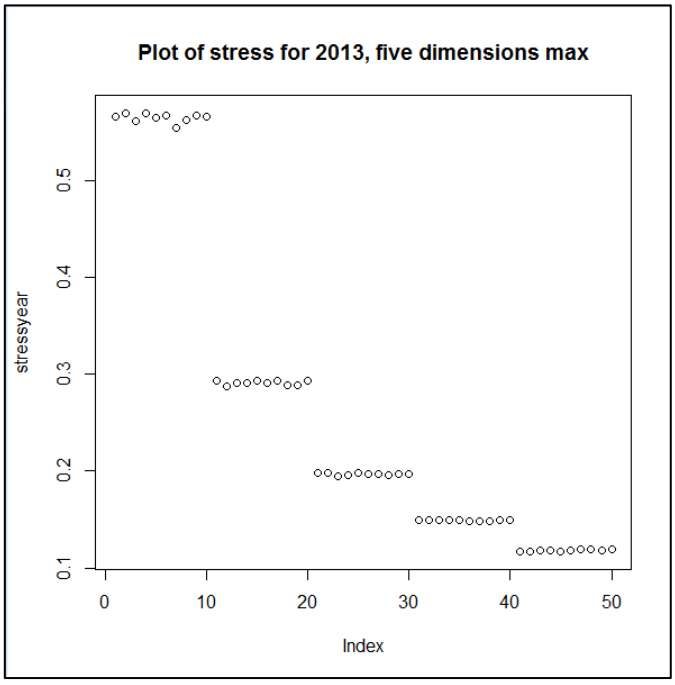

Figure 5.34. NMDS plot of stress for aboveground arthropod community structure (by Genus and species) collected via sticky traps in summer 2013 at Snook, Texas (Stress test 0.1937; $r^2 = 0.8066$).

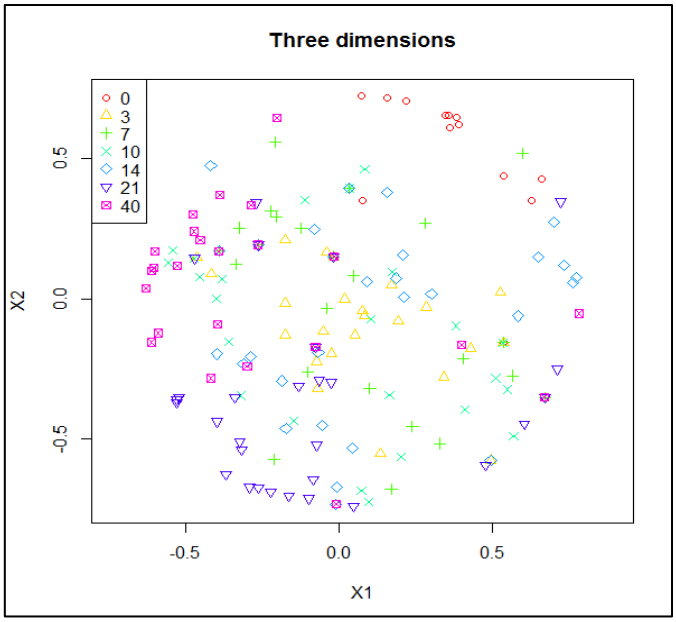

Figure 5.35. NMDS ordinations for aboveground arthropod community structure by carrion decomposition days (by Genus and species) collected via sticky traps in summer 2013 at Snook, Texas.

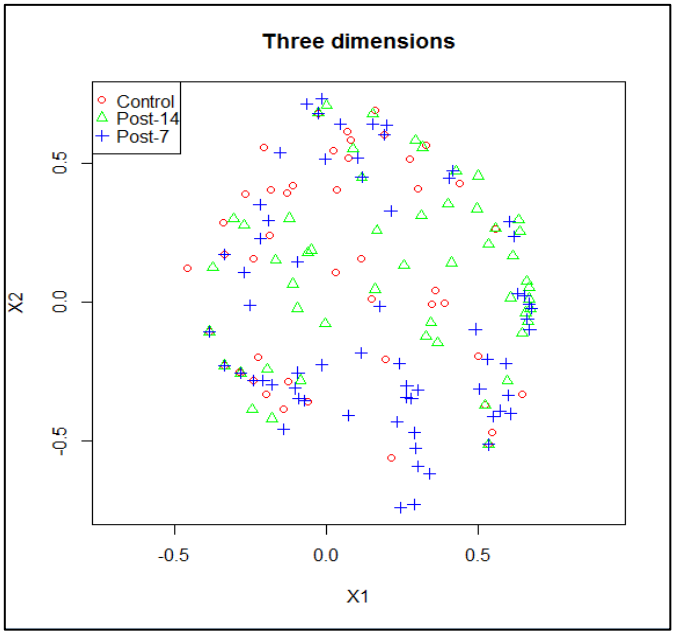

Figure 5.36. NMDS ordinations for aboveground arthropod community structure by treatments (by Genus and species) collected via sticky traps in summer 2013 at Snook, Texas.

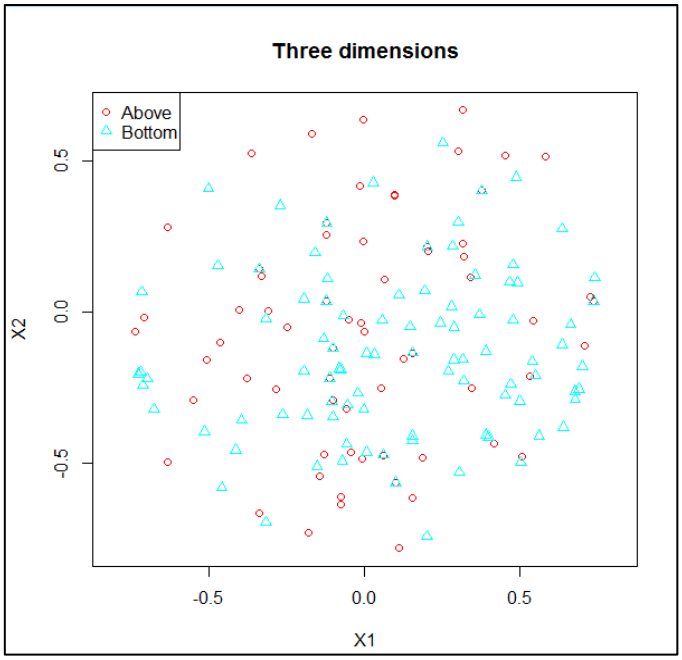

Figure 5.37. NMDS ordinations for aboveground arthropod community structure by heights (by Genus and species) of sticky traps in summer 2013 at Snook, Texas.

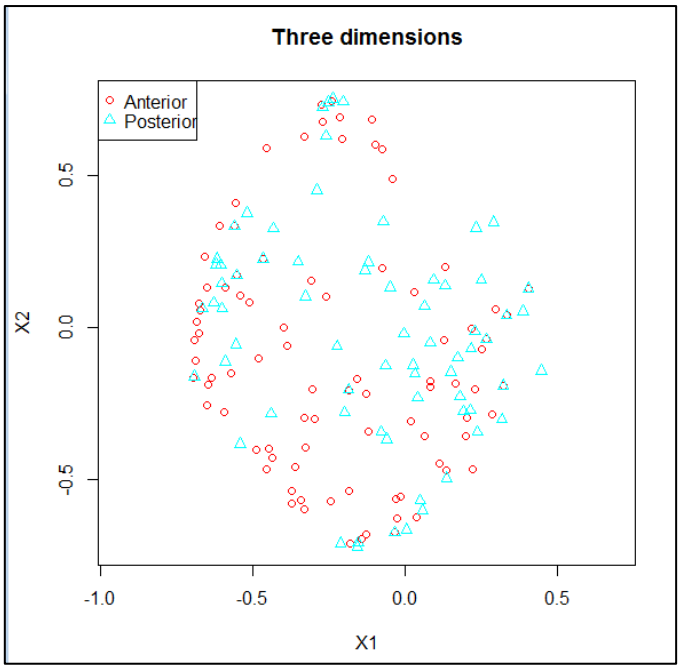

Figure 5.38. NMDS ordinations for aboveground arthropod community structure by positions (by Genus and species) of sticky traps in summer 2013 at Snook, Texas.

For ISA, results demonstrated seven indicator Genera among aboveground arthropods trapped by sticky traps in summer 2013 at Snook, Texas (Table 5.23).

Table 5.23. Indicator species analysis by Genus and species for aboveground arthropods collected via sticky traps in summer 2013 at Snook, Texas.

Type	Genus and species	Indicator value	P value
Sticky traps	*Medetera* sp.	0.5600	0.002*
	O. aenescens	0.2571	0.001*
	M. domestica	0.1364	0.014*
	Fannia sp.	0.3333	0.029*
	Co. macellaria	0.2302	0.033*
	Ataenius sp.	0.1961	0.018*
	P. fulvescens	0.1538	0.011*

Abundance

The full model showed a significant difference in Height ($p < 0.0001$), Position ($p = 0.0413$) and interactions between Treatment x Position ($p = 0.0473$) and Height x Position ($p = 0.0144$). There was a significant difference on Day 0 between Control x Post-7 with $p = 0.0011$ and between Post-7 x Post-14 with $p = 0.0007$. Also, on Day 3, there was significant difference between Control x Post-7 ($p = 0.0389$) and Control x Post-14 ($p = 0.0238$). There was a marginal significant on Day 14 ($p = 0.0553$) (Figure 5.39). Resilience was tested for all treatments and resilience was observed on Day 21 for Control carcasses while there was resistance for Post-7 and Post-14 carcasses throughout decomposition days (Table 5.24). Average abundance of arthropods according to Genera and species collected at sticky trap in 2013 trial were demonstrated in Figure 5.40. For *Co. macellaria*, significant difference was detected on Day 3 between Control x Post-7 ($p = 0.0336$) and Control x Post-14 ($p = 0.0008$). For *Ch. rufifacies*, there was a significant difference on Day 3 between Control x Post-14 ($p = 0.0012$),

Post-7 x Post-14 (p = 0.0053), as well as on Day 14 (p = 0.0353). *Hydrotaea aenescens* also demonstrated a significant difference on Day 3 between Control x Post-7 (p = 0.0115). Furthermore, *Oligosita* sp. showed a significant difference on Day 3 between Control x Post-14 (p = 0.0189).

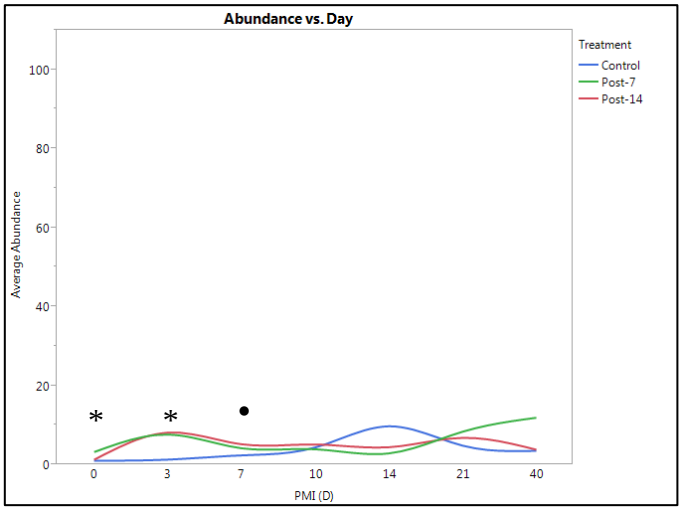

Figure 5.39. Aboveground arthropod community abundance (by Genus and species) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (* denotes significant difference; • represents marginal significant difference).

Table 5.24. Resilience for aboveground arthropod community (by Genus and species) abundance collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 14	0.0042*	21
Post-7	None	0.4988	Resistance
Post-14	None	0.0555•	Resistance

• Marginal significant difference.

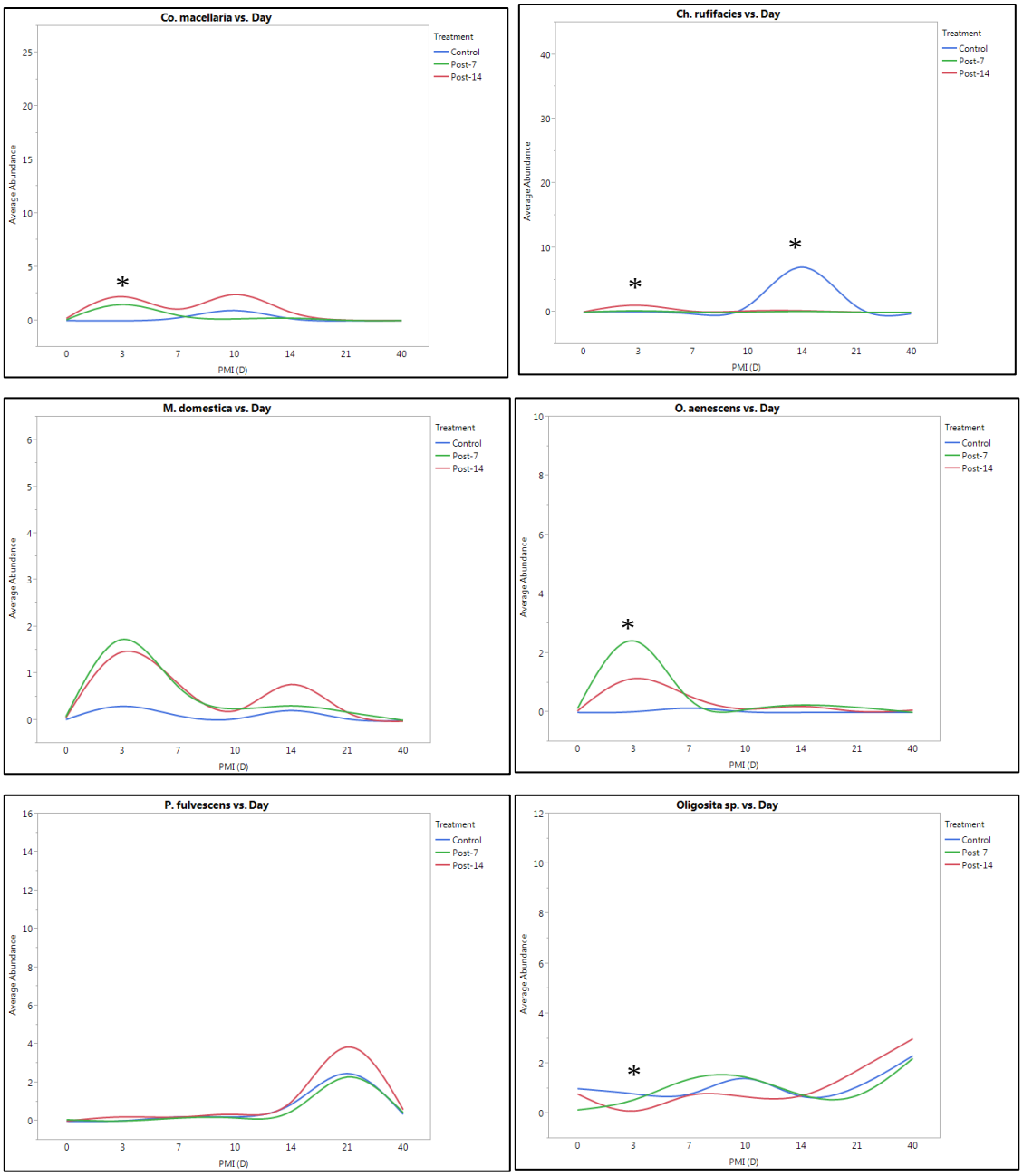

Figure 5.40. Average abundance of arthropods according to Genus and species collected via sticky trap in summer 2013 at Snook, Texas. Upper Left. Abundance of *Co. macellaria* across Treatments over time. Upper Right. Abundance of *Ch. rufifacies* across Treatments over time. Middle Left. Abundance of *M. domestica* across Treatments over time. Middle Right. Abundance of *O. aenescens* across Treatments over time. Lower Left. Abundance of *P. fulvescens* across Treatments over time. Lower Right. Abundance of *Oligosita* sp. across Treatments over time (* represents significantly different).

Richness

The full model showed a significant difference in Day ($p = 0.0002$), Treatment ($p = 0.0010$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Treatment ($p = 0.0008$), Day x Position ($p < 0.0001$), Height x Position ($p = 0.0006$) and Treatment x Position ($p = 0.0099$). There was a significant difference (divergence) in richness between Post-7 x Post-14 on Day 0 ($p = 0.0249$). Furthermore, significant difference was also detected on Day 3 between Control x Post-7 ($p = 0.0058$) and Control x Post-14 ($p = 0.0029$) (Figure 5.41). Resilience was tested for all treatments and resilience occurred on Day 21 for Control carcasses while for Post-14 carcasses, first resilience was observed on Day 7, and second resilience was observed on Day 14. Post-7 carcasses were resistance throughout the decomposition days (Table 5.25).

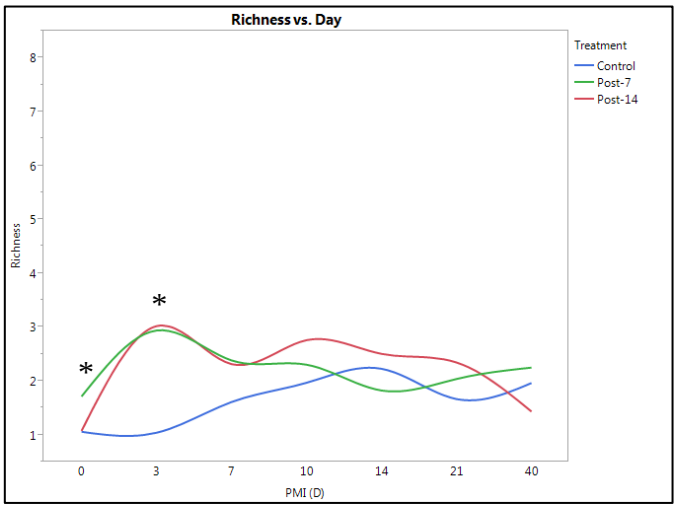

Figure 5.41. Aboveground arthropod community richness (by Genus and species) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.25. Resilience for aboveground arthropod community (by Genus and species) richness collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 14	0.0306*	21
Post-7	None	0.2838	Resistance
Post-14	0 x 3	0.0086*	7
	0 x 10	0.0415*	14

Simpson's diversity index

The full model showed a significant difference in Day ($p = 0.0004$), Treatment ($p = 0.0012$), Height ($p < 0.0001$) and Position ($p = 0.0005$) and interactions between Day x Treatment ($p = 0.0001$), Day x Position ($p < 0.0001$), Height x Position ($p = 0.0464$) and Day x Treatment x Position ($p = 0.0278$). There was a significant difference in Simpson's Diversity on Day 0 between Post-7 x Post-14 ($p = 0.0183$) and Day 3 between Control x Post-7 ($p = 0.0003$) and Control x Post-14 ($p = 0.0001$) (Figure 5.42). Resilience was tested for all treatments and resilience was observed on Day 7 and Day 40 for Post-14 carcasses while Control and Post-7 carcasses were resistance throughout the decomposition days (Table 5.26).

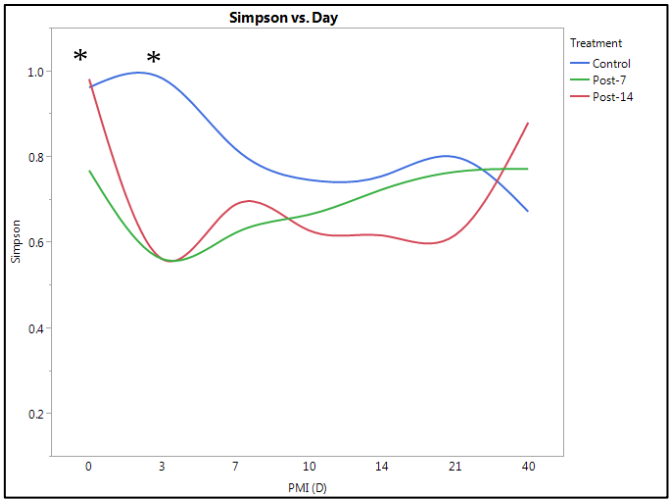

Figure 5.42. Simpson's diversity of the aboveground arthropod community (by Genus and species) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.26. Resilience for Simpson's Diversity of the aboveground arthropod community (by Genus and species) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0150*	Resistance[#]
Post-7	None	0.3630	Resistance
Post-14	0 x 3	0.0010*	7
	0 x 10	0.0133*	40
	0 x 14	0.0264*	
	0 x 21	0.0108*	

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p = 0.0001$), Treatment ($p = 0.0007$), Height ($p < 0.0001$) and Position ($p = 0.0001$) and interactions between Day x

Treatment ($p = 0.0001$), Day x Position ($p < 0.0001$), Height x Position ($p = 0.0167$) and Day x Treatment x Position ($p = 0.0437$). There was significant difference found in Shannon-Wiener's Diversity between Post-7 x Post-14 ($p = 0.0200$) on Day 0. Also, significant difference was found on Day 3 between Control x Post-7 ($p = 0.0008$) and Control x Post-14 ($p = 0.0003$) (Figure 5.43). Resilience was tested for all treatments and resilience was observed on Day 7 and Day 40 for Post-14 carcasses while Control and Post-7 carcasses were resistance throughout all decomposition days (Table 5.27).

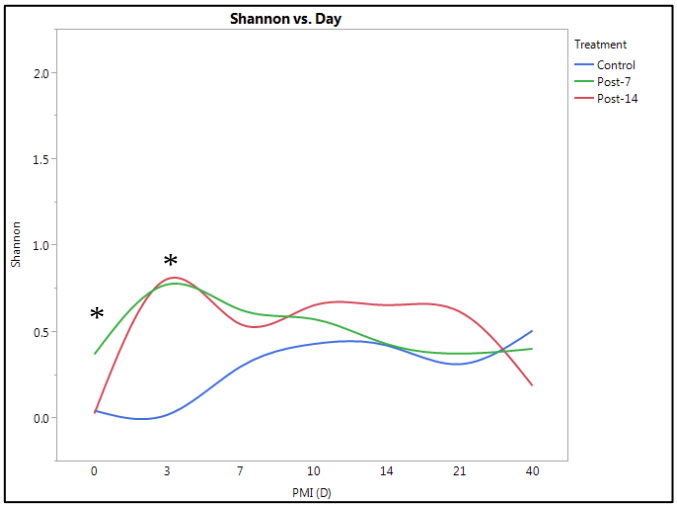

Figure 5.43. Shannon-Wiener's diversity of the aboveground arthropod community (by Genus and species) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.27. Resilience for Shannon-Wiener's Diversity of the aboveground arthropod community (by Genus and species) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0185*	Resistance[#]
Post-7	None	0.2708	Resistance
Post-14	0 x 3	0.0010*	7
	0 x 14	0.0499*	40
	0 x 21	0.0407*	

 # = There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Evenness

The full model showed a significant difference in Day ($p = 0.0021$), Treatment ($p = 0.0039$), Height ($p = 0.0005$) and Position ($p = 0.0168$) and interactions between Day x Treatment ($p = 0.0002$), and Day x Position ($p = 0.0002$). There was a significant difference in evenness on Day 0 between Post-7 x Post-14 ($p = 0.0169$), as well as on Day 3 between Control x Post-7 ($p < 0.0001$) and Control x Post-14 ($p < 0.0001$) (Figure 5.44). Resilience was tested for all treatments and resilience was observed on Day 40 for Post-14 carcasses. However, there was loss of resistance on Day 40 for Control carcasses while Post-7 carcasses demonstrated resistance throughout the decomposition days (Table 5.28).

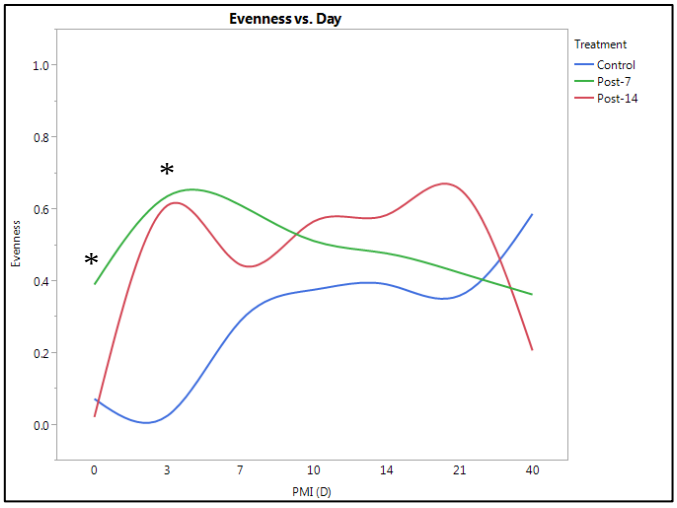

Figure 5.44. Evenness of the aboveground arthropod community (by Genus and species) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.28. Resilience for evenness of the aboveground arthropod community (by Genus and species) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 40	0.0348*	Loss of resistance on Day 40
Post-7	None	0.6409	Resistance
Post-14	0 x 3	0.0018*	40
	0 x 10	0.0069*	
	0 x 14	0.0204*	
	0 x 21	0.0011*	

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Effective number of species

The full model showed a significant difference in Day ($p = 0.0002$), Treatment ($p = 0.0012$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Treatment ($p = 0.0014$), Day x Position ($p < 0.0001$), and Height x Position ($p = 0.0161$). There was significant difference found in ENS between Post-7 x Post-14 ($p = 0.0349$) on Day 0, as well as on Day 3 between Control x Post-7 ($p = 0.0058$) and Control x Post-14 ($p = 0.0013$) (Figure 5.45). Resilience was tested for all treatments and resilience was observed on Day 7 for Post-14 carcasses while Control and Post-7 carcasses demonstrated resistance throughout the decomposition days (Table 5.29).

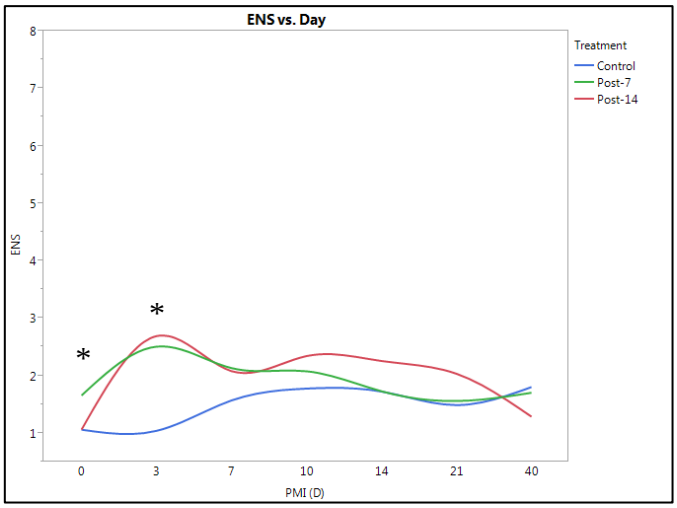

Figure 5.45. Effective Number of Species of the aboveground arthropod community (by Genus and species) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.29. Resilience for ENS of the aboveground arthropod community (by Genus and species) collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0621	Resistance
Post-7	None	0.1836	Resistance
Post-14	0 x 3	0.0041*	7

Function in 2013

PERMANOVA was performed on aboveground arthropod data by functional groups. Results showed that there was Day, Treatment, Height, and Position effects ($p < 0.05$). There were interactions between Day x Height ($p = 0.002$), Day x Position ($p = 0.001$) and Height x Position ($p = 0.001$) (Table 5.30).

Table 5.30. Analysis of the aboveground arthropod community functions collected via sticky traps in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	16.5700	0.001*
Treatment	2	2.0236	0.022*
Height	1	13.5509	0.001*
Position	1	17.2984	0.001*
Day x Treatment	2	1.3963	0.190
Day x Height	1	3.6154	0.003*
Treatment x Height	2	0.9367	0.465
Day x Position	1	4.2518	0.001*
Treatment x Position	2	0.5602	0.845
Height x Position	1	7.3122	0.001*
Day x Treatment x Height	2	0.3934	0.946
Day x Treatment x Position	2	0.2943	0.985
Day x Height x Position	1	0.7190	0.614
Treatment x Height x Position	2	0.5316	0.887
Day x Treatment x Height x Position	2	0.3698	0.963

Since there was significant effect in Day and Treatment, further analyses were carried out. For day of decomposition, all day to day comparisons were significantly different, except Day 3 x Day 7 and Day 7 x Day 10 where there were no significant difference (Table 5.31). As for Treatment effect, significant difference was found between Control x Post-7 ($p = 0.028$) (Table 5.32). The NMDS plot of stress for aboveground arthropod community structure (Figure 5.46) and NMDS ordinations for Day, Treatment, Height, and Position were provided for visualization about data distribution (Figure 5.47, 5.48, 5.49 and 5.50, respectively). Minimum stress for given dimensionality was 0.1308 with $r^2 = 0.9055$. The MRPP analysis for day showed a

significant difference (A value = 0.1086; Significant of Delta = 0.001 based on 999 permutations). The MRPP for treatment demonstrated significant difference with A value 0.0037 and Significant of Delta 0.064. The MRPP for height also showed a significant difference with A value 0.0252 and Significant of Delta 0.001 while MRPP for position was significantly different with A value 0.0322 and Significant of Delta 0.001.

Table 5.31. Pairwise comparisons of aboveground arthropod community functions collected via sticky traps between carrion decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.001*	0.047*	0.005*	0.001*	0.001*	0.001*
3	0.001*	-	0.094	0.002*	0.001*	0.001*	0.001*
7	0.047*	0.094	-	0.138	0.001*	0.001*	0.001*
10	0.005*	0.002*	0.138	-	0.001*	0.003*	0.001*
14	0.001*	0.001*	0.001*	0.001*	-	0.001*	0.001*
21	0.001*	0.001*	0.001*	0.003*	0.001*	-	0.001*
40	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	-

Table 5.32. Pairwise comparisons of aboveground arthropod community function collected via sticky traps between treatments in summer 2013 at Snook, Texas after Bonferroni's correction.

Treatment	df	SS	MS	F model	R2	P value
Control x Post-7	1	0.3555	0.3554	2.6004	0.0154	0.028*
Residual	166	22.6922	0.1367		0.9846	
Total	167	23.0476			1.0000	
Control x Post-14	1	0.1962	0.1962	1.5196	0.0091	0.184
Residual	166	21.4339	0.1291		0.9909	
Total	167	21.6301			1.0000	
Post-7 x Post-14	1	0.1301	0.1300	0.9259	0.0056	0.434
Residual	166	23.3183	0.1404		0.9944	
Total	167	23.4484			1.0000	

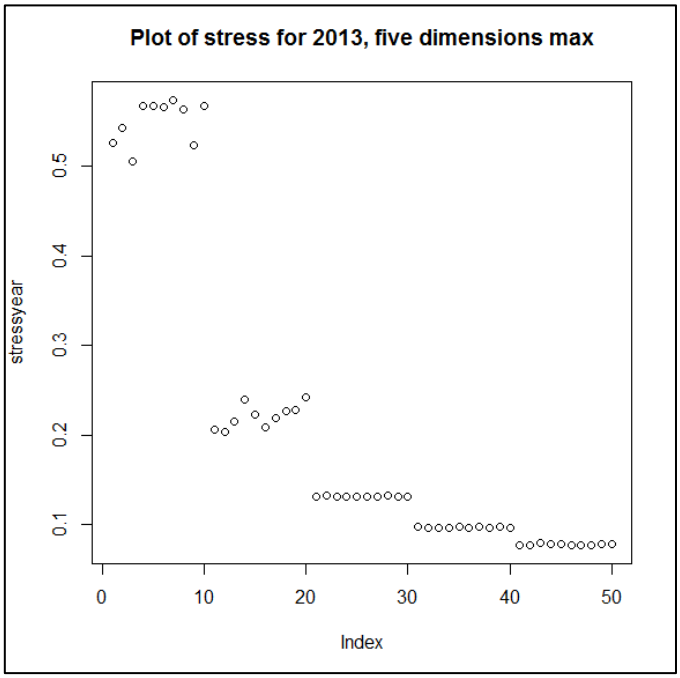

Figure 5.46. NMDS plot of stress for aboveground arthropod community functions collected via sticky traps in summer 2013 at Snook, Texas (Stress test 0.1308; r^2 = 0.9055).

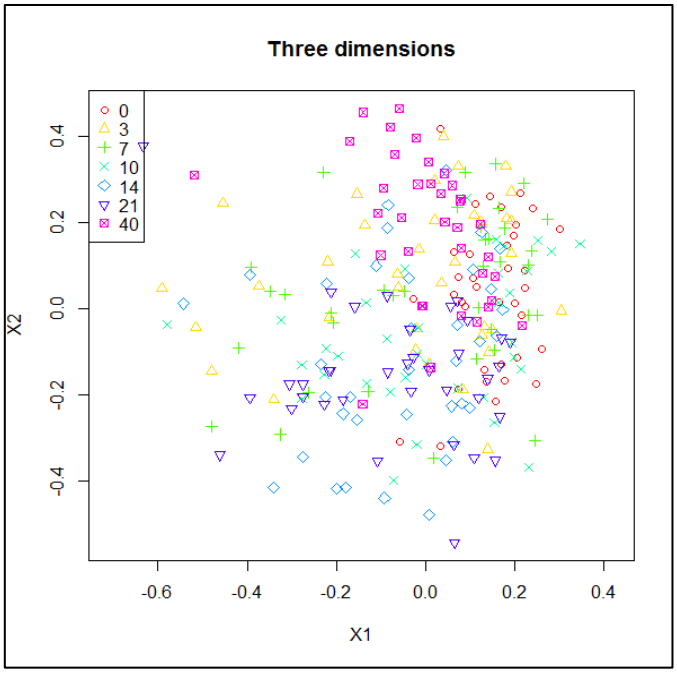

Figure 5.47. NMDS ordinations for aboveground arthropod community function by carrion decomposition days collected via sticky traps in summer 2013 at Snook, Texas.

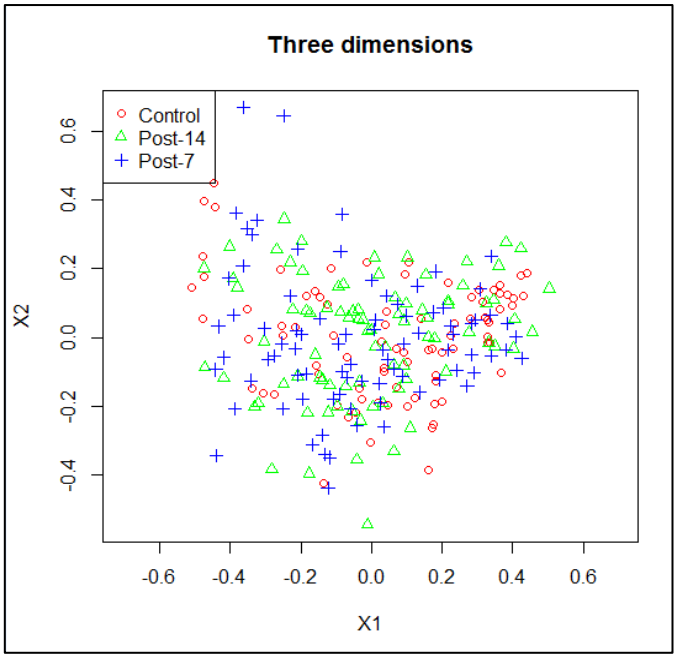

Figure 5.48. NMDS ordinations for aboveground arthropod community function by treatments collected via sticky traps in summer 2013 at Snook, Texas.

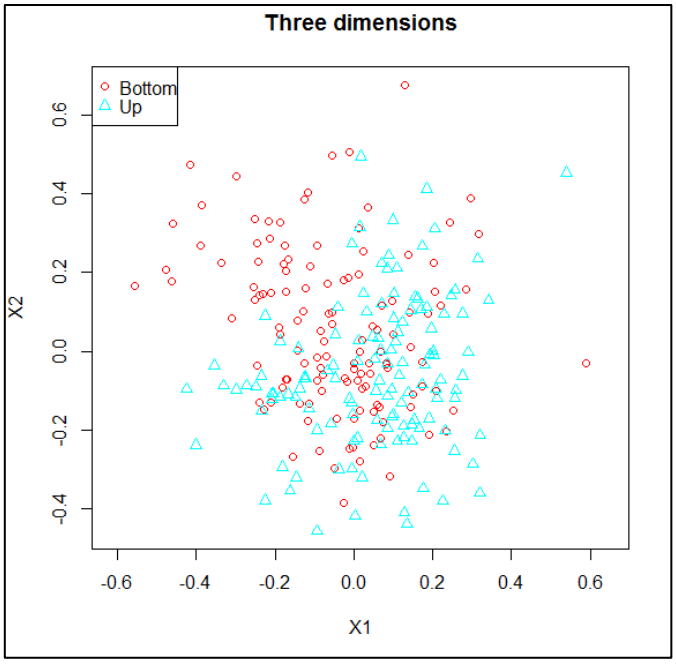

Figure 5.49. NMDS ordinations for aboveground arthropod community function by heights of sticky traps in summer 2013 at Snook, Texas.

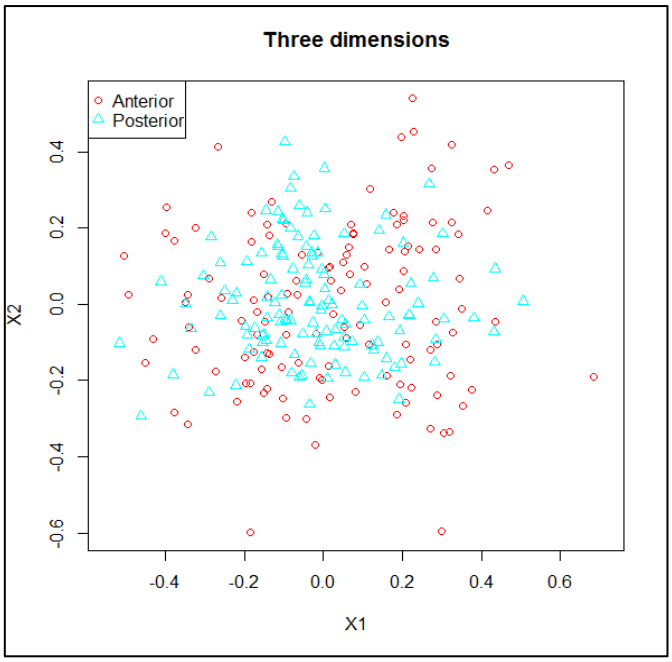

Figure 5.50. NMDS ordinations for aboveground arthropod community function by positions of sticky traps in summer 2013 at Snook, Texas.

For ISA, results demonstrated one indicator functional group (herbivores) among aboveground arthropods collected via sticky traps in summer 2013 (Table 5.33).

Table 5.33. Indicator species analysis by functional groups for aboveground arthropods trapped by sticky traps in summer 2013 at Snook, Texas.

Type	Functional groups	Indicator value	P value
Sticky traps	Herbivores	0.0449	0.022*

Abundance

Six functional groups were highlighted individually (Figure 5.51). For necrophagous guild, significant difference was found on Day 3 between Control x Post-7 ($p = 0.0212$) and Control x Post-14 ($p = 0.0121$). For herbivores, there was a significant difference on Day 0 between Control x Post-7 ($p = 0.0013$). Also, on Day 10, there was a marginal significant difference ($p = 0.0516$). It is interesting to note that abundance of herbivores increased after Day 21 (when decomposition process had been completed in all groups). For detritivores, significant difference was found on Day 0 ($p = 0.0398$). Fungivore also demonstrated significant difference on Day 0 between Control x Post-7 ($p = 0.0018$) and Post-7 x Post-14 ($p = 0.0018$). For predators/parasites and nectarivores guilds, both groups showed resistance between treatments in all sampling days.

Resilience was tested for all treatments in six functional groups. The results showed all functional groups were resistance to perturbations or having resilience on or before Day 40, except for herbivores at Post-7 and Post-14 carcasses, which loss the resilience on Day 40 (Table 5.34).

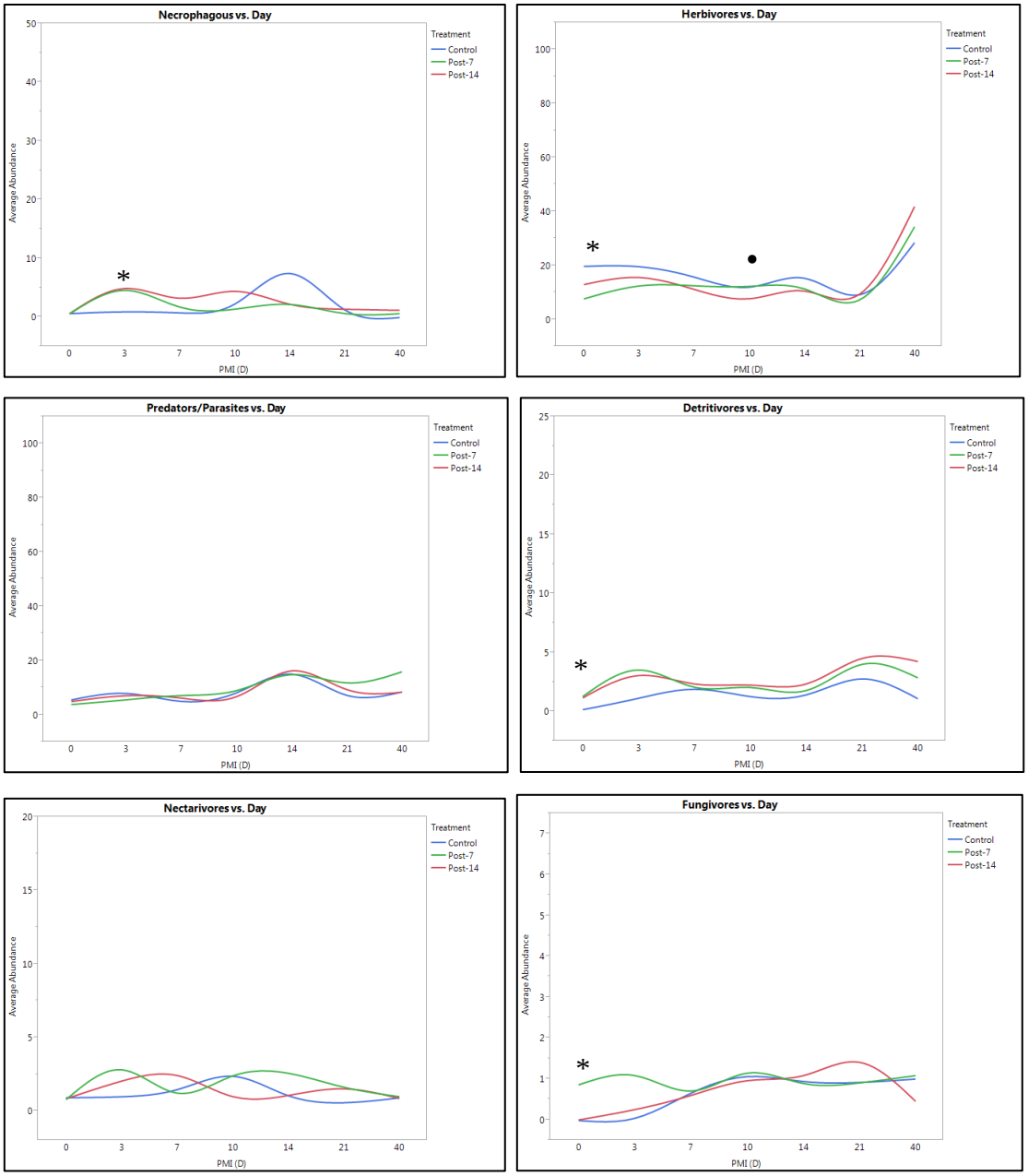

Figure 5.51. Average abundance of arthropods according to functional groups collected via sticky traps in summer 2013 at Snook, Texas. Upper Left. Abundance of necrophagous across Treatments over time. Upper Right. Abundance of herbivores across Treatments over time. Middle Left. Abundance of predators/parasites across Treatments over time. Middle Right. Abundance of detritivores across Treatments over time. Lower Left. Abundance of nectarivores across Treatments over time. Lower Right. Abundance of fungivores across Treatments over time (* represent significantly different; • represents marginal significant difference).

Table 5.34. Resilience for aboveground arthropod community function collected via sticky traps for each treatment in summer 2013 at Snook, Texas.

Function	Treatment	Significant difference	P value	Resilience on Day
Necrophagous	Control	0 x 14	0.0143*	21
	Post-7	0 x 3	<0.0001*	7
	Post-14	None	0.1008	Resistance
Detritivores	Control	0 x 21	0.0106*	40
	Post-7	None	0.1665	Resistance
	Post-14	None	0.2411	Resistance
Predators/Parasites	Control	0 x 14	0.0012*	21
	Post-7	None	0.1222	Resistance
	Post-14	0 x 14	<0.0001*	21
Fungivores	Control	None	0.0217*	Resistance[#]
	Post-7	None	0.9112	Resistance
	Post-14	0 x 21	0.0496*	40
Herbivores	Control	None	0.0002*	Resistance[#]
	Post-7	0 x 40	<0.0001*	Loss of resistance on Day 40
	Post-14	0 x 40	<0.0001*	Loss of resistance on Day 40
Nectarivores	Control	0 x 10	0.0359*	14
	Post-7	None	0.3434	Resistance
	Post-14	None	0.0974*	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Pitfall trap in 2013

Year effect

There was a year effect (df =1; F = 2.5248; p = 0.015) between two trials by Order of crawling arthropods collected by pitfall traps (Figure 5.52 showed NMDS plot between years). Furthermore, when function of crawling arthropods was analyzed for Year effect, the results showed that there was significant difference between years (df = 1; F = 4.84; p = 0.002). Hence, data were analyzed separately.

Replicate effect

There was no replicate effect (df = 1; F = 1.7474; p = 0.086) among the replicates by Order of crawling arthropods collected by pitfall traps. Therefore, all data in the replicates were pooled together and analyzed.

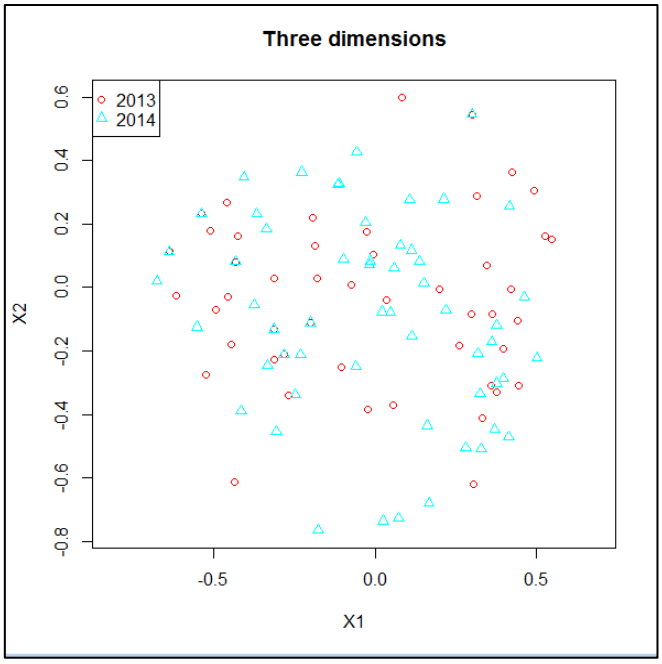

Figure 5.52. NMDS ordinations of crawling arthropod data (by Order) between 2013 and 2014 trials. Minimum stress for given dimensionality 0.1859 and r^2 for minimum stress configuration was 0.7594.

Total Order in 2013

A total of seven Orders of Class Insecta, two Orders of Class Arachnida (Araneae and Scorpiones), one Order of Class Malacostraca (Isopoda), and one Class of Diplopoda have been recovered from all pitfall traps in 2013 trial. There was an individual of Anura collected in one of the pitfall traps. However, it was excluded from the analysis due to non-arthropod origin. Table 5.35 showed the Orders identified in summer 2013 and the most dominant crawling arthropods were the Hymenoptera (60.44%), followed by Araneae (16.50%), Coleoptera (11.89%) and others (less than 3%).

Table 5.35. Total abundance and dominance of Orders in the Class Insecta, Class Arachnida and other arthropod classes identified from all pitfall trap samples in summer 2013 at Snook, Texas.

Order	Total abundance	Dominance
Hymenoptera	249	60.44
Araneae	68	16.50
Coleoptera	49	11.89
Isopoda	12	2.91
Orthoptera	10	2.43
Hemiptera	7	1.70
Blattodea	7	1.70
Diptera	5	1.21
Collembola	3	0.73
Diplopoda (Class)	1	0.24
Scorpiones	1	0.24
Total	412	100

Total Family in 2013

A total of 23 families of arthropods (including three families from the Order Araneae and one family from the Order Isopoda) were identified from all pitfall traps in summer 2013 (Table 5.36). Total abundance of all arthropods identified to Family level was 385 individuals. The dominant family was Formicidae (65.19%), followed by Lycosidae (15.06%) and other families (less than 3%).

Table 5.36. Total abundance and dominance of Families in the Class Insecta, Arachnida and Malacostraca identified from all pitfall trap samples in summer 2013 at Snook, Texas.

Family	Total abundance	Dominance
Formicidae	251	65.19
Lycosidae	58	15.06
Armadillidiidae	11	2.86
Gryllidae	9	2.34
Carabidae	8	2.08
Ectobiidae	7	1.82
Oxyopidae	6	1.56
Miridae	4	1.04
Trogidae	4	1.04
Dermestidae	4	1.04
Phoridae	4	1.04
Salticisae	3	0.78
Staphylinidae	3	0.78
Entomobryidae	3	0.78
Buthidae	2	0.52
Apidae	1	0.26
Psyllidae	1	0.26
Cicadellidae	1	0.26
Silphidae	1	0.26
Muscidae	1	0.26
Cucurlionidae	1	0.26
Coccinellidae	1	0.26
Acrididae	1	0.26
Total	385	100

Total Genus and species in 2013

A total of six genera and species of crawling arthropods have been identified from pitfall traps in summer 2013. They were *S. invicta* (Formicidae), *P. fulvescens* (Ectobiidae), *O. suberosus* (Trogidae), *D. caninus* (Dermestidae), *Centruroides vittatus* (Buthidae), and *Hydroteae* sp. (Muscidae). The most dominant genus or species collected was *S. invicta* (92.20%), followed by *P. fulvescens* (3.03%) and others (all less than 3%).

Total function in 2013

Five functional groups were identified from 385 crawling arthropods collected in pitfall traps in summer 2013. The most dominant group was predators (85.45%), followed by necrophagous guild (6.23%), herbivores (4.42%), detritivores (3.64%), and nectarivores (0.26%).

Order in 2013

PERMANOVA was performed on crawling arthropod structural data by Order level. Results showed that there was no significant difference in Day, and Treatment, or any interaction ($p < 0.05$) (Table 5.37). There was no significant difference in Replicate ($p = 0.08$) as well.

Table 5.37. Analysis of the crawling arthropod community structure (by Order) collected via pitfall traps in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	1.9252	0.091*
Treatment	2	0.7032	0.697
Day x Treatment	2	1.0496	0.390

For ISA, results showed that there was no significant indicator among crawling arthropods in summer 2013 at Snook, Texas.

Abundance

The full model showed no significant difference in Day, Treatment or any interaction ($p > 0.05$). Resilience was tested and results showed resistance in all sampling days for every treatment group (Table 5.38). Average abundance of crawling arthropods according to Orders collected at pitfall traps in 2013 trial was demonstrated in Figure 5.53. For Araneae, there was a significant difference on Day 7 between Post-7 x Post-14 ($p = 0.0285$). There was no significant difference detected between treatments in all sampling days for other Orders (Figure 5.54).

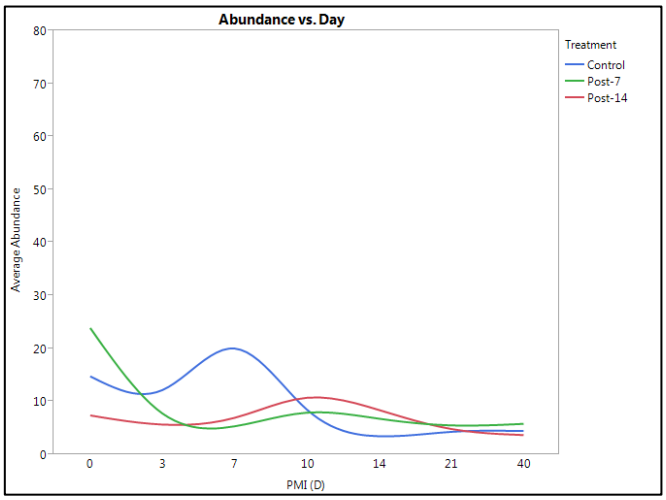

Figure 5.53. Crawling arthropod community abundance (by Order) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.38. Resilience for crawling arthropod community (by Order) abundance collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4004	Resistance
Post-7	None	0.0602•	Resistance
Post-14	None	0.1261	Resistance

• Marginal significant difference.

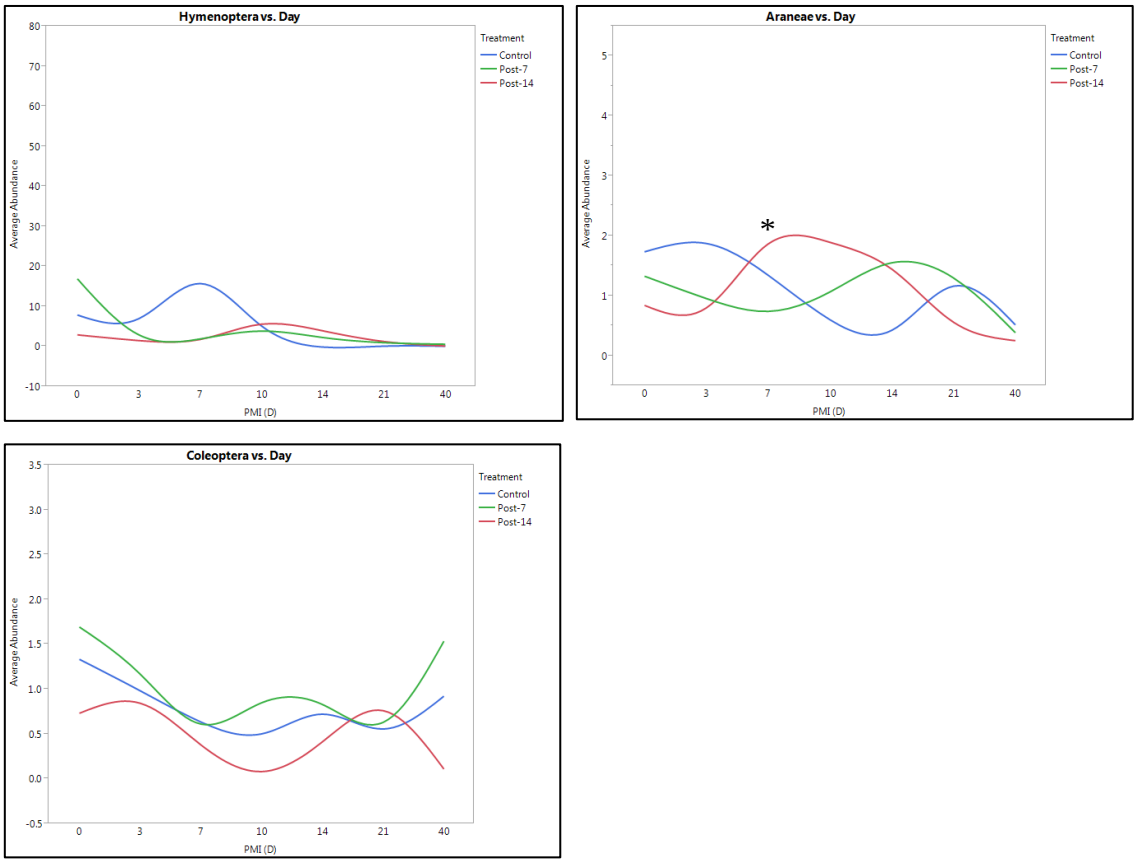

Figure 5.54. Average abundance of arthropods according to Orders collected via pitfall traps in summer 2013 at Snook, Texas. Upper Left. Abundance of Hymenoptera across Treatments over time. Upper Right. Abundance of Araneae across Treatments over time. Lower Left. Abundance of Coleoptera across Treatments over time (* represents significant difference).

Richness

The full model showed a significant difference in Day ($p = 0.0045$). There was significant difference found in richness between Control x Post-7 ($p = 0.0242$) and Control x Post-14 ($p = 0.0021$) on Day 10 (Figure 5.55). Resilience was tested for all treatments and Post-7 and Post-7 carcasses demonstrated resistance throughout the days while there was loss of resistance on Day 40 for Control carcasses (Table 5.39).

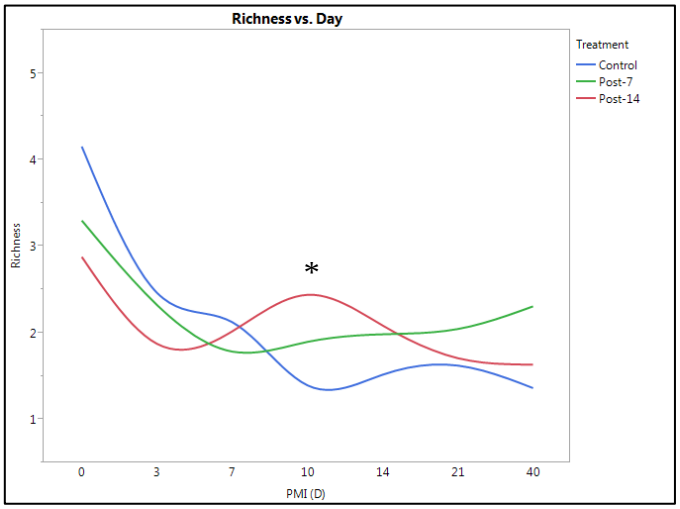

Figure 5.55. Crawling arthropod community richness (by Order) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.39. Resilience for crawling arthropod community (by Order) richness collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 10	0.0065*	Loss of resistance on
	0 x 14	0.0340*	Day 40
	0 x 21	0.0340*	
	0 x 40	0.0149*	
Post-7	None	0.6404	Resistance
Post-14	None	0.4931	Resistance

Simpson's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference was found in Simpson's Diversity between treatments in all sampling days ($p > 0.05$), although there was a marginal significant difference on Day 21 ($p = 0.0604$) (Figure 5.56). In other words, the system was resistance that no divergence or convergence was observed. Resilience was tested for all treatments and the results demonstrated that all carcasses were resistance throughout the sampling days (Table 5.40).

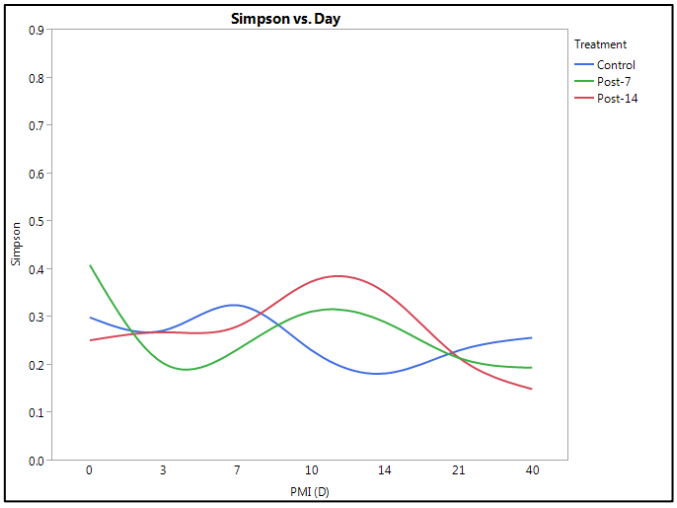

Figure 5.56. Simpson's diversity of the crawling arthropod community (by Order) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.40. Resilience for Simpson's Diversity of the crawling arthropod community (by Order) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.9076	Resistance
Post-7	None	0.6598	Resistance
Post-14	None	0.5404	Resistance

Shannon-Wiener's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was significant difference found in Shannon-Wiener's Diversity between Control x Post-14 ($p = 0.0362$) on Day 10 (Figure 5.57). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.41).

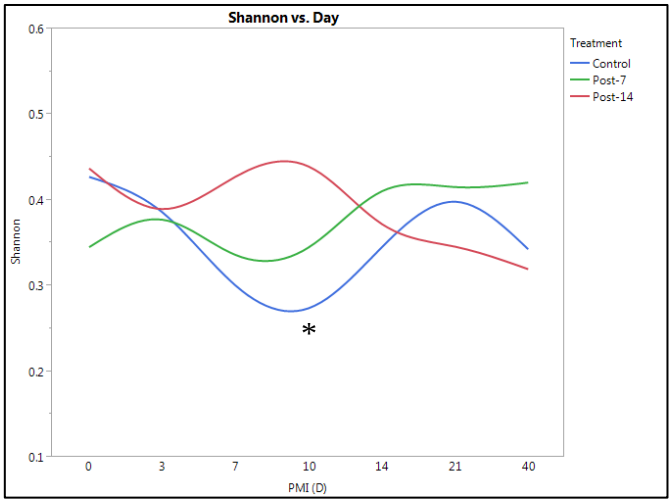

Figure 5.57. Shannon-Wiener's diversity of the crawling arthropod community (by Order) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.41. Resilience for Shannon-Wiener's Diversity of the crawling arthropod community (by Order) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.7770	Resistance
Post-7	None	0.8742	Resistance
Post-14	None	0.6318	Resistance

Evenness

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. However, there was significant difference found in evenness between Control x Post-7 ($p = 0.0040$) and Control x Post-14 ($p = 0.0030$) on Day 10 (Figure 5.58). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.42).

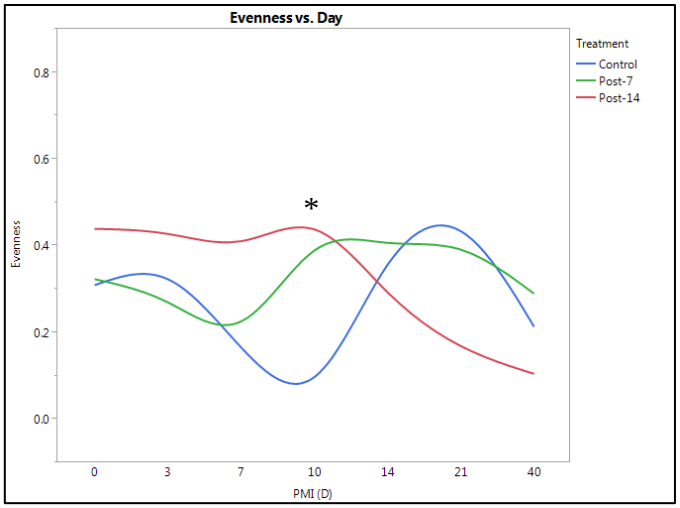

Figure 5.58. Evenness of the crawling arthropod community (by Order) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.42. Resilience for evenness of the crawling arthropod community (by Order) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4817	Resistance
Post-7	None	0.7978	Resistance
Post-14	None	0.4324	Resistance

Effective number of species

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. However, there was significant difference found in ENS between Control x Post-14 ($p = 0.0313$) on Day 10 (Figure 5.59). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.43).

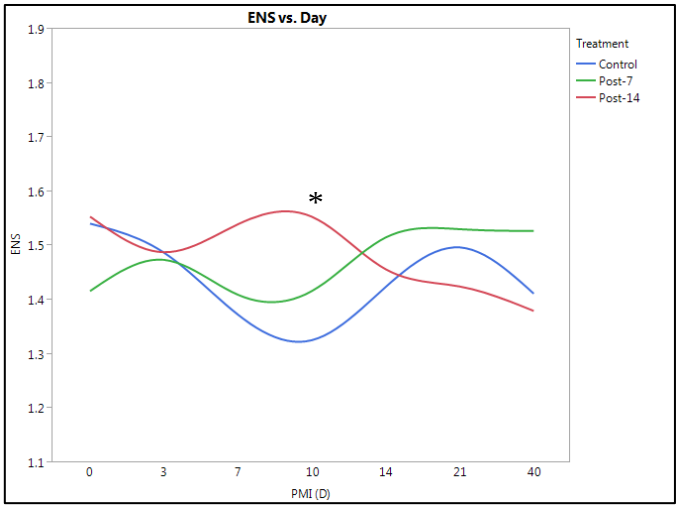

Figure 5.59. Effective Number of Species of the crawling arthropod community (by Order) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.43. Resilience for ENS of the crawling arthropod community (by Order) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.8073	Resistance
Post-7	None	0.8505	Resistance
Post-14	None	0.6271	Resistance

Family in 2013

PERMANOVA was performed on crawling arthropod structural data by Family level. Results showed that there was significant difference in Day ($p = 0.023$), but no significant difference in Treatment, Replicate or any interaction ($p < 0.05$) (Table 5.44).

Table 5.44. Analysis of the crawling arthropod community structure (by Family) collected via pitfall traps in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	2.4869	0.023*
Treatment	2	0.8402	0.629
Day x Treatment	2	1.0473	0.414

Since there was significant difference in Day, further analyses were performed. For days of decomposition, most of the day to day comparisons were not significantly different, except Day 0 x Day 40 where there was a significant difference ($p = 0.024$) (Table 5.45). The NMDS plot of stress for crawling arthropod community structure (Figure 5.60) and NMDS ordinations for Day was provided for visualization about data distribution (Figure 5.61). Minimum stress for given dimensionality was 0.1812 with $r^2 = 0.7717$. The MRPP analysis for day showed A value = -0.002 and Significant of Delta = 0.527 based on 999 permutations.

Table 5.45. Pairwise comparisons of crawling arthropod community structure (by Family) collected via pitfall traps between carrion decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.191	0.456	0.628	0.612	0.144	0.024*
3	0.191	-	0.672	0.778	0.679	0.404	0.318
7	0.456	0.672	-	0.618	0.952	0.418	0.118
10	0.628	0.778	0.618	-	0.699	0.400	0.135
14	0.612	0.679	0.952	0.699	-	0.989	0.354
21	0.144	0.404	0.418	0.400	0.989	-	0.385
40	0.024*	0.318	0.118	0.135	0.354	0.385	-

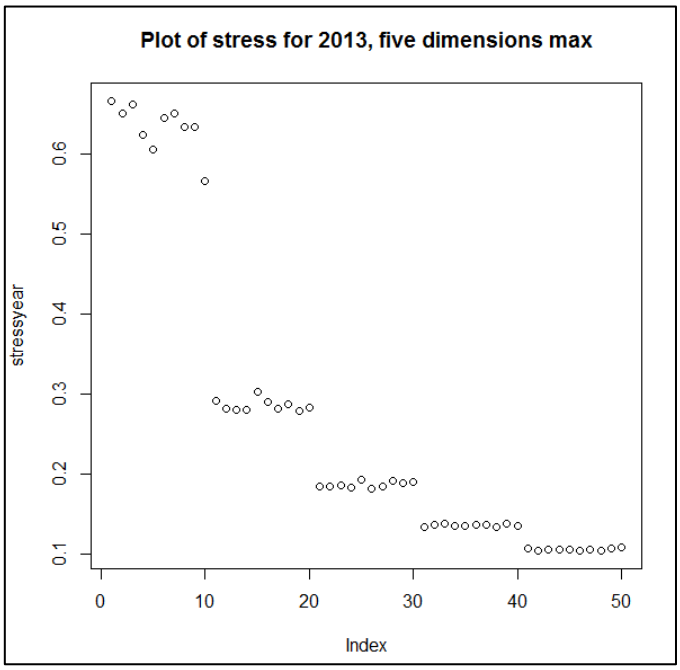

Figure 5.60. NMDS plot of stress for crawling arthropod community structure (by Family) collected via pitfall traps in summer 2013 at Snook, Texas (Stress test 0.1812; r^2 = 0.7717).

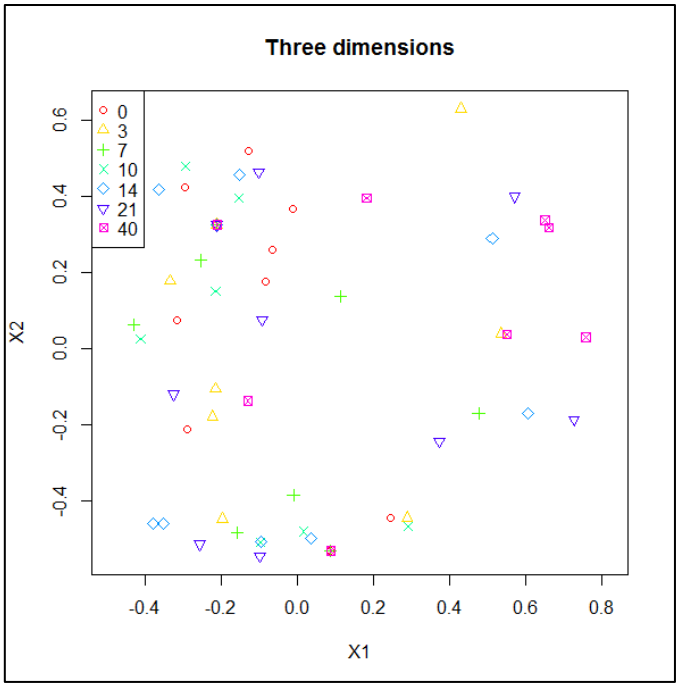

Figure 5.61. NMDS ordinations for crawling arthropod community structure by carrion decomposition days (Family level) collected via pitfall traps in summer 2013 at Snook, Texas.

The ISA results showed that there was no significant indicator among crawling arthropods by Family in summer 2013 at Snook, Texas.

Abundance

The full model showed no significant difference in Day, Treatment or any interaction ($p > 0.05$). Resilience was tested and results showed resistance in all sampling days for every treatment group (Table 5.46). Average abundance of crawling arthropods according to Family collected at pitfall traps in 2013 trial was demonstrated in Figure 5.62. There was no significant difference detected between treatments in all sampling days for families Lycosidae and Formicidae (Figure 5.63).

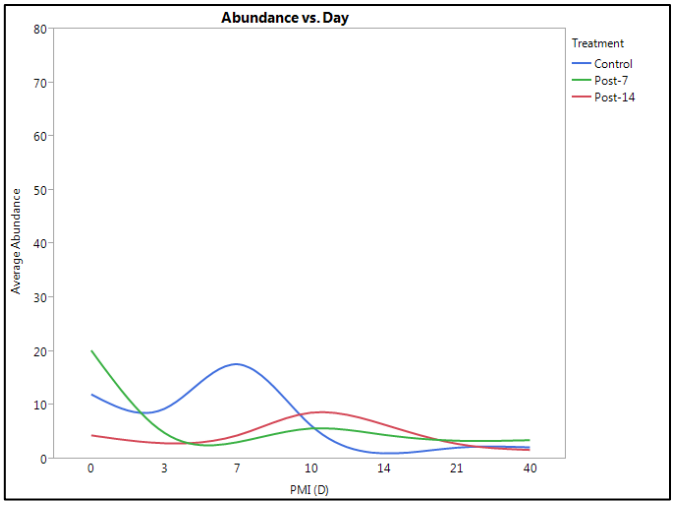

Figure 5.62. Crawling arthropod community abundance (by Family) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.46. Resilience for crawling arthropod community (by Family) abundance collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4146	Resistance
Post-7	None	0.0962	Resistance
Post-14	None	0.1189	Resistance

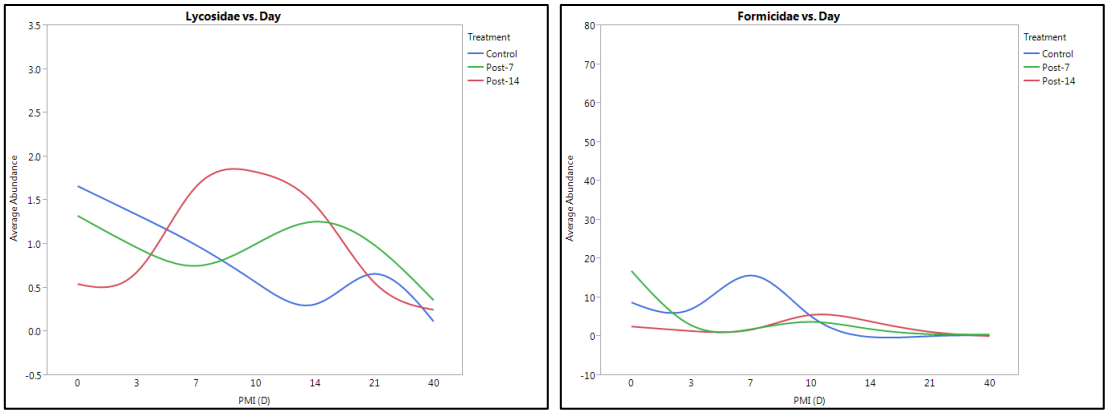

Figure 5.63. Average abundance of arthropods according to Families collected via pitfall traps in summer 2013 at Snook, Texas. Left. Abundance of Lycosidae across Treatments over time. Right. Abundance of Formicidae across Treatments over time.

Richness

The full model showed no significant difference in Day, Treatment or any interaction ($p > 0.05$). There was significant difference found in richness between Control x Post-7 ($p = 0.0242$) and Control x Post-14 ($p = 0.0021$) on Day 10 (Figure 5.64). Resilience was tested for all treatments, and the results showed that all treatments were resistance throughout the sampling days (Table 5.47).

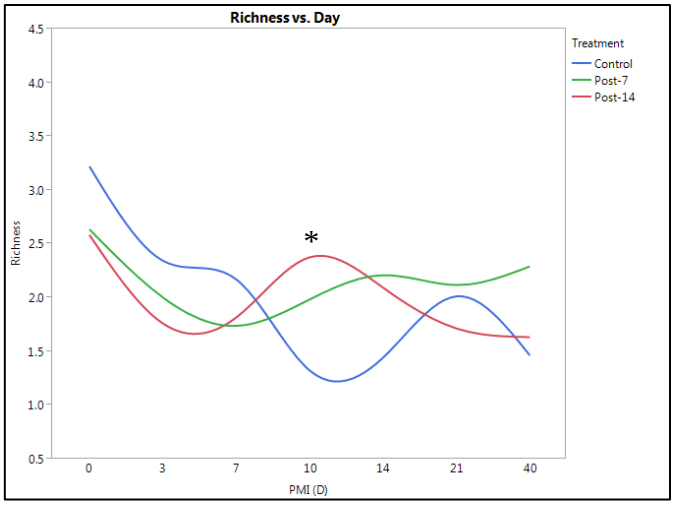

Figure 5.64. Crawling arthropod community richness (by Family) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.47. Resilience for crawling arthropod community (by Family) richness collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0654•	Resistance
Post-7	None	0.9565	Resistance
Post-14	None	0.6007	Resistance

• Marginal significant difference.

Simpson's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. However, there was a significant difference found in Simpson's Diversity between Control x Post-14 ($p = 0.0322$) on Day 10 (Figure 5.65). Resilience was tested and results showed resistance in all sampling days for all treatment group (Table 5.48).

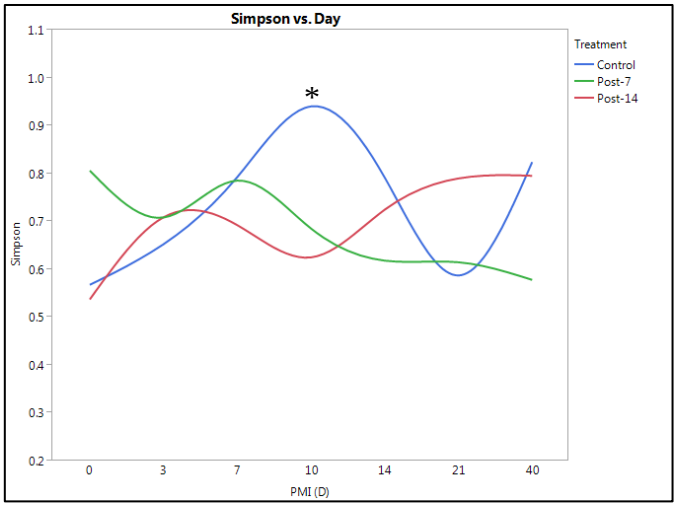

Figure 5.65. Simpson's diversity of the crawling arthropod community (by Family) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.48. Resilience for Simpson's Diversity of the crawling arthropod community (by Family) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2067	Resistance
Post-7	None	0.7996	Resistance
Post-14	None	0.8297	Resistance

Shannon-Wiener's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was significant difference found in Shannon-Wiener's Diversity between Control x Post-14 ($p = 0.0153$) on Day 10 (Figure 5.66). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.49).

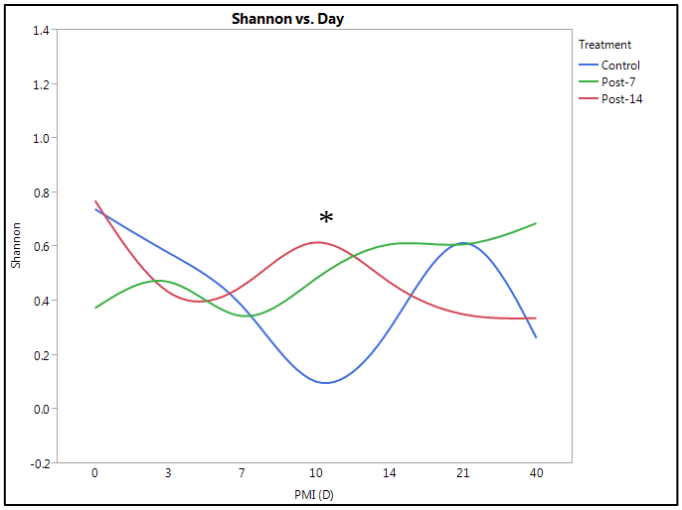

Figure 5.66. Shannon-Wiener's diversity of the crawling arthropod community (by Family) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.49. Resilience for Shannon-Wiener's Diversity of the crawling arthropod community (by Family) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2204	Resistance
Post-7	None	0.8900	Resistance
Post-14	None	0.7941	Resistance

Evenness

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. However, there was significant difference found in evenness between Control x Post-7 ($p = 0.0183$) and Control x Post-14 ($p = 0.0187$) on Day 10 (Figure 5.67). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.50).

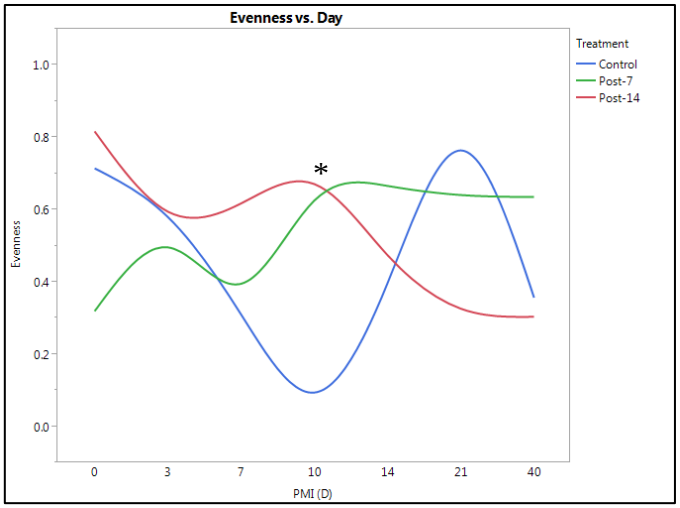

Figure 5.67. Evenness of the crawling arthropod community (by Family) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.50. Resilience for evenness of the crawling arthropod community (by Family) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1758	Resistance
Post-7	None	0.7288	Resistance
Post-14	None	0.7018	Resistance

Effective number of species

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. However, there was significant difference found in ENS between Control x Post-14 ($p = 0.0419$) on Day 10 (Figure 5.68). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.51).

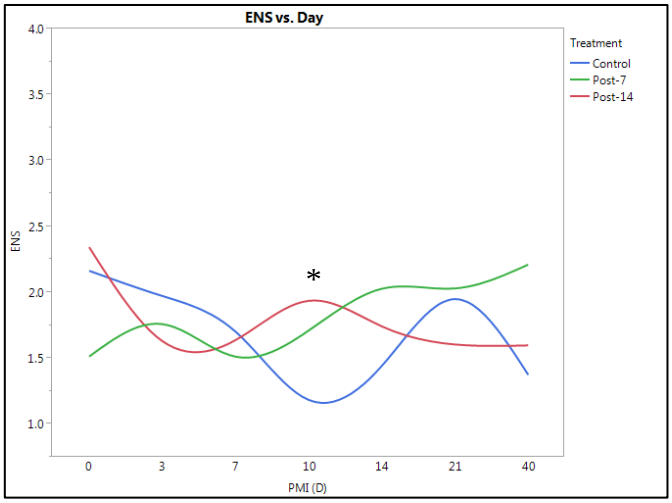

Figure 5.68. Effective Number of Species of the crawling arthropod community (by Family) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.51. Resilience for ENS of the crawling arthropod community (by Family) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.3614	Resistance
Post-7	None	0.8100	Resistance
Post-14	None	0.8233	Resistance

Genus and species in 2013

PERMANOVA was performed on crawling arthropod structural data by Genus and species level. Results showed that there was significant difference in Treatment ($p = 0.015$) and Day ($p = 0.050$). There was no significant difference in Replicate or any interaction ($p < 0.05$) (Table 5.52).

Table 5.52. Analysis of the crawling arthropod community structure (by Genus and species) collected via pitfall traps in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	2.3638	0.050*
Treatment	2	2.4571	0.015*
Day x Treatment	2	0.4797	0.853

Since there was significant difference in Treatment and Day, further analyses were performed. For days of decomposition, most of the day to day comparisons were not significantly different, except Day 0 x Day 40 and Day 10 x Day 40 where there were significant differences ($p < 0.05$) (Table 5.53). Comparison between treatments demonstrated that Control x Post-7 was significantly different ($p = 0.014$) (Table 5.54). The NMDS plot of stress for crawling arthropod community structure (Figure 5.69) and NMDS ordinations for Day and for Treatment were provided for visualization about data distribution (Figure 5.70 and 5.71). Minimum stress for given dimensionality was 0.0890 with $r^2 = 0.9799$. However, the MRPP analysis for day showed A value = 0.0290; Significant of Delta = 0.12 based on 999 permutations) while the MRPP for treatments showed A value 0.0378 and Significant of Delta 0.021.

Table 5.53. Pairwise comparisons of crawling arthropod community structure (by Genus and species) collected via pitfall traps between carrion decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.249	0.756	0.594	0.679	0.316	0.029*
3	0.249	-	0.520	0.332	0.635	0.422	0.204
7	0.756	0.520	-	0.255	0.926	0.370	0.206
10	0.594	0.332	0.255	-	0.616	0.342	0.006*
14	0.679	0.635	0.926	0.616	-	0.922	0.076
21	0.316	0.422	0.370	0.342	0.922	-	0.088
40	0.029*	0.204	0.206	0.006*	0.076	0.088	-

Table 5.54. Pairwise comparisons of aboveground arthropod community structure (by Genus and species) collected via pitfall traps between treatments in summer 2013 at Snook, Texas after Bonferroni's correction.

Treatment	df	SS	MS	F model	R2	P value
Control x Post-7	1	0.8141	0.8141	3.6429	0.0835	0.014*
Residual	40	8.9394	0.2234		0.9165	
Total	41	9.7535			1.0000	
Control x Post-14	1	0.2915	0.2915	1.8674	0.0446	0.104
Residual	40	6.2444	0.1561		0.9554	
Total	41	6.5359			1.0000	
Post-7 x Post-14	1	0.4623	0.4623	1.7648	0.0423	0.108
Residual	40	10.4791	0.2619		0.9577	
Total	41	10.9414			1.0000	

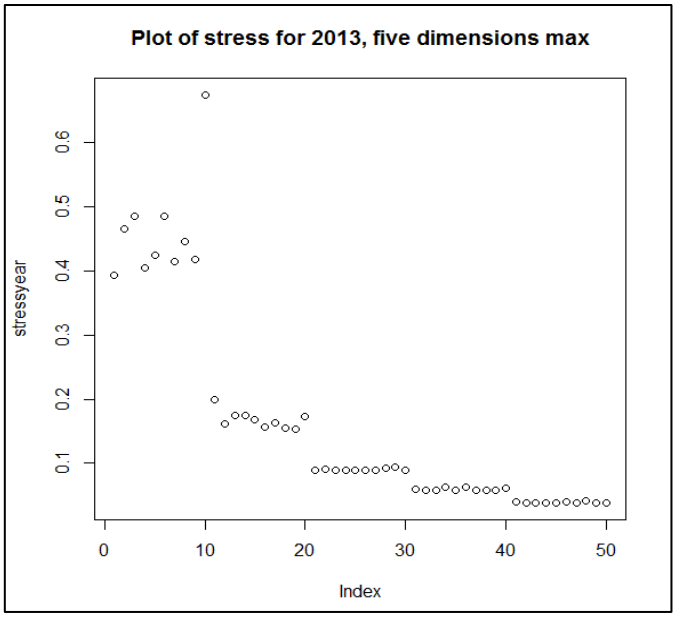

Figure 5.69. NMDS plot of stress for crawling arthropod community structure (by Genus and species) collected via pitfall traps in summer 2013 at Snook, Texas (Stress test 0.0890; $r^2 = 0.9799$.).

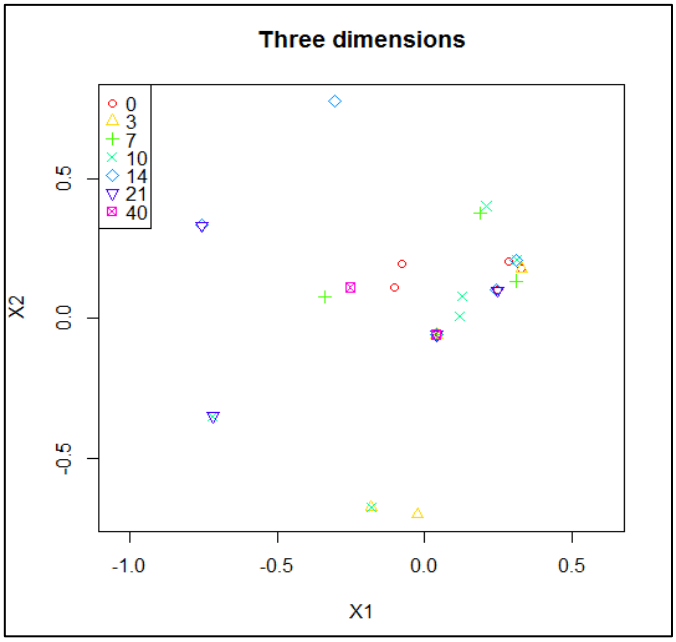

Figure 5.70. NMDS ordinations for crawling arthropod community structure by carrion decomposition days (by Genus and species) collected via pitfall traps in summer 2013 at Snook, Texas.

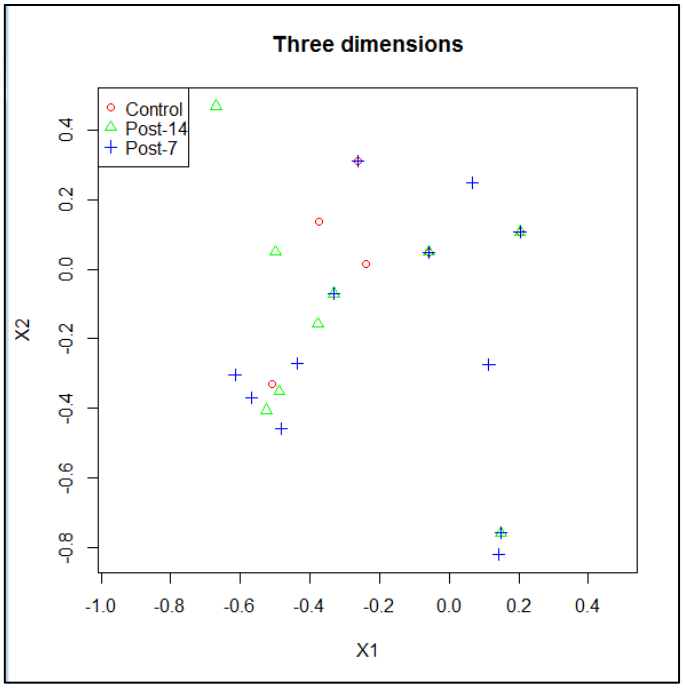

Figure 5.71. NMDS ordinations for crawling arthropod community structure by treatments (by Genus and species) collected via pitfall traps in summer 2013 at Snook, Texas.

The ISA results showed that there was no significant indicator among crawling arthropods by Genus and species in summer 2013 at Snook, Texas.

Abundance

The full model showed no significant difference in Day, Treatment or any interaction ($p > 0.05$). Resilience was tested and results showed resistance in all sampling days for every treatment group (Table 5.55). There was no significant difference detected in arthropod abundance between treatments in all sampling days (Figure 5.72).

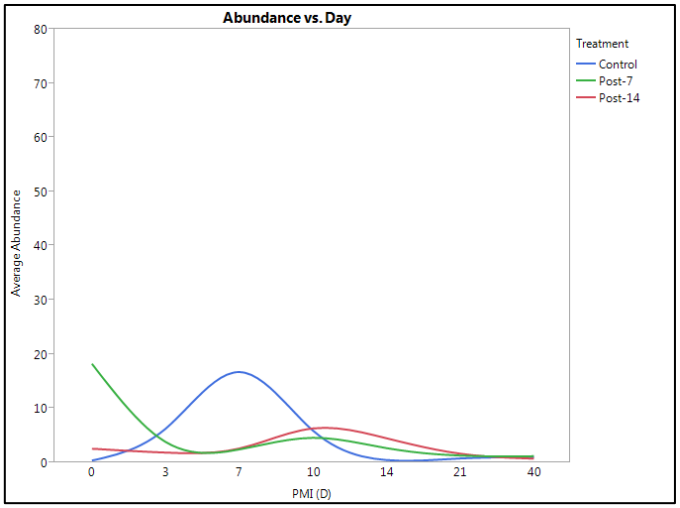

Figure 5.72. Crawling arthropod community abundance (by Genus and species) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.55. Resilience for crawling arthropod community (by Genus and species) abundance collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.3996	Resistance
Post-7	None	0.0611•	Resistance
Post-14	None	0.1338	Resistance

• Marginal significant difference.

Richness

The full model showed no significant difference in Day, Treatment or any interaction ($p > 0.05$). There was no significant difference found in richness between treatments in all sampling days (Figure 5.73). Resilience was tested for all treatments, and the results showed that all treatments were resistance throughout the sampling days (Table 5.56).

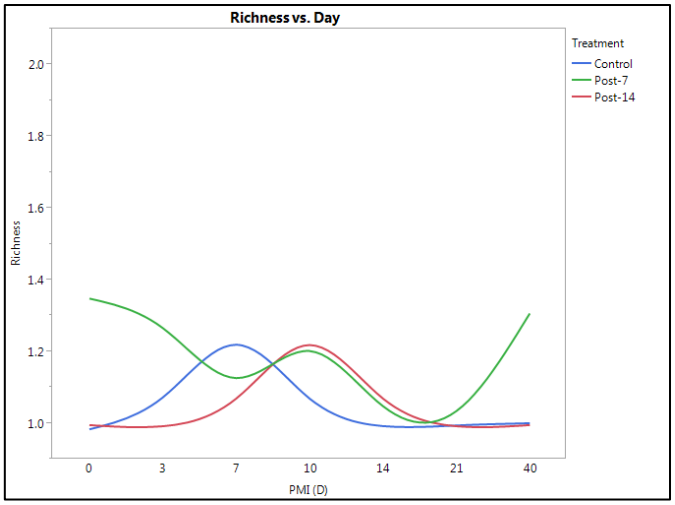

Figure 5.73. Crawling arthropod community richness (by Genus and species) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.56. Resilience for crawling arthropod community (by Genus and species) richness collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.7982	Resistance
Post-14	None	0.4628	Resistance

Simpson's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference found in Simpson's diversity between treatments in all sampling days (Figure 5.74). Resilience was tested and results showed resistance in all sampling days for all treatment group (Table 5.57).

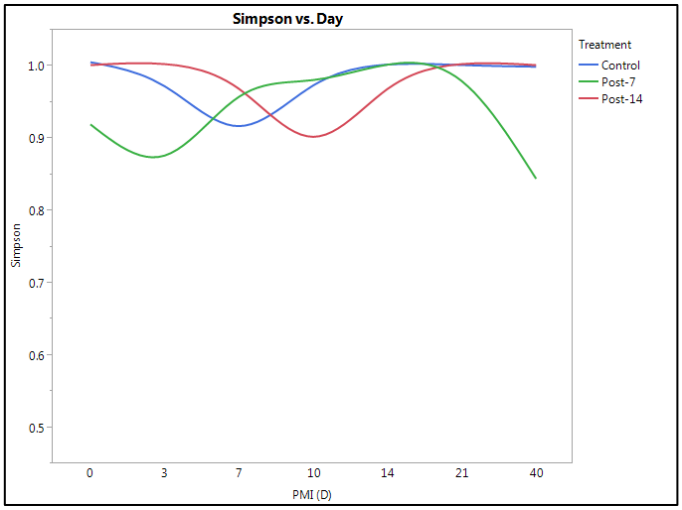

Figure 5.74. Simpson's diversity of the crawling arthropod community (by Genus and species) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.57. Resilience for Simpson's Diversity of the crawling arthropod community (by Genus and species) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.6914	Resistance
Post-14	None	0.4628	Resistance

Shannon-Wiener's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference found in Shannon-Wiener's Diversity between treatments in all sampling days (Figure 5.75). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.58).

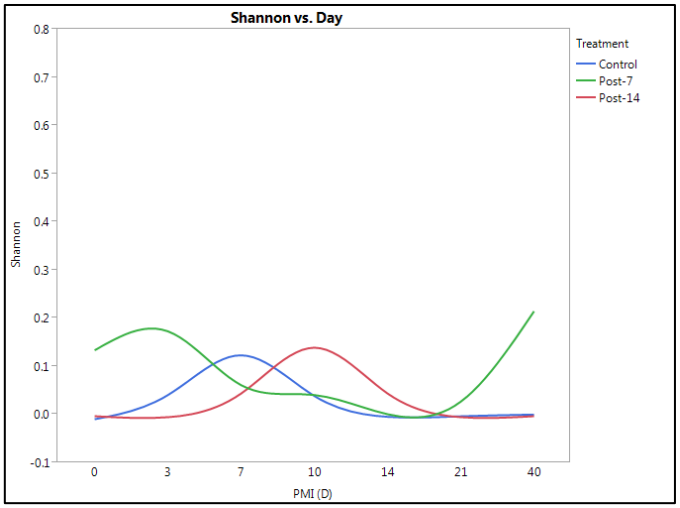

Figure 5.75. Shannon-Wiener's diversity of the crawling arthropod community (by Genus and species) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.58. Resilience for Shannon-Wiener's Diversity of the crawling arthropod community (by Genus and species) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.7258	Resistance
Post-14	None	0.4628	Resistance

Evenness

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference found in evenness between treatments in all sampling days (Figure 5.76). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.59).

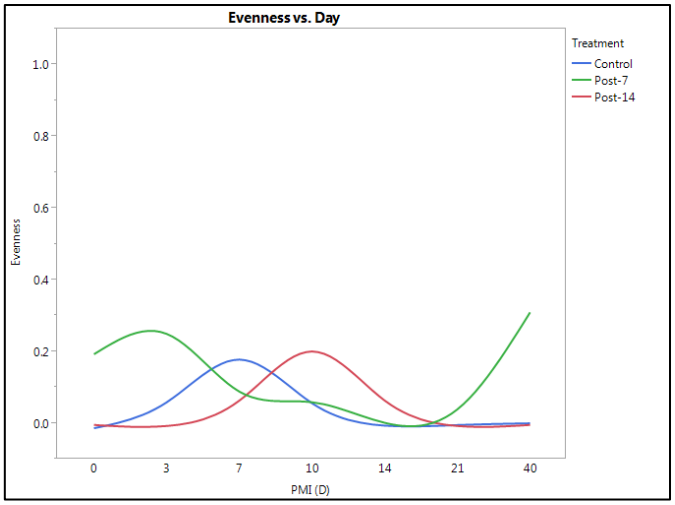

Figure 5.76. Evenness of the crawling arthropod community (by Genus and species) collected via sticky traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.59. Resilience for evenness of the crawling arthropod community (by Genus and species) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.7258	Resistance
Post-14	None	0.4628	Resistance

Effective number of species

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference found in ENS between treatments in all sampling days (Figure 5.77). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.60).

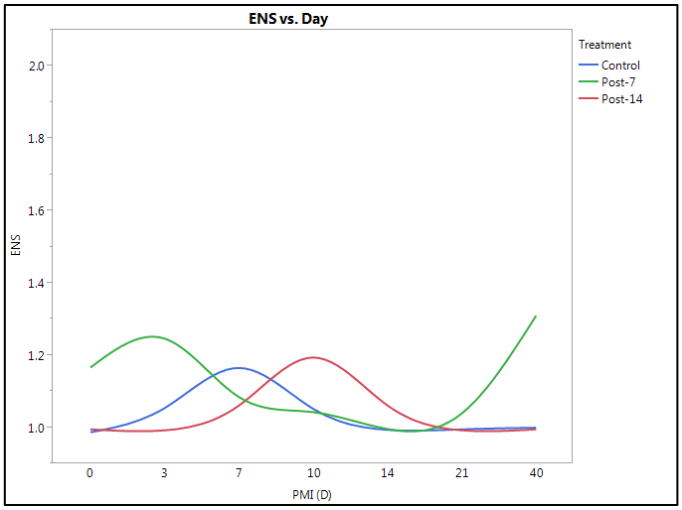

Figure 5.77. Effective Number of Species of the crawling arthropod community (by Genus and species) collected via pitfall traps across Treatments over time in summer 2013 at Snook, Texas.

Table 5.60. Resilience for ENS of the crawling arthropod community (by Genus and species) collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.7007	Resistance
Post-14	None	0.4628	Resistance

Function in 2013

PERMANOVA was performed on crawling arthropod functional data. Results showed that there was significant difference in Day ($p = 0.014$). Note that there was significant difference in Replicate as well ($p = 0.026$) (Table 5.61).

Table 5.61. Analysis of the crawling arthropod community functions collected via pitfall traps in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	3.4532	0.014*
Treatment	2	0.8461	0.551
Replicate	1	2.7924	0.026*
Day x Treatment	2	0.4210	0.899
Day x Replicate	1	1.9471	0.107
Treatment x Replicate	2	0.3443	0.929
Day x Treatment x Replicate	2	1.4214	0.189

Since there was significant difference in Day and Replicate, further analyses were performed. For day of decomposition, most of the day to day comparisons were not significantly different, except Day 0 x Day 21 and Day 0 x Day 40 where there were significant differences ($p < 0.05$) (Table 5.62). Comparison between replicate demonstrated that Replicate 1 x Replicate 3 was significantly different ($p = 0.034$) (Table 5.63). The NMDS plot of stress for crawling arthropod community function (Figure 5.78) and NMDS ordinations for Day and Replicate were provided for visualization about data distribution (Figure 5.79 and 5.80, respectively). Minimum stress for given dimensionality was 0.1170 with $r^2 = 0.9312$. However, the MRPP analysis for day showed A value = 0.0116; Significant of Delta = 0.266 based on 999 permutations) while the MRPP for replicate showed A value 0.0125 and Significant of Delta 0.141.

Table 5.62. Pairwise comparisons of crawling arthropod community function collected via pitfall traps between carrion decomposition days in summer 2013 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.161	0.150	0.189	0.258	0.016*	0.006*
3	0.161	-	0.839	0.820	0.946	0.729	0.599
7	0.150	0.839	-	0.940	0.952	0.872	0.083
10	0.189	0.820	0.940	-	0.855	0.629	0.071
14	0.258	0.946	0.952	0.855	-	0.739	0.217
21	0.016*	0.729	0.872	0.629	0.739	-	0.096
40	0.006*	0.599	0.083	0.071	0.217	0.096	-

Table 5.63. Pairwise comparisons between replicates for crawling arthropod community function collected via pitfall traps in summer 2013 at Snook, Texas after Bonferroni's correction.

Replicate	df	SS	MS	F model	R2	P value
1 x 2	1	0.1638	0.1637	0.7466	0.0183	0.541
Residual	40	8.7729	0.2193		0.9817	
Total	41	8.9367			1.0000	
1 x 3	1	0.5627	0.5626	2.6661	0.0625	0.034*
Residual	40	8.4418	0.2110		0.9375	
Total	41	9.0045			1.0000	
2 x 3	1	0.3425	0.3425	1.7887	0.0428	0.124
Residual	40	7.6592	0.1914		0.9672	
Total	41	8.0017			1.0000	

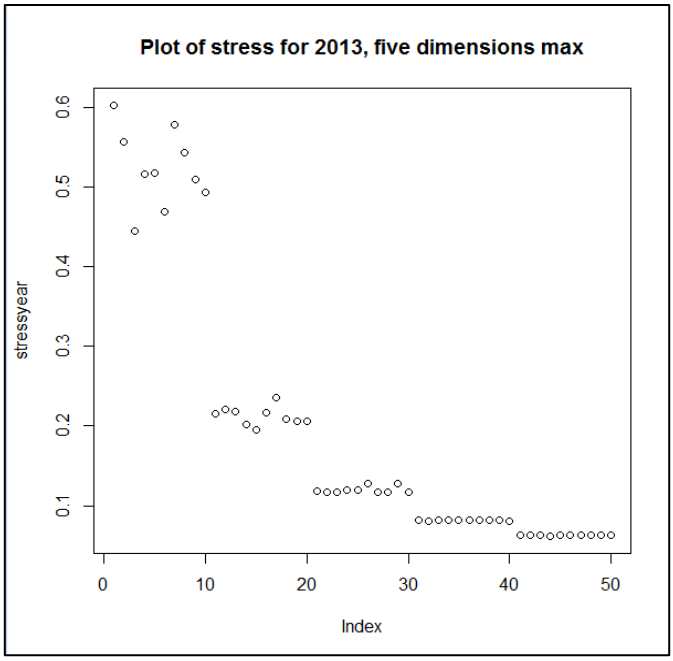

Figure 5.78. NMDS plot of stress for crawling arthropod community function collected via pitfall traps in summer 2013 at Snook, Texas (Stress test 0.1170; $r^2 = 0.9312$).

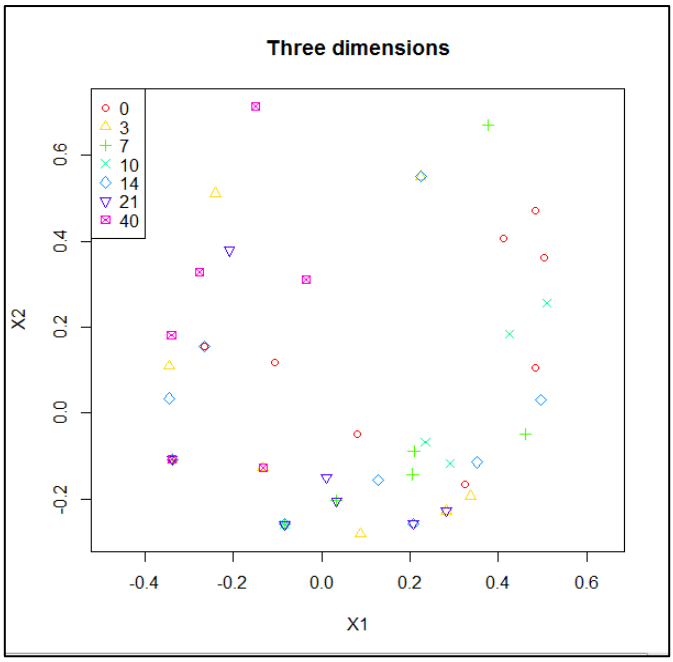

Figure 5.79. NMDS ordinations for crawling arthropod community function by carrion decomposition days collected via pitfall traps in summer 2013 at Snook, Texas.

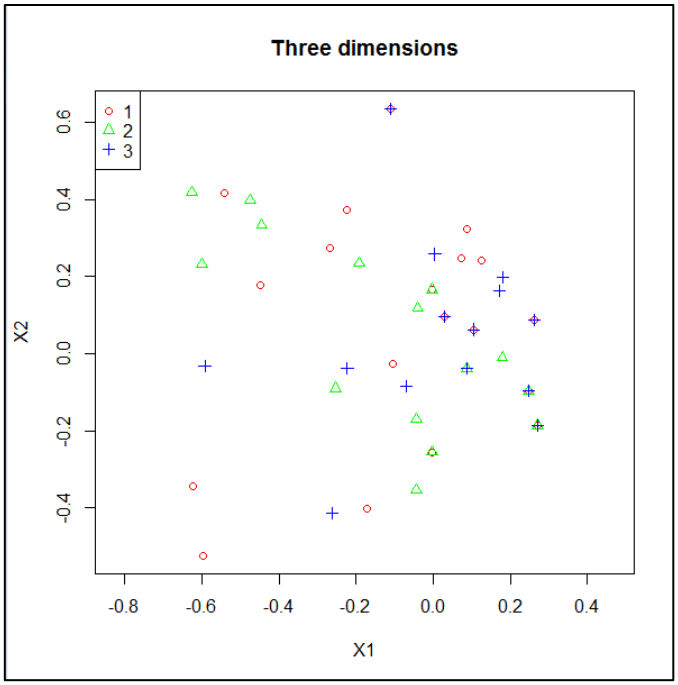

Figure 5.80. NMDS ordinations for crawling arthropod community function by replicates collected via pitfall traps in summer 2013 at Snook, Texas.

For ISA, results showed that there was no significant indicator among crawling arthropods by functional group in summer 2013 at Snook, Texas.

Abundance

Five functional groups namely necrophagous, herbivores, predators/parasites, nectarivores and detritivores were highlighted individually (Figure 5.81). Statistical tests showed no difference ($p > 0.05$) was detected between treatments in all sampling days for all functional groups.

Resilience was tested for all treatments in four functional groups (nectarivores was excluded due to the absence of arthropod in Control and Post-14 groups). The results showed all functional groups were resistance to perturbations throughout all sampling days except for herbivores of Control carcasses, which the resilience was observed on Day 7 and then loss the resistance again on Day 40 (Table 5.64).

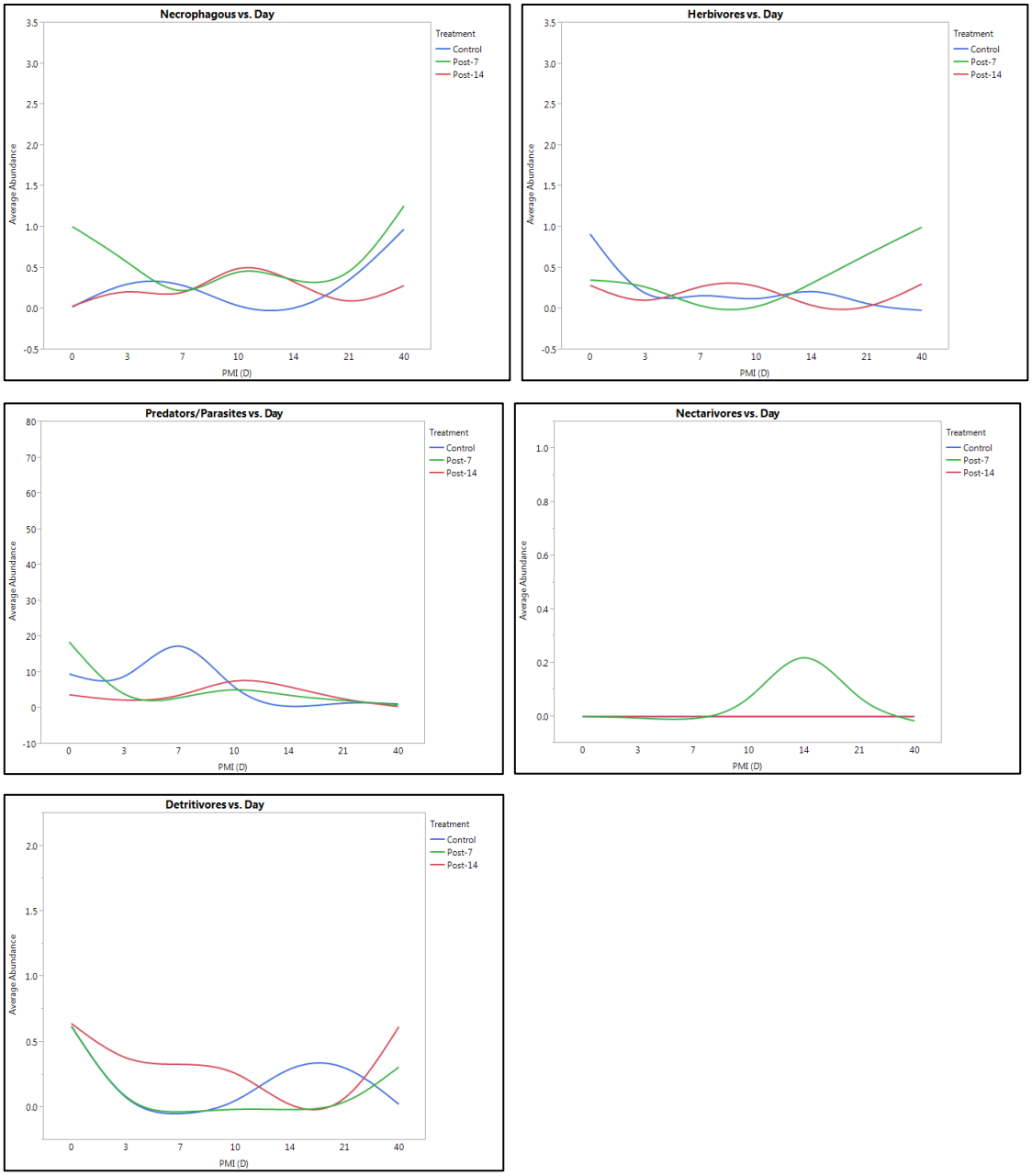

Figure 5.81. Average abundance of arthropods according to functional groups collected via pitfall traps in summer 2013 at Snook, Texas. Upper Left. Abundance of necrophagous across Treatments over time. Upper Right. Abundance of herbivores across Treatments over time. Middle Left. Abundance of predators/parasites across Treatments over time. Middle Right. Abundance of nectarivores across Treatments over time. Lower Left. Abundance of detritivores across Treatments over time.

Table 5.64. Resilience for crawling arthropod community function collected via pitfall traps for each treatment in summer 2013 at Snook, Texas.

Function	Treatment	Significant difference	P value	Resilience on Day
Necrophagous	Control	None	0.6781	Resistance
	Post-7	None	0.7618	Resistance
	Post-14	None	0.7468	Resistance
Detritivores	Control	None	0.2664	Resistance
	Post-7	None	0.1095	Resistance
	Post-14	None	0.7382	Resistance
Predators/Parasites	Control	None	0.4237	Resistance
	Post-7	None	0.0723	Resistance
	Post-14	None	0.1632	Resistance
Herbivores	Control	0 x 3	0.0184*	7
		0 x 10	0.0184*	Loss of
		0 x 21	0.0184*	resistance on
		0 x 40	0.0184*	Day 40
	Post-7	None	0.7982	Resistance
	Post-14	None	0.7982	Resistance

Sweep nets in 2013

Year effect

There was a year effect (df =1; F = 3.0478; p = 0.022) between two trials by Order of arthropods collected by sweep nets (Figure 5.82 showed NMDS ordinations between years). However, when Function of arthropods was analyzed for Year effect, the results showed no significant difference between years (df = 1; F = 1.7226; p = 0.156).

Replicate effect

There was no replicate effect (df = 1; F = 0.3877; p = 0.856) among the replicates by Order of arthropods collected by sweep nets. Furthermore, there was no replicate effect by arthropod functional between trials (df = 1; F = 0.2976; p = 0.841).

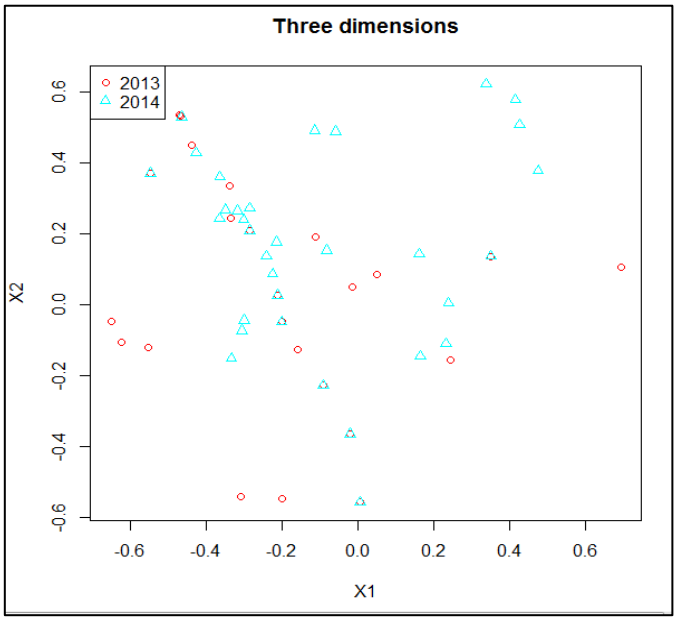

Figure 5.82. NMDS ordinations of arthropod data (by Order) collected by sweep nets between 2013 and 2014 trials at Snook, Texas. Minimum stress for given dimensionality 0.1156 and r^2 for minimum stress configuration was 0.9350.

Total Order in 2013

A total of five Orders of Class Insecta and one Order of Class Arachnida (Araneae) have been collected from sweep nets in 2013 trial. Table 5.65 showed the Orders identified in summer 2013 and the most dominant arthropod collected was the Diptera (87.54%), followed by Hemiptera (6.23%), Orthoptera (3.74%), and others (less than 3%).

Table 5.65. Total abundance and sominance of Orders in the Class Insecta, Class Arachnida and other arthropod classes identified from all sweep net samples in summer 2013 at Snook, Texas.

Order	Total abundance	Dominance
Diptera	281	87.54
Hemiptera	20	6.23
Orthoptera	12	3.74
Hymenoptera	4	1.25
Coleoptera	2	0.62
Araneae	2	0.62
Total	321	100

Total Family in 2013

A total of 13 families of arthropods (including two families from the Order Araneae) were identified from sweep nets in 2013 (Table 5.66). Total abundance of all arthropods identified to Family level was 303 individuals. The dominant family was Calliphoridae (70.30%), followed by Muscidae (14.19%), Cicadellidae (7.92%) and other families (less than 3%).

Table 5.66. Total abundance and sominance of Families in the Class Insecta, Arachnida and Malacostraca identified from all sweep net samples in summer 2013 at Snook, Texas.

Family	Total abundance	Dominance
Calliphoridae	213	70.30
Muscidae	43	14.19
Cicadellidae	24	7.92
Sarcophagidae	6	1.98
Tettigoniidae	6	1.98
Acrididae	4	1.32
Cercopidae	1	0.33
Oxyopidae	1	0.33
Silphidae	1	0.33
Asilidae	1	0.33
Lycosidae	1	0.33
Gryllidae	1	0.33
Halictidae	1	0.33
Total	303	100

Total Genus and species in 2013

A total of ten genera and species of arthropods have been identified from sweep nets in 2013 trial (included an unidentified genus of Cicadellidae) (Table 5.67). The most dominant genus or species collected was *Co. macellaria* (68.81%), followed by *O. aenescens* (9.83%), Cicadellidae sp. (9.15%), *M. domestica* (4.75%), *Ch. rufifacies* (3.05%) and others (all less than 3%).

Table 5.67. Total abundance and sominance of Genera in the Class Insecta identified from sweep net samples in summer 2013 at Snook, Texas.

Family	Total abundance	Dominance
Cochliomyia macellaria	203	68.81
Hydrotaea aenescens	29	9.83
Cicadellidae sp.	27	9.15
Musca domestica	14	4.75
Chrysomya rufifacies	9	3.05
Conocephalus sp.	6	2.03
Melanopus differentialis	4	1.36
Chrysomya megacephala	1	0.34
Necrophorus marginatus	1	0.34
Gryllus texensis	1	0.34
Total	295	100

Total function in 2013

Four functional groups were identified from 305 arthropods collected in sweep nets in summer 2013. The most dominant group was the necrophagous guild (85.90%), followed by herbivores (12.79%), predators/parasites (0.98%), and nectarivores (0.33%).

Order in 2013

PERMANOVA was performed on arthropod structural data by Order level. Results showed that there was no significant difference ($p > 0.05$) in Day, and Treatment. However, there was a significant interaction between Day x Treatment ($p = 0.015$) (Table 5.68).

Table 5.68. Analysis of the arthropod community structure (by Order) collected via sweep nets in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	1.9701	0.081
Treatment	2	1.5863	0.131
Day x Treatment	2	2.5052	0.015*

For ISA, results demonstrated that the Diptera was the only indicator among arthropod Orders collected via sweep nets in summer 2013 at Snook, Texas (Table 5.69).

Table 5.69. Indicator species analysis by Order for arthropods caught by sweep nets in summer 2013 at Snook, Texas.

Type	Order	Indicator value	P value
Sweep nets	Diptera	0.4093	0.013*

Abundance

The full model showed a significant difference in Day ($p = 0.0002$). There was a significant difference ($p = 0.0302$) in average abundance detected between Control x Post-14 on Day 21. Resilience was tested and results showed resilience on Day 7 for Post-7 carcasses while Control and Post-14 carcasses were resistance throughout the decomposition days (Table 5.70). Average abundance of Diptera collected via sweep nets in 2013 trial were demonstrated in Figure 5.83. There was a significant difference in Diptera abundance on Day 21 between Control x Post-14 ($p = 0.0084$) (Figure 5.84).

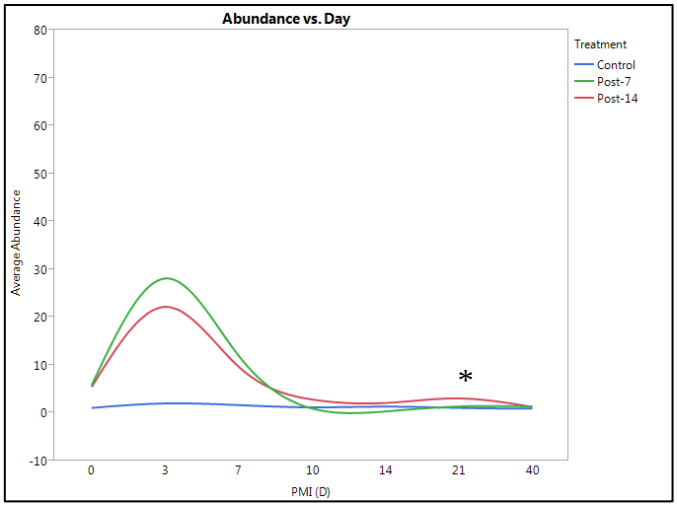

Figure 5.83. Arthropod community abundance (by Order) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.70. Resilience for arthropod community (by Order) abundance collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1465	Resistance
Post-7	0 x 3	0.0268*	7
Post-14	None	0.1571	Resistance

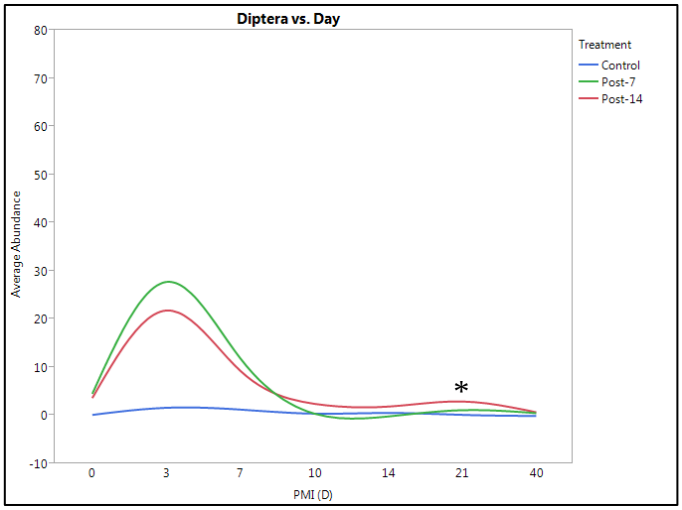

Figure 5.84. Average abundance of Diptera collected via sweep nets in summer 2013 at Snook, Texas trial across Treatments over time (* represents significant difference).

Richness

The full model showed no significant difference ($p > 0.05$) in Day, Treatment, or any interaction. Moreover, there was no significant difference ($p > 0.05$) in richness between treatments in all sampling days (Figure 5.85). Resilience was tested for all treatments and results showed resistance in richness throughout the days for Control and both treatments (Table 5.71).

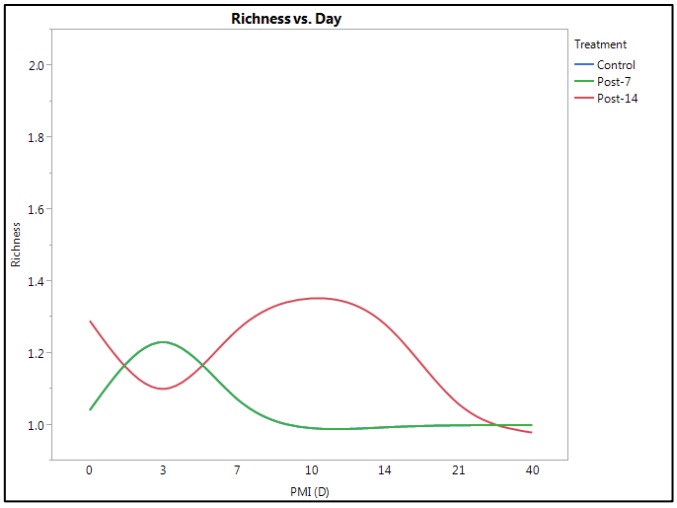

Figure 5.85. Arthropod community richness (by Order) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.71. Resilience for arthropod community (by Order) richness collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.4628	Resistance
Post-14	None	0.7982	Resistance

Simpson's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference was found in Simpson's Diversity between treatments in all sampling days ($p > 0.05$) (Figure 5.86). In other words, the system was resistance that no divergence or convergence was observed. Resilience was tested for all treatments and the results demonstrated that all carcasses were resistance throughout all sampling days (Table 5.72).

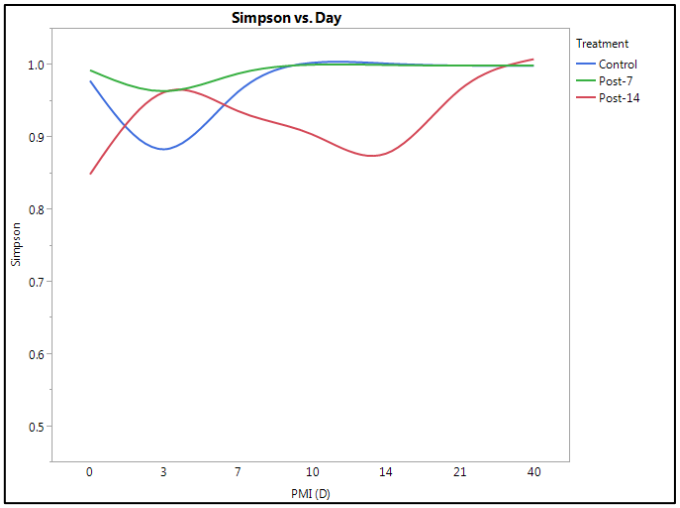

Figure 5.86. Simpson's diversity of the arthropod community (by Order) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.72. Resilience for Simpson's Diversity of the arthropod community (by Order) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.4628	Resistance
Post-14	None	0.7421	Resistance

Shannon-Wiener's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) was found in Shannon-Wiener's Diversity between treatments in all sampling days (Figure 5.87). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.73).

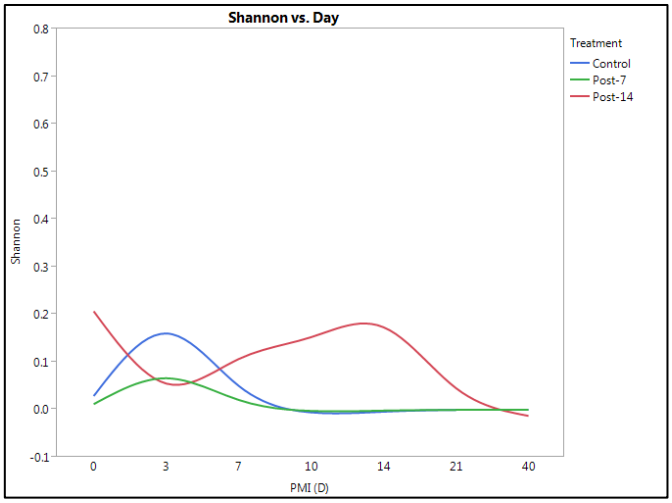

Figure 5.87. Shannon-Wiener's diversity of the arthropod community (by Order) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.73. Resilience for Shannon-Wiener's Diversity of the arthropod community (by Order) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.4628	Resistance
Post-14	None	0.7643	Resistance

Evenness

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) found in evenness between treatments in all sampling days (Figure 5.88). Resilience was tested for all treatments and all of them demonstrated resistance throughout all sampling days (Table 5.74).

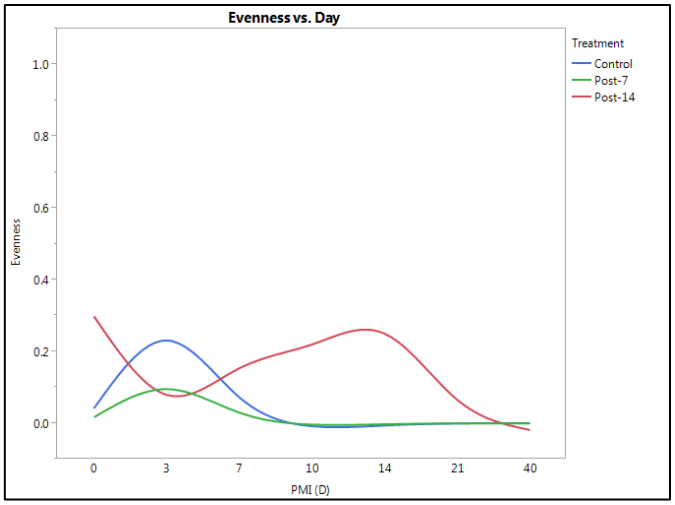

Figure 5.88. Evenness of the arthropod community (by Order) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.74. Resilience for evenness of the arthropod community (by Order) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.4628	Resistance
Post-14	None	0.7643	Resistance

Effective number of species

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) found in ENS between treatments in all sampling days (Figure 5.89). Resilience was tested for all treatments and all of them demonstrated resistance throughout all sampling days (Table 5.75).

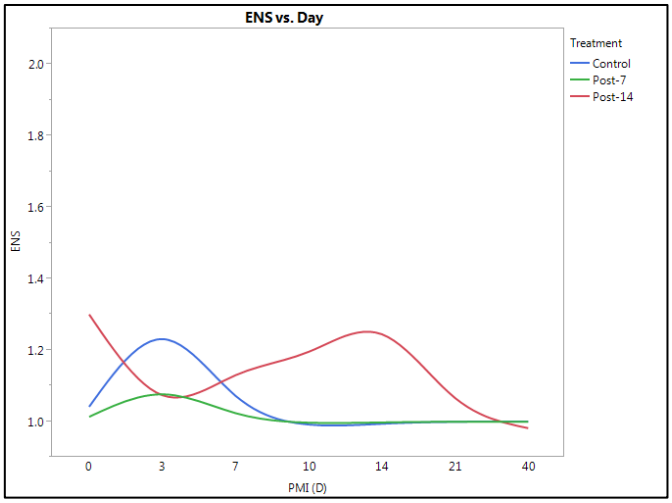

Figure 5.89. Effective Number of Species of the arthropod community (by Order) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.75. Resilience for ENS of the arthropod community (by Order) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.4628	Resistance
Post-14	None	0.7467	Resistance

Family in 2013

PERMANOVA was performed on arthropod structural data by Family level. Results showed that there was significant difference in Treatment ($p = 0.027$), but no significant difference in Day, Replicate or any interaction ($p < 0.05$) (Table 5.76).

Table 5.76. Analysis of the arthropod community structure (by Family) collected via sweep nets in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	1.5011	0.156
Treatment	2	2.1661	0.027*
Day x Treatment	2	1.4648	0.163

Since there was significant difference in Treatment, further analyses were performed (Table 5.77). Results showed Control x Post-14 was significantly different ($p = 0.007$). The NMDS plot of stress for crawling arthropod community structure (Figure 5.90) and NMDS ordinations for Treatments was provided for visualization about data distribution (Figure 5.91). Minimum stress for given dimensionality was 0.1618 with $r^2 = 0.8614$. The MRPP analysis for treatments showed a significant difference (A value = 0.0338; Significant of Delta = 0.019 based on 999 permutations).

Table 5.77. Pairwise comparisons of arthropod community structure (by Family) collected via sweep nets between treatments in summer 2013 at Snook, Texas after Bonferroni's correction.

Treatment	df	SS	MS	F model	R2	P value
Control x Post-7	1	0.5967	0.5966	1.6333	0.0392	0.143
Residual	40	14.6131	0.3653		0.9608	
Total	41	15.2098			1.0000	
Control x Post-14	1	1.3073	1.3073	3.726	0.0852	0.007*
Residual	40	14.0350	0.3508		0.9148	
Total	41	15.3423			1.0000	
Post-7 x Post-14	1	0.4527	0.4527	1.1382	0.0277	0.303
Residual	40	15.9103	0.3977		0.9723	
Total	41	16.3630			1.0000	

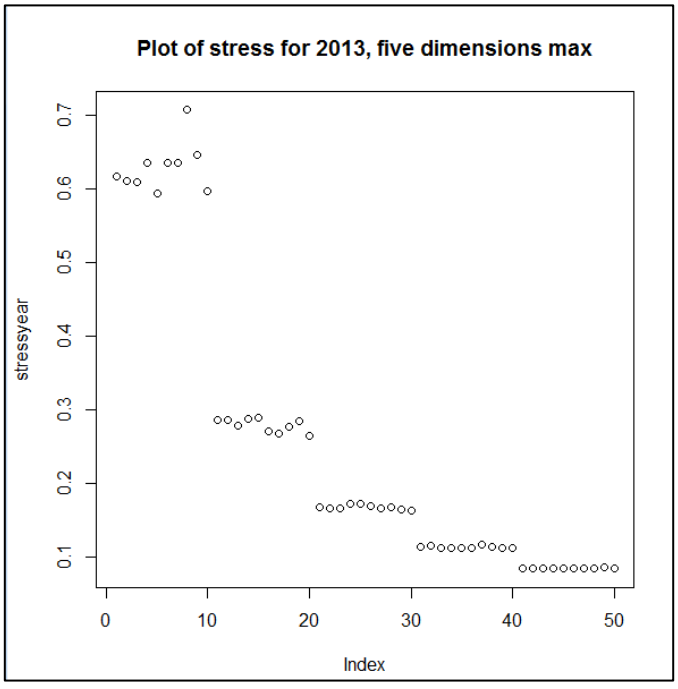

Figure 5.90. NMDS plot of stress for arthropod community structure (by Family) collected via sweep nets in summer 2013 at Snook, Texas (Stress test 0.1618; r^2 = 0.8614).

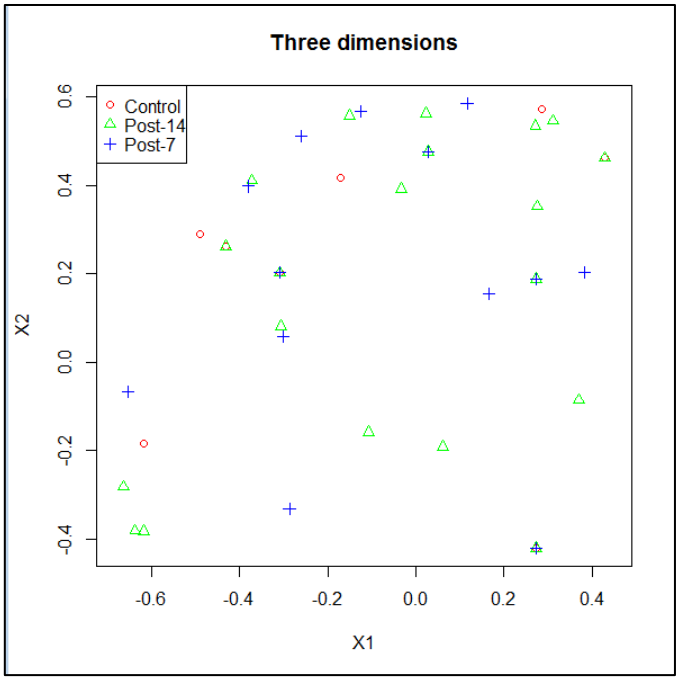

Figure 5.91. NMDS ordinations for arthropod community structure by treatments (by Family) collected via sweep nets in summer 2013 at Snook, Texas.

For ISA, results showed that the adults of Calliphoridae and Muscidae were the indicators among arthropod families collected via sweep nets in summer 2013 at Snook, texas (Table 5.78).

Table 5.78. Indicator species analysis by Family for arthropods caught by sweep nets in summer 2013 at Snook, Texas.

Type	Family	Indicator value	P value
Sweep nets	Calliphoridae	0.4648	0.007*
	Muscidae	0.3488	0.006*

Abundance

The full model showed there was significant difference in Day ($p = 0.0002$), but no significant difference in Treatment or any interaction ($p > 0.05$). There was no significant difference ($p > 0.05$) between treatments in all sampling days. Resilience was tested and results showed Post-7 carcasses had resilience on Day 7 while Control and Post-14 carcasses were resistance throughout all sampling days (Table 5.79). Average abundance of arthropods according to Family collected at sweep nets in summer 2013 were demonstrated in Figure 5.92. There was significant difference in Muscidae abundance on Day 3 between Control x Post-7 ($p = 0.0336$). No significant difference ($p > 0.05$) in abundance was detected for Calliphoridae across treatments in all sampling days (Figure 5.93).

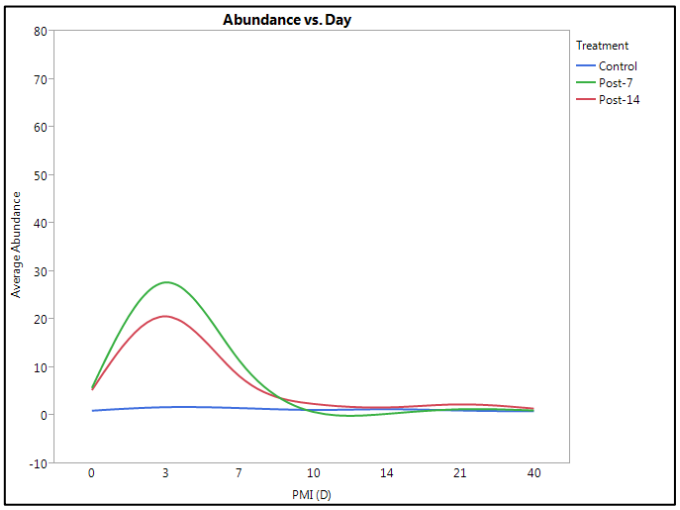

Figure 5.92. Arthropod community abundance (by Family) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.79. Resilience for arthropod community (by Family) abundance collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4299	Resistance
Post-7	0 x 3	0.0286*	7
Post-14	None	0.1311	Resistance

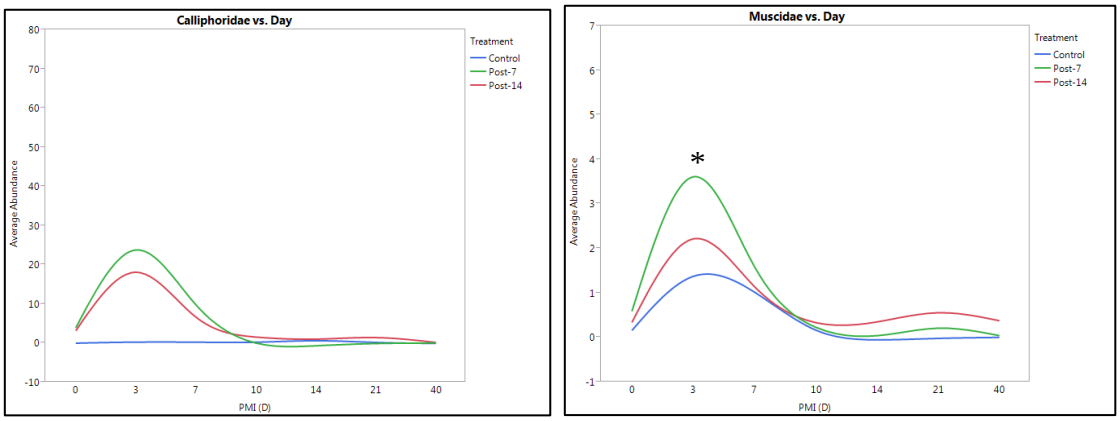

Figure 5.93. Average abundance of arthropods according to Families collected via sweep nets in summer 2013 at Snook, Texas. Left. Abundance of Calliphoridae across Treatments over time. Right. Abundance of Muscidae across Treatments over time (* represents significant difference).

Richness

The full model showed a significant difference in Day ($p = 0.0018$), and Treatment ($p = 0.0005$) without any significant interaction ($p > 0.05$). There was no significant difference ($p > 0.05$) between treatments in all sampling days (Figure 5.94). Resilience was tested and results showed Post-7 carcasses had resilience on Day 7 while Control and Post-14 carcasses were resistance throughout all sampling days (Table 5.80).

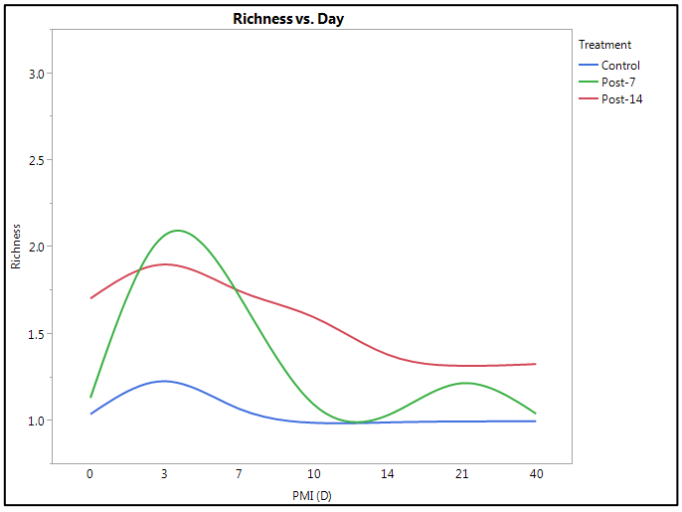

Figure 5.94. Arthropod community richness (by Family) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.80. Resilience for arthropod community (by Family) richness collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	0 x 3	0.0097*	7
Post-14	None	0.6781	Resistance

Simpson's diversity index

The full model showed a significant difference in Treatment ($p = 0.0034$). There was no significant difference ($p > 0.05$) in Simpson's diversity between treatments in all sampling days (Figure 5.95). Resilience was tested and results showed resistance in all sampling days for all treatment groups (Table 5.81).

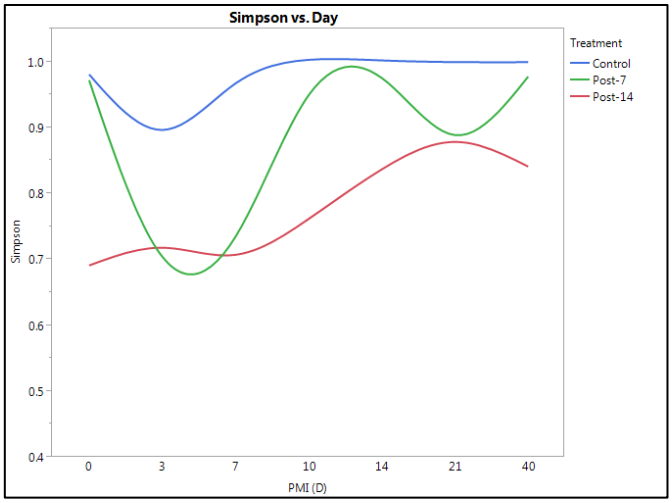

Figure 5.95. Simpson's diversity of the arthropod community (by Family) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.81. Resilience for Simpson's Diversity of the arthropod community (by Family) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.0771	Resistance
Post-14	None	0.9220	Resistance

Shannon-Wiener's diversity index

The full model showed a significant difference in Treatment ($p = 0.0024$). There was no significant difference ($p > 0.05$) in Shannon-Wiener's diversity between treatments in all sampling days (Figure 5.96). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.82).

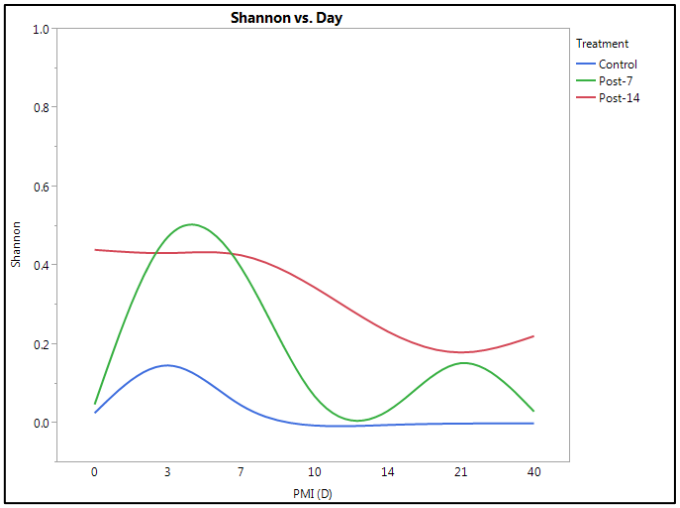

Figure 5.96. Shannon-Wiener's diversity of the arthropod community (by Family) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.82. Resilience for Shannon-Wiener's Diversity of the arthropod community (by Family) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.0488*	Resistance
Post-14	None	0.9105	Resistance

Evenness

The full model showed a significant difference in Treatment ($p = 0.0017$). There was no significant difference ($p > 0.05$) in evenness between treatments in all sampling days (Figure 5.97). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days, although marginal significant difference was detected on Post-7 carcasses (Table 5.83).

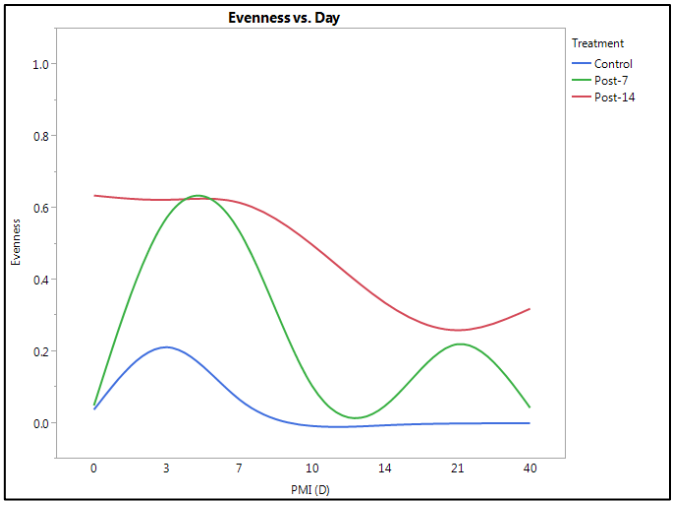

Figure 5.97. Evenness of the arthropod community (by Family) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.83. Resilience for evenness of the arthropod community (by Family) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.0551•	Resistance
Post-14	None	0.9105	Resistance

• Marginal significant difference.

Effective number of species

The full model showed a significant difference in Treatment ($p = 0.0042$). There was no significant difference ($p > 0.05$) in ENS between treatments in all sampling days (Figure 5.98). Resilience was tested for all treatments and all of them demonstrated resistance throughout all sampling days (Table 5.84).

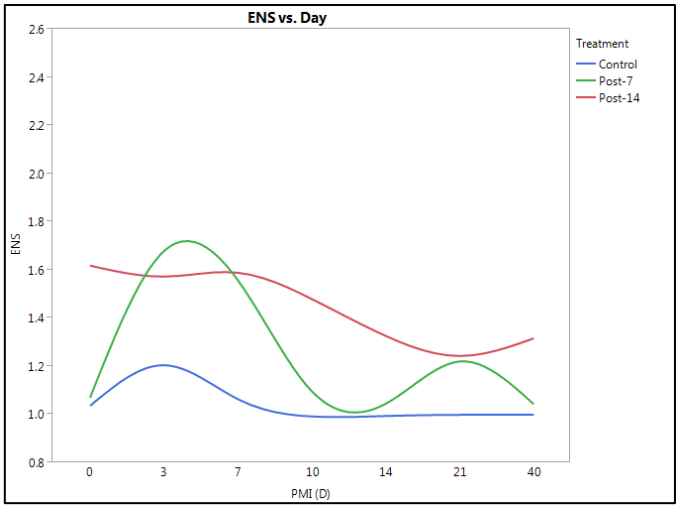

Figure 5.98. Effective Number of Species of the arthropod community (by Family) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.84. Resilience for ENS of the arthropod community (by Family) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.0749	Resistance
Post-14	None	0.9198	Resistance

Genus and species in 2013

PERMANOVA was performed on arthropod structural data by Genus and species level. Results showed that there was significant difference in Treatment ($p = 0.011$), and a marginal significant difference in Day ($p = 0.070$). There was no significant difference in Replicate or any interaction ($p < 0.05$) (Table 5.85).

Table 5.85. Analysis of the arthropod community structure (by Genus and species) collected via sweep nets in summer 2013 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	2.1362	0.070•
Treatment	2	2.6311	0.011*
Day x Treatment	2	0.6230	0.817

• Marginal significant difference.

Since there was significant difference in Treatment, further analyses were performed. Comparison between treatments demonstrated that Control x Post-14 and Post-7 x Post-14 was significantly different ($p < 0.05$) (Table 5.86). The NMDS plot of stress for crawling arthropod community structure (Figure 5.99) and NMDS ordinations for Treatments was provided for visualization about data distribution (Figure 5.100). Minimum stress for given dimensionality was 0.1493 with $r^2 = 0.9061$. The MRPP for treatments showed A value 0.0431 and Significant of Delta 0.015.

Table 5.86. Pairwise comparisons of arthropod community structure (by Genus and species) collected via sweep nets between treatments in summer 2013 at Snook, Texas after Bonferroni's correction.

Treatment	df	SS	MS	F model	R2	P value
Control x Post-7	1	0.2561	0.2561	0.7624	0.0187	0.512
Residual	40	13.4370	0.3359		0.9813	
Total	41	13.6931			1.0000	
Control x Post-14	1	1.5191	1.5191	4.1444	0.0939	0.003*
Residual	40	14.6619	0.3665		0.9061	
Total	41	16.1811			1.0000	
Post-7 x Post-14	1	1.0411	1.0410	2.7782	0.0650	0.025*
Residual	40	14.9893	0.3747		0.9350	
Total	41	16.0303			1.0000	

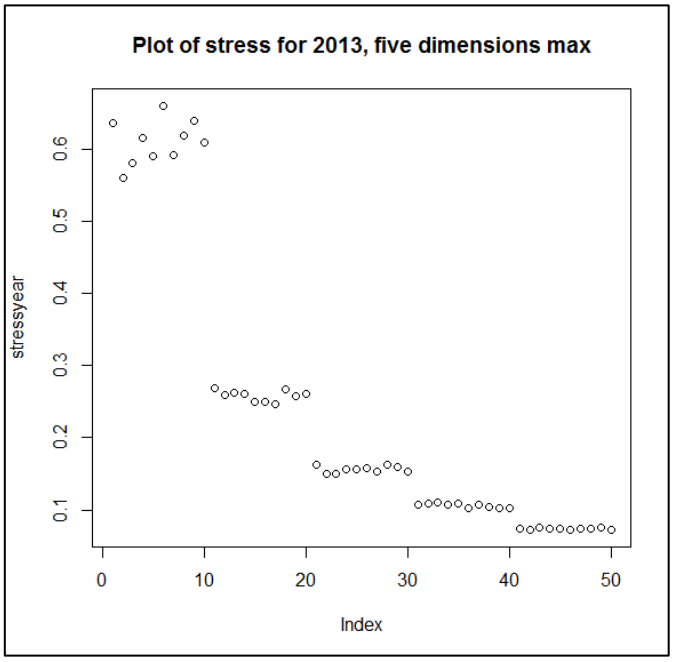

Figure 5.99. NMDS plot of stress for arthropod community structure (by Genus and species) collected via sweep nets in summer 2013 at Snook, Texas (Stress test 0.1493; r^2 = 0.9061).

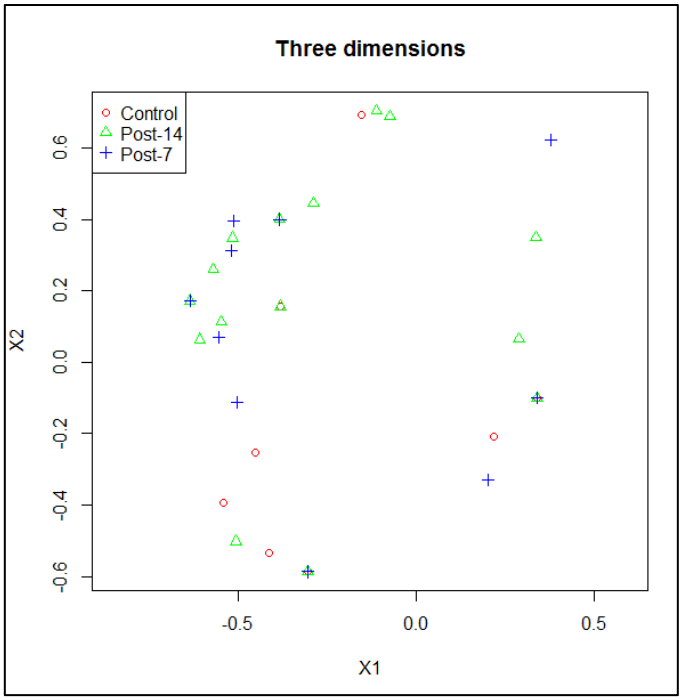

Figure 5.100. NMDS ordinations for arthropod community structure by treatments (by Genus and species) collected via sweep nets in summer 2013 at Snook, Texas.

For ISA, results showed that the *Co. macellaria*, *O. aenescens*, and *M. domestica* were the indicators among arthropods collected via sweep nets in summer 2013 at Snook, Texas (Table 5.87).

Table 5.87. Indicator species analysis by Genus and species for arthropods caught by sweep nets in summer 2013 at Snook, Texas.

Type	Genus and species	Indicator value	P value
Sweep nets	*M. domestica*	0.3571	0.015*
	Co. macellaria	0.4729	0.006*
	O. aenescens	0.4138	0.006*

Abundance

The full model showed a significant difference in Day ($p = 0.0001$), but not in Treatment or any interaction ($p > 0.05$). There was significant difference detected between treatments on Day 21 ($p = 0.0341$). Resilience was observed on Day 7 for Post-7 carcasses while Control and Post-14 carcasses were resistance in all sampling days (Table 5.88). The average abundance of several important genera was highlighted in Figure 5.101. For *O. aenescens*, significant difference in abundance was noted on Day 3 between Control x Post-7 ($p = 0.0242$). There was no significant difference ($p > 0.05$) detected in abundance between treatments in all sampling days for other genera (Figure 5.102).

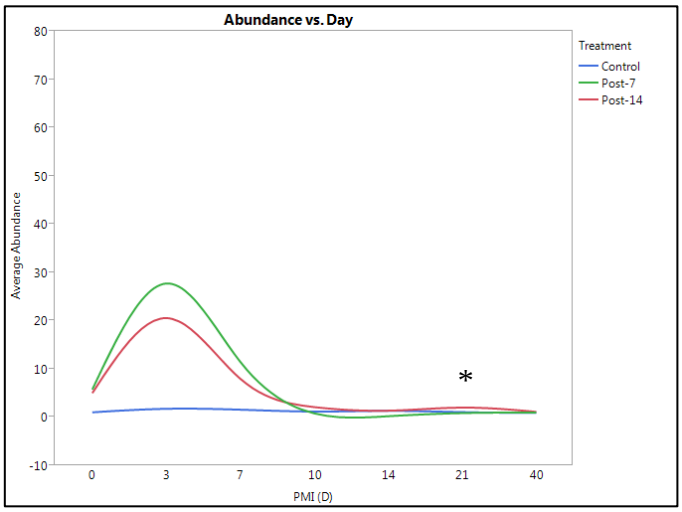

Figure 5.101. Arthropod community abundance (by Genus and species) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.88. Resilience for arthropod community (by Genus and species) abundance collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4299	Resistance
Post-7	0 x 3	0.0285*	7
Post-14	None	0.1216	Resistance

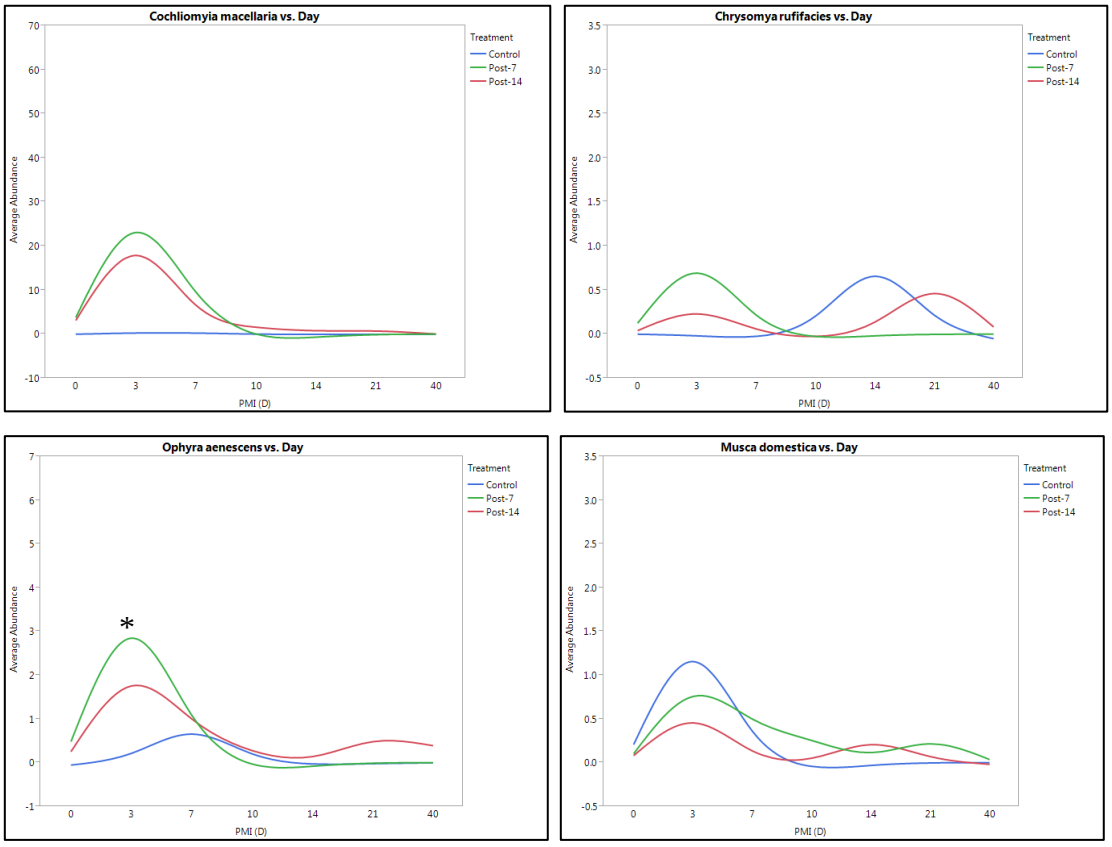

Figure 5.102. Average abundance of arthropods according to Genus and species collected via sweep nets in summer 2013 at Snook, Texas. Upper Left. Abundance of *Co. macellaria* across Treatments over time. Upper Right. Abundance of *Ch. rufifacies* across Treatments over time. Lower Left. Abundance of *O. aenescens* across Treatments over time. Lower Right. Abundance of *M. domestica* across Treatments over time (* represents significant difference).

Richness

The full model showed a significant difference in Day ($p < 0.0001$), Treatment ($p = 0.0009$) and interaction between Day x Treatment ($p = 0.0022$). There was significant difference found in richness between Control x Post-7 ($p = 0.0383$) on Day 3 (Figure 5.103). Resilience was observed on Day 7 for Post-7 and Post-14 carcasses while Control carcasses were resistance in all sampling days (Table 5.89).

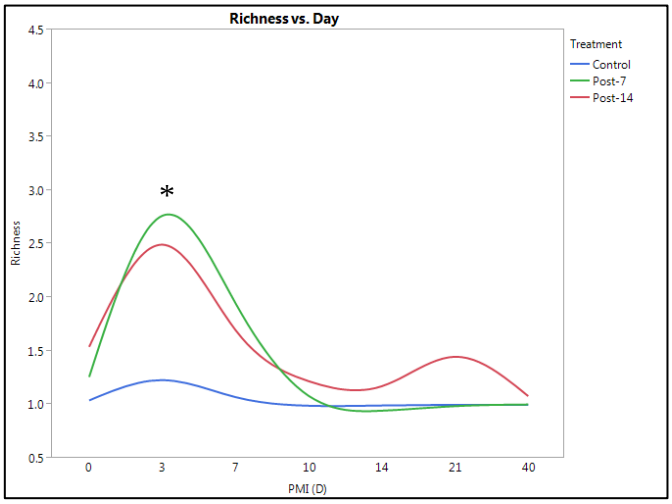

Figure 5.103. Arthropod community richness (by Genus and species) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas (* represents significant difference).

Table 5.89. Resilience for arthropod community (by Genus and species) richness collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	0 x 3	<0.0001*	7
Post-14	0 x 3	0.0403*	7

Simpson's diversity index

The full model showed a significant difference in Day (p = 0.0058), and Treatment (p = 0.0311) and there was no significant interaction. There was no significant difference (p > 0.05) found in Simpson's diversity between treatments in all sampling days (Figure 5.104). Resilience was tested and resilience was observed on Day 7 for Post-7 carcasses while Control and Post-14 carcasses were resistance in all sampling days (Table 5.90).

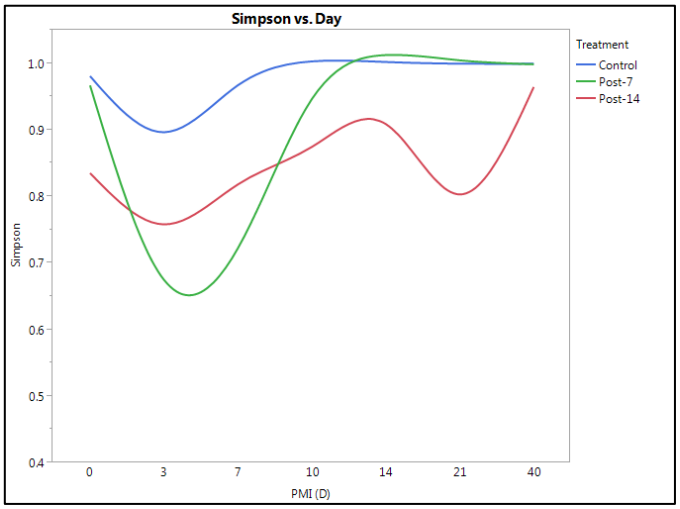

Figure 5.104. Simpson's diversity of the arthropod community (by Genus and species) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.90. Resilience for Simpson's Diversity of the arthropod community (by Genus and species) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	0 x 3	0.0284*	7
Post-14	None	0.4898	Resistance

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p = 0.0005$), and Treatment ($p = 0.0162$), but no significant in interaction. There was no significant difference ($p > 0.05$) found in Shannon-Wiener's Diversity between treatments in all sampling days (Figure 5.105). Resilience was observed on Day 7 for Post-7 carcasses while Control and Post-14 carcasses were resistance in all sampling days (Table 5.91).

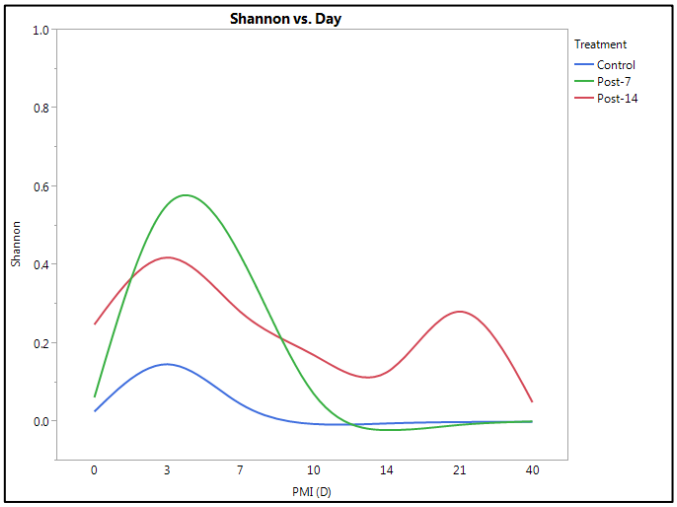

Figure 5.105. Shannon-Wiener's diversity of the arthropod community (by Genus and species) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.91. Resilience for Shannon-Wiener's Diversity of the arthropod community (by Genus and species) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	0 x 3	0.0047*	7
Post-14	None	0.3351	Resistance

Evenness

The full model showed a significant difference in Day ($p = 0.0175$), and Treatment ($p = 0.0324$), but no significant interaction. There was no significant difference ($p > 0.05$) found in evenness between treatments in all sampling days (Figure 5.106). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days, although Post-7 showed significant values ($p = 0.0066$) but pairwise comparisons did not reveal any significant pairs (Table 5.92).

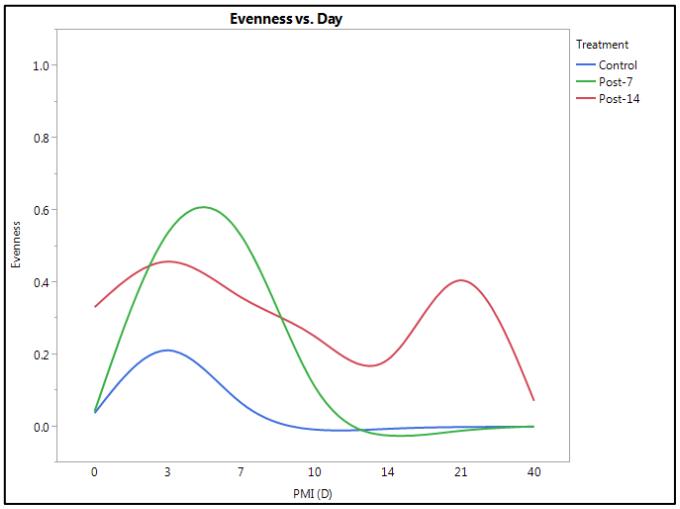

Figure 5.106. Evenness of the arthropod community (by Genus and species) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.92. Resilience for evenness of the arthropod community (by Genus and species) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	None	0.0066*	Resistance
Post-14	None	0.5142	Resistance

Effective number of species

The full model showed a significant difference in Day ($p = 0.0007$), and Treatment ($p = 0.0227$) without any significant interaction. There was no significant difference ($p > 0.05$) found in ENS between treatments in all sampling days (Figure 5.107). Resilience was observed on Day 7 for Post-7 carcasses while Control and Post-14 carcasses were resistance in all sampling days (Table 5.93).

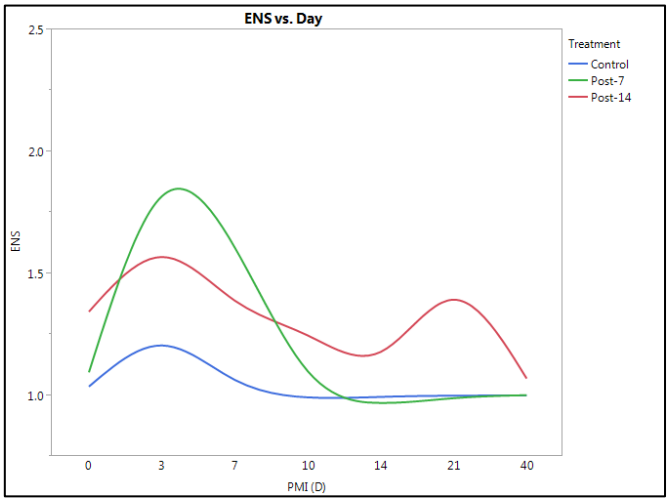

Figure 5.107. Effective Number of Species of the arthropod community (by Genus and species) collected via sweep nets across Treatments over time in summer 2013 at Snook, Texas.

Table 5.93. Resilience for ENS of the arthropod community (by Genus and species) collected via sweep nets for each treatment in summer 2013 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4628	Resistance
Post-7	0 x 3	0.0076*	7
Post-14	None	0.3910	Resistance

Function in 2013 and 2014

Due to no year effect was observed in arthropod functions between two trials, thus two years data (summers 2013 and 2014) was pooled together for statistical analyses. PERMANOVA was performed on arthropod functional data. Results showed that there was significant difference in Day ($p = 0.004$), Treatment ($p = 0.019$) and a marginal significant interaction Day x Treatment ($p = 0.054$). Note that there was no significant difference in Replicate ($p = 0.864$) (Table 5.94).

Table 5.94. Analysis of the arthropod community functions collected via sweep nets in summers 2013 and 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	5.4453	0.004*
Treatment	2	2.7027	0.019*
Day x Treatment	2	2.1955	0.054•

• Marginal significant difference.

Since there was significant difference in Day and Treatment, further analyses were performed. For day of decomposition, most of the day to day comparisons were significantly different, except Day 3 x Day 7, Day 10 x Day 14, Day 10 x Day 21, Day 14 x Day 21, and Day 14 x Day 40 (Table 5.95). Comparison between treatments demonstrated that Control x Post-14 was significantly different ($p = 0.007$) (Table 5.96). The NMDS plot of stress for arthropod community function (Figure 5.108) and NMDS ordinations for Day and Treatment were provided for visualization about data distribution (Figure 5.109 and 5.110, respectively). Minimum stress for given dimensionality was 0.0819 with $r^2 = 0.9718$. The MRPP analysis for day showed A value = 0.1929; Significant of Delta = 0.001 based on 999 permutations) while MRPP for treatments showed A value 0.0277 and Significant of Delta 0.012.

Table 5.95. Pairwise comparisons of arthropod community function collected via sweep nets between carrion decomposition days in both summers 2013 and 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.001*	0.001*	0.001*	0.004*	0.004*	0.011*
3	0.001*	-	0.228	0.002*	0.001*	0.001*	0.001*
7	0.001*	0.288	-	0.039*	0.001*	0.003*	0.001*
10	0.001*	0.002*	0.039*	-	0.195	0.441	0.016*
14	0.004*	0.001*	0.001*	0.195	-	0.580	0.125
21	0.004*	0.001*	0.003*	0.441	0.580	-	0.037*
40	0.011*	0.001*	0.001*	0.016*	0.125	0.037*	-

Table 5.96. Pairwise comparisons between treatments for arthropod community function collected via sweep nets in both summers 2013 and 2014 at Snook, Texas after Bonferroni's correction.

Treatments	df	SS	MS	F model	R2	P value
Control x Post-7	1	0.6656	0.6655	2.2554	0.0268	0.08
Residual	82	24.1981	0.2951		0.9732	
Total	83	24.8637			1.0000	
Control x Post-14	1	1.4005	1.4005	5.0796	0.0583	0.007*
Residual	82	22.6082	0.2751		0.9417	
Total	83	24.0087			1.0000	
Post-7 x Post-14	1	0.1775	0.1774	0.5810	0.0070	0.644
Residual	82	25.0462	0.3054		0.9930	
Total	83	25.2237			1.0000	

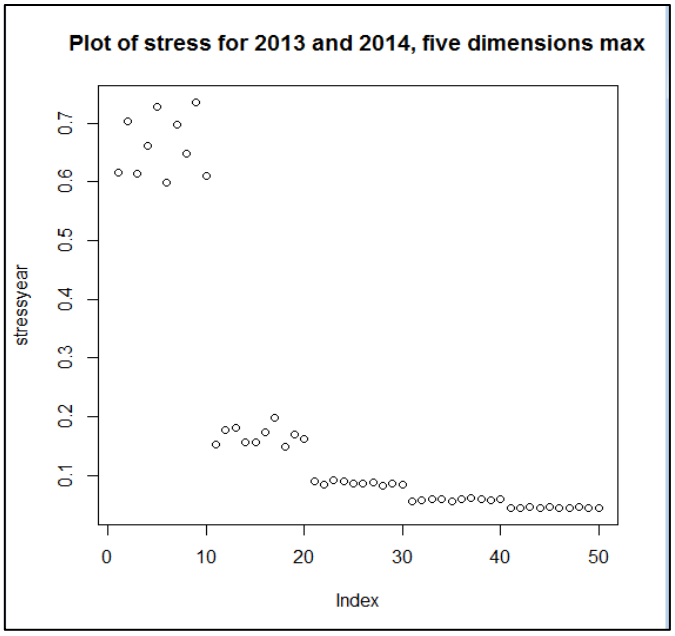

Figure 5.108. NMDS plot of stress for arthropod community function collected via sweep nets in summers 2013 and 2014 at Snook, Texas (Stress test 0.0819; $r^2 = 0.9718$).

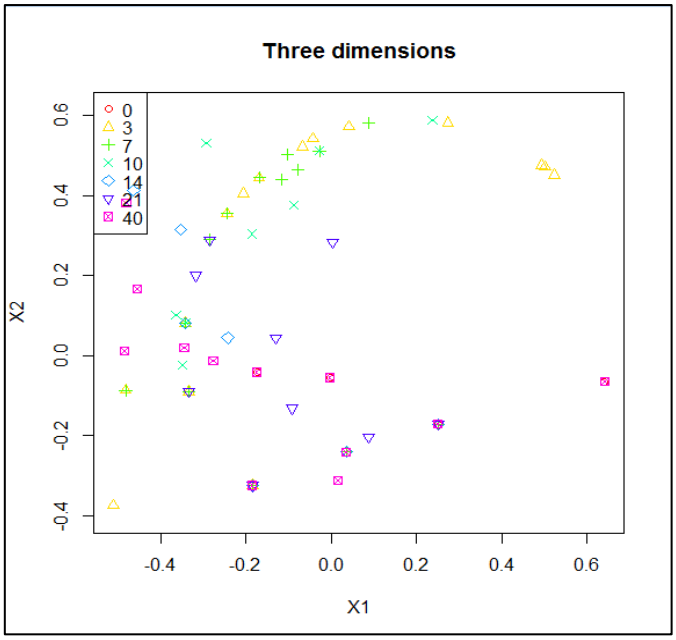

Figure 5.109. NMDS ordinations for arthropod community function by carrion decomposition days collected via sweep nets in summers 2013 and 2014 at Snook, Texas.

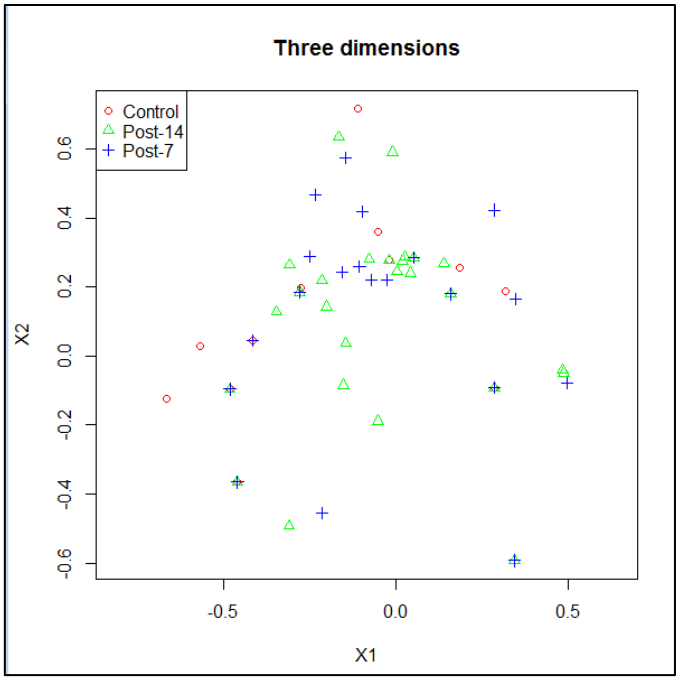

Figure 5.110. NMDS ordinations for arthropod community function by treatments collected via sweep nets in summers 2013 and 2014 at Snook, Texas.

For ISA, results showed one significant indicator (necrophagous) among arthropods by functional groups in both 2013 and 2014 trials at Snook, Texas (Table 5.97).

Table 5.97. Indicator species analysis by functional groups for arthropods caught by sweep nets in summers 2013 and 2014 at Snook, Texas.

Type	Functional group	Indicator value	P value
Sweep nets	Necrophagous	0.3277	0.003*

Abundance in both trials

Four functional groups namely necrophagous, herbivores, predators/parasites, and detritivores were highlighted individually (Figure 5.111). There was significant difference in the average abundance of necrophagous guild between Control x Post-14 ($p = 0.0375$) and Post-7 x Post-14 ($p = 0.0153$) on Day 14, as well as on Day 21 (Control x Post-14 ($p = 0.0004$) and Post-7 x Post-14 ($p = 0.0041$)). For other functional groups, no significant difference ($p > 0.05$) was detected between treatments in all sampling days.

Resilience was tested for all treatments in four functional groups (nectarivores was excluded due to low numbers of individual collected). The results showed all functional groups were resistance to perturbations throughout all sampling days except for necrophagous guild of Post-7 and Post-14 carcasses, which the resilience was observed on Day 7 (Table 5.98).

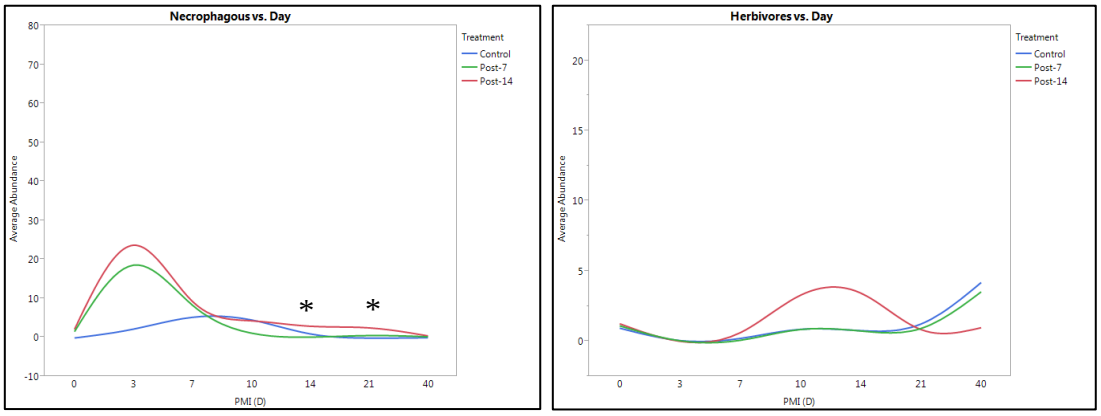

Figure 5.111. Average abundance of arthropods according to functional groups collected via sweep nets in both summers 2013 and 2014 at Snook, Texas. Upper Left. Abundance of necrophagous across Treatments over time. Upper Right. Abundance of herbivores across Treatments over time. Lower Left. Abundance of predators/parasites across Treatments over time. Lower Right. Abundance of detritivores across Treatments over time (* represents significant difference).

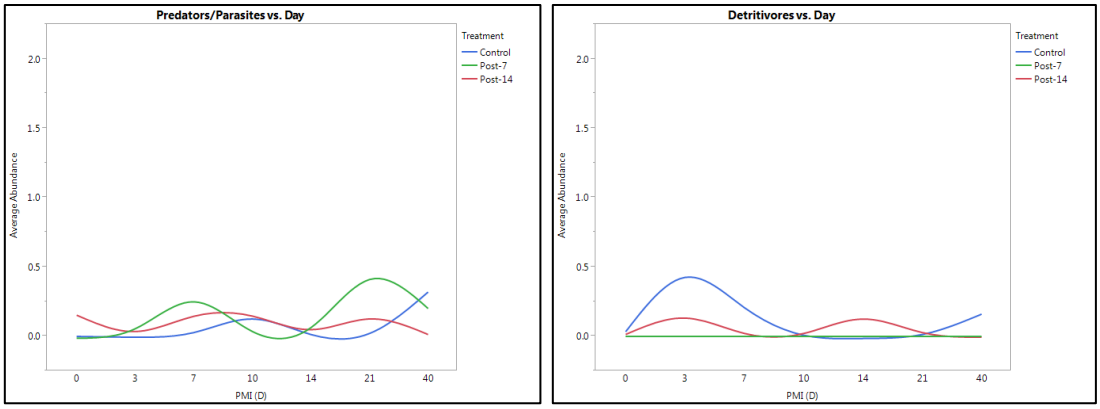

Figure 5.111. (Continued).

Table 5.98. Resilience for arthropod community functions collected via sweep nets for each treatment in both summers 2013 and 2014 at Snook, Texas.

Function	Treatment	Significant difference	P value	Resilience on Day
Necrophagous	Control	None	0.1900	Resistance
	Post-7	0 x 3	0.0106*	7
	Post-14	0 x 3	0.0010*	7
Detritivores	Control	None	0.2431	Resistance
	Post-7			Resistance
	Post-14	None	0.5525	Resistance
Predators/Parasites	Control	None	0.5289	Resistance
	Post-7	None	0.2006	Resistance
	Post-14	None	0.8040	Resistance
Herbivores	Control	None	0.1192	Resistance
	Post-7	None	0.0041*	Resistance[#]
	Post-14	None	0.4550	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Community structure and function of aboveground arthropods collected by sticky traps in 2014

Total Order in 2014

A total of 12 Orders of Insecta, one Order of Arachnida (Araneae) and one subclass of Arachnida (Acari) have been recovered from all sticky traps in 2014 trial. Figure 5.112 showed the Orders/Subclass identified in 2014 trial and the most dominant group was the Diptera (30.49%), followed by Hymenoptera (25.47%), Thysanoptera (24.30%), Hemiptera (11.39%), Coleoptera (4.18%) and others (all less than 3 %).

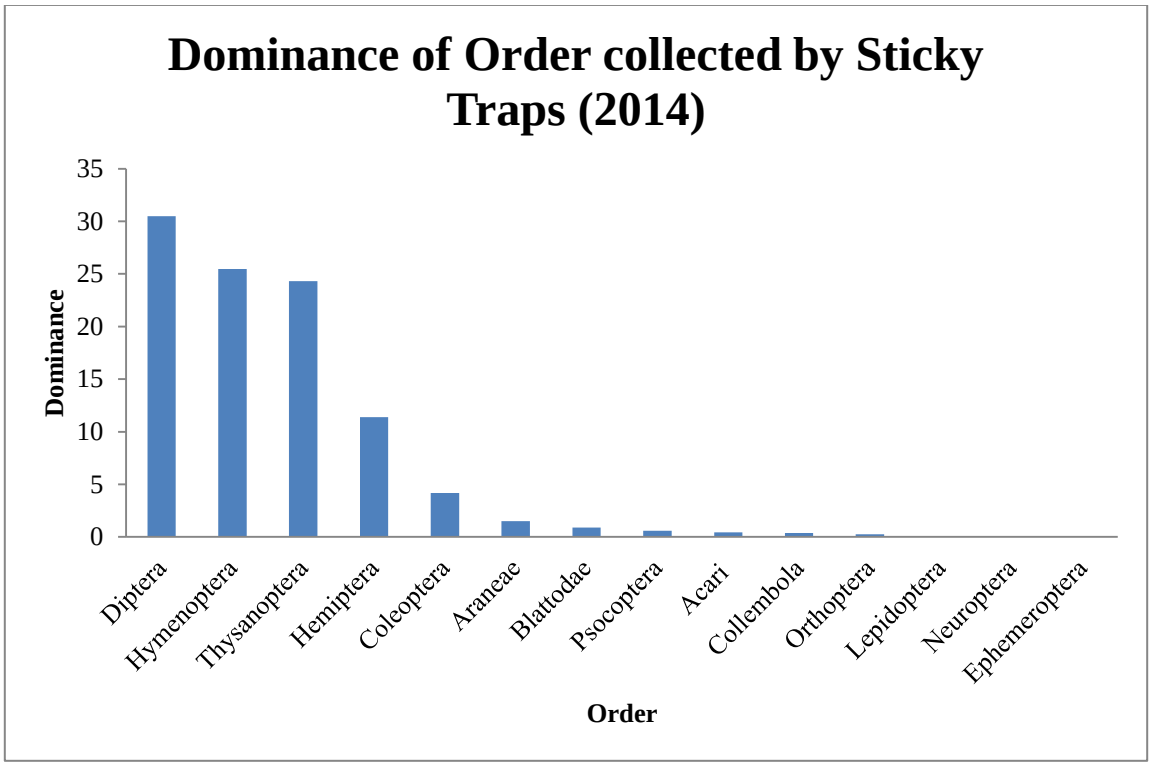

Figure 5.112. Dominance by Order of the aboveground arthropods collected using sticky traps in 2014 trial at Snook, Texas.

Total collection of arthropod (by Order) by height and position in 2014

Out of 12448 arthropods collected in 2014 trial, 8129 arthropods (65.30%) were trapped by the sticky traps at the bottom (~0.3 m from ground surface) compared to 4319 arthropods (34.70%) by the sticky trap placed at above (~1 m from ground surface). For the location of sticky traps, anterior position of the pig carcasses collected 62.87% of total arthropods while posterior position caught 37.13%. In detail, sticky traps with position and height of "Anterior Bottom" collected 49.92% of the total arthropods, sticky trap with "Anterior Above" collected 12.95%, and sticky traps at "Posterior Bottom" collected 15.38%, while sticky traps with "Posterior Above" collected 21.75% of total arthropods (Figure 5.113).

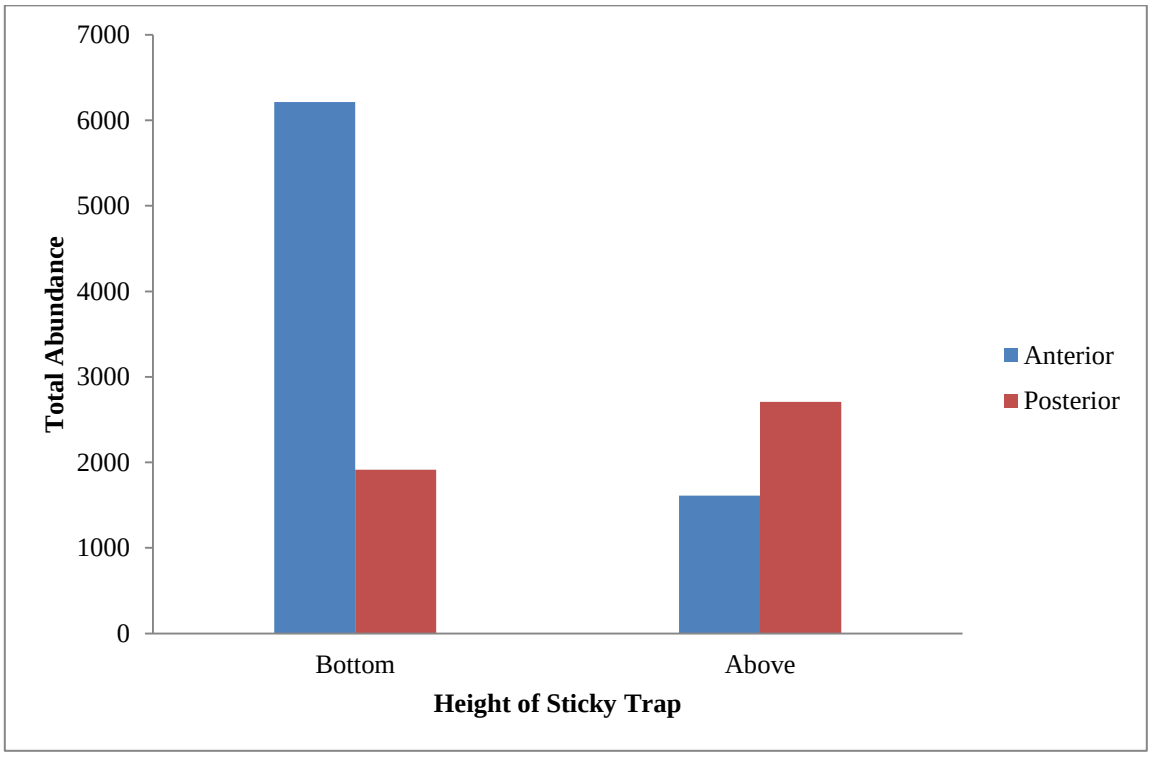

Figure 5.113. Total arthropods collected according to locations (anterior or posterior) and heights (above or bottom) of sticky traps in 2014 trial at Snook, Texas.

Total Family in 2014

A total of 103 families of arthropods (including seven families from the Order Araneae) were identified from all sticky traps in 2014 trial. Total abundance of all arthropods identified to Family level was 12027 individuals. The dominant family was Thripidae (24.01%), followed by Formicidae (18.50%), Calliphoridae (16.11%), Aphididae (7.28%), Chloropidae (6.66%), Trichogrammatidae (2.98%), Sarcophagidae (2.49%), Muscidae (2.12%) and Staphylinidae (2.00%). The other families were not shown as their dominance was less than 2%. Figure 5.114 showed the dominance of the some families collected in 2014 trial.

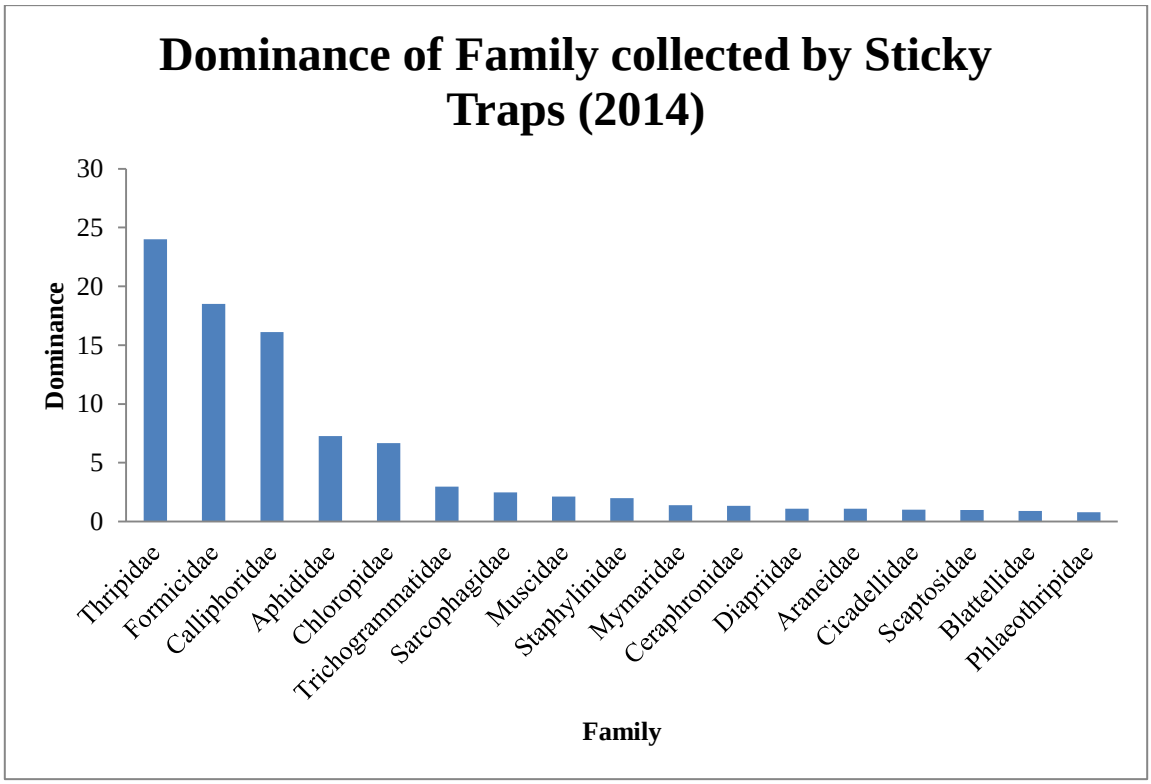

Figure 5.114. Dominance by Family of the aboveground arthropods collected using sticky traps in summer 2014 at Snook, Texas.

Total Genus and species in 2014

A total of 112 genera and species of aboveground arthropods have been identified in 2014 trial at the field site located at Snook, Texas (Figure 5.115). The most dominant genus and species collected was *S. invicta* (33.58%), followed by *Co. macellaria* (31.36%), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) (4.89%), *Oligosita* sp. (4.83%), *Incertella* sp. (Diptera: Chloropidae) (2.27%), *Frankliniella fusca* (Hinds) (Thysanoptera: Thripidae) (2.17%), *M. domestica* (2.14%) and others (all less than 2%).

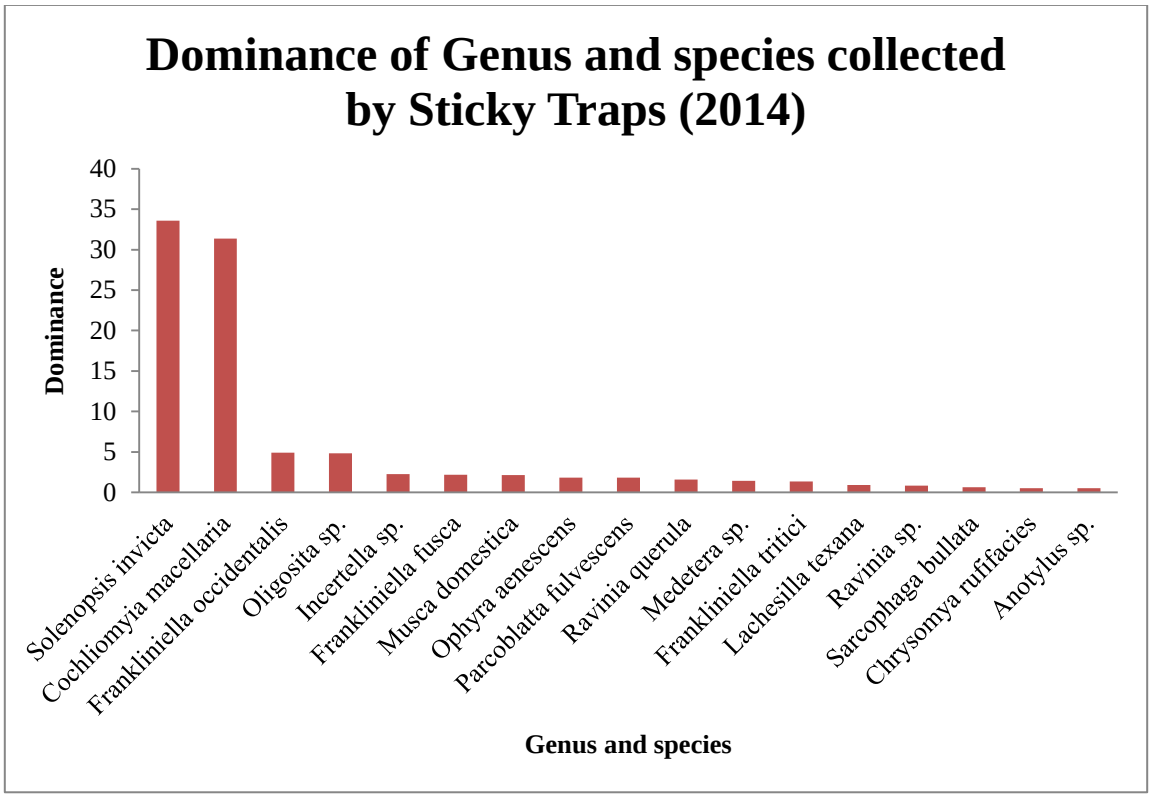

Figure 5.115. Dominance by Genera and species of the aboveground arthropods collected using sticky traps in summer 2014 at Snook, Texas.

Total function in 2014

Eight functional groups were identified from aboveground arthropods collected on sticky traps in 2014 trial. The most dominant group was herbivores (42.72%), followed by predators/parasites (30.54%), necrophagous (18.61%), detritivores (6.23%), nectarivores (0.81%), haematophagous (0.07%), and non-feeding group (0.07%) (Figure 5.116).

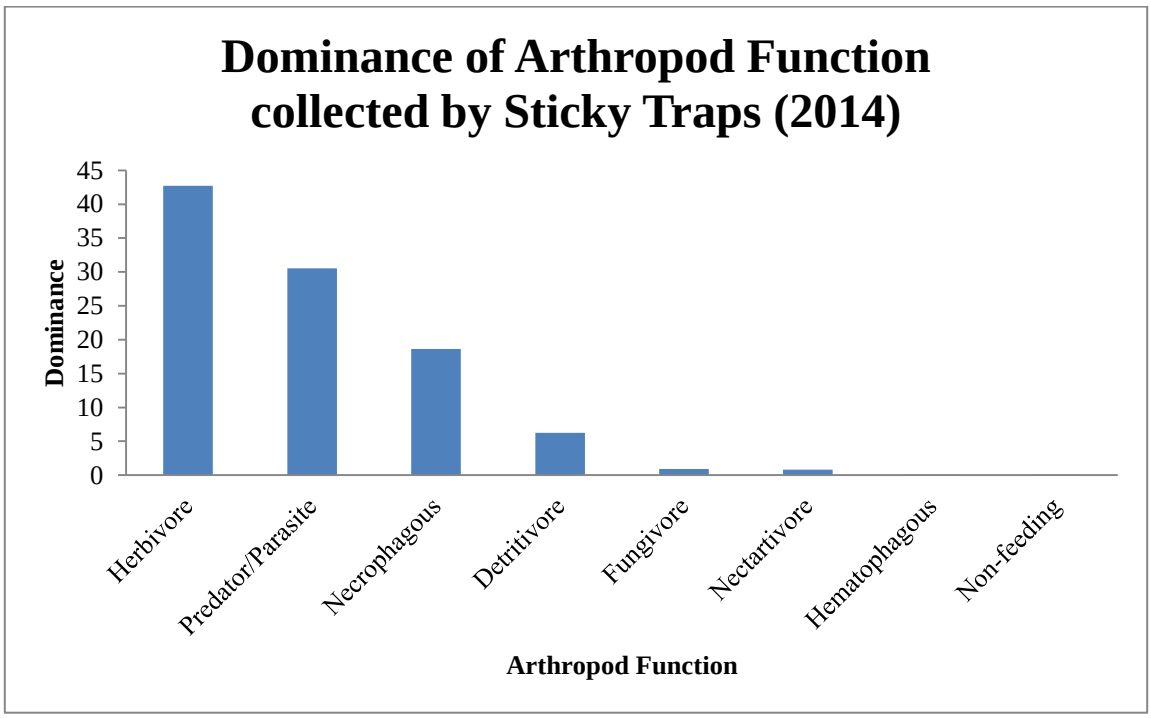

Figure 5.116. Dominance by functional groups of the aboveground arthropods collected using sticky traps in summer 2014 at Snook, Texas.

Order in 2014

PERMANOVA was performed on aboveground arthropod data by Order level. Results showed that there was Day, Treatment, Height, and Position effects ($p < 0.05$).There were interactions between Day x Height, Day x Position, Treatment x

Position, Height x Position, Day x Height x Position and Treatment x Height x Position. (Table 5.99).

Table 5.99. Analysis of the aboveground arthropod community structure (by Order) collected via sticky traps in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	56.556	0.001*
Treatment	2	2.231	0.012*
Height	1	23.125	0.001*
Position	1	57.256	0.001*
Day x Treatment	2	1.491	0.095
Day x Height	1	3.341	0.003*
Treatment x Height	2	0.823	0.602
Day x Position	1	5.339	0.001*
Treatment x Position	2	1.758	0.039*
Height x Position	1	27.111	0.001*
Day x Treatment x Height	2	0.661	0.807
Day x Treatment x Position	2	0.944	0.480
Day x Height x Position	1	4.135	0.002*
Treatment x Height x Position	2	1.786	0.048*
Day x Treatment x Height x Position	2	0.603	0.856

Since there was significant effect in Day, Treatment, Height, and Position, further analyses were carried out. For day of decomposition, all day to day comparisons were significantly different, except Day 0 x Day 14 where there was no significant difference (Table 5.100). For comparison between treatments, Control x Post-14 was found to be significantly different in terms of aboveground arthropod community

structure by Order (Table 5.101). The NMDS plot of stress for aboveground arthropod community structure (Figure 5.117) and NMDS ordinations for Day, Treatment, Height, and Position were provided for visualization about data distribution (Figure 5.118, 5.119, 5.120 and 5.121, respectively). Minimum stress for given dimensionality was 0.1538 with $r^2 = 0.8629$. The MRPP analysis for day showed a significant difference (A value = 0.1245; Significant of Delta = 0.001 based on 999 permutations), the MRPP for Treatment showed A value 0.0014 and Significant of Delta 0.219. The MRPP for height also showed a significant difference with A value 0.0303 and Significant of Delta 0.001 while the MRPP for position was significantly different with A value 0.0827 and Significant of Delta 0.001.

Table 5.100. Pairwise comparisons of aboveground arthropod community structure (by Order) collected via sticky traps between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.011*	0.038*	0.001*	0.103	0.003*	0.001*
3	0.001*	-	0.017*	0.003*	0.003*	0.001*	0.001*
7	0.038*	0.017*	-	0.007*	0.041*	0.001*	0.001*
10	0.001*	0.003*	0.007*	-	0.002*	0.001*	0.001*
14	0.103	0.003*	0.041*	0.002*	-	0.016*	0.001*
21	0.003*	0.001*	0.001*	0.001*	0.016*	-	0.001*
40	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	-

Table 5.101. Pairwise comparisons of aboveground arthropod community structure (by Order) collected via sticky traps between treatments in summer 2014 at Snook, Texas after Bonferroni's correction.

Treatment	df	SS	MS	F model	R2	P value
Control x Post-7	1	0.206	0.2055	0.8886	0.0053	0.507
Residual	166	38.390	0.2312		0.9947	
Total	167	38.596			1.0000	
Control x Post-14	1	0.572	0.572	2.5028	0.0148	0.024*
Residual	166	37.961	37.961		0.9852	
Total	167	38.534	38.534		1.0000	
Post-7 x Post-14	1	0.147	0.1466	0.6094	0.0037	0.729
Residual	166	39.943	0.2406		0.9963	
Total	167	40.089			1.0000	

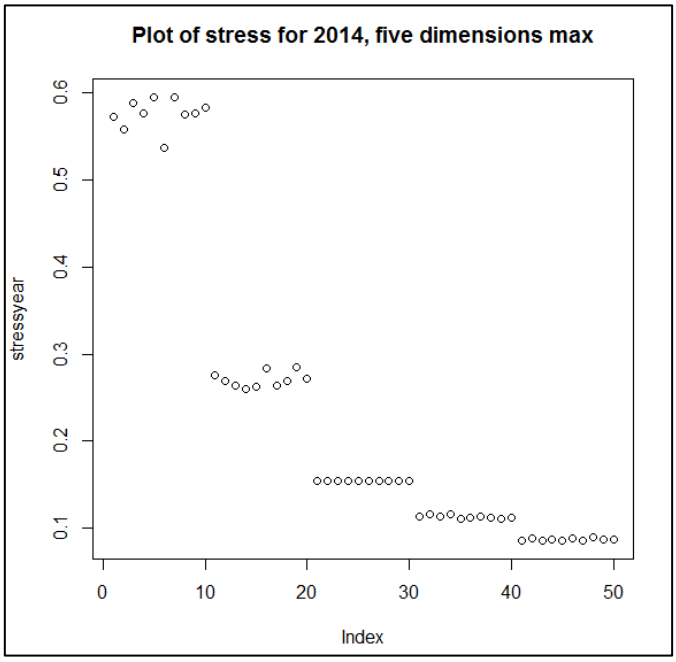

Figure 5.117. NMDS plot of stress for aboveground arthropod community structure (by Order) collected via sticky traps in summer 2014 at Snook, Texas (Stress test 0.1538; r^2 = 0.8629).

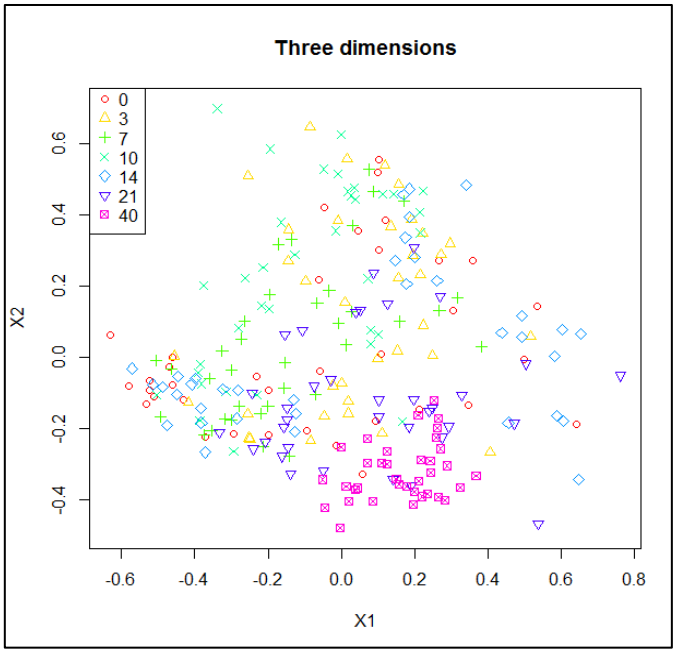

Figure 5.118. NMDS ordinations for aboveground arthropod community structure (by Order) by carrion decomposition days collected via sticky traps in summer 2014 at Snook, Texas.

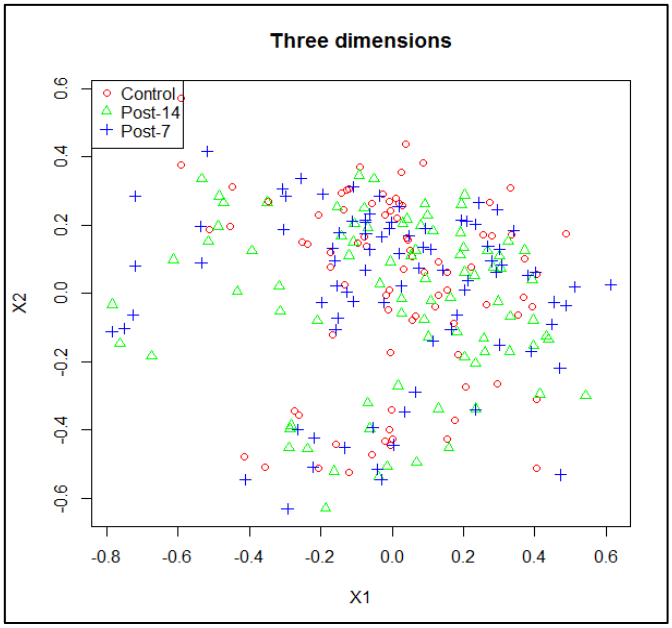

Figure 5.119. NMDS ordinations for aboveground arthropod community structure (by Order) by treatments of sticky traps in summer 2014 at Snook, Texas.

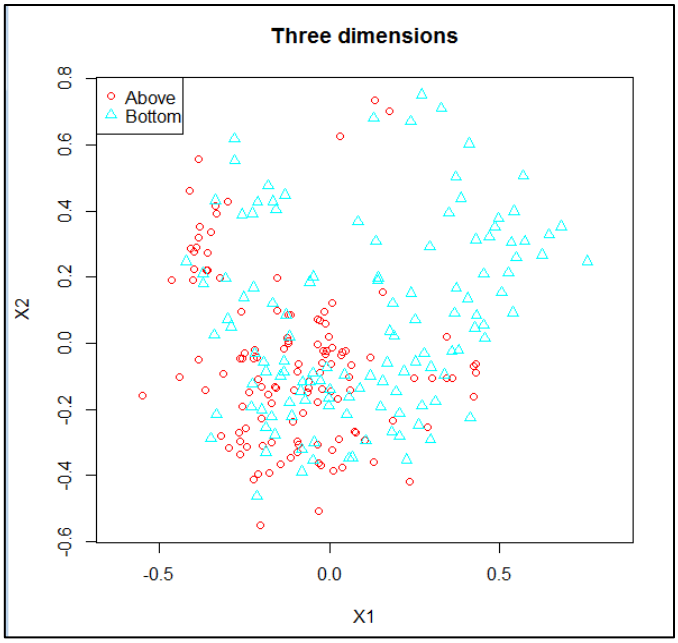

Figure 5.120. NMDS ordinations for aboveground arthropod community structure (by Order) by heights of sticky traps in summer 2014 at Snook, Texas.

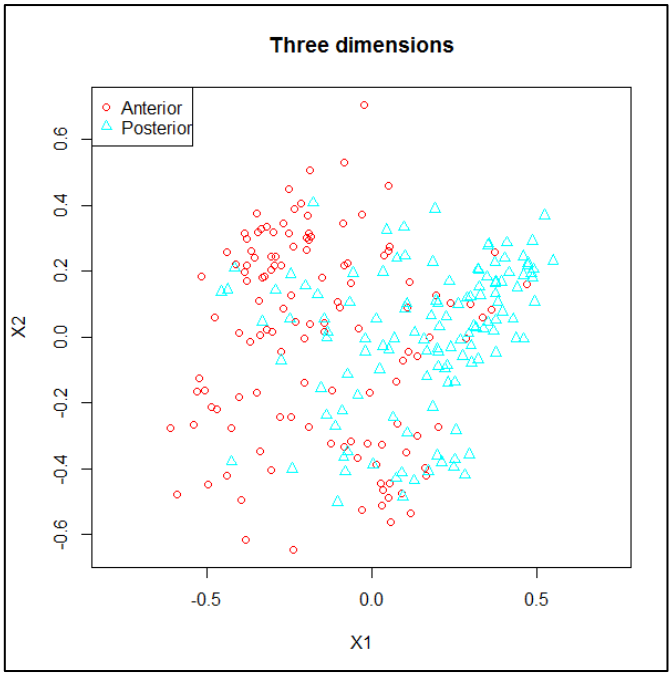

Figure 5.121. NMDS ordinations for aboveground arthropod community structure (by Order) by positions of sticky traps in summer 2014 at Snook, Texas.

For ISA, results demonstrated six indicative Orders among aboveground arthropods in summer 2014 at Snook, Texas. They were Thysanoptera, Hymenoptera, Coleoptera, Araneae, Diptera, and Hemiptera (Table 5.102).

Table 5.102. Indicator species analysis by Order for aboveground arthropods collected via sticky traps in summer 2014 at Snook, Texas.

Type	Order	Indicator value	P value
Sticky traps	Thysanoptera	0.0744	0.003*
	Hymenoptera	0.2284	0.009*
	Coleoptera	0.0942	0.001*
	Araneae	0.0691	0.010*
	Diptera	0.1567	0.005*
	Hemiptera	0.0952	0.005*

Abundance

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p = 0.0002$), Position ($p < 0.0001$) and interactions between Day x Treatment ($p = 0.0041$), Day x Height ($p = 0.0493$), Day x Position ($p < 0.0001$), Treatment x Position ($p = 0.0282$), Height x Position ($p < 0.0001$), Day x Treatment x Position ($p = 0.0049$), Day x Treatment x Height ($p = 0.0239$), Day x Height x Position ($p < 0.0001$). There was no significant difference found in abundance between treatments in all other sampling days ($p > 0.05$). Resilience was tested and results showed all treatments were resistant throughout all sampling days (Table 5.103). Average abundance of arthropods according to Orders collected at sticky trap in 2014 trial was demonstrated in Figure 5.122. Thysanoptera was difference significantly on Day 7 between Control x Post-14 ($p = 0.0044$) and the abundance of Hymenoptera was significantly different on Day 10 ($p = 0.0483$). No significant difference was detected between treatments in all sampling days for Diptera, Coleoptera and Hemiptera (Figure 5.123).

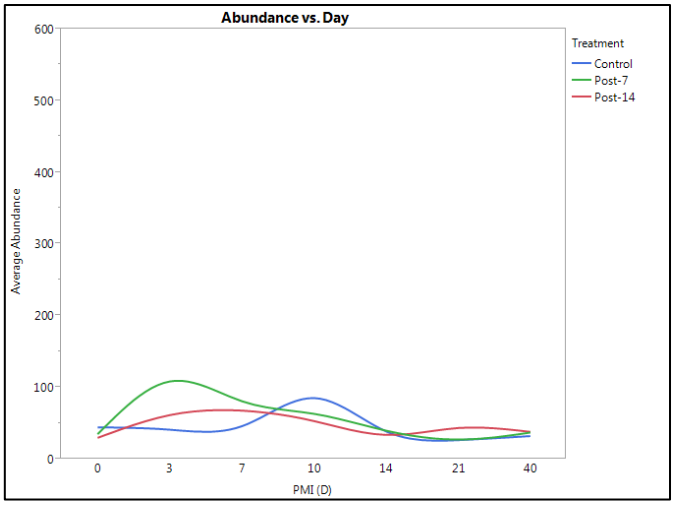

Figure 5.122. Aboveground arthropod community abundance (by Order) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.103. Resilience for aboveground arthropod community (by Order) abundance collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0129*	Resistance[#]
Post-7	None	0.0729	Resistance
Post-14	None	0.5628	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

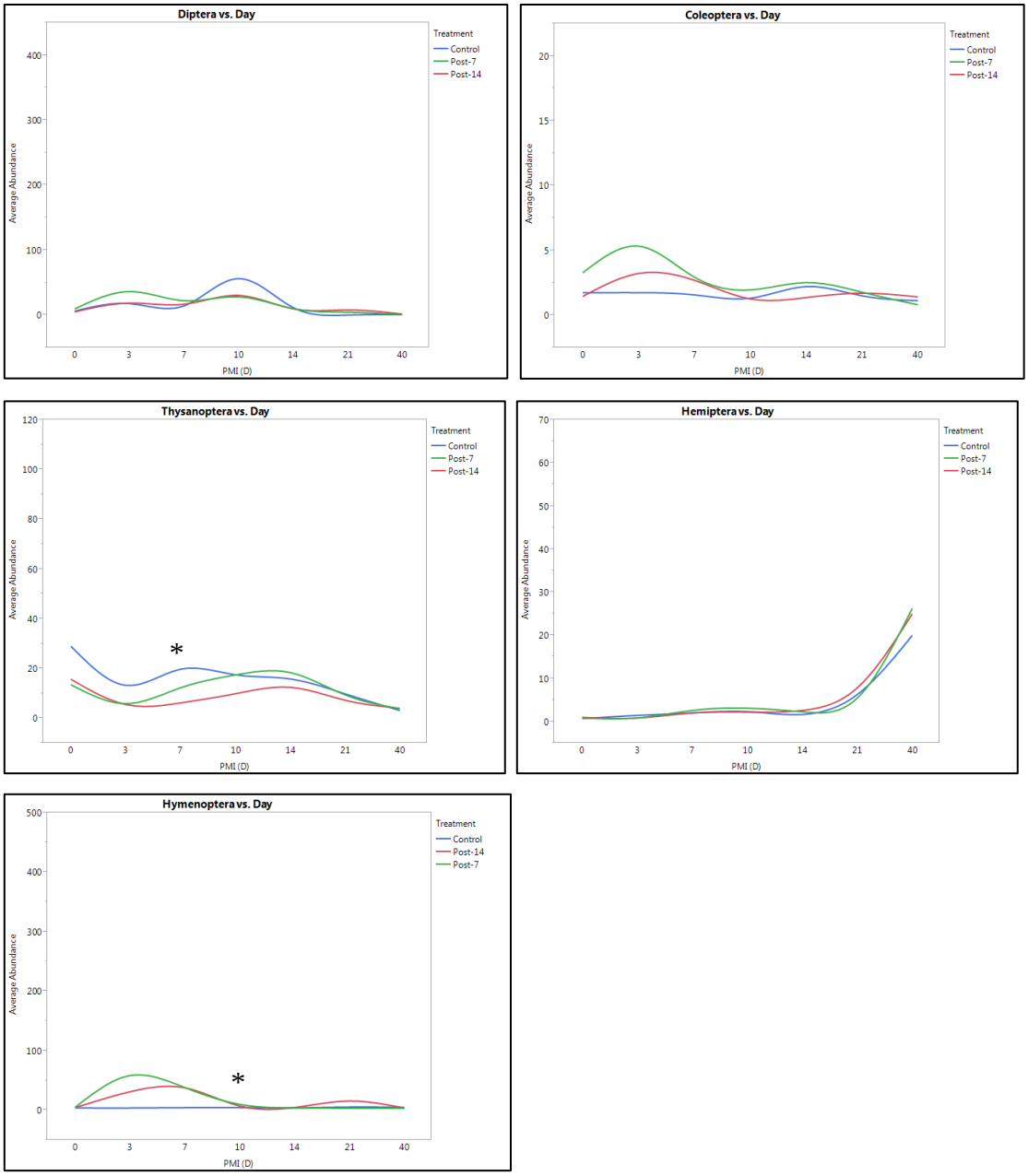

Figure 5.123. Average abundance of arthropods according to Orders collected at sticky traps in summer 2014 at Snook, Texas. Upper Left. Abundance of Diptera across Treatments over time. Upper Right. Abundance of Coleoptera across Treatments over time. Middle Left. Abundance of Thysanoptera across Treatments over time. Middle Right. Abundance of Hemiptera across Treatments over time. Lower Left. Abundance of Hymenoptera across Treatments over time (* represents significant difference).

Richness

The full model showed a significant difference in Day ($p = 0.0292$), Height ($p < 0.0001$) and Position ($p = 0.0389$) with no interaction observed. There was no significant difference found in richness between treatments in all sampling days ($p > 0.05$) (Figure 5.124). In other words, the system was stable that no divergence or convergence was observed. Resilience was tested and results showed for all treatments were resistant throughout the days (Table 5.104).

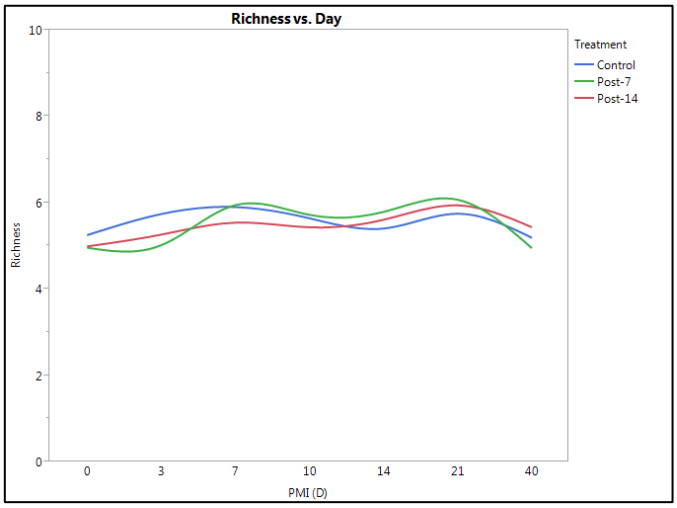

Figure 5.124. Aboveground arthropod community richness (by Order) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.104. Resilience for aboveground arthropod community (by Order) richness collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.8347	Resistance
Post-7	None	0.1170	Resistance
Post-14	None	0.7497	Resistance

Simpson's diversity index

The full model showed a significant difference in Day ($p < 0.0001$), and interactions between Day x Height ($p = 0.0458$), Day x Position ($p < 0.0001$), Treatment x Position ($p = 0.0289$), Height x Position ($p < 0.0001$) and Day x Height x Position ($p = 0.0011$). There was no significant difference ($p > 0.05$) found in Simpson's Diversity between treatments in all sampling days (Figure 5.125). In other words, the system was resistance. Resilience was tested and results showed all treatments were resistant throughout the sampling days (Table 5.105).

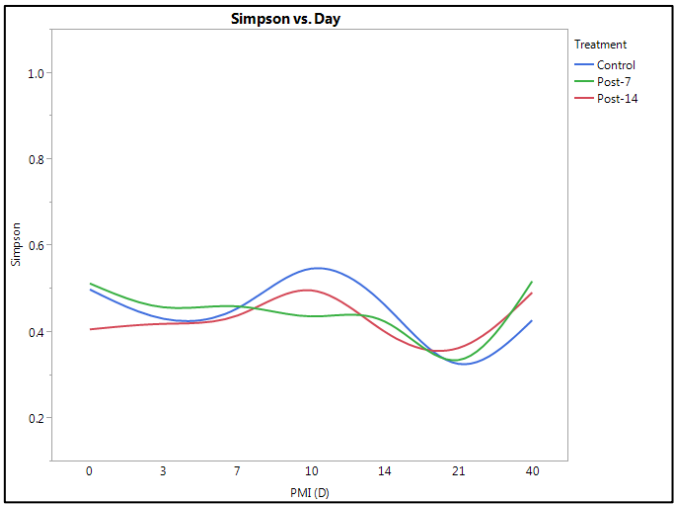

Figure 5.125. Simpson's diversity of the aboveground arthropod community (by Order) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.105. Resilience for Simpson's Diversity of the aboveground arthropod community (by Order) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0656•	Resistance
Post-7	None	0.1078	Resistance
Post-14	None	0.3453	Resistance

• Marginal significant difference.

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p < 0.0166$), and interactions between Day x Height ($p = 0.0415$), Day x Position ($p < 0.0001$), Treatment x Height ($p = 0.0457$), Height x Position ($p < 0.0001$), and Day x Height x Position ($p = 0.0030$). There was no significant difference ($p > 0.05$) found in Shannon-Wiener's Diversity between treatments in all sampling days (Figure 5.126). In other words, the system was resistance. Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.106).

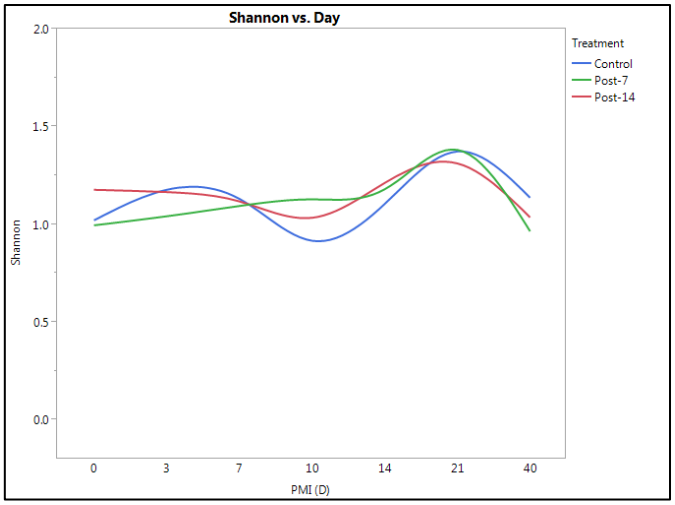

Figure 5.126. Shannon-Wiener's diversity of the aboveground arthropod community (by Order) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.106. Resilience for Shannon-Wiener's Diversity of the aboveground arthropod community (by Order) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0752	Resistance
Post-7	None	0.0535•	Resistance
Post-14	None	0.4304	Resistance

• Marginal significant difference.

Evenness

The full model showed a significant difference in Day ($p < 0.0001$), and interactions between Day x Height ($p = 0.0097$), Day x Position ($p < 0.0001$), Treatment x Position ($p = 0.0084$), Height x Position ($p < 0.0001$) and Day x Height x Position ($p = 0.0007$). There was no significant difference ($p > 0.05$) was found in evenness between treatments in all sampling days (Figure 5.127). In other words, the system was

resistance. Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.107).

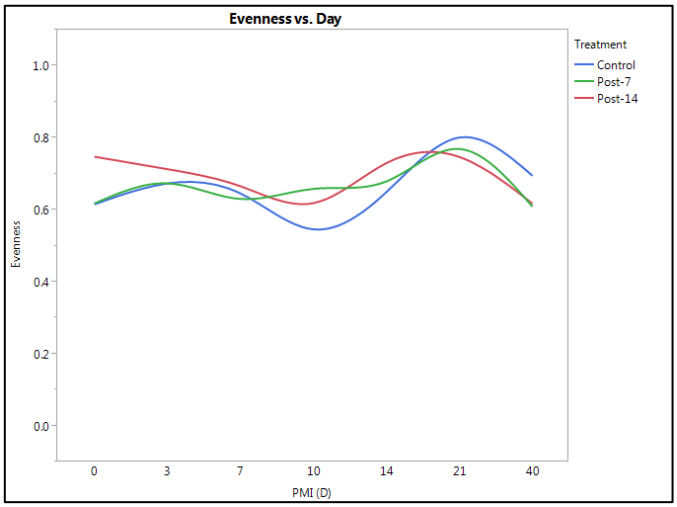

Figure 5.127. Evenness of the aboveground arthropod community (by Order) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.107. Resilience for evenness of the aboveground arthropod community (by Order) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0396*	Resistance[#]
Post-7	None	0.2782	Resistance
Post-14	None	0.3095	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Effective number of species

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p = 0.0139$) and Position ($p = 0.0457$) and interactions between Treatment x Height ($p =$

0.0092), Day x Position ($p < 0.0001$), Height x Position ($p < 0.0001$), Day x Height x Position ($p = 0.0079$), Treatment x Height x Position ($p = 0.0054$). There was no significant difference ($p > 0.05$) was found in ENS between treatments in all sampling days (Figure 5.128). In other words, the system was resistance. Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.108).

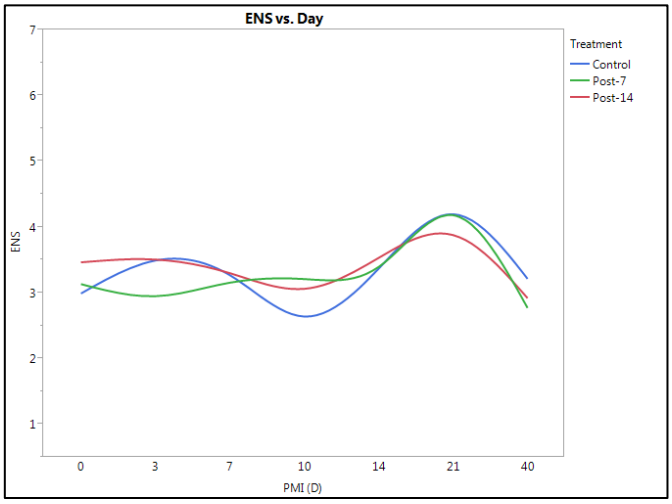

Figure 5.128. Effective Number of Species of the aboveground arthropod community (by Order) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.108. Resilience for ENS of the aboveground arthropod community (by Order) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0311*	Resistance#
Post-7	None	0.0232*	Resistance#
Post-14	None	0.4326	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Family in 2014

PERMANOVA was performed on aboveground arthropod data by Family level. Results showed that there was Day, Treatment, Height, and Position effects ($p < 0.05$).There were interactions ($p < 0.05$) between Day x Treatment, Day x Height, Day x Position, Treatment x Position, Height x Position and Day x Height x Position (Table 5.109).

Table 5.109. Analysis of the aboveground arthropod community structure (by Family) collected via sticky traps in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	37.919	0.001*
Treatment	2	2.371	0.003*
Height	1	14.716	0.001*
Position	1	37.963	0.001*
Day x Treatment	2	1.626	0.028*
Day x Height	1	2.294	0.017*
Treatment x Height	2	0.953	0.481
Day x Position	1	3.607	0.001*
Treatment x Position	2	1.703	0.028
Height x Position	1	14.041	0.001*
Day x Treatment x Height	2	0.486	0.994
Day x Treatment x Position	2	0.717	0.849
Day x Height x Position	1	3.274	0.004*
Treatment x Height x Position	2	1.425	0.098
Day x Treatment x Height x Position	2	0.602	0.946

Since there was significant effect in Day and Treatment, further analyses were carried out. For days of decomposition, all day to day comparisons was significantly different ($p < 0.05$) (Table 5.110). As for Treatment effect, significant difference was found between Control x Post-14 ($p = 0.005$) (Table 5.111). The NMDS plot of stress for aboveground arthropod community structure (Figure 5.129) and NMDS ordinations for Day, Treatment, Height, and Position were provided for visualization about data distribution (Figure 5.130, 5.131, 5.132 and 5.133, respectively). Minimum stress for given dimensionality was 0.2130 with $r^2 = 0.7256$. The MRPP analysis for day showed a significant difference (A value = 0.1075; Significant of Delta = 0.001 based on 999 permutations). The MRPP for treatment demonstrated significant difference with A value 0.0028 and Significant of Delta 0.046. The MRPP for height also showed a significant difference with A value 0.0218 and Significant of Delta 0.001 while the MRPP for position was significantly different with A value 0.0616 and Significant of Delta 0.001.

Table 5.110. Pairwise comparisons of aboveground arthropod community structure (by Family) collected via sticky traps between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.002*	0.002*	0.001*	0.017*	0.001*	0.001*
3	0.002*	-	0.015*	0.001*	0.001*	0.001*	0.001*
7	0.002*	0.015*	-	0.001*	0.007*	0.001*	0.001*
10	0.001*	0.001*	0.001*	-	0.001*	0.001*	0.001*
14	0.017*	0.001*	0.007*	0.001*	-	0.001*	0.001*
21	0.001*	0.001*	0.001*	0.001*	0.001*	-	0.001*
40	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	-

Table 5.111. Pairwise comparisons of aboveground arthropod community structure (by Family) collected via sticky traps between treatments in summer 2014 at Snook, Texas after Bonferroni's correction.

Treatment	df	SS	MS	F model	R2	P value
Control x Post-7	1	0.450	0.4504	1.4882	0.0089	0.124
Residual	166	50.206	0.3024		0.9911	
Total	167	50.656			1.0000	
Control x Post-14	1	0.852	0.8518	2.7892	0.0165	0.005*
Residual	166	50.696	0.3054		0.9835	
Total	167	51.548			1.0000	
Post-7 x Post-14	1	0.226	0.2261	0.7163	0.0043	0.728
Residual	166	52.410	0.3157		0.9957	
Total	167	52.636			1.0000	

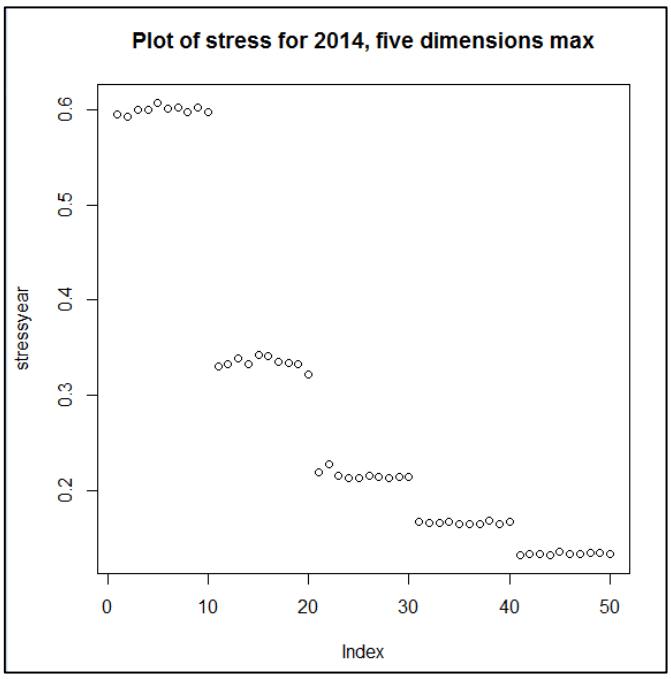

Figure 5.129. NMDS plot of stress for aboveground arthropod community structure (by Family) collected via sticky traps in summer 2014 at Snook, Texas (Stress test 0.2130; r^2 = 0.7256).

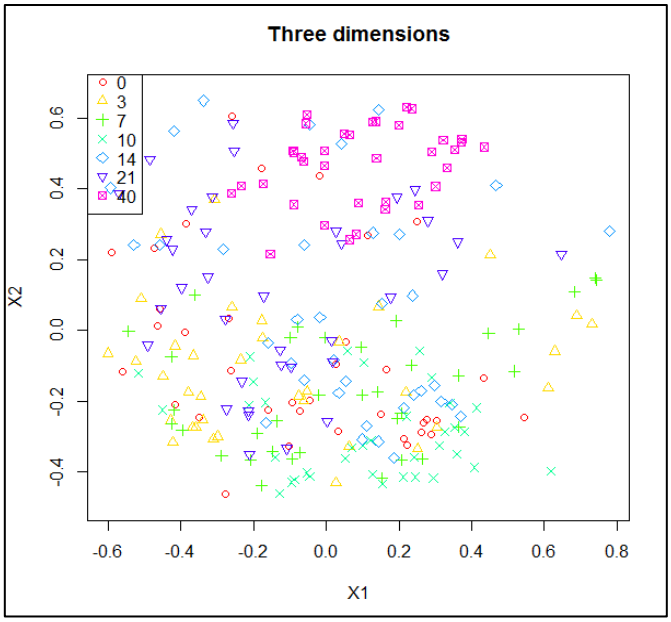

Figure 5.130. NMDS ordinations for aboveground arthropod community structure (by Family) by carrion decomposition days collected via sticky traps in summer 2014 at Snook, Texas

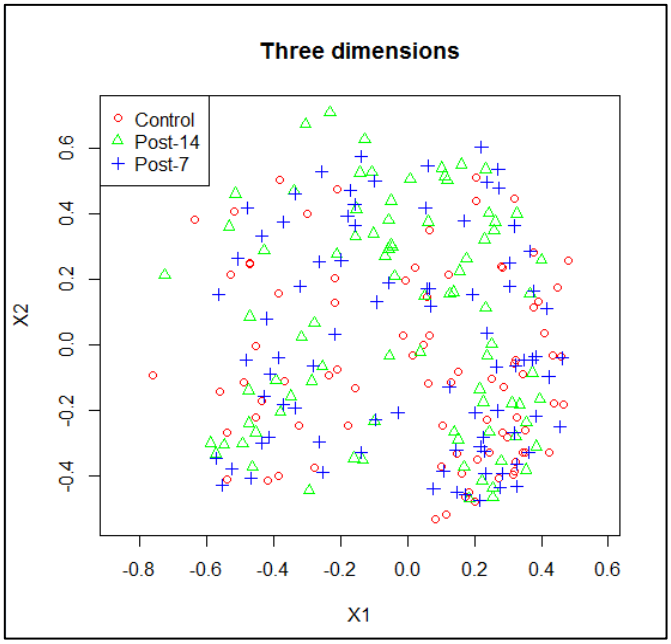

Figure 5.131. NMDS ordinations for aboveground arthropod community structure (by Family) by treatments collected via sticky traps in summer 2014 at Snook, Texas.

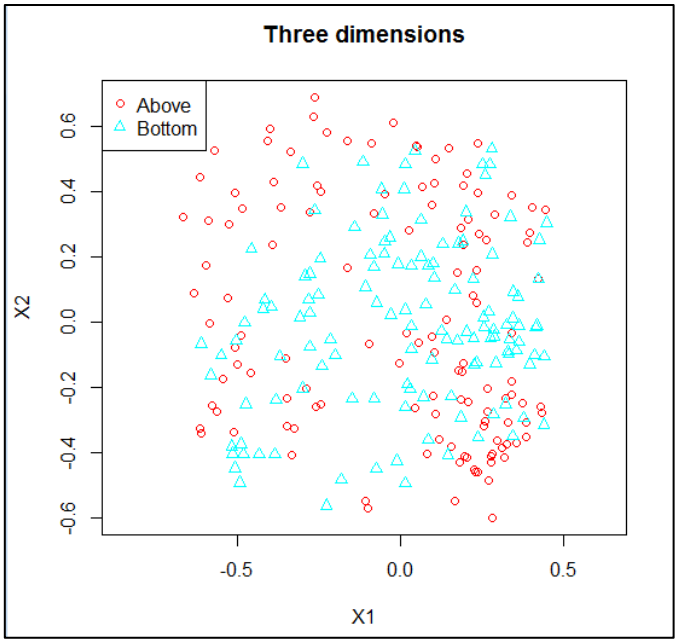

Figure 5.132. NMDS ordinations for aboveground arthropod community structure (by Family) by heights of sticky traps in summer 2014 at Snook, Texas.

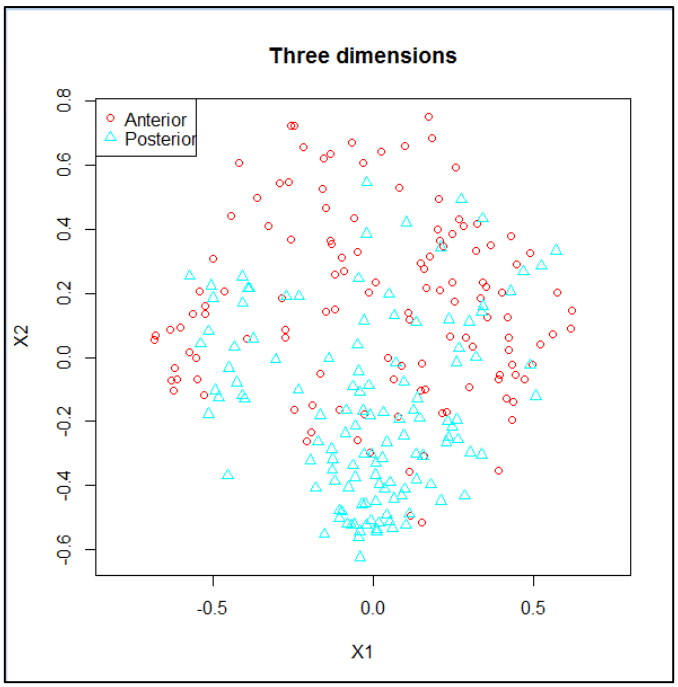

Figure 5.133. NMDS ordinations for aboveground arthropod community structure (by Family) by positions of sticky traps in summer 2014 at Snook, Texas.

For ISA, results showed 22 indicative families among aboveground arthropods collected via sticky traps in summer 2014 at Snook, Texas (Table 5.112).

Table 5.112. Indicator species analysis by Family for aboveground arthropods in summer 2014 at Snook, Texas.

Type	Family	Indicator value	P value
Sticky traps	Thripidae	0.0772	0.002*
	Therevidae	0.2254	0.008*
	Cecidomyiidae	0.1250	0.035*
	Culicidae	0.3750	0.001*
	Piophilidae	0.3810	0.016*
	Chloropidae	0.2122	0.032*
	Sepsidae	0.3333	0.009*
	Formicidae	0.3222	0.006*
	Muscidae	0.1804	0.001*
	Staphylinidae	0.1535	0.002*
	Sarcophagidae	0.1137	0.035*
	Anthicidae	0.1667	0.022*
	Corylophidae	0.1481	0.022*
	Carabidae	0.4444	0.024*
	Calliphoridae	0.2762	0.001*
	Hybotidae	0.2222	0.007*
	Ceraphronidae	0.1000	0.023*
	Ceratopogonidae	0.3030	0.015*
	Cicadidae	0.4444	0.003*
	Diapriidae	0.0752	0.045*
	Aphididae	0.1050	0.002*
	Sciaridae	0.3111	0.021*

Abundance

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p < 0.0001$), Position ($p < 0.0001$) and interactions between Day x Treatment ($p = 0.0046$), Day x Height ($p < 0.0001$), Height x Position ($p < 0.0001$), Day x Position ($p < 0.0001$), Treatment x Position ($p = 0.0273$), Day x Treatment x Height ($p = 0.0210$), Day x Treatment x Position ($p = 0.0051$) and Day x Height x Position ($p < 0.0001$). There was no significant difference was found in abundance between treatments in all other sampling days ($p > 0.05$). Resilience was tested and the results showed all treatments were resistant throughout all sampling days, although there was a marginal significant difference for Post-7 carcasses (Table 5.113). Average abundance of arthropods according to Families collected at sticky trap in 2014 trial was demonstrated in Figure 5.134. Thripidae abundance was significantly different on Day 7 between Control x Post-14 ($p = 0.0044$). For Muscidae, there was a marginal significant difference on Day 14 ($p = 0.0632$) (Figure 5.135).

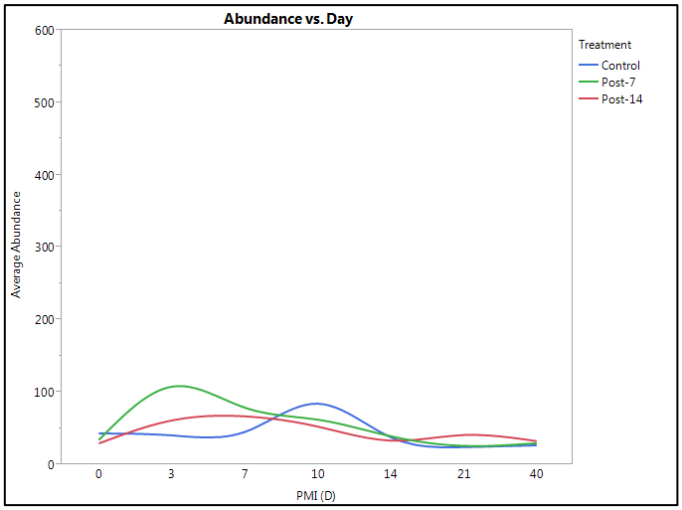

Figure 5.134. Aboveground arthropod community abundance (by Family) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.113. Resilience for aboveground arthropod community (by Family) abundance collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0085*	Resistance#
Post-7	None	0.0592•	Resistance
Post-14	None	0.5109	Resistance

• Marginal significant difference.
= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

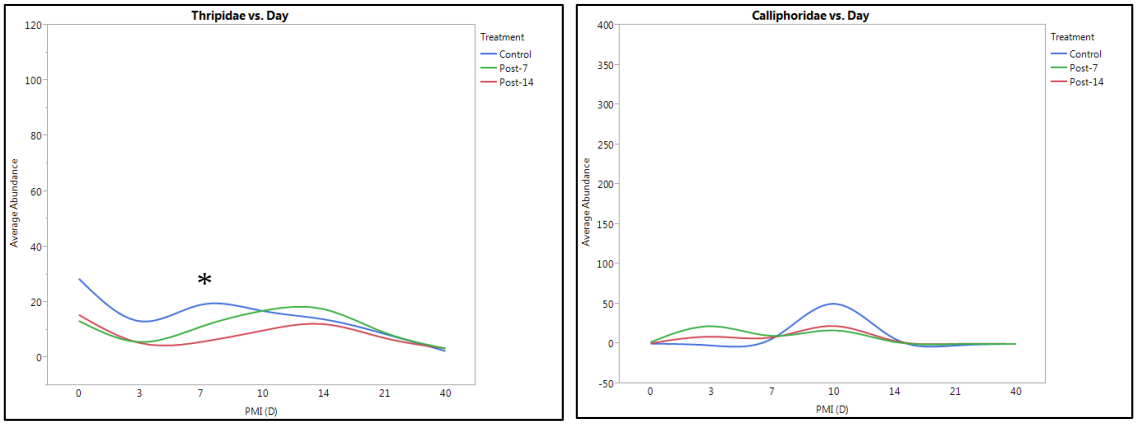

Figure 5.135. Average abundance of arthropods according to Families collected at sticky trap in summer 2014 at Snook, Texas. Upper Left. Abundance of Thripidae across Treatments over time. Upper Right. Abundance of Calliphoridae across Treatments over time. Middle Left. Abundance of Sarcophagidae across Treatments over time. Middle Right. Abundance of Muscidae across Treatments over time. Lower Left. Abundance of Formicidae across Treatments over time. Lower Right. Abundance of Aphididae across Treatments over time (* represent significantly different; • denotes marginal significant difference).

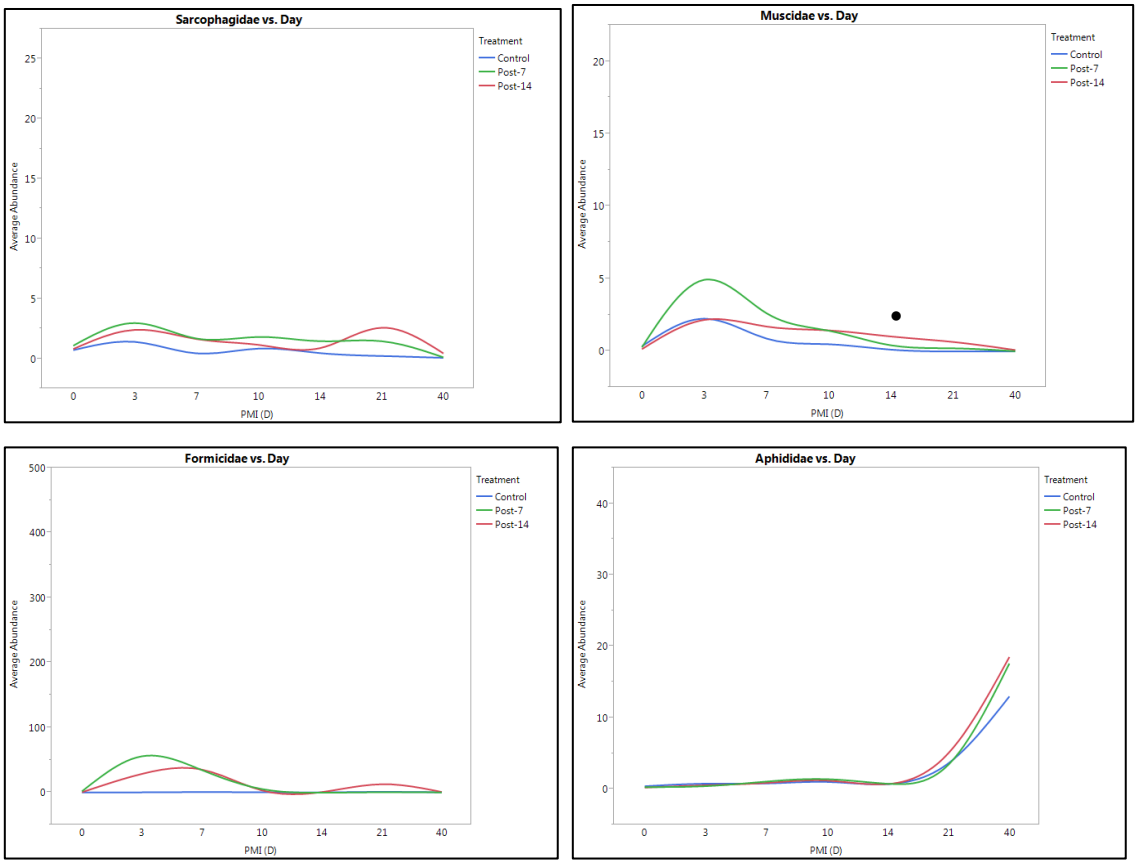

Figure 5.135 (Continued).

Richness

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Height ($p = 0.0372$), Height x Position ($p < 0.0001$) and Day x Height x Position ($p = 0.0487$). There was no significant difference ($p > 0.05$) was found in richness between treatments in all other sampling days (Figure 5.136). Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.114).

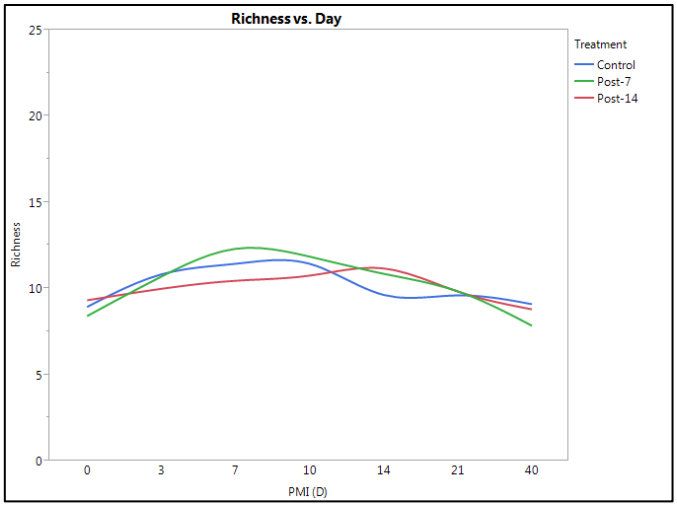

Figure 5.136. Aboveground arthropod community richness (by Family) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.114. Resilience for aboveground arthropod community (by Family) richness collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.5809	Resistance
Post-7	None	0.0935	Resistance
Post-14	None	0.7742	Resistance

Simpson's diversity index

The full model showed a significant difference in Day ($p = 0.0054$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Position ($p < 0.0001$), Height x Position ($p < 0.0001$) and Day x Height x Position ($p = 0.0046$). There was no significant difference ($p > 0.05$) was found in Simpson's Diversity between treatments in all sampling days (Figure 5.137). In other words, the system was resistant. Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.115).

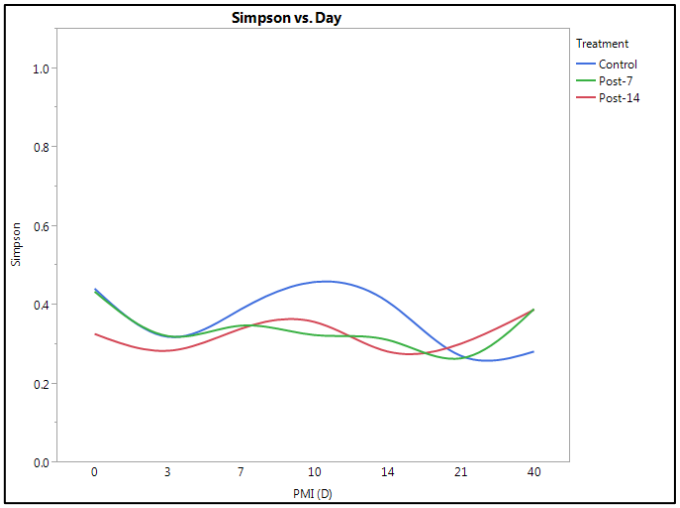

Figure 5.137. Simpson's diversity of the aboveground arthropod community (by Family) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.115. Resilience for Simpson's Diversity of the aboveground arthropod community (by Family) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0896	Resistance
Post-7	None	0.3534	Resistance
Post-14	None	0.6788	Resistance

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p < 0.0201$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Position ($p < 0.0001$), Day x Height x Position ($p = 0.0008$) and Treatment x Height x Position ($p = 0.0233$). There was no significant difference ($p > 0.05$) was found in Shannon-Wiener's Diversity between treatments in all sampling days (Figure 5.138). In other words, the system was resistance. Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.116).

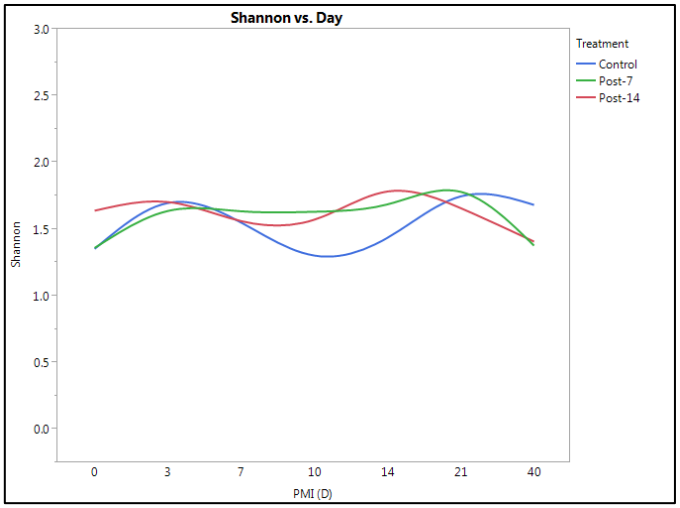

Figure 5.138. Shannon-Wiener's diversity of the aboveground arthropod community (by Family) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.116. Resilience for Shannon-Wiener's Diversity of the aboveground arthropod community (by Family) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2671	Resistance
Post-7	None	0.3342	Resistance
Post-14	None	0.5915	Resistance

Evenness

The full model showed a significant difference in Day ($p < 0.0001$), and Position ($p < 0.0001$) and interactions between Day x Position ($p < 0.0001$), Treatment x Position ($p = 0.0361$), Height x Position ($p < 0.0001$) and Day x Height x Position ($p = 0.0035$). There was a marginal significant difference in evenness on Day 10 ($p = 0.0651$) (Figure 5.139). Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.117).

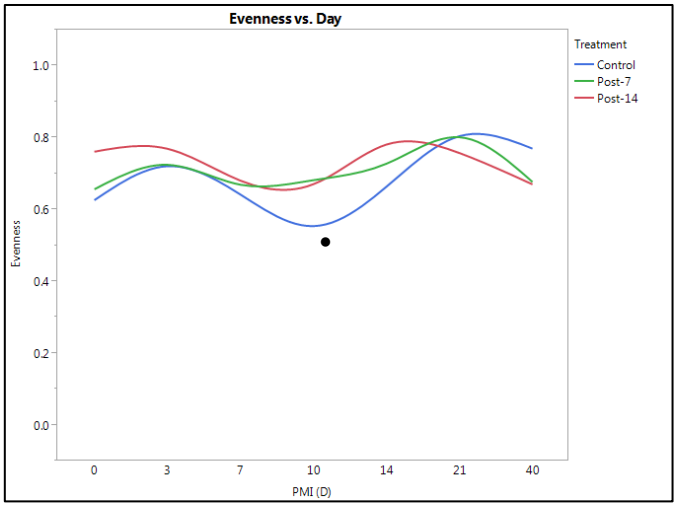

Figure 5.139. Evenness of the aboveground arthropod community (by Family) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas (• represents marginal significant difference).

Table 5.117. Resilience for evenness of the aboveground arthropod community (by Family) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0258*	Resistance[#]
Post-7	None	0.4341	Resistance
Post-14	None	0.4589	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Effective number of species

The full model showed a significant difference in Day ($p = 0.0020$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Height ($p = 0.0027$), Day x Position ($p < 0.0001$), Day x Height x Position ($p < 0.0001$) and Treatment x Height x Position ($p = 0.0010$). There was no significant difference ($p > 0.05$) was found in ENS between treatments in all sampling days (Figure 5.140), although there was

marginal significant difference on Day 40 (p = 0.0654). In other words, the system was resistance. Resilience was tested for all treatments and all of them demonstrated resistance throughout the days (Table 5.118).

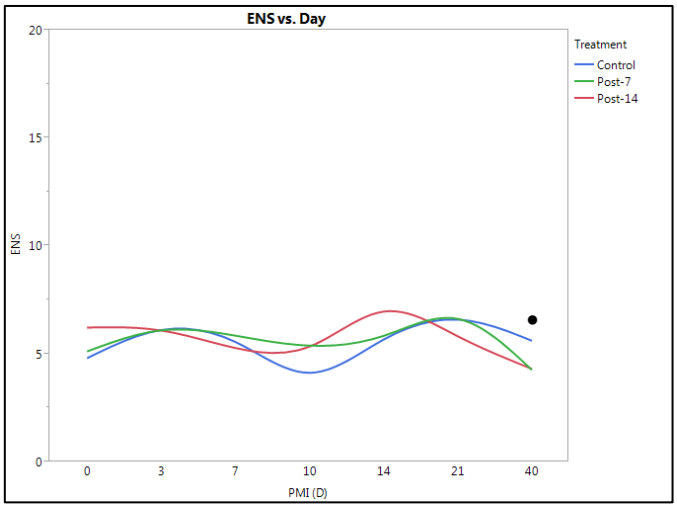

Figure 5.140. Effective Number of Species of the aboveground arthropod community (by Family) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas (• denotes marginal significant difference).

Table 5.118. Resilience for ENS of the aboveground arthropod community (by Family) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4625	Resistance
Post-7	None	0.3956	Resistance
Post-14	None	0.3539	Resistance

Genus and species in 2014

PERMANOVA was performed on aboveground arthropod data by Genus and species level. Results showed that there was Day, Treatment, Height, and Position effects ($p < 0.05$) and significant interactions between Day x Height, Day x Position, and Height x Position (Table 5.119).

Table 5.119. Analysis of the aboveground arthropod community structure (by Genus and species) collected via sticky traps in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	6.6415	0.001*
Treatment	2	2.3753	0.001*
Height	1	7.6716	0.001*
Position	1	8.6044	0.001*
Day x Treatment	2	1.1314	0.260
Day x Height	1	2.4478	0.002*
Treatment x Height	2	0.9810	0.463
Day x Position	1	2.5479	0.002*
Treatment x Position	2	1.3530	0.095
Height x Position	1	2.6383	0.003*
Day x Treatment x Height	2	0.9791	0.479
Day x Treatment x Position	2	1.1870	0.186
Day x Height x Position	1	1.3508	0.147
Treatment x Height x Position	2	1.3583	0.084
Day x Treatment x Height x Position	2	0.8469	0.732

Since there was significant effect in Day, Treatment, Height and Position, further analyses were carried out. For day of decomposition, all day to day comparisons were

significantly different, except Day 0 x Day 14 and Day 14 x Day 21 where there were no significant difference (Table 5.120). As for Treatment effect, significant difference was found between Control x Post-7 ($p = 0.005$), and Control x Post-14 ($p = 0.001$) (Table 5.121). The NMDS plot of stress for aboveground arthropod community structure (Figure 5.141) and NMDS ordinations for Day, Treatment, Height, and Position were provided for visualization about data distribution (Figure 5.142, 5.143, 5.144 and 5.145, respectively). Minimum stress for given dimensionality was 0.2552 with $r^2 = 0.5305$. The MRPP analysis for day showed a significant difference (A value = 0.0653; Significant of Delta = 0.001 based on 999 permutations). The MRPP for treatment demonstrated significant difference with A value 0.0057 and Significant of Delta = 0.003. The MRPP for height also showed a significant difference with A value 0.0148 and Significant of Delta 0.001 while the MRPP for position was significantly different with A value 0.0176 and Significant of Delta 0.001.

Table 5.120. Pairwise comparisons of aboveground arthropod community structure (by Genus and species) collected via sticky traps between decomposition carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.001*	0.001*	0.001*	0.127	0.030*	0.025*
3	0.001*	-	0.001*	0.001*	0.001*	0.001*	0.001*
7	0.001*	0.001*	-	0.001*	0.001*	0.001*	0.001*
10	0.001*	0.001*	0.001*	-	0.001*	0.001*	0.001*
14	0.127	0.001*	0.001*	0.001*	-	0.993	0.013*
21	0.030*	0.001*	0.001*	0.001*	0.993	-	0.021*
40	0.025*	0.001*	0.001*	0.001*	0.013*	0.021*	-

Table 5.121. Pairwise comparisons of aboveground arthropod community structure (by Genus and species) collected via sticky traps between treatments in summer 2014 at Snook, Texas after Bonferroni's correction.

Treatment	df	SS	MS	F model	R2	P value
Control x Post-7	1	1.018	1.0805	2.6626	0.0158	0.005*
Residual	166	67.366	0.4058		0.9842	
Total	167	68.446			1.0000	
Control x Post-14	1	1.284	1.2836	3.2003	0.0189	0.001*
Residual	166	66.584	0.4011		0.9811	
Total	167	67.867			1.0000	
Post-7 x Post-14	1	0.248	0.2483	0.6051	0.0036	0.868
Residual	166	68.110	0.4103		0.9964	
Total	167	68.358			1.0000	

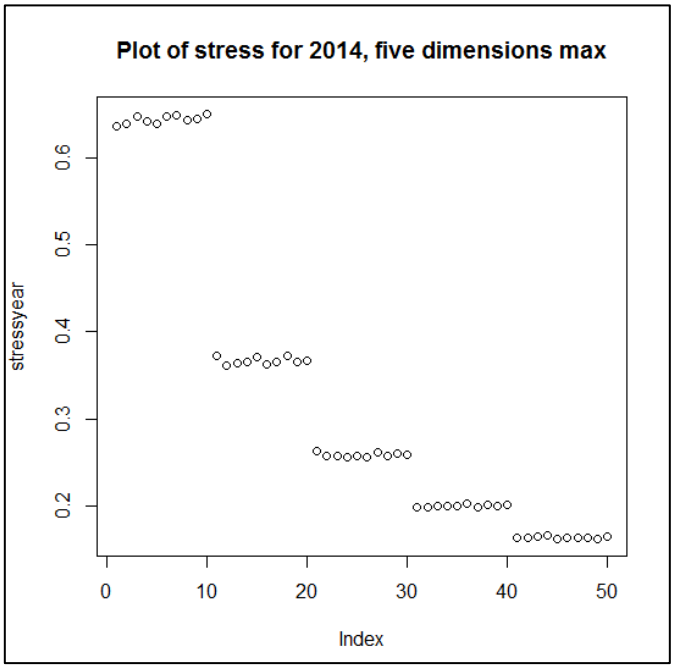

Figure 5.141. NMDS plot of stress for aboveground arthropod community structure (by Genus and species) collected via sticky traps in summer 2014 at Snook, Texas (Stress test 0.2552; $r^2 = 0.5305$).

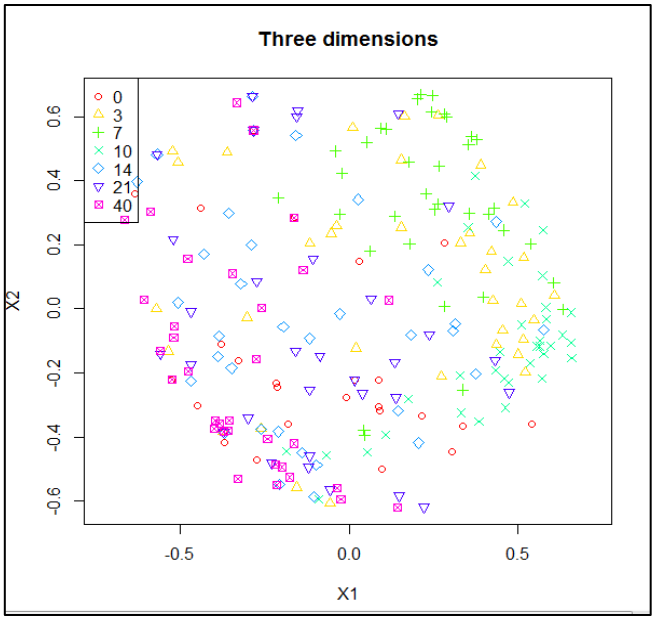

Figure 5.142. NMDS ordinations for aboveground arthropod community structure (by Genus and species) by carrion decomposition days collected via sticky traps in summer 2014 at Snook, Texas.

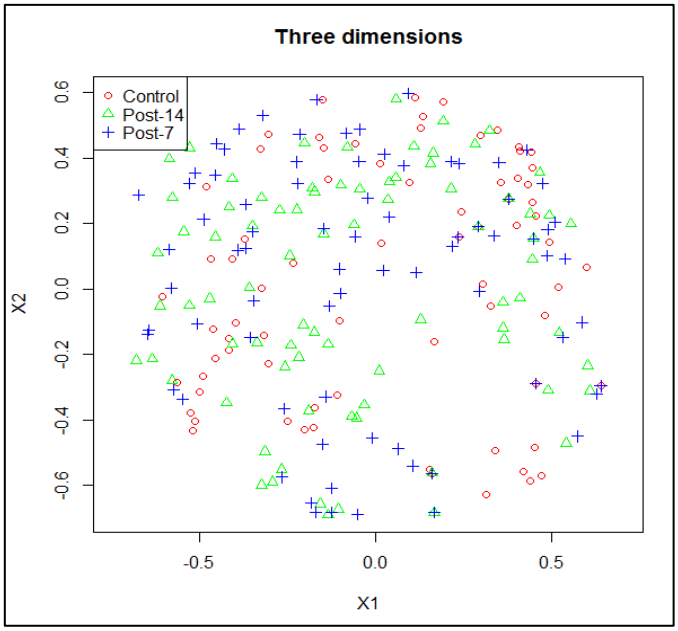

Figure 5.143. NMDS ordinations for aboveground arthropod community structure (by Genus and species) by treatments collected via sticky traps in summer 2014 at Snook, Texas.

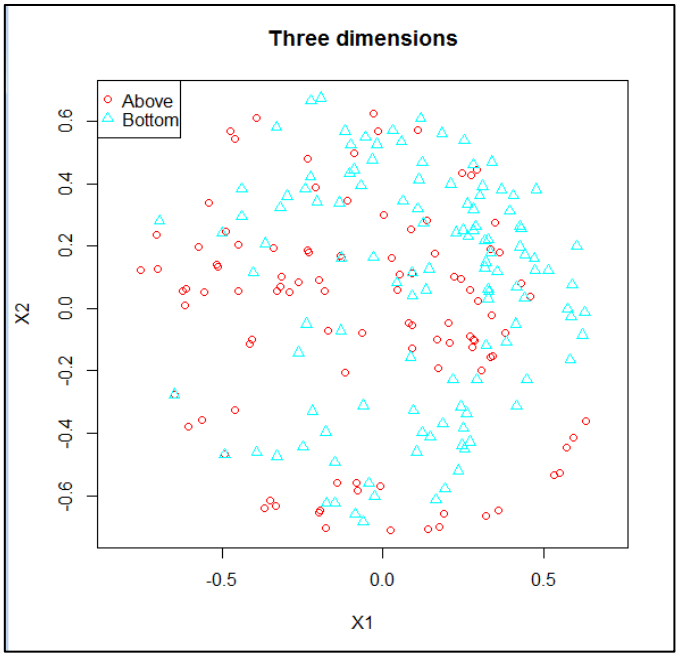

Figure 5.144. NMDS ordinations for aboveground arthropod community structure (by Genus and species) by heights of sticky traps in summer 2014 at Snook, Texas.

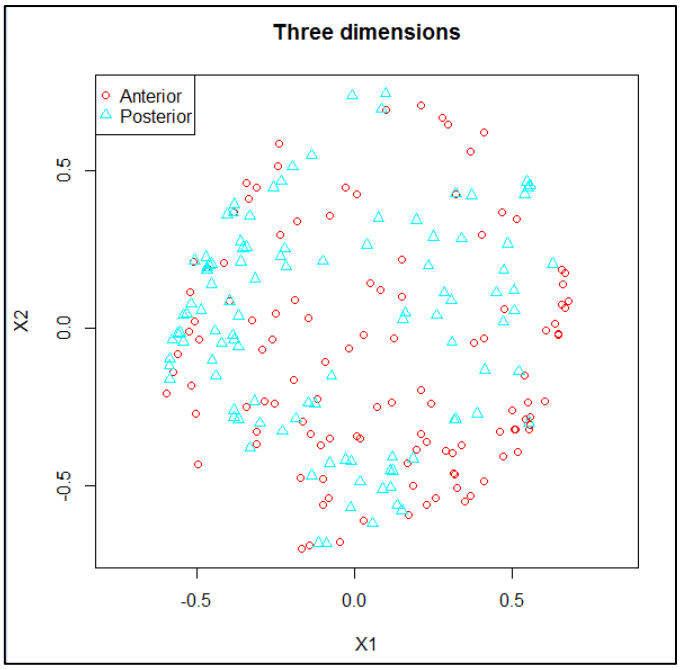

Figure 5.145. NMDS ordinations for aboveground arthropod community structure (by Genus and species) by positions of sticky traps in summer 2014 at Snook, Texas.

For ISA, results demonstrated 14 indicative Genera and species among aboveground arthropods in summer 2014 at Snook, Texas (Table 5.122).

Table 5.122. Indicator species analysis by Genus and species for aboveground arthropods collected via sticky traps in summer 2014 at Snook, Texas.

Type	Genus and species	Indicator value	P value
Sticky traps	*Incertella* sp.	0.6324	0.002*
	O. aenescens	0.2545	0.001*
	M. domestica	0.1406	0.009*
	F. occidentalis	0.2799	0.002*
	Frankliniella tritici	0.1852	0.007*
	Frankliniela fusca	0.1538	0.020*
	Sericoderus sp.	0.1905	0.009*
	Vacusus vicinus	0.1754	0.029*
	Co. macellaria	0.2781	0.003*
	Ravinia derelicta	0.1724	0.016*
	Lucilia cuprina	0.3333	0.046*
	Oligosita sp.	0.0657	0.031*
	Euxesta sp.	0.3333	0.050*
	Ravinia querula	0.1263	0.039*

Abundance

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p < 0.0001$), Position ($p < 0.0001$) and interactions between Day x Treatment ($p = 0.0074$), Day x Height ($p < 0.0001$), Day x Position ($p < 0.0001$), Height x Position ($p < 0.0001$) and Day x Height x Position ($p < 0.0001$). There was no significant difference between treatments in all sampling days ($p > 0.05$). Resilience was tested for all treatments and resilience was observed on Day 14 for Control carcasses while there was resistance for

Post-7 and Post-14 carcasses throughout decomposition days (Table 5.123). Average abundance of arthropods according to Genera and species collected at sticky trap in 2014 trial were demonstrated in Figure 5.146. For *Ch. rufifacies*, significant difference in abundance was detected on Day 10 between Control x Post-7 ($p = 0.0366$). For *F. occidentalis* (Thysanoptera: Thripidae), significant difference in abundant was detected on Day 7 between Control x Post-7 ($p = 0.0236$) and Control x Post-14 ($p = 0.0047$), and again on Day 10 (Control x Post-7 ($p = 0.0224$) and Control x Post-14 ($p = 0.0224$)) (Figure 5.147).

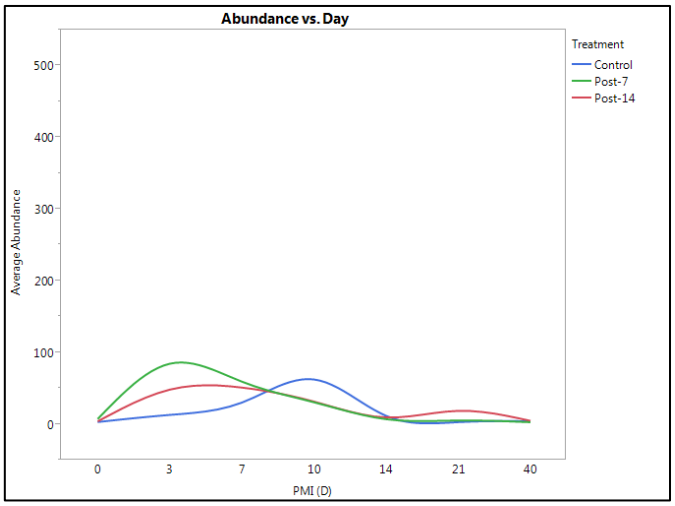

Figure 5.146. Aboveground arthropod community abundance (by Genus and species) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.123. Resilience for aboveground arthropod community (by Genus and species) abundance collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 10	0.0028*	14
Post-7	None	0.0165*	Resistance[#]
Post-14	None	0.1017	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

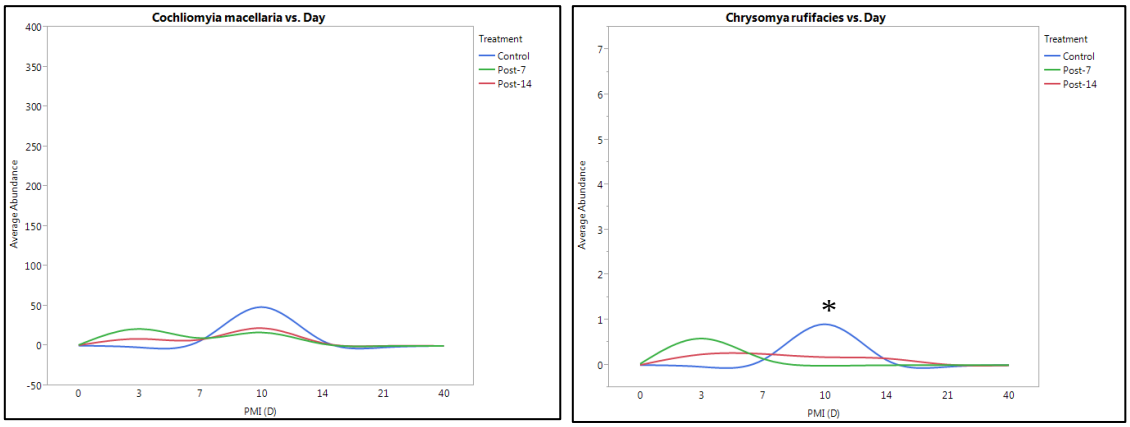

Figure 5.147. Average abundance of arthropods according to Genus and species collected via sticky traps in summers 2014 at Snook, Texas. Upper Left. Abundance of *Co. macellaria* across Treatments over time. Upper Right. Abundance of *Ch. rufifacies* across Treatments over time. Middle Left. Abundance of *O. aenescens* across Treatments over time. Middle Right. Abundance of *F. occidentalis* across Treatments over time. Lower Left. Abundance of *Oligosita sp.* across Treatments over time. Lower Right. Abundance of *S. invicta* across Treatments over time (* represent significantly different).

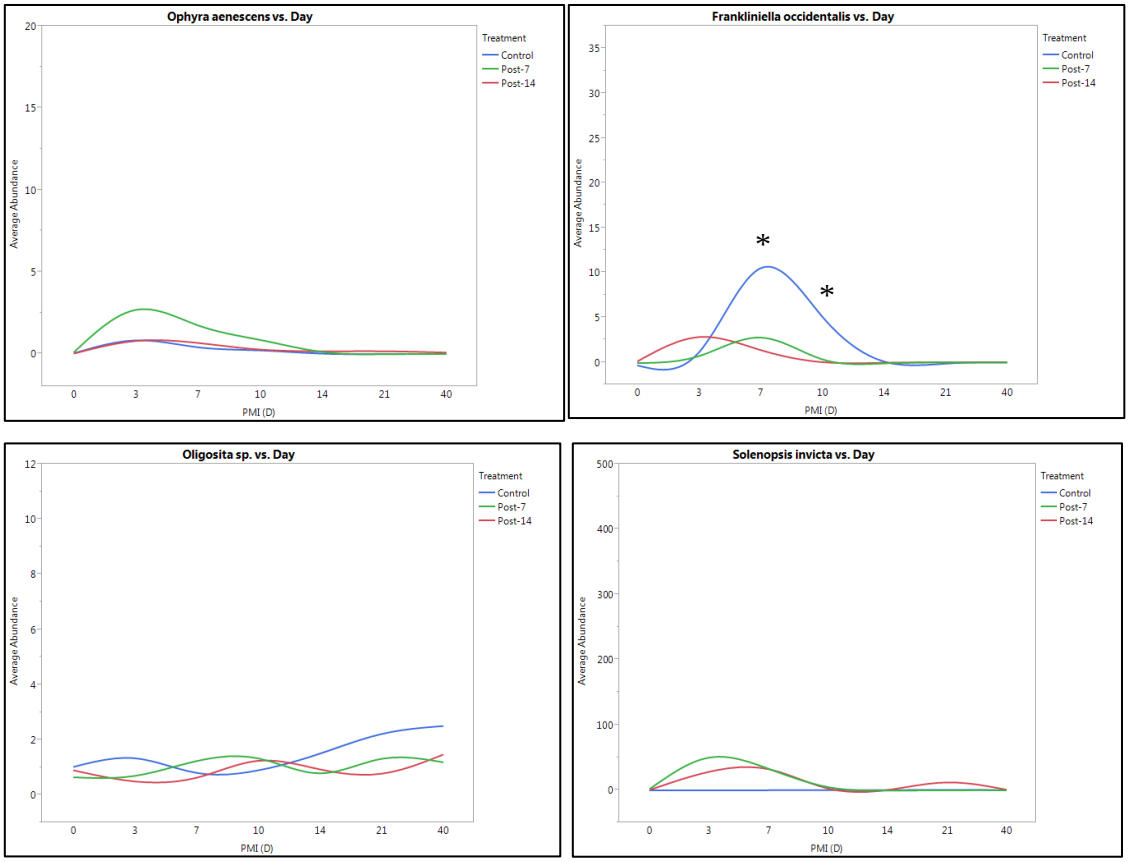

Figure 5.147 (Continued).

Richness

The full model showed a significant difference in Day ($p < 0.0001$), Treatment ($p = 0.0070$), Height ($p < 0.0001$) and Position ($p < 0.0001$) and interactions between Day x Height ($p = 0.0164$), Day x Position ($p = 0.0100$), Height x Position ($p < 0.0001$), Treatment x Position ($p = 0.0211$) and Day x Height x Position ($p = 0.0329$). There was no significant difference between treatments in all sampling days ($p > 0.05$) (Figure 5.148). Resilience was tested for all treatments and resilience occurred on Day 10 for Post-7 and Post-14 carcasses while resilience occurred on Day 14 for Control carcasses (Table 5.124).

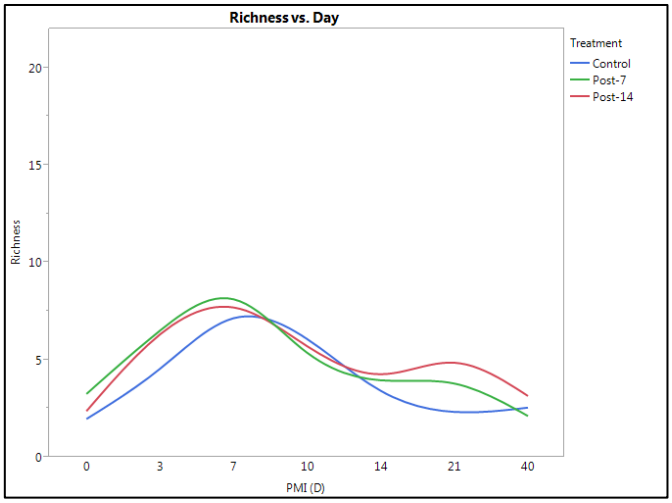

Figure 5.148. Aboveground arthropod community richness (by Genus and species) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.124. Resilience for aboveground arthropod community (by Genus and species) richness collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 7	<0.0001*	14
	0 x 10	0.0024*	
Post-7	0 x 7	0.0122*	10
Post-14	0 x 7	0.0020*	10

Simpson's diversity index

The full model showed a significant difference in Day ($p < 0.0001$), Height ($p < 0.0001$) and Position ($p = 0.0456$) and interactions between Day x Height ($p = 0.0006$), Day x Position ($p = 0.0106$), and Day x Height x Position ($p = 0.0280$). There was no significant difference ($p > 0.05$) in Simpson's diversity between treatments in all sampling days (Figure 5.149). Resilience was tested for all treatments and resilience was observed on Day 10 for Control carcasses while Post-7 and Post-14 carcasses were resistance throughout the decomposition days (Table 5.125).

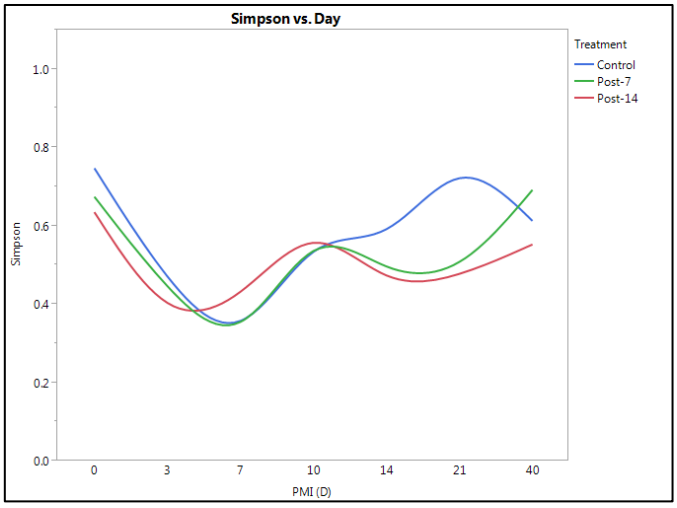

Figure 5.149. Simpson's diversity of the aboveground arthropod community (by Genus and species) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.125. Resilience for Simpson's Diversity of the aboveground arthropod community (by Genus and species) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 7	0.0065*	10
Post-7	None	0.0571•	Resistance
Post-14	None	0.3266	Resistance

• Marginal significant difference.

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p = 0.0076$), Height ($p = 0.0006$) and interactions between Day x Position ($p = 0.0022$). There was no significant difference ($p > 0.05$) in Shannon-Wiener's diversity between treatments in all sampling days (Figure 5.150). Resilience was tested and the results showed all treatments were resistance throughout all decomposition days (Table 5.126).

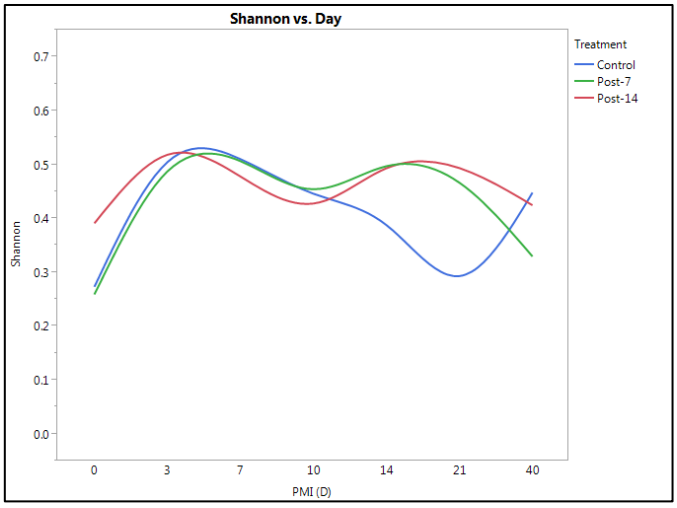

Figure 5.150. Shannon-Wiener's diversity of the aboveground arthropod community (by Genus and species) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.126. Resilience for Shannon-Wiener's Diversity of the aboveground arthropod community (by Genus and species) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1020	Resistance
Post-7	None	0.1351	Resistance
Post-14	None	0.8226	Resistance

Evenness

The full model showed a significant difference in interactions between Day x Position ($p = 0.0063$), and Height x Position ($p = 0.0020$). There was no significant difference ($p > 0.05$) in evenness between treatments in all sampling days (Figure 5.151). Resilience was tested and the results showed all treatments were resistance throughout the decomposition days (Table 5.127).

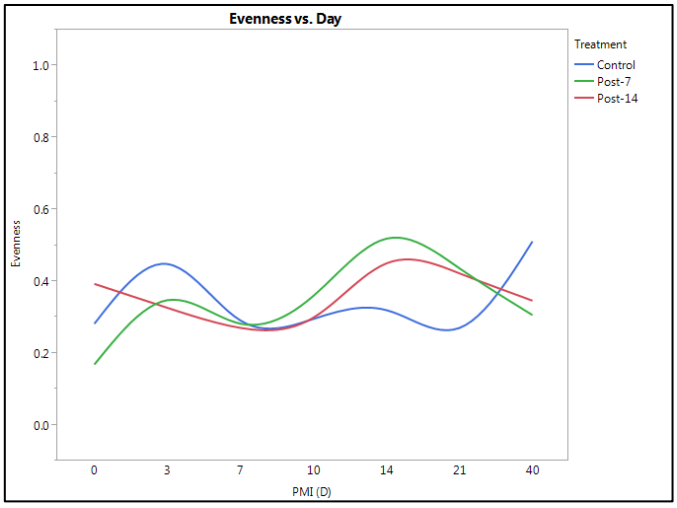

Figure 5.151. Evenness of the aboveground arthropod community (by Genus and species) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.127. Resilience for evenness of the aboveground arthropod community (by Genus and species) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1511	Resistance
Post-7	None	0.0891	Resistance
Post-14	None	0.6188	Resistance

Effective number of species

The full model showed a significant difference in Day ($p = 0.0327$), Height ($p = 0.0038$) and interactions between Day x Height ($p = 0.0203$), and Day x Position ($p < 0.0015$). There was no significant difference ($p > 0.05$) in ENS between treatments in all sampling days (Figure 5.152). Resilience was tested and the results showed all treatments were resistance throughout the decomposition days (Table 5.128).

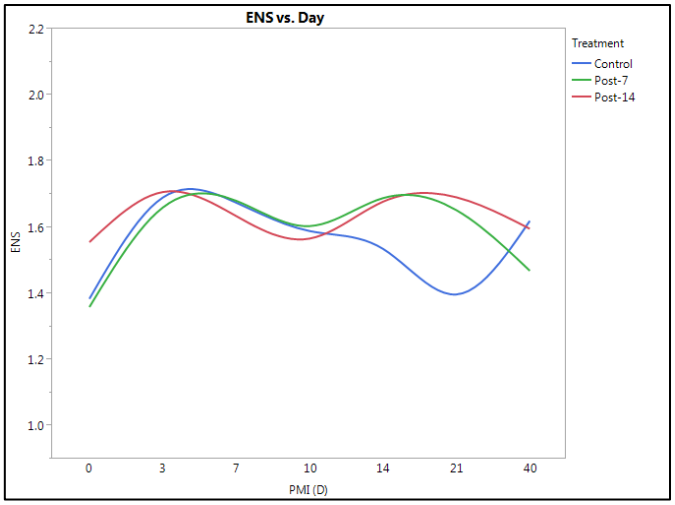

Figure 5.152. Effective Number of Species of the aboveground arthropod community (by Genus and species) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.128. Resilience for ENS of the aboveground arthropod community (by Genus and species) collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1640	Resistance
Post-7	None	0.2161	Resistance
Post-14	None	0.8691	Resistance

Function in 2014

PERMANOVA was performed on aboveground arthropod data by functional groups. Results showed that there was Day, Treatment, Height, and Position effects ($p < 0.05$).There were interactions between Day x Treatment ($p = 0.003$), Day x Height ($p = 0.041$), Day x Position ($p = 0.001$) and Height x Position ($p = 0.001$) (Table 5.129).

Table 5.129. Analysis of the aboveground arthropod community functions collected via sticky traps in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	13.071	0.001*
Treatment	2	2.512	0.011*
Height	1	14.340	0.001*
Position	1	21.446	0.001*
Day x Treatment	2	3.100	0.003*
Day x Height	1	2.350	0.041*
Treatment x Height	2	1.080	0.351
Day x Position	1	4.858	0.001*
Treatment x Position	2	1.386	0.184
Height x Position	1	40.127	0.001*
Day x Treatment x Height	2	0.468	0.921
Day x Treatment x Position	2	1.083	0.357
Day x Height x Position	1	1.823	0.107
Treatment x Height x Position	2	1.655	0.089
Day x Treatment x Height x Position	2	1.724	0.077

Since there was significant effect in Day and Treatment, further analyses were carried out. For days of decomposition, all day to day comparisons were significantly different, except Day 0 x Day 14, Day 0 x Day 21, Day 7 x Day 14 and Day 14 x Day 21 where there were no significant difference (Table 5.130). As for Treatment effect, significant difference was found between Control x Post-7 ($p = 0.028$) (Table 5.131). The NMDS plot of stress for aboveground arthropod community structure (Figure 5.153) and NMDS ordinations for Day, Treatment, Height, and Position were provided for visualization about data distribution (Figure 5.154, 5.155, 5.156 and 5.157, respectively). Minimum stress for given dimensionality was 0.1244 with $r^2 = 0.9267$. MRPP analysis

for day showed a significant difference (A value = 0.0677; Significant of Delta = 0.001 based on 999 permutations). The MRPP for treatment demonstrated significant difference with A value 0.0060 and Significant of Delta 0.019. MRPP for height also showed a significant difference with A value 0.0231 and Significant of Delta 0.001 while the MRPP for position was significantly different with A value 0.0387 and Significant of Delta 0.001.

Table 5.130. Pairwise comparisons of aboveground arthropod community functions collected via sticky traps between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.028*	0.062*	0.001*	0.996	0.284	0.003*
3	0.028*	-	0.001*	0.001*	0.028*	0.016*	0.001*
7	0.062*	0.001*	-	0.001*	0.107	0.002*	0.001*
10	0.001*	0.001*	0.001*	-	0.001*	0.001*	0.001*
14	0.996	0.028*	0.107	0.001*	-	0.137	0.002*
21	0.284	0.016*	0.002*	0.001*	0.137	-	0.001*
40	0.003*	0.001*	0.001*	0.001*	0.002*	0.001*	-

Table 5.131. Pairwise comparisons of aboveground arthropod community function collected via sticky traps between treatments in summer 2014 at Snook, Texas after Bonferroni's correction.

Treatment	df	SS	MS	F model	R2	P value
Control x Post-7	1	0.2636	0.2636	1.6688	0.0099	0.137
Residual	166	26.2247	0.1579		0.9901	
Total	167	26.4884			1.0000	
Control x Post-14	1	0.4621	0.4621	2.9193	0.0173	0.014*
Residual	166	26.2767	0.1582		0.9827	
Total	167	26.7388			1.0000	
Post-7 x Post-14	1	0.158	0.1580	0.9081	0.0054	0.45
Residual	166	28.886	0.1740		0.9946	
Total	167	29.004			1.0000	

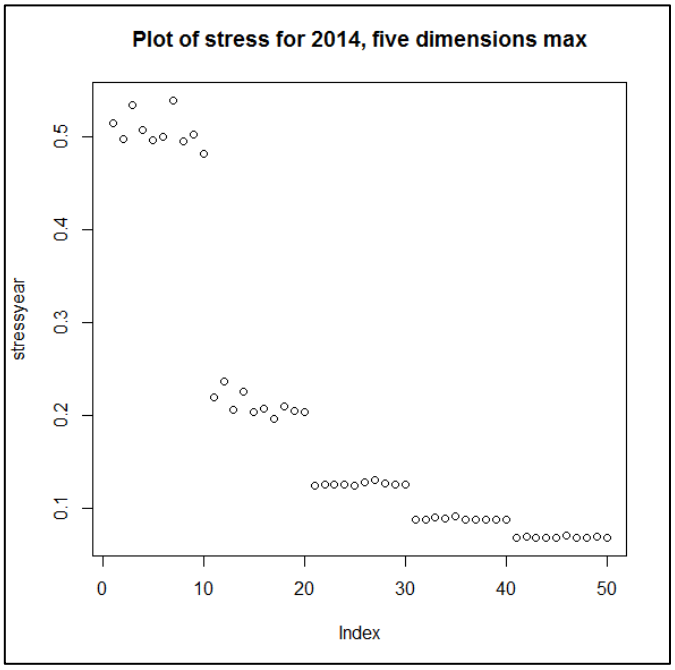

Figure 5.153. NMDS plot of stress for aboveground arthropod community functions collected via sticky traps in summer 2014 at Snook, Texas (Stress test 0.1244; r^2 = 0.9267).

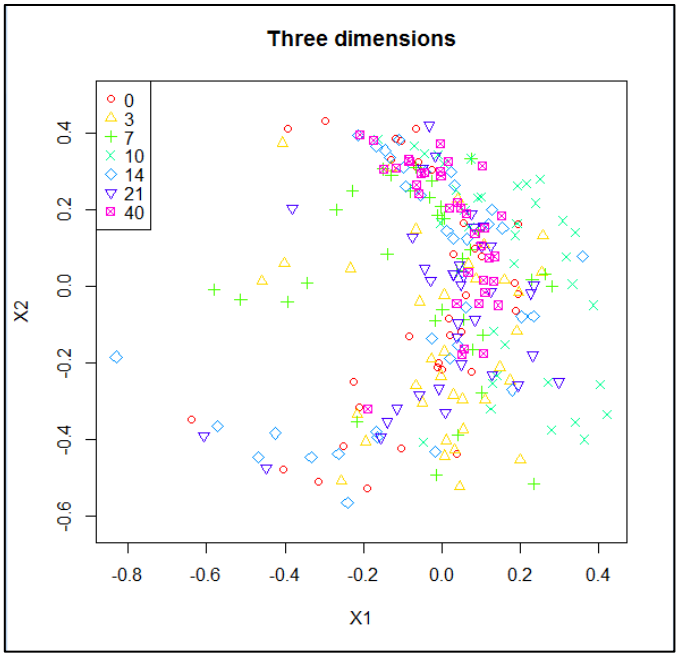

Figure 5.154. NMDS ordinations for aboveground arthropod community function by carrion decomposition days collected via sticky traps in summer 2014 at Snook, Texas.

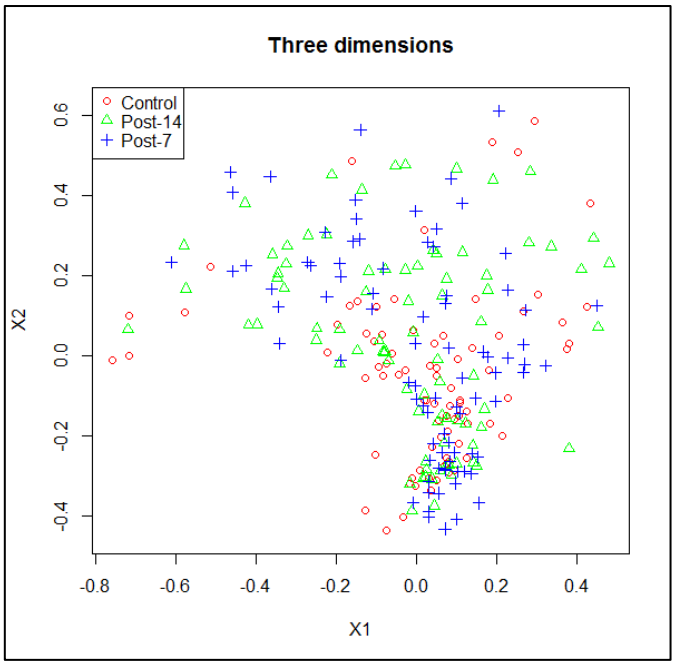

Figure 5.155. NMDS ordinations for aboveground arthropod community function by treatments collected via sticky traps in summer 2014 at Snook, Texas.

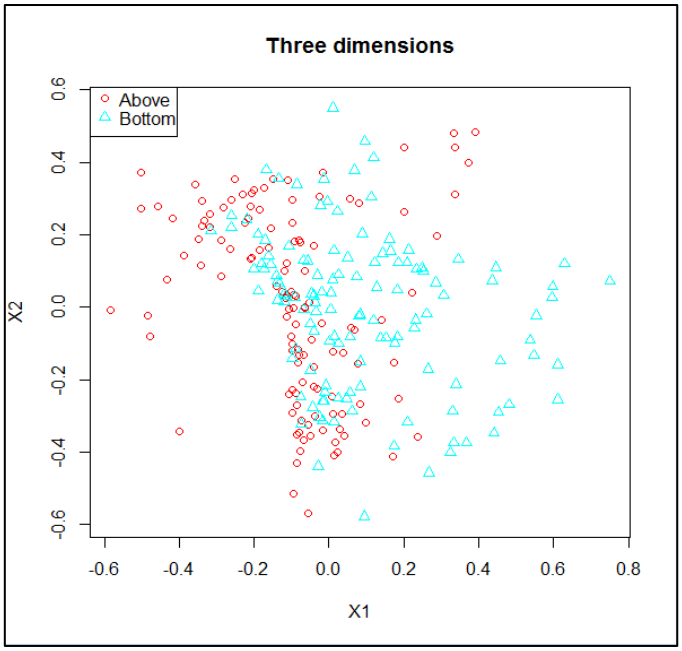

Figure 5.156. NMDS ordinations for aboveground arthropod community function by heights of sticky traps in summer 2014 at Snook, Texas.

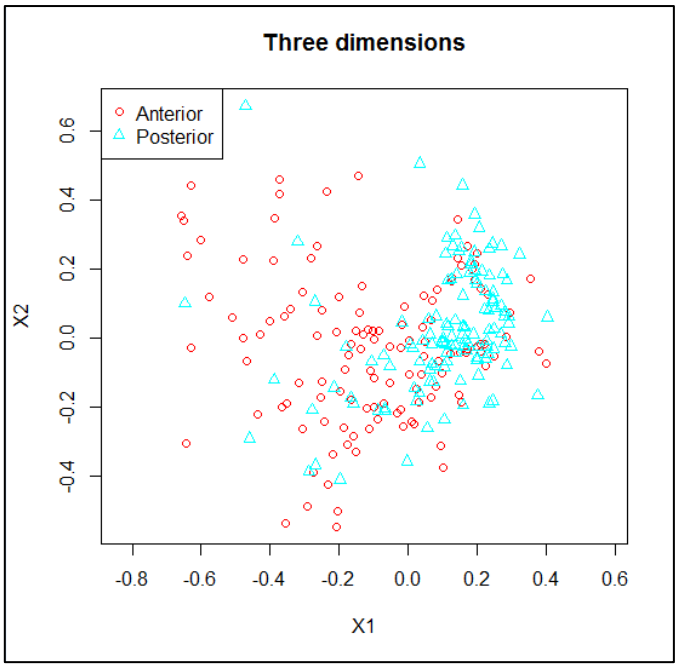

Figure 5.157. NMDS ordinations for aboveground arthropod community function by positions of sticky traps in summer 2014 at Snook, Texas.

For ISA, results demonstrated three indicative functional groups among aboveground arthropods in summer 2014 at Snook, Texas (Table 5.132).

Table 5.132. Indicator species analysis by functional groups for aboveground arthropods trapped by sticky traps in summer 2014 at Snook, Texas.

Type	Functional groups	Indicator value	P value
Sticky traps	Herbivores	0.0438	0.044*
	Predators/Parasites	0.2044	0.010*
	Necrophagous	0.2339	0.004*

Abundance

Six functional groups were highlighted individually (excluding hematophagous and non-feeding groups) (Figure 5.158). For nectarivores, significant difference was detected on Day 3 ($p = 0.0365$). For necrophagous guild, a marginal significant difference was found on Day 21 ($p = 0.0627$). For herbivores, there was a significant difference on Day 7 between Control x Post-14 ($p = 0.0149$). For predators / parasites, a marginal significant difference was observed on Day 10 ($p = 0.0558$). Detritivores and fungivores both showed resistance between treatments in all sampling days.

Resilience was tested for all treatments in six functional groups. The results showed four functional groups were resistant to perturbations. For necrophagous group, resilience was observed on Day 7 for Control carcasses and Day 14 for Post-14 groups. For nectarivores, resilience was observed on Day 10 for Post-7 carcasses (Table 5.133).

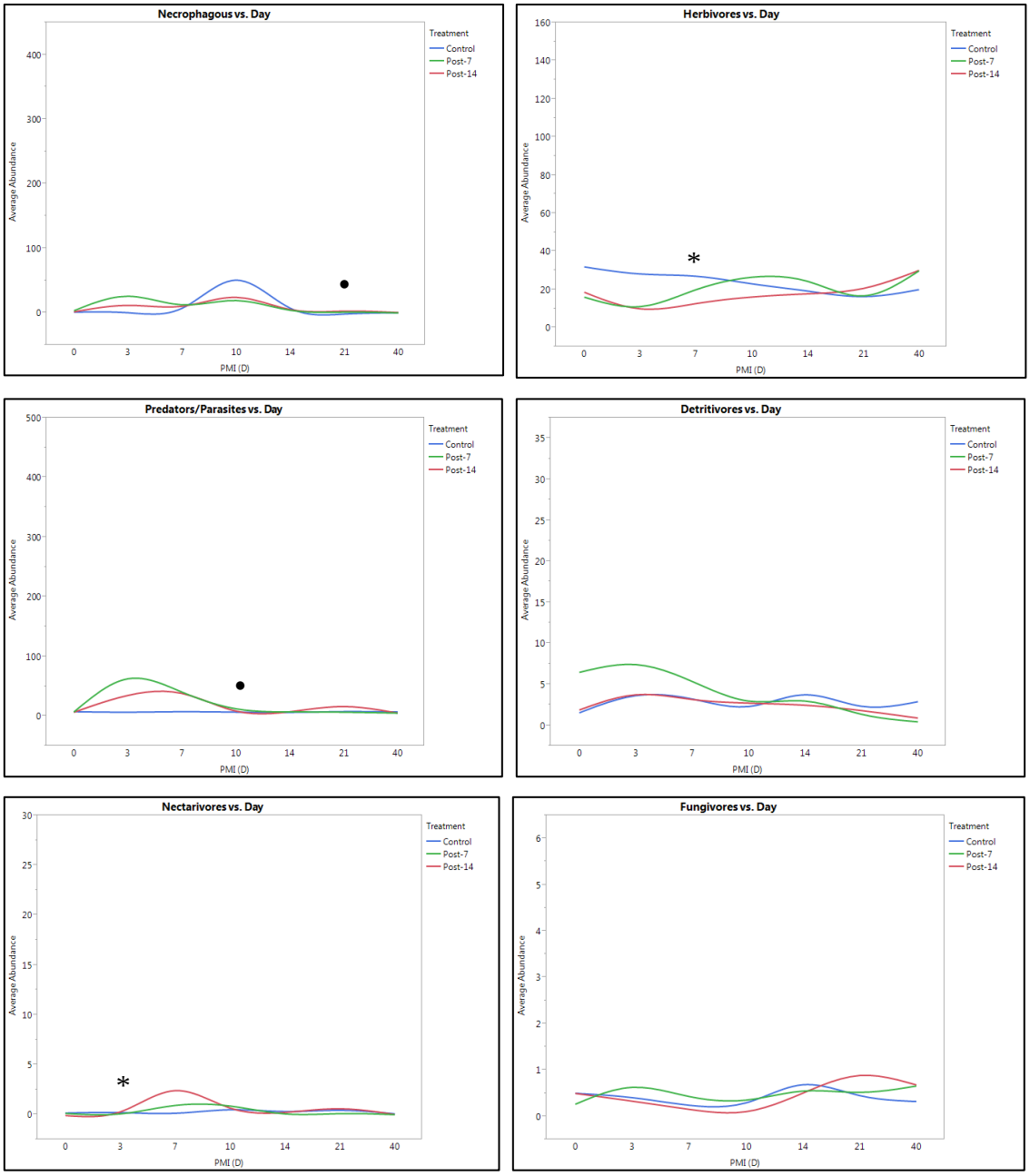

Figure 5.158. Average abundance of arthropods according to functional groups collected via sticky traps in summer 2014 at Snook, Texas. Upper Left. Abundance of necrophagous across Treatments over time. Upper Right. Abundance of herbivores across Treatments over time. Middle Left. Abundance of predators / parasites across Treatments over time. Middle Right. Abundance of detritivores across Treatments over time. Lower Left. Abundance of nectarivores across Treatments over time. Lower Right. Abundance of fungivores across Treatments over time (* represent significantly different; • represents marginal significant difference).

Table 5.133. Resilience for aboveground arthropod community functions collected via sticky traps for each treatment in summer 2014 at Snook, Texas.

Function	Treatment	Significant difference	P value	Resilience on Day
Necrophagous	Control	0 x 3	0.0112	7
	Post-7	None	0.0540•	Resistance
	Post-14	0 x 10	0.0036*	14
Detritivores	Control	None	0.8019	Resistance
	Post-7	None	0.0518•	Resistance
	Post-14	None	0.1270	Resistance
Predators/Parasites	Control	None	0.9631	Resistance
	Post-7	None	0.0990	Resistance
	Post-14	None	0.3287	Resistance
Fungivores	Control	None	0.5981	Resistance
	Post-7	None	0.8678	Resistance
	Post-14	None	0.4494	Resistance
Herbivores	Control	None	0.6020	Resistance
	Post-7	None	0.0111*	Resistance#
	Post-14	None	0.0210*	Resistance#
Nectarivores	Control	None	0.1499	Resistance
	Post-7	0 x 7	0.0356*	10
	Post-14	None	0.2020	Resistance

• Marginal significant difference.
= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Pitfall trap in 2014

Total Order in 2014

A total of eight Orders of Class Insecta, one Order and one Subclass of Arachnida (Araneae and Acari, respectively), one Order of Class Malacostraca (Isopoda), and one Class of Diplopoda, and one Class of Chilopoda have been recovered from all pitfall traps in 2014 trial. Table 5.134 showed the Orders identified in 2014 trial and the most dominant crawling arthropods was the Diptera larvae (94.86%), followed by Hymenoptera (3.67%) and others (less than 3%).

Table 5.134. Total abundance and dominance of Orders in the Class Insecta, Class and Subclass Arachnida and other arthropod classes identified from all pitfall trap samples in summer 2014 at Snook, Texas.

Order	Total abundance	Dominance
Diptera (larvae)	22334	94.86
Hymenoptera	863	3.67
Araneae	161	0.68
Coleoptera	97	0.41
Hemiptera	47	0.20
Orthoptera	17	0.07
Blattodea	10	0.04
Collembola	7	0.03
Acari	4	0.02
Chilopoda	2	0.01
Isopoda	1	0.00
Diplopoda	1	0.00
Psocoptera	1	0.00
Total	23545	100

Total Family in 2014

A total of 33 families of arthropods (including three families from the Order Araneae, two families in the Subclass Acari, and one family from the Order Isopoda) were identified from all pitfall traps in 2014 trial (Table 5.135). Total abundance of all arthropods identified to Family level was 23508 individuals. The dominant family was Calliphoridae (94.73%), followed by Formicidae (3.62%) and other families (less than 3%).

Table 5.135. Total abundance and dominance of Families in the Class Insecta, Arachnida and Malacostraca identified from all pitfall trap samples in summer 2014 at Snook, Texas.

Family/Suborder	Total abundance	Dominance
Calliphoridae (larvae)	22270	94.73
Formicidae	852	3.62
Lycosidae	145	0.62
Carabidae	48	0.20
Sarcophagidae (larvae)	43	0.18
Aphididae	40	0.17
Araneidae	16	0.07
Gryllidae	15	0.06
Tenebrionidae	9	0.04
Scarabaeidae	8	0.03
Dermestidae	8	0.03
Ectobiidae	7	0.03
Isotomidae	6	0.03
Trogidae	5	0.02
Phoridae	5	0.02
Cucurlionidae	4	0.02
Staphylinidae	3	0.01

Table 5.135 (Continued).

Family/Suborder	Total abundance	Dominance
Anthicidae	3	0.01
Oribatida	3	0.01
Cicadellidae	2	0.01
Histeridae	2	0.01
Anthocoridae	2	0.01
Chironomidae	2	0.01
Apidae	1	0.00
Armadillidiidae	1	0.00
Oxyopidae	1	0.00
Silphidae	1	0.00
Chloropidae	1	0.00
Sminthuridae	1	0.00
Acrididae	1	0.00
Mymaridae	1	0.00
Acaridae	1	0.00
Laemophloeidae	1	0.00
Total	23508	100

Total Genus and species in 2014

A total of 22 genera and species of crawling arthropods have been identified from pitfall traps in 2014 trial (Table 5.136). The most dominant genus or species collected was *Co. macellaria* larvae (95.29%), followed by *S. invicta* (2.52%) and others (all less than 2%).

Table 5.136. Total abundance and dominance of Genus and species of arthropods identified from all pitfall trap samples in summer 2014 at Snook, Texas.

Genus and species	Total abundance	Dominance
Cochliomyia macellaria (larvae)	22269	95.29
Solenopsis Invicta	590	2.52
Monomorium sp.	256	1.10
Hogna sp.	143	0.61
Sarcophaga bullata (larvae)	43	0.18
Pterostichus sp.	17	0.07
Gryllus sp.	14	0.06
Parcoblatta fulvescens	7	0.03
Dermestes caninus	6	0.03
Omorgus suberosus	5	0.02
Phyllophaga sp.	4	0.02
Ataeneus sp.	3	0.01
Pogonomyrmes sp.	2	0.01
Vacusus sp.	2	0.01
Megaselia scalaris	2	0.01
Harpalus affinis	1	0.00
Melanoplus differentialis	1	0.00
Necrodes surinamensis	1	0.00
Tapinoma sp.	1	0.00
Cryptolestes sp.	1	0.00
Apis mellifera	1	0.00
Dichromorpha viridis	1	0.00
Total	23370	100

Total function in 2014

Six functional groups were identified from 23539 crawling arthropods collected in pitfall traps in summer 2014. The most dominant group was necrophagous guild (94.93%), followed by predators/parasites (4.59%), herbivores (0.31%), detritivores (0.15%), nectarivores (0.01%) and arthropods that do not feed (0.01%).

Order in 2014

PERMANOVA was performed on crawling arthropod structural data by Order level. Results showed that there was significant difference in Day ($p = 0.004$), but no significant difference in Treatment, or any interaction ($p < 0.05$) (Table 5.137). There was no significant difference in Replicate ($p = 0.096$) as well.

Table 5.137. Analysis of the crawling arthropod community structure (by Order) collected via pitfall traps in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	2.8656	0.004*
Treatment	2	1.1960	0.264
Day x Treatment	2	0.8044	0.692

Since there was significant effect in Day, further analyses were carried out. For days of decomposition, all day to day comparisons were significantly different, except Day 0 x Day 14, Day 3 x Day 7, Day 3 x Day 21, Day 7 x Day 10, Day 7 x Day 21, Day 7 x Day 40, Day 10 x Day 21, Day 14 x Day 21, and Day 21 x Day 40 where there were no significant difference (Table 5.138). The NMDS plot of stress for crawling arthropod community structure (Figure 5.159) and NMDS ordinations for Day was provided for visualization about data distribution (Figure 5.160). Minimum stress for given dimensionality was 0.1946 with $r^2 = 0.7227$. The MRPP analysis for day showed a

significant difference (A value = 0.0809; Significant of Delta = 0.001 based on 999 permutations).

Table 5.138. Pairwise comparisons of crawling arthropod community structure (by Order) collected via pitfall traps between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.002*	0.001*	0.002*	0.407	0.003*	0.001*
3	0.002*	-	0.479	0.023*	0.020*	0.225	0.040*
7	0.001*	0.479	-	0.473	0.023*	0.723	0.126
10	0.002*	0.023*	0.473	-	0.003*	0.639	0.013*
14	0.407	0.020*	0.023*	0.003*	-	0.234	0.023*
21	0.003*	0.225	0.723	0.639	0.234	-	0.200
40	0.001*	0.040*	0.126	0.013*	0.023*	0.200	-

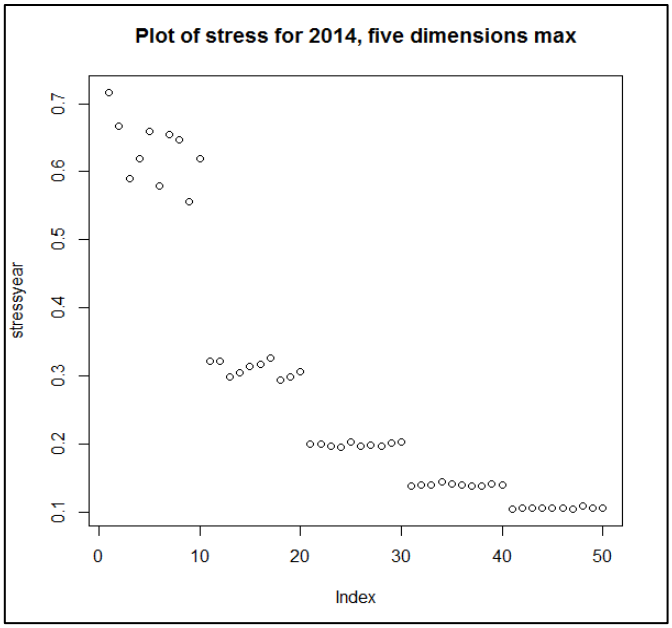

Figure 5.159. NMDS plot of stress for crawling arthropod community structure (by Order) collected via pitfall traps in summer 2014 at Snook, Texas (Stress test 0.1946; r^2 = 0.7227).

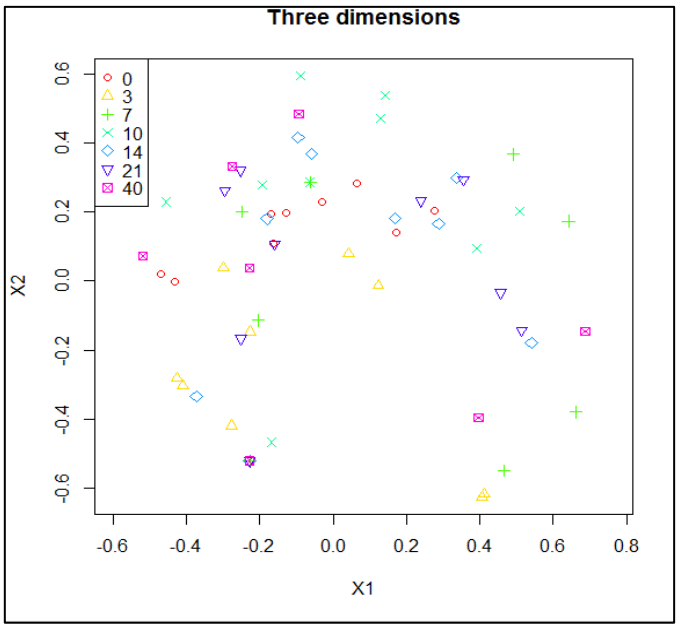

Figure 5.160. NMDS ordinations for crawling arthropod community structure (by Order) by carrion decomposition days collected via pitfall traps in summer 2014 at Snook, Texas.

For ISA, results demonstrated that there were two significant Order indicators among crawling arthropods in summer 2014 namely Diptera and Coleoptera (Table 5.139).

Table 5.139. Indicator species analysis by Order for crawling arthropods trapped by pitfall traps in summer 2014 at Snook, Texas.

Type	Order	Indicator value	P value
Pitfall traps	Diptera	0.6454	0.032*
	Coleoptera	0.1856	0.016*

Abundance

The full model showed a significant difference in Day ($p = 0.0038$), Treatment ($p = 0.0205$) and an interaction between Treatment x Day ($p = 0.0005$). Resilience was tested and results showed resilience was observed on Day 7 for Control carcasses while Post-7 and Post-14 carcasses were resistant in all sampling days (Table 5.140). Average abundance of crawling arthropods according to Orders collected at pitfall traps in 2014 trial was demonstrated in Figure 5.161. For Coleoptera, there was a significant difference on Day 10 between Control x Post-7 ($p = 0.0350$) and Post-7 x Post-14 ($p = 0.0350$). There was no significant difference detected between treatments in all sampling days for other Orders (Figure 5.162).

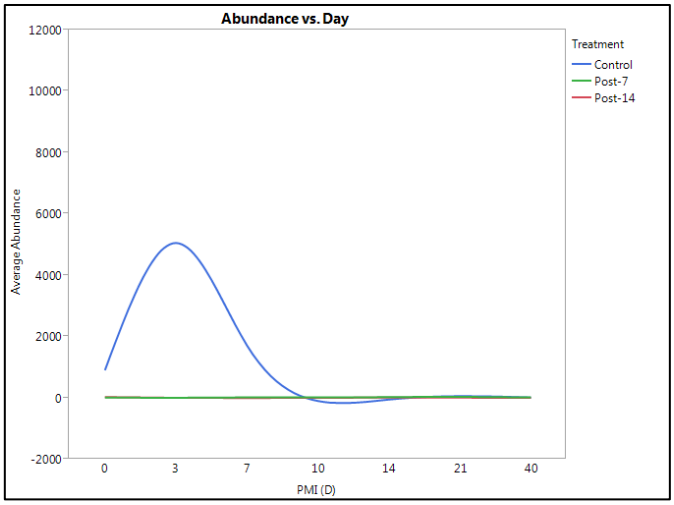

Figure 5.161. Crawling arthropod community abundance (by Order) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.140. Resilience for crawling arthropod community (by Order) abundance collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 3	0.0295*	7
Post-7	None	0.8111	Resistance
Post-14	None	0.1708	Resistance

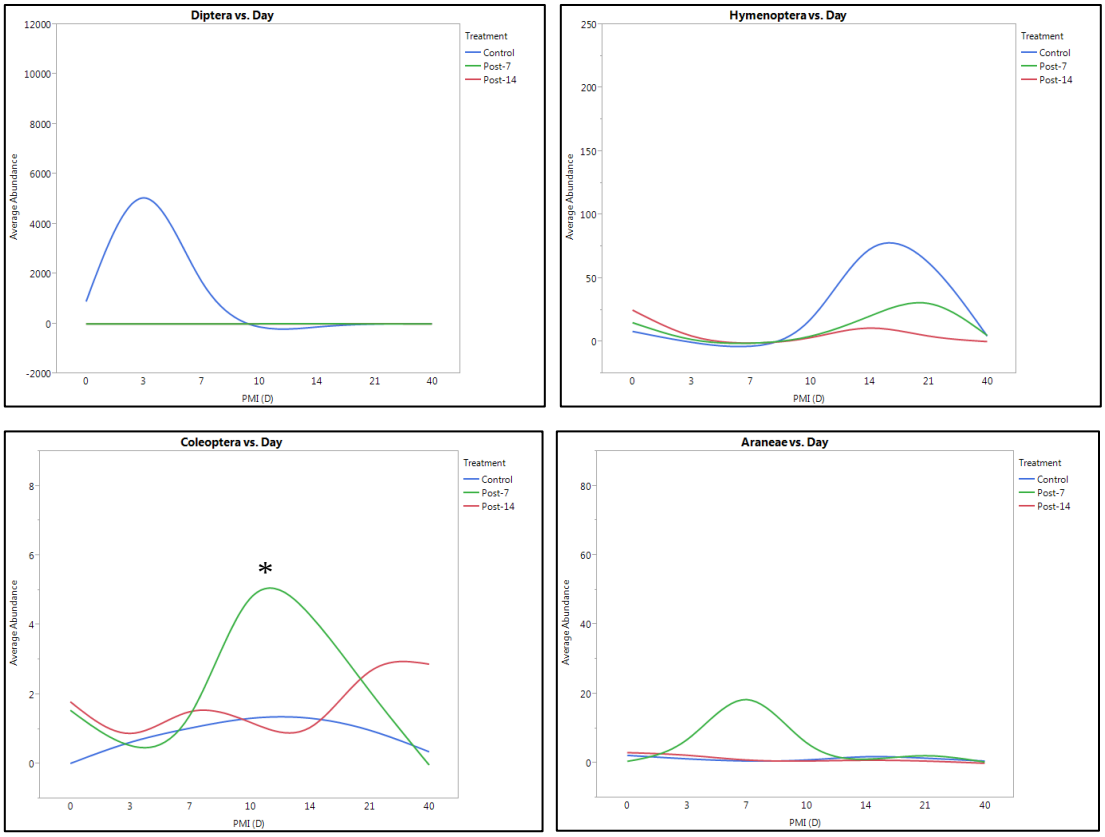

Figure 5.162. Average abundance of arthropods according to Orders collected via pitfall traps in summer 2014 at Snook, Texas. Upper Left. Abundance of Diptera across Treatments over time. Upper Right. Abundance of Hymenoptera across Treatments over time. Lower Left. Abundance of Coleoptera across Treatments over time. Lower Right. Abundance of Araneae across Treatments over time (* represents significant difference).

Richness

The full model showed no significant difference in Day, Treatment or any interaction ($p > 0.05$). There was significant difference found in richness between Control x Post-7 ($p = 0.0062$) and Control x Post-14 ($p = 0.0062$) on Day 7 (Figure 5.163). Resilience was tested and the results showed resistance in all treatment groups in all sampling days (Table 5.141).

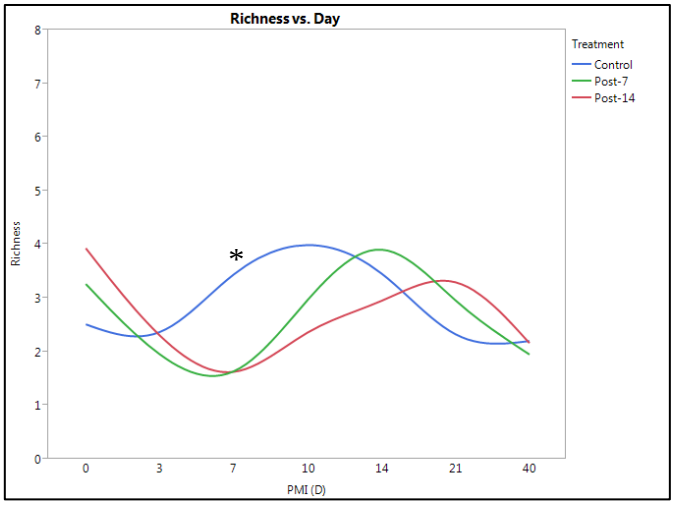

Figure 5.163. Crawling arthropod community richness (by Order) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.141. Resilience for crawling arthropod community (by Order) richness collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2536	Resistance
Post-7	None	0.0991	Resistance
Post-14	None	0.5158	Resistance

Simpson's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) found in Simpson's Diversity between treatments in all sampling days (Figure 5.164). In other words, the system was resistance that no divergence or convergence was observed. Resilience was tested for all treatments and the results demonstrated that all carcasses were resistance throughout the sampling days (Table 5.142).

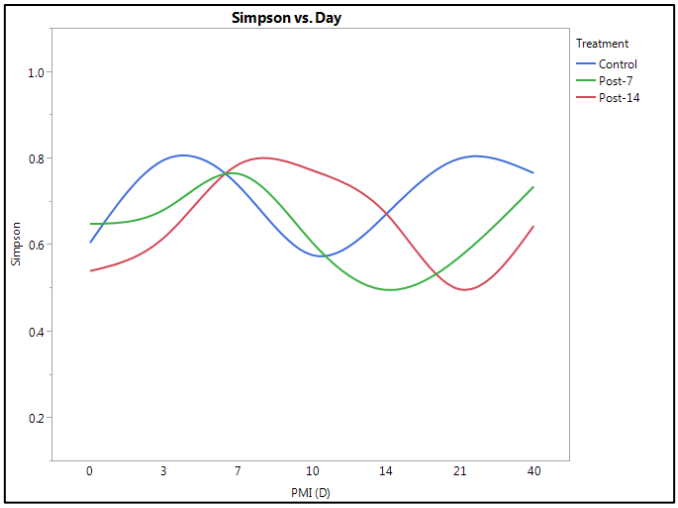

Figure 5.164. Simpson's diversity of the crawling arthropod community (by Order) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.142. Resilience for Simpson's Diversity of the crawling arthropod community (by Order) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.7954	Resistance
Post-7	None	0.7016	Resistance
Post-14	None	0.6014	Resistance

Shannon-Wiener's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was significant difference found in Shannon-Wiener's Diversity between Control x Post-14 ($p = 0.0366$) on Day 21 (Figure 5.165). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.143).

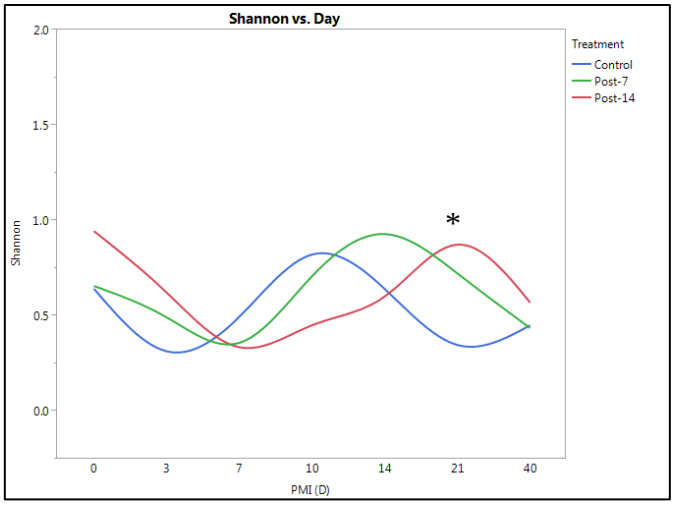

Figure 5.165. Shannon-Wiener's diversity of the crawling arthropod community (by Order) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.143. Resilience for Shannon-Wiener's Diversity of the crawling arthropod community (by Order) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.6989	Resistance
Post-7	None	0.5096	Resistance
Post-14	None	0.6272	Resistance

Evenness

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) found in evenness between treatments in all sampling days (Figure 5.166). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.144).

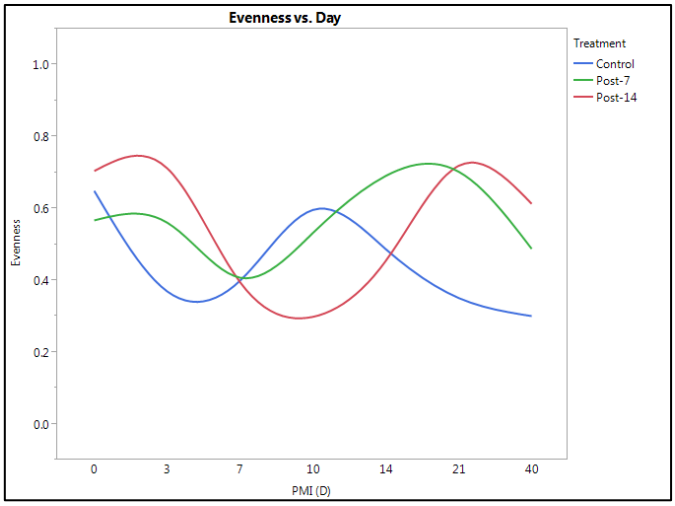

Figure 5.166. Evenness of the crawling arthropod community (by Order) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.144. Resilience for evenness of the crawling arthropod community (by Order) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.8344	Resistance
Post-7	None	0.8832	Resistance
Post-14	None	0.5192	Resistance

Effective number of species

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. However, there was significant difference found in ENS between Control x Post-14 ($p = 0.0319$) on Day 21 (Figure 5.167). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.145).

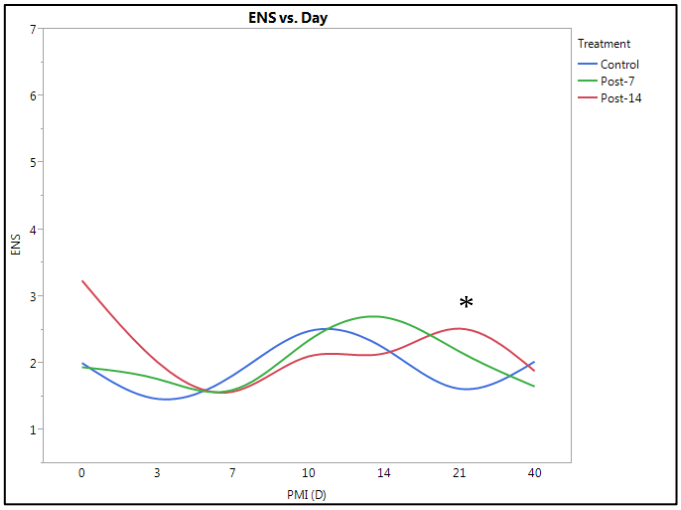

Figure 5.167. Effective Number of Species of the crawling arthropod community (by Order) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.145. Resilience for ENS of the crawling arthropod community (by Order) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.7903	Resistance
Post-7	None	0.4561	Resistance
Post-14	None	0.7301	Resistance

Family in 2014

PERMANOVA was performed on crawling arthropod structural data by Family level. Results showed that there was significant difference in Day ($p = 0.003$), but no significant difference in Treatment, Replicate or any interaction ($p < 0.05$) (Table 5.146).

Table 5.146. Analysis of the crawling arthropod community structure (by Family) collected via pitfall traps in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	2.4777	0.003*
Treatment	2	0.1019	0.333
Day x Treatment	2	0.8080	0.732

Since there was significant difference in Day, further analyses were performed. For days of decomposition, most of the day to day comparisons were significantly different, except Day 0 x Day 14, Day 3 x Day 7, Day 3 x Day 10, Day 3 x Day 21, Day 3 x Day 40, Day 7 x Day 21, Day 7 x Day 40, Day 10 x Day 21, Day 14 x Day 21 and Day 21 x Day 40 where there were no significant difference (Table 5.147). The NMDS plot of stress for crawling arthropod community structure (Figure 5.168) and the NMDS ordination for Day was provided for visualization of data distribution (Figure 5.169). Minimum stress for given dimensionality was 0.2165 with $r^2 = 0.6509$. The MRPP analysis for day showed a significant difference (A value = 0.0799; Significant of Delta = 0.001 based on 999 permutations).

Table 5.147. Pairwise comparisons of crawling arthropod community structure (by Family) collected via pitfall traps between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.001*	0.001*	0.001*	0.411	0.002*	0.001*
3	0.001*	-	0.285	0.085	0.012*	0.446	0.069•
7	0.001*	0.285	-	0.049*	0.012*	0.241	0.080
10	0.001*	0.085	0.049*	-	0.004*	0.193	0.014*
14	0.411	0.012*	0.012*	0.004*	-	0.243	0.025*
21	0.002*	0.446	0.241	0.193	0.243	-	0.197
40	0.001*	0.069•	0.080	0.014*	0.025*	0.197	-

• Marginal significant difference.

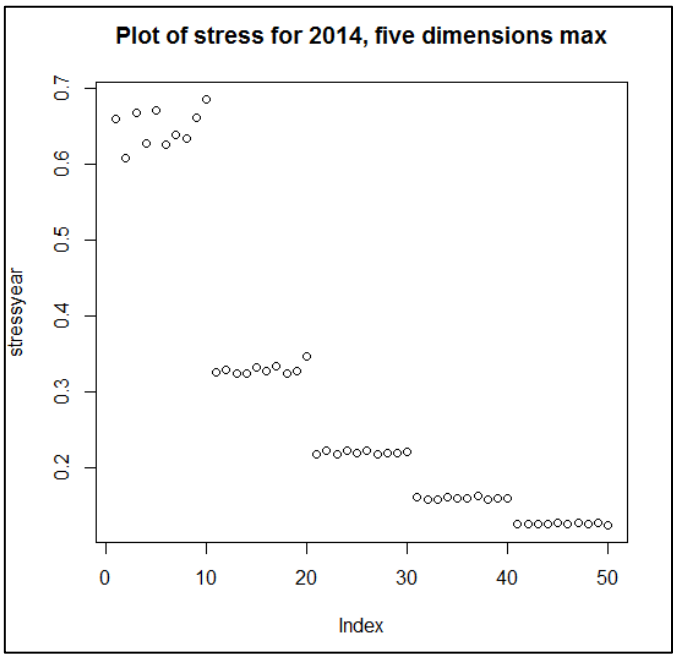

Figure 5.168. NMDS plot of stress for crawling arthropod community structure (by Family) collected via pitfall traps in summer 2014 at Snook, Texas (Stress test 0.2165; r^2 = 0.6509).

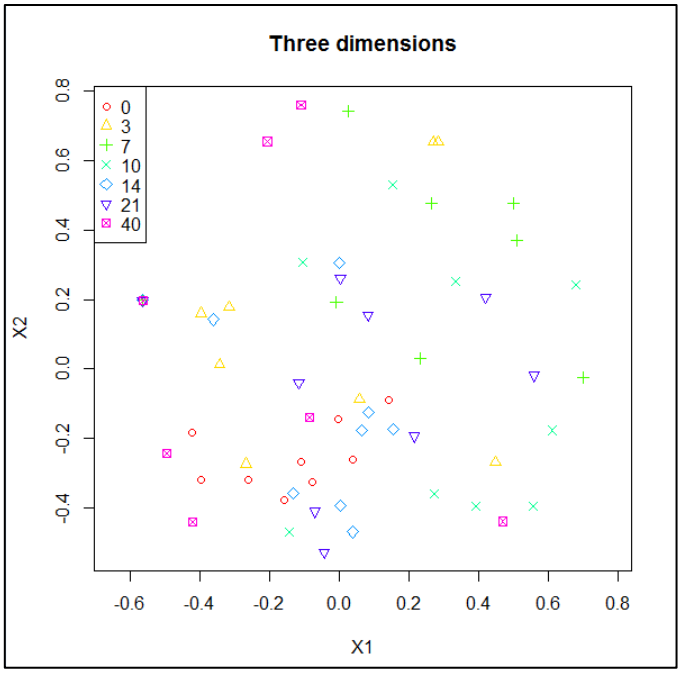

Figure 5.169. NMDS ordinations for crawling arthropod community structure (by Family) by carrion decomposition days collected via pitfall traps in summer 2014 at Snook, Texas.

For ISA, results showed that there were two significant indicators among crawling arthropods by Family in summer 2014 at Snook, Texas (Table 5.148).

Table 5.148. Indicator species analysis by Family for crawling arthropods trapped by pitfall traps in summer 2014 at Snook, Texas.

Type	Family	Indicator value	P value
Pitfall traps	Calliphoridae	0.6472	0.028*
	Histeridae	0.6667	0.036*

Abundance

The full model showed a significant difference in Day ($p = 0.0038$), Treatment ($p = 0.0205$) and an interaction Day x Treatment ($p = 0.0005$). Resilience was tested and resilience was observed on Day 7 for Control carcasses while there was resistance in Post-7 and Post-14 carcasses (Table 5.149). Average abundance of crawling arthropods according to Family collected at pitfall traps in 2014 trial was demonstrated in Figure 5.170. There was significant difference in the abundance of Carabidae between Control x Post-7 ($p = 0.0242$) and Post-7 x Post-14 ($p = 0.0393$) on Day 10. There was no significant difference detected between treatments in all sampling days for other Orders (Figure 5.171).

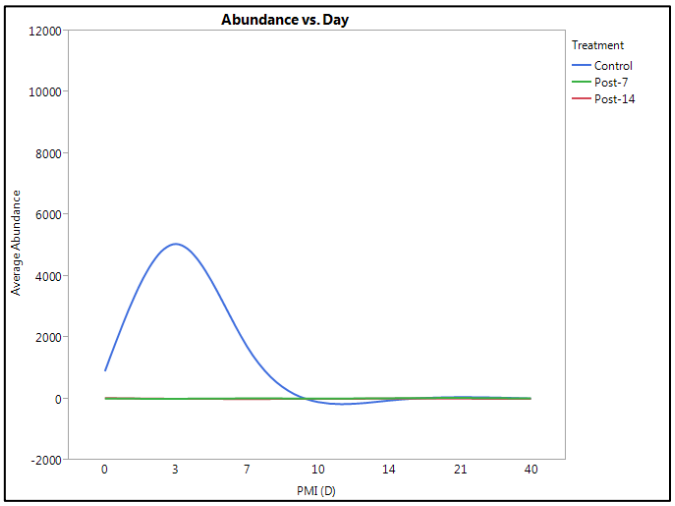

Figure 5.170. Crawling arthropod community abundance (by Family) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.149. Resilience for crawling arthropod community (by Family) abundance collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 3	0.0295*	7
Post-7	None	0.8091	Resistance
Post-14	None	0.1579	Resistance

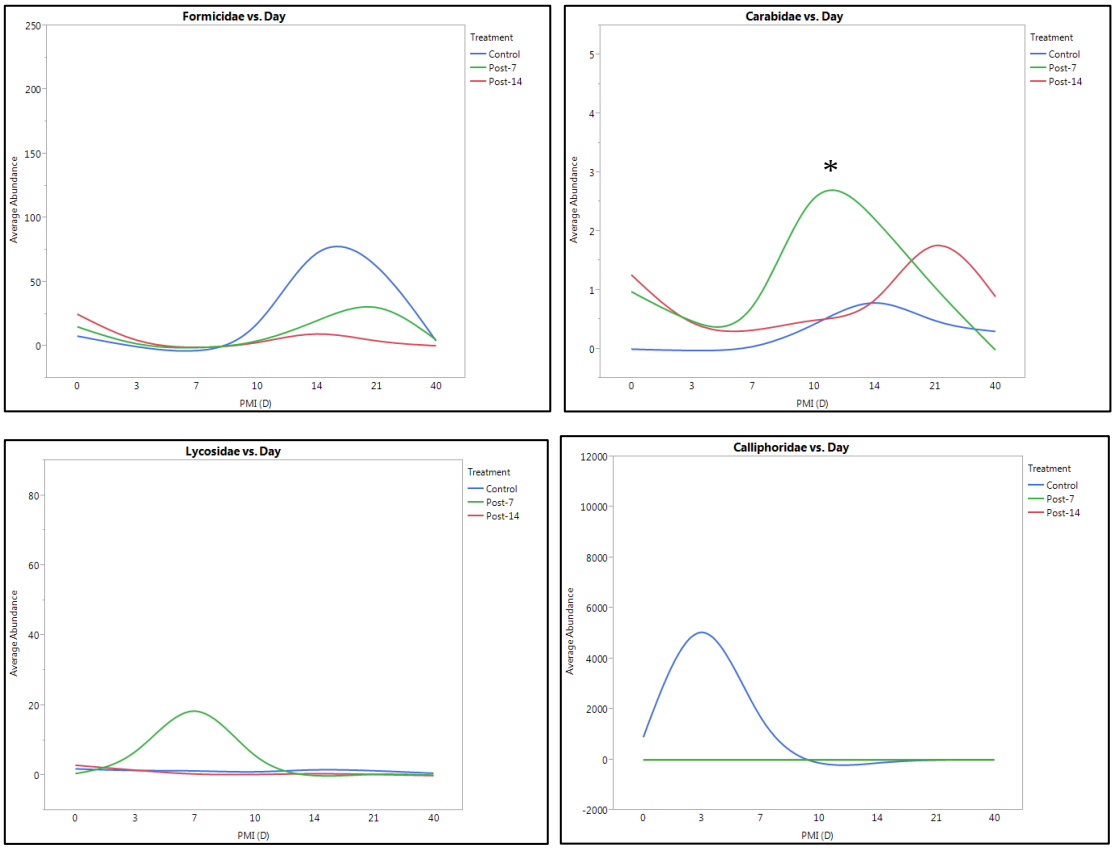

Figure 5.171. Average abundance of arthropods according to Families collected via pitfall traps in summer 2014 at Snook, Texas. Upper Left. Abundance of Formicidae across Treatments over time. Upper Right. Abundance of Carabidae across Treatments over time. Lower Left. Abundance of Lycosidae across Treatments over time. Lower Right. Abundance of Calliphoridae (larvae) across Treatments over time (* represents significant difference).

Richness

The full model showed a significant difference in Day ($p = 0.0115$), but not in Treatment or any interaction ($p > 0.05$). There was no significant difference ($p > 0.05$) in richness between treatments in all sampling days (Figure 5.172). Resilience was tested for all treatments, and the results showed that all treatments were resistance throughout the sampling days (Table 5.150).

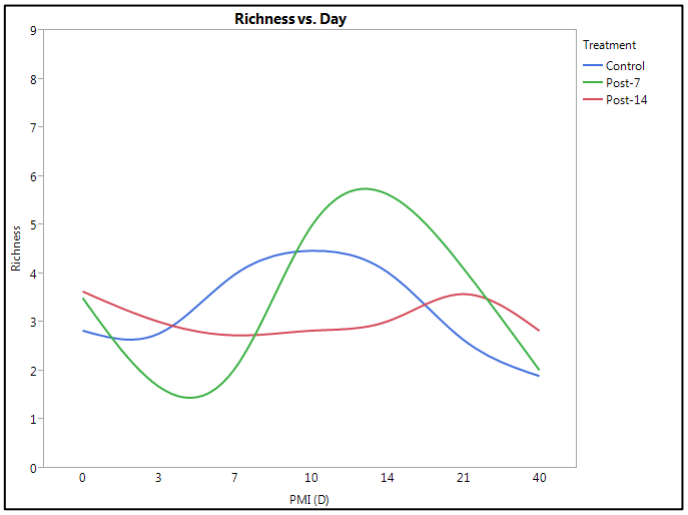

Figure 5.172. Crawling arthropod community richness (by Family) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.150. Resilience for crawling arthropod community (by Family) richness collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1263	Resistance
Post-7	None	0.0028*	Resistance[#]
Post-14	None	0.9211	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Simpson's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) in Simpson's Diversity between treatments in all sampling days (Figure 5.173). Resilience was tested and results showed resistance in all sampling days for all treatment group (Table 5.151).

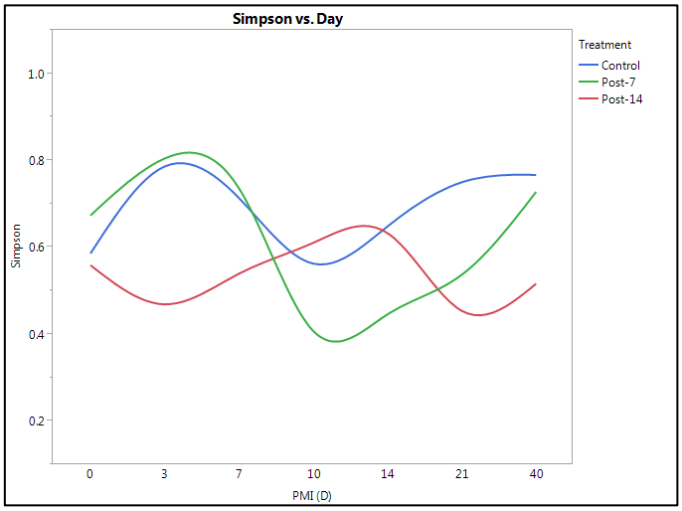

Figure 5.173. Simpson's diversity of the crawling arthropod community (by Family) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.151. Resilience for Simpson's Diversity of the crawling arthropod community (by Family) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.8538	Resistance
Post-7	None	0.0961	Resistance
Post-14	None	0.8599	Resistance

Shannon-Wiener's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) in Shannon-Wiener's diversity between treatments in all sampling days (Figure 5.174). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.152).

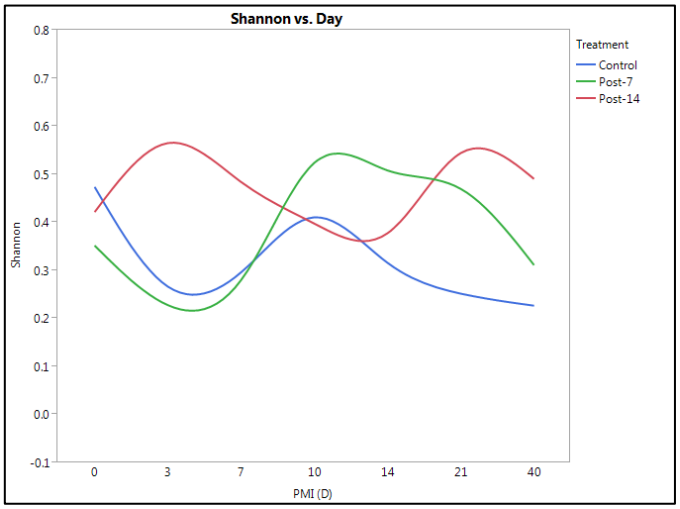

Figure 5.174. Shannon-Wiener's diversity of the crawling arthropod community (by Family) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.152. Resilience for Shannon-Wiener's Diversity of the crawling arthropod community (by Family) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.8732	Resistance
Post-7	None	0.4268	Resistance
Post-14	None	0.8186	Resistance

Evenness

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) in evenness between treatments in all sampling days (Figure 5.175). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.153).

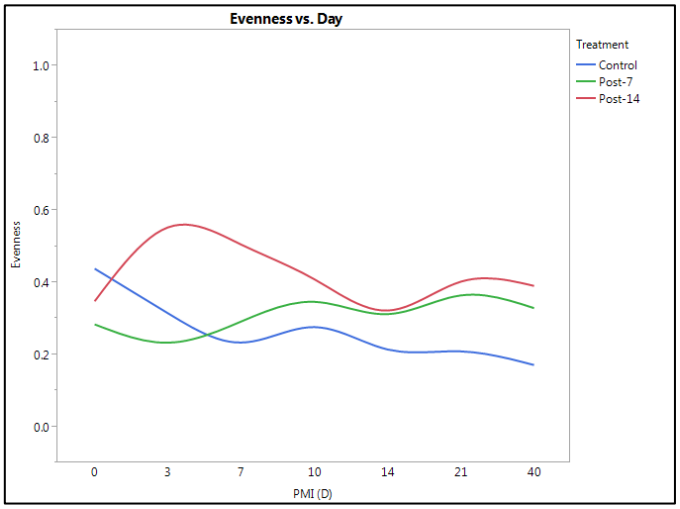

Figure 5.175. Evenness of the crawling arthropod community (by Family) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.153. Resilience for evenness of the crawling arthropod community (by Family) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.8848	Resistance
Post-7	None	0.9764	Resistance
Post-14	None	0.8909	Resistance

Effective number of species

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) in ENS between treatments in all sampling days (Figure 5.176). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.154).

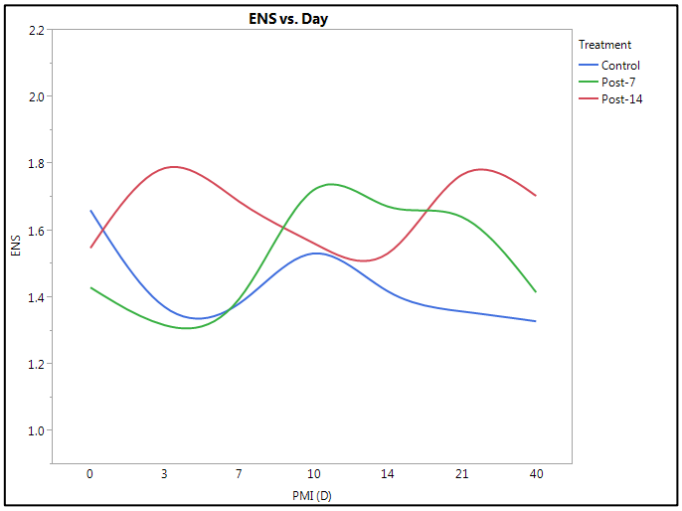

Figure 5.176. Effective Number of Species of the crawling arthropod community (by Family) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.154. Resilience for ENS of the crawling arthropod community (by Family) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.9104	Resistance
Post-7	None	0.4501	Resistance
Post-14	None	0.8334	Resistance

Genus and species in 2014

PERMANOVA was performed on crawling arthropod structural data by Genus and species level. Results showed that there was significant difference in Day ($p = 0.002$). There was no significant difference in Treatment, Replicate or any interaction ($p < 0.05$) (Table 5.155).

Table 5.155. Analysis of the crawling arthropod community structure (by Genus and species) collected via pitfall traps in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	2.8663	0.002*
Treatment	2	0.9583	0.526
Day x Treatment	2	0.9721	0.498

Since there was significant difference in Day, further analyses were performed. For days of decomposition, most of the day to day comparisons were significantly different, except Day 0 x Day 14, Day 3 x Day 7, Day 3 x Day 10, Day 3 x Day 14, Day 3 x Day 21, Day 7 x Day 21, Day 10 x Day 21, Day 14 x Day 21, Day 21 x Day 40 where there were no significant differences ($p > 0.05$) (Table 5.156). The NMDS plot of stress for crawling arthropod community structure (Figure 5.177) and the NMDS ordination for Day was provided for visualization about data distribution (Figure 5.178). Minimum stress for given dimensionality was 0.2149 with $r^2 = 0.6628$. The MRPP analysis for day showed A value = 0.0686; Significant of Delta = 0.001 based on 999 permutations).

Table 5.156. Pairwise comparisons of crawling arthropod community structure (by Genus and species) collected via pitfall traps between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.015*	0.001*	0.001*	0.637	0.038*	0.001*
3	0.015*	-	0.348	0.062•	0.057•	0.482	0.048*
7	0.001*	0.348	-	0.012*	0.015*	0.646	0.028*
10	0.001*	0.062•	0.012*	-	0.005*	0.070•	0.002*
14	0.637	0.057•	0.015*	0.005*	-	0.267	0.035*
21	0.038*	0.482	0.646	0.070•	0.267	-	0.108
40	0.001*	0.048*	0.028*	0.002*	0.035*	0.108	-

• Marginal significant difference.

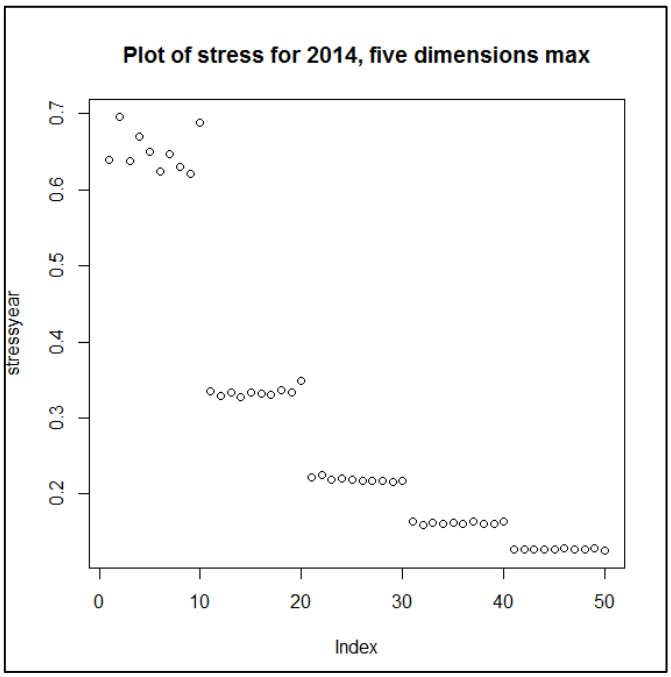

Figure 5.177. NMDS plot of stress for crawling arthropod community structure (by Genus and species) collected via pitfall traps in summer 2014 at Snook, Texas (Stress test 0.2149; $r^2 = 0.6628$).

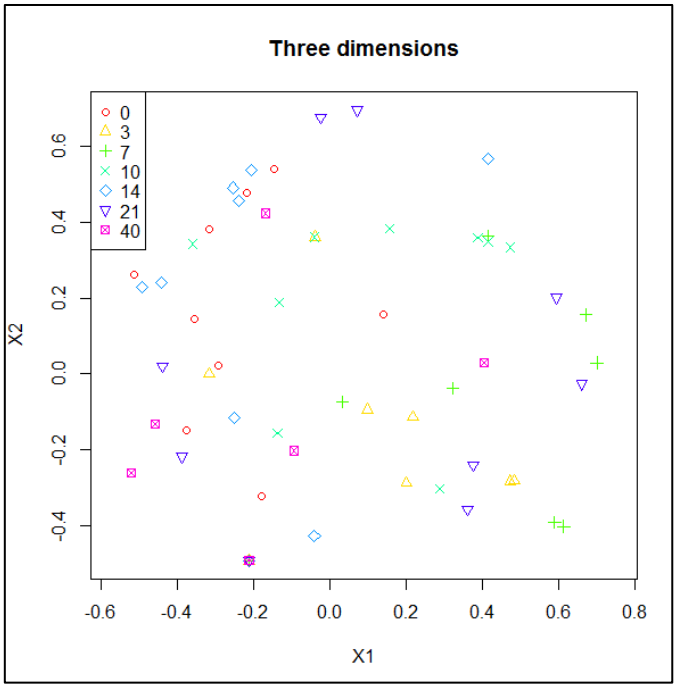

Figure 5.178. NMDS ordinations for crawling arthropod community structure by carrion decomposition days (by Genus and species) collected via pitfall traps in summer 2014 at Snook, Texas.

For ISA, results showed that there was a significant indicator (i.e., *Co. macellaria*) among crawling arthropods by Genus and species in summer 2014 at Snook, Texas (Table 5.157).

Table 5.157. Indicator species analysis by Genus and species for crawling arthropods trapped by pitfall traps in summer 2014 at Snook, Texas.

Type	Genus and species	Indicator value	P value
Pitfall traps	*Co. macellaria*	0.6472	0.030*

Abundance

The full model showed a significant difference in Day ($p = 0.0038$), Treatment ($p = 0.0203$) and an interaction Day x Treatment ($p = 0.0005$). Resilience was tested and resilience was observed on Day 7 for Control carcasses while there was resistance in Post-7 and Post-14 carcasses in all sampling days (Table 5.158). There was no significant difference ($p > 0.05$) in average arthropod abundance detected between treatments in all sampling days (Figure 5.179). Abundance of *S. invicta* and *Co. macellaria* were highlighted individually in Figure 5.180. No significant difference ($p > 0.05$) was observed in abundance between treatments across all sampling days for both species.

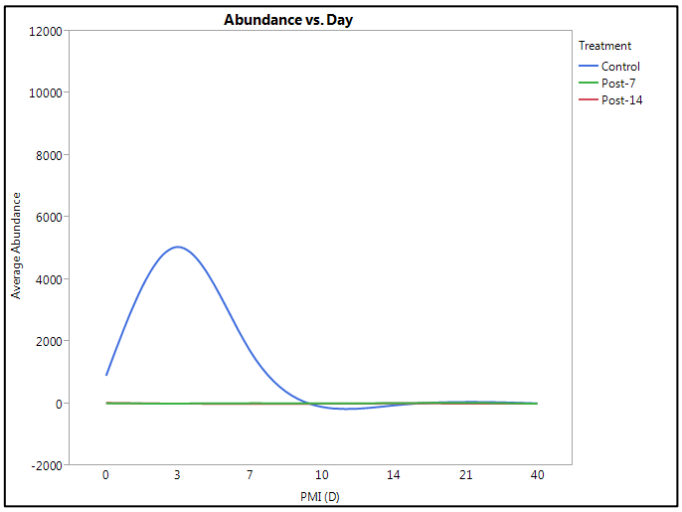

Figure 5.179. Crawling arthropod community abundance (by Genus and species) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.158. Resilience for crawling arthropod community (by Genus and species) abundance collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 3	0.0294*	7
Post-7	None	0.6976	Resistance
Post-14	None	0.1359	Resistance

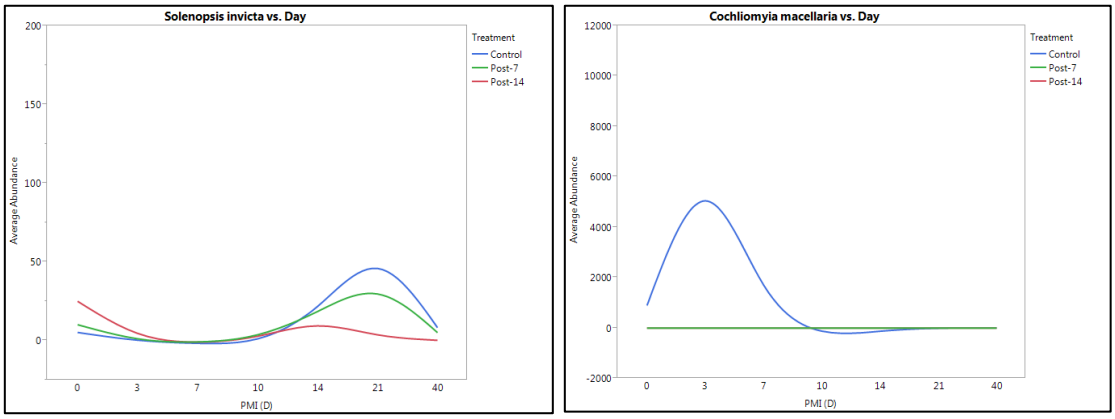

Figure 5.180. Average abundance of arthropods according to Genus and species collected via pitfall traps in summer 2014 at Snook, Texas. Left. Abundance of *S. invicta* across Treatments over time. Right. Abundance of *Co. macellaria* (larvae) across Treatments over time.

Richness

The full model showed a significant difference in Day (p = 0.0025) and an interaction Day x Treatment (p = 0.0006). There was significant difference found in richness between Control x Post-7 (p = 0.0110) and Control x Post-14 (p = 0.0202) on Day 7. Furthermore, significant difference was found on Day 14 between Post-7 x Post-14 (p = 0.0234) (Figure 5.181). Resilience was tested for all treatments, and resilience was observed in Day 10 for Post-7 carcasses while Control and Post-14 carcasses exhibited resistant throughout the sampling days (Table 5.159).

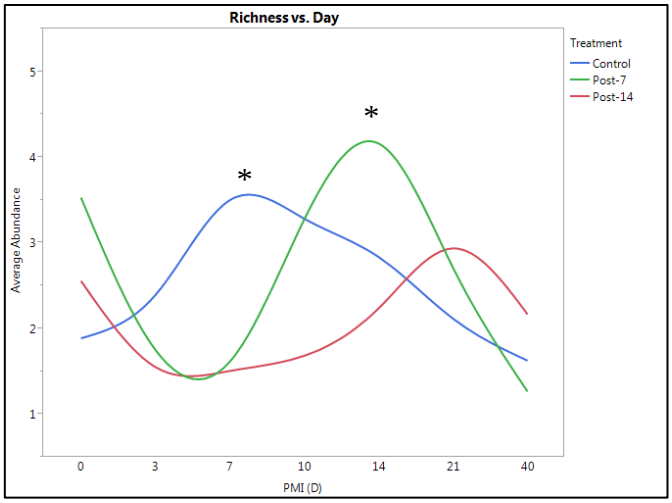

Figure 5.181. Crawling arthropod community richness (by Genus and species) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.159. Resilience for crawling arthropod community (by Genus and species) richness collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1338	Resistance
Post-7	0 x 3	0.0390*	10
	0 x 7	0.0134*	
Post-14	None	0.1159	Resistance

Simpson's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) found in Simpson's diversity between treatments in all sampling days (Figure 5.182). Resilience was tested and results showed resistance in all sampling days for all treatment group (Table 5.160).

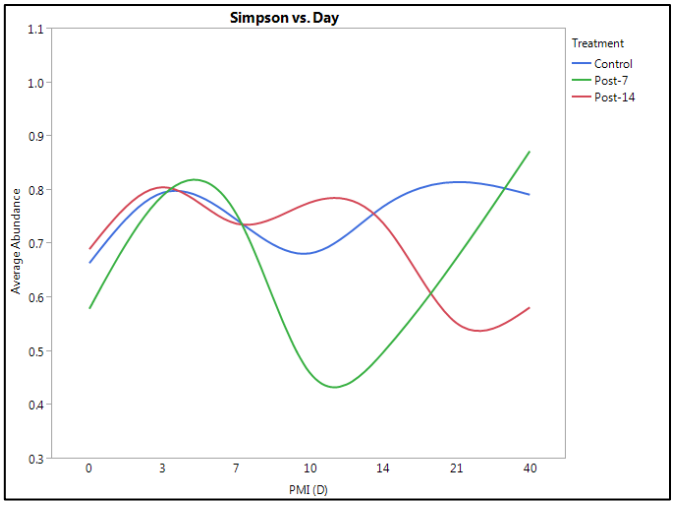

Figure 5.182. Simpson's diversity of the crawling arthropod community (by Genus and species) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.160. Resilience for Simpson's Diversity of the crawling arthropod community (by Genus and species) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.9428	Resistance
Post-7	None	0.1009	Resistance
Post-14	None	0.6765	Resistance

Shannon-Wiener's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) found in Shannon-Wiener's Diversity between treatments in all sampling days (Figure 5.183). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.161).

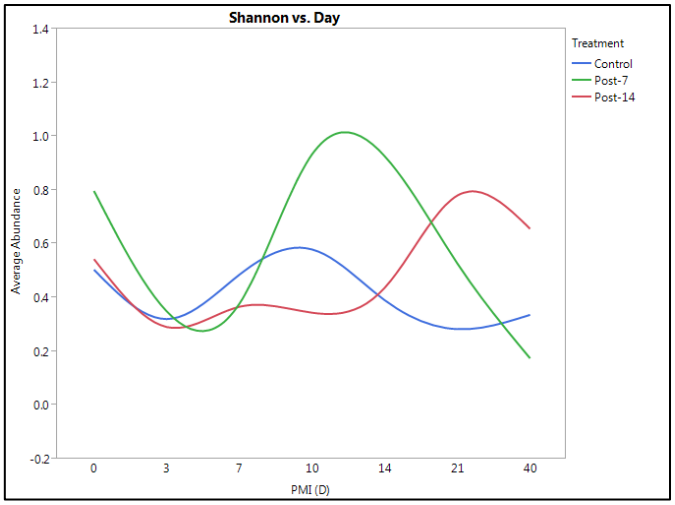

Figure 5.183. Shannon-Wiener's diversity of the crawling arthropod community (by Genus and species) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.161. Resilience for Shannon-Wiener's Diversity of the crawling arthropod community (by Genus and species) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.8640	Resistance
Post-7	None	0.0255*	Resistance
Post-14	None	0.4941	Resistance

Evenness

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) found in evenness between treatments in all sampling days (Figure 5.184). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.162).

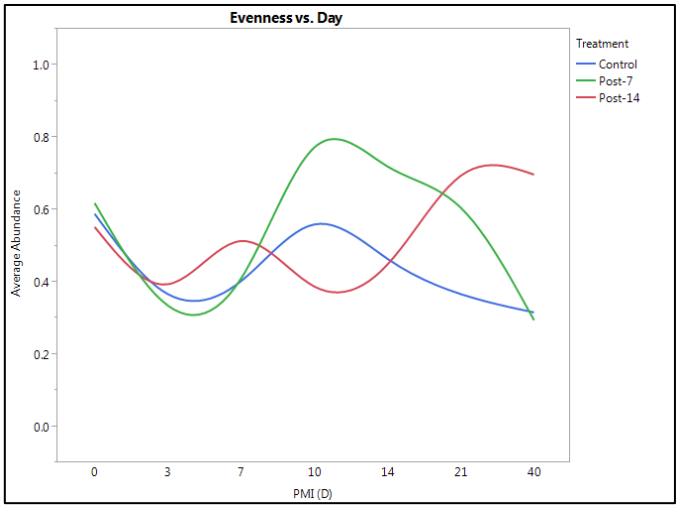

Figure 5.184. Evenness of the crawling arthropod community (by Genus and species) collected via sticky traps across Treatments over time in summer 2014 at Snook, Texas.

Table 5.162. Resilience for evenness of the crawling arthropod community (by Genus and species) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.9512	Resistance
Post-7	None	0.3813	Resistance
Post-14	None	0.8018	Resistance

Effective number of species

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) found in ENS between treatments in all sampling days, although there were marginal significant differences on Day 10 ($p = 0.0647$) and Day 14 ($p = 0.0576$) (Figure 5.185). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.163).

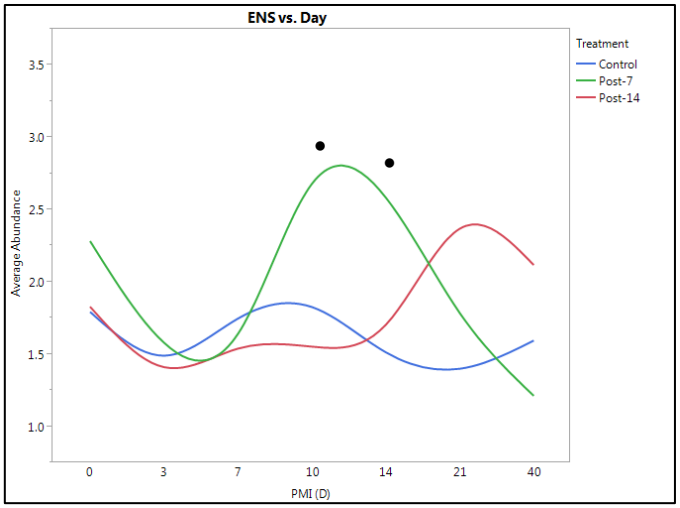

Figure 5.185. Effective Number of Species of the crawling arthropod community (by Genus and species) collected via pitfall traps across Treatments over time in summer 2014 at Snook, Texas (• represents marginal significant difference).

Table 5.163. Resilience for ENS of the crawling arthropod community (by Genus and species) collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.8954	Resistance
Post-7	None	0.0150*	Resistance[#]
Post-14	None	0.4309	Resistance

= There was no significant difference between Day 0 with other sampling days. The significant p value in the model indicates significant difference between other sampling days.

Function in 2014

PERMANOVA was performed on crawling arthropod functional data. Results showed that there was significant difference in Day ($p = 0.009$) while Treatment and Replicate was not significantly different ($p > 0.05$) (Table 5.164).

Table 5.164. Analysis of the crawling arthropod community functions collected via pitfall traps in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	2.5949	0.009*
Treatment	2	1.2050	0.269
Day x Treatment	2	0.5587	0.894

Since there was significant difference in Day, further analyses were performed. For days of decomposition, no significant differences ($p > 0.05$) were found on these comparisons: Day 0 x Day 14, Day 3 x Day 7, Day 3 x Day 10, Day 3 x Day 21, Day 3 x Day 40, Day 7 x Day 10, Day 7 x Day 14, Day 7 x Day 21, Day 7 x Day 40, Day 10 x Day 21, Day 10 x Day 40, Day 14 x Day 21, and Day 21 x Day 40 (Table 5.165). The NMDS plot of stress for crawling arthropod community function was demonstrated in Figure 5.186 and the NMDS ordination for Day was provided for visualization of data distribution (Figure 5.187). Minimum stress for given dimensionality was 0.1495 with $r^2 = 0.8639$. The MRPP analysis for day showed A value = 0.0688; Significant of Delta = 0.002 based on 999 permutations).

Table 5.165. Pairwise comparisons of crawling arthropod community function collected via pitfall traps between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.001*	0.002*	0.001*	0.343	0.008*	0.001*
3	0.001*	-	0.263	0.090	0.008*	0.224	0.115
7	0.002*	0.263	-	0.774	0.099	0.890	0.714
10	0.001*	0.090	0.774	-	0.040*	0.681	0.512
14	0.343	0.008*	0.099	0.040*	-	0.255	0.021*
21	0.008*	0.224	0.890	0.681	0.255	-	0.424
40	0.001*	0.115	0.714	0.512	0.021*	0.424	-

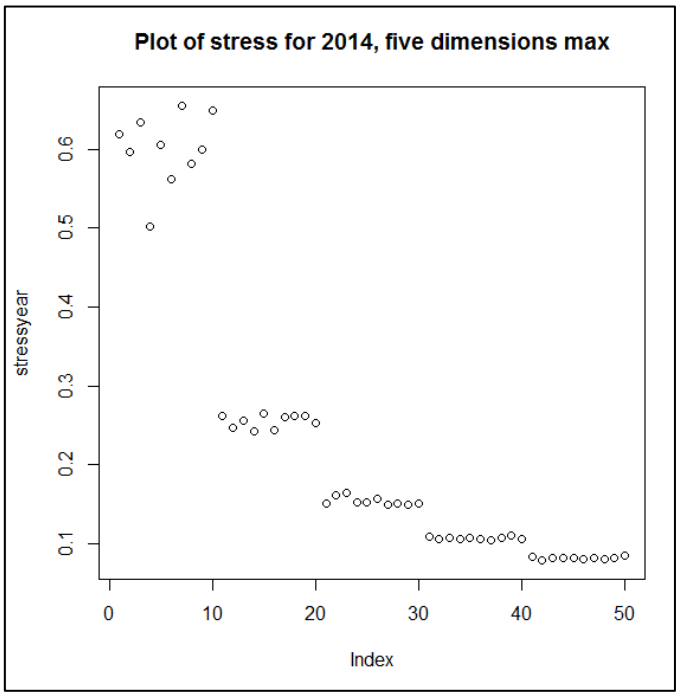

Figure 5.186. NMDS plot of stress for crawling arthropod community function collected via pitfall traps in summer 2014 at Snook, Texas (Stress test 0.1495; $r^2 = 0.8639$).

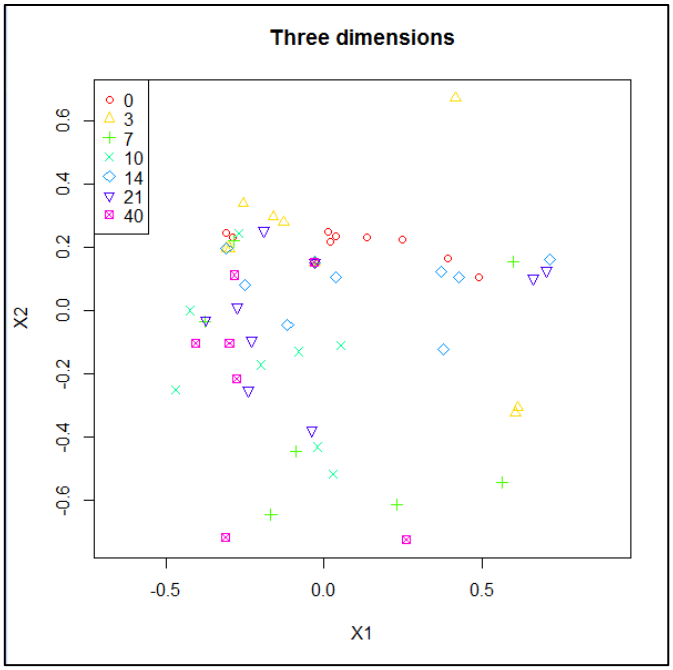

Figure 5.187. NMDS ordinations for crawling arthropod community function by carrion decomposition days collected via pitfall traps in summer 2014 at Snook, Texas.

For ISA, results showed that there was only one significant indicator (i.e., necrophagous) among crawling arthropods by functional group in summer 2014 at Snook, Texas (Table 5.166).

Table 5.166. Indicator species analysis by functional groups for crawling arthropods trapped by pitfall traps in summer 2014 at Snook, Texas.

Type	Functional group	Indicator value	P value
Pitfall traps	Necrophagous	0.645	0.032*

Abundance

Five functional groups namely necrophagous, herbivores, predators/parasites, nectarivores and detritivores were highlighted individually (Figure 5.187). Statistical tests showed there was significant difference in the abundance of detritivores on Day 7 between Control x Post-14 ($p = 0.0242$). Furthermore, significant difference in abundance of herbivores was found on Day 14 between Control x Post-7 ($p = 0.0310$) and Post-7 x Post-14 ($p = 0.0117$). Marginal significant difference was also detected in the abundance of predators/parasites on Day 3 ($p = 0.0534$) (Figure 5.188).

Resilience was tested for all treatments in four functional groups (nectarivores was excluded due to the low number of arthropods). The results showed all functional groups were resistance to perturbations throughout all sampling days except for necrophagous guild of Control carcasses, where the resilience was observed on Day 7 (Table 5.167).

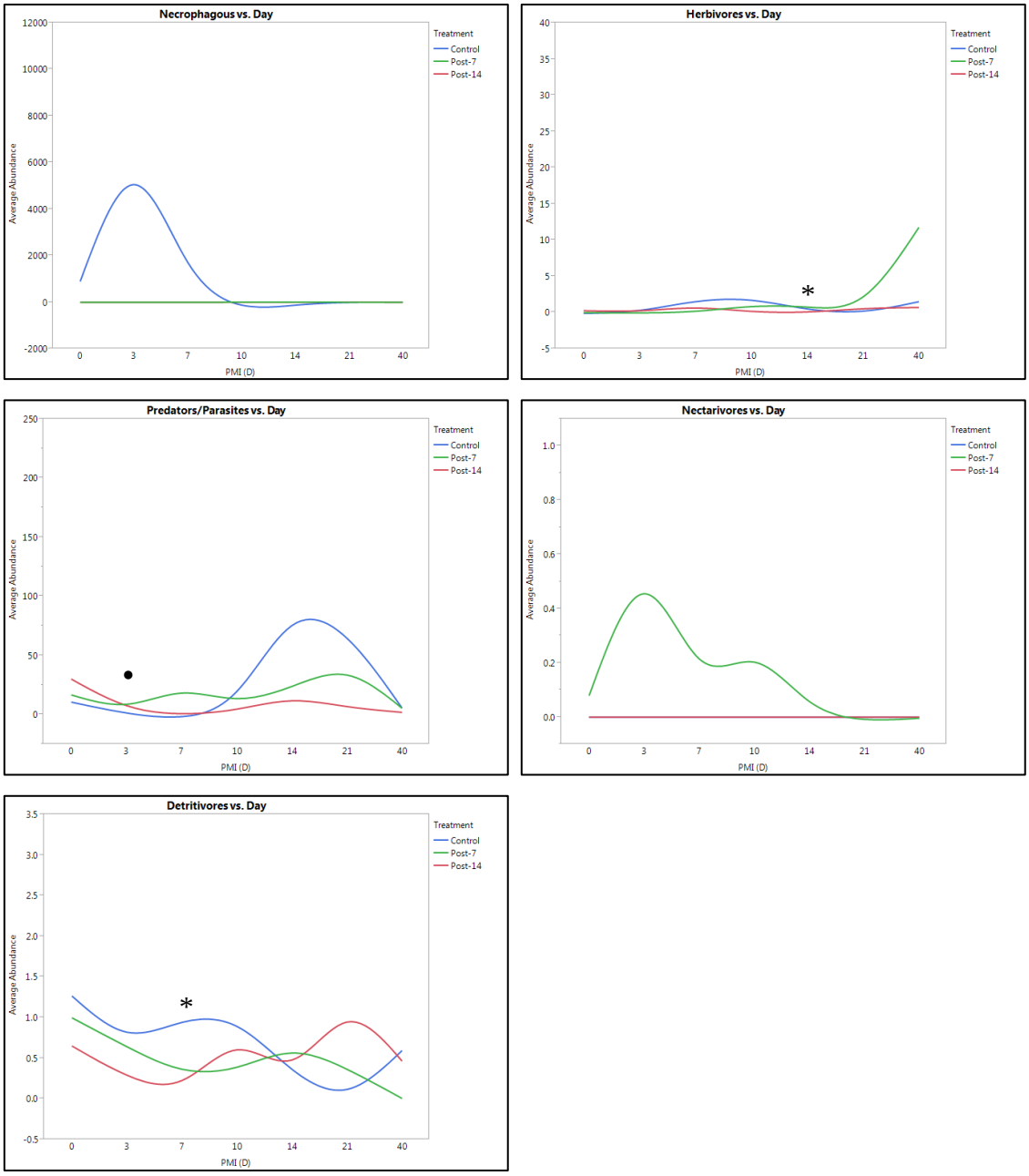

Figure 5.188. Average abundance of arthropods according to functional groups collected via pitfall traps in summer 2014 at Snook, Texas. Upper Left. Abundance of necrophagous across Treatments over time. Upper Right. Abundance of herbivores across Treatments over time. Middle Left. Abundance of predators/parasites across Treatments over time. Middle Right. Abundance of nectarivores across Treatments over time. Lower Left. Abundance of detritivores across Treatments over time (* represents significant difference; • denotes marginal significant difference).

Table 5.167. Resilience for crawling arthropod community function collected via pitfall traps for each treatment in summer 2014 at Snook, Texas.

Function	Treatment	Significant difference	P value	Resilience on Day
Necrophagous	Control	0 x 3	0.0291*	7
	Post-7	None	0.3173	Resistance
	Post-14	None	0.4199	Resistance
Detritivores	Control	None	0.7437	Resistance
	Post-7	None	0.5638	Resistance
	Post-14	None	0.6353	Resistance
Predators/Parasites	Control	None	0.4221	Resistance
	Post-7	None	0.6664	Resistance
	Post-14	None	0.1033	Resistance
Herbivores	Control	None	0.2149	Resistance
	Post-7	None	0.4092	Resistance
	Post-14	None	0.1883	Resistance

Sweep nets in 2014

Total Order in 2014

A total of seven Orders of Class Insecta and one Order of Class Arachnida (Araneae) have been collected from sweep nets in 2014 trial. Table 5.168 showed the Orders identified in summer 2014 and the most dominant arthropod collected was the Diptera (75.96%), followed by Hemiptera (20.67%), and others (all less than 1%).

Table 5.168. Total abundance and dominance of Orders in the Class Insecta and Arachnida identified from all sweep net samples in summer 2014 at Snook, Texas.

Order	Total abundance	Dominance
Diptera	316	75.96
Hemiptera	86	20.67
Hymenoptera	4	0.96
Coleoptera	3	0.72
Orthoptera	2	0.48
Araneae	2	0.48
Odonata	2	0.48
Psocoptera	1	0.24
Total	416	100

Total Family in 2014

A total of 20 families of arthropods (including two families from the Order Araneae) were identified from sweep nets in summer 2014 (Table 5.169). Total abundance of all arthropods identified to Family level was 406 individuals. The dominant family was Calliphoridae (42.86%), followed by Chloropidae (20.44%), Cicadellidae (13.30%), Aphididae (7.88%), Muscidae (7.39%), Fanniidae (3.69%) and other families (all less than 1%).

Table 5.169. Total abundance and dominance of Families in the Class Insecta and Arachnida identified from all sweep net samples in summer 2014 at Snook, Texas.

Family	Total abundance	Dominance
Calliphoridae	174	42.86
Chloropidae	83	20.44
Cicadellidae	54	13.30
Aphididae	32	7.88
Muscidae	30	7.39
Fanniidae	15	3.69
Ephydridae	2	0.49
Chironomidae	2	0.49
Formicidae	2	0.49
Coenagrionidae	2	0.49
Sarcophagidae	1	0.25
Therevidae	1	0.25
Acrididae	1	0.25
Membracidae	1	0.25
Oxyopidae	1	0.25
Cynipidae	1	0.25
Diapriidae	1	0.25
Histeridae	1	0.25
Staphylinidae	1	0.25
Araneidae	1	0.25
Total	406	100

Total Genus and species in 2014

A total of seven genera and species of arthropods have been identified from sweep nets in summer 2014 (included an unidentified genus of Cicadellidae) (Table 5.170). The most dominant genus or species collected was *Co. macellaria* (67.86%), followed by Cicadellidae sp. (11.90%), *O. aenescens* (11.11%), *F. pusio* (5.95%), *M. domestica* (1.98%), and others (all less than 1%).

Table 5.170. Total abundance and dominance of Genera and species in the Class Insecta identified from sweep net samples in summer 2014 at Snook, Texas.

Family	Total abundance	Dominance
Cochliomyia macellaria	171	67.86
Cicadellidae sp.	30	11.90
Hydrotaea aenescens	28	11.11
Fannia pusio	15	5.95
Musca domestica	5	1.98
Argia apicalis	2	0.79
Blaesoxipha plinthopyga	1	0.40
Total	252	100

Total function in 2014

Five functional groups were identified from 406 arthropods collected in sweep nets in summer 2014. The most dominant group was the necrophagous guild (66.26%), followed by herbivores (29.06%), predators/parasites (2.46%), detritivores (1.72%), and nectarivores (0.49%).

Order in 2014

PERMANOVA was performed on arthropod structural data by Order level. Results showed that there was significant difference in Day ($p = 0.015$) while there was no significant difference in replicate, treatment or any interaction (Table 5.171).

Table 5.171. Analysis of the arthropod community structure (by Order) collected via sweep nets in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	3.9410	0.015*
Treatment	2	1.2097	0.301
Day x Treatment	2	0.8896	0.462

Since there was significant difference in Day, further analyses were performed. For day of decomposition, most of the day to day comparisons were significantly different, except Day 0 x Day 14, Day 3 x Day 7, Day 3 x Day 10, Day 7 x Day 10, Day 10 x Day 21, Day 14 x Day 21, Day 14 x Day 40, Day 21 x Day 40 where there were no significant differences ($p > 0.05$) (Table 5.172). The NMDS plot of stress for arthropod community structure (by Order) was demonstrated in Figure 5.189 and the NMDS ordination for Day was also provided for data visualization (Figure 5.190). Minimum stress for given dimensionality was 0.0695 with $r^2 = 0.9783$. The MRPP analysis for day showed A value = 0.314; Significant of Delta = 0.001 based on 999 permutations).

Table 5.172. Pairwise comparisons of arthropod community structure (by Order) collected via sweep nets between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.001*	0.001*	0.001*	0.084	0.044*	0.003*
3	0.001*	-	0.156	0.809	0.012*	0.004*	0.001*
7	0.001*	0.156	-	0.202	0.004*	0.001*	0.001*
10	0.001*	0.809	0.202	-	0.018*	0.002*	0.001*
14	0.084	0.012*	0.004*	0.018*	-	0.859	0.270
21	0.044*	0.004*	0.001*	0.002*	0.859	-	0.153
40	0.003*	0.001*	0.001*	0.001*	0.270	0.153	-

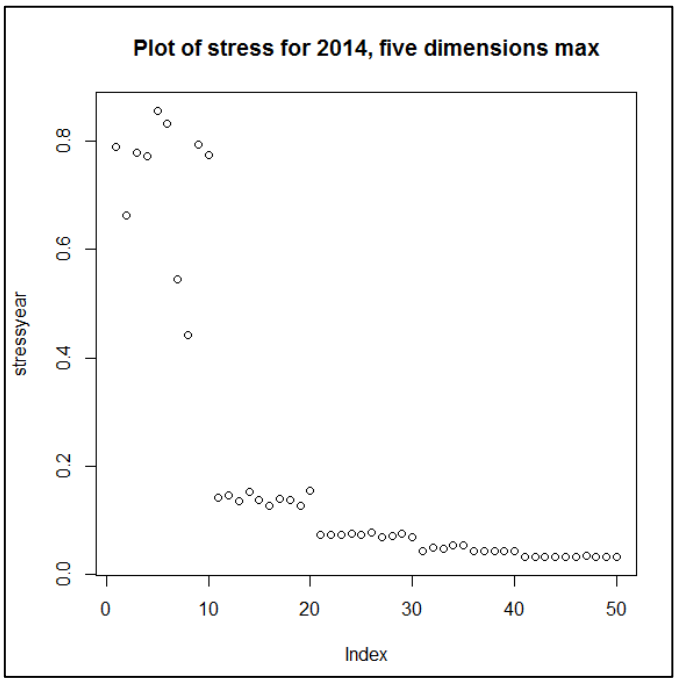

Figure 5.189. NMDS plot of stress for arthropod community structure (by Order) collected via sweep nets in summer 2014 at Snook, Texas (Stress test 0.0695; r^2 = 0.9783).

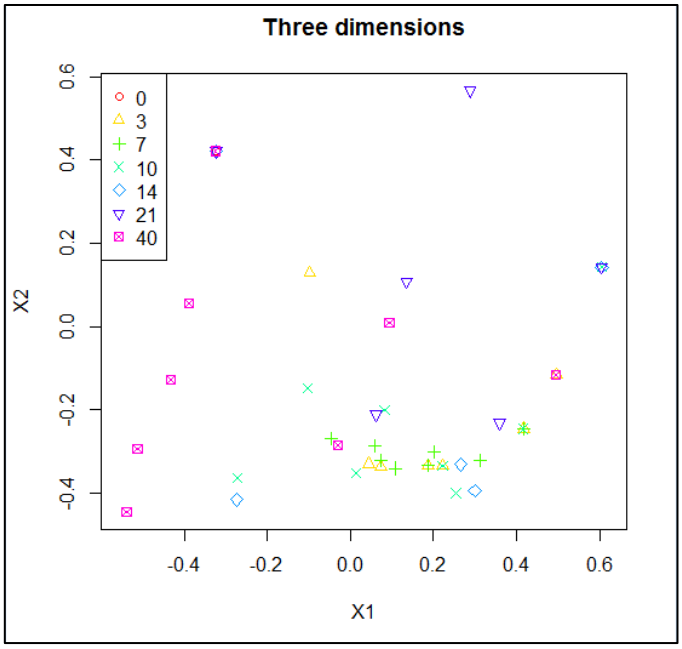

Figure 5.190. NMDS ordinations for arthropod community structure (by Order) by carrion decomposition days collected via sweep nets in summer 2014 at Snook, Texas.

For ISA, results showed that the Diptera and Orthoptera were the indicators among arthropod Orders collected via sweep nets in summer 2014 at Snook, Texas (Table 5.173).

Table 5.173. Indicator species analysis by Order for arthropods caught by sweep nets in summer 2014 at Snook, Texas.

Type	Order	Indicator value	P value
Sweep nets	Diptera	0.2785	0.034*
	Orthoptera	0.6667	0.049*

Abundance

The full model showed marginal significant difference in Day ($p = 0.0559$), Treatment ($p = 0.0594$). There was no significant difference ($p > 0.05$) in average abundance between treatments in all sampling days. Resilience was tested and results showed resilience was observed on Day 10 for Post-7 carcasses while Control and Post-14 carcasses were resistant throughout the decomposition days (Table 5.174). Average abundance of Diptera and Hemiptera collected via sweep nets in 2014 trial were demonstrated in Figure 5.191. There was significant difference in Diptera abundance on Day 14 between Control x Post-14 ($p = 0.0066$) and Post-7 x Post-14 ($p = 0.0082$) (Figure 5.192).

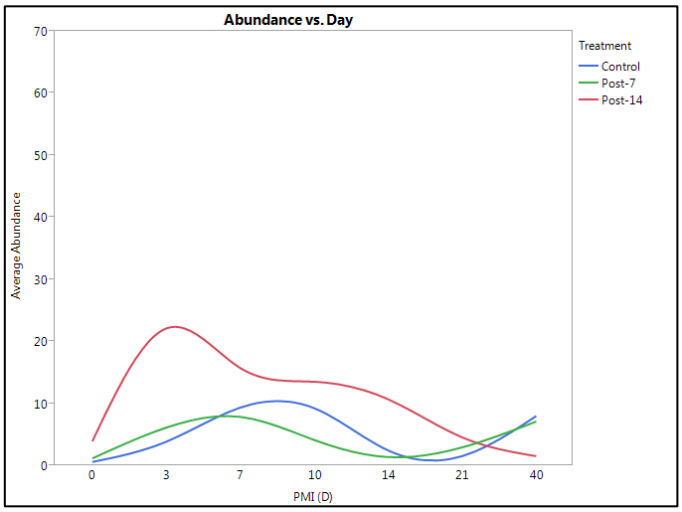

Figure 5.191. Arthropod community abundance (by Order) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas.

Table 5.174. Resilience for arthropod community (by Order) abundance collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.3139	Resistance
Post-7	0 x 7	0.0291*	10
Post-14	None	0.1708	Resistance

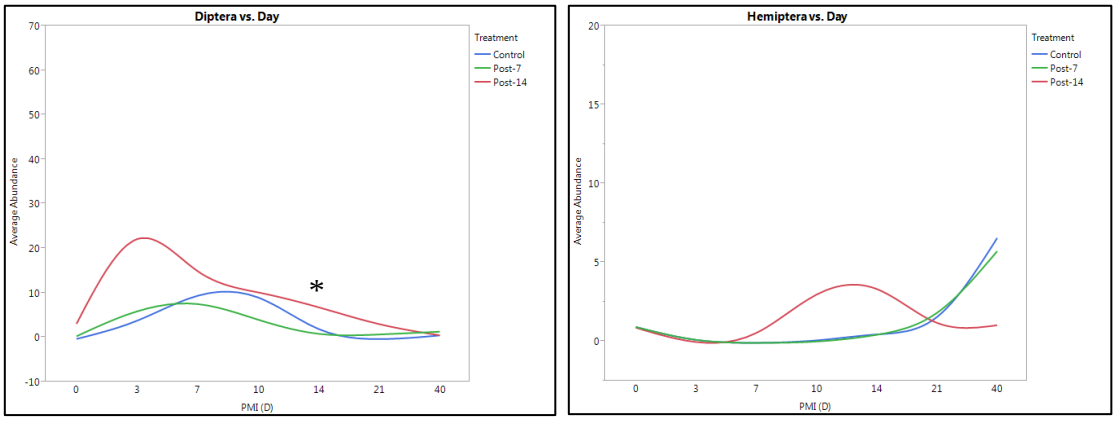

Figure 5.192. Average abundance of Diptera (Left) and Hemiptera (Right) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Richness

The full model showed no significant difference ($p > 0.05$) in Day, Treatment, or any interaction. There was significant difference in richness between Post-7 x Post-14 ($p = 0.0242$) on Day 10 (Figure 5.193). Resilience was tested for all treatments and results showed resistance in richness throughout the days for Control and both treatments (Table 5.175).

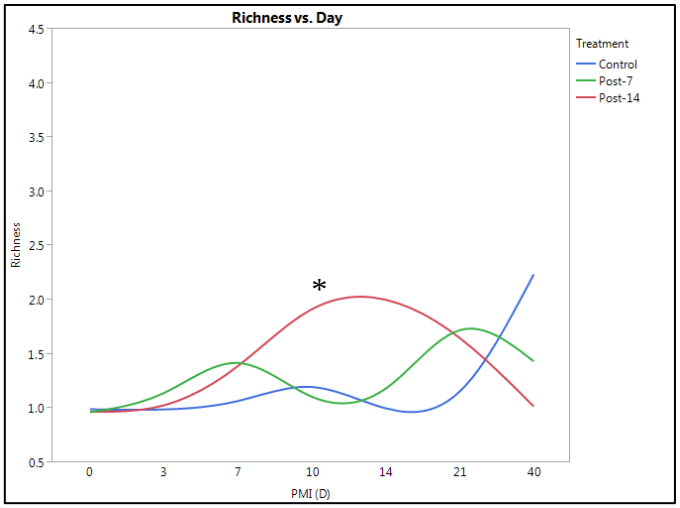

Figure 5.193. Arthropod community richness (by Order) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.175. Resilience for arthropod community (by Order) richness collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1408	Resistance
Post-7	None	0.4978	Resistance
Post-14	None	0.0612•	Resistance

• Marginal significant difference.

Simpson's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) was found in Simpson's Diversity between treatments in all sampling days (Figure 5.194). In other words, the system was resistance that no divergence or convergence was observed. Resilience was tested for all treatments and the results demonstrated that all carcasses were resistant throughout all sampling days (Table 5.176).

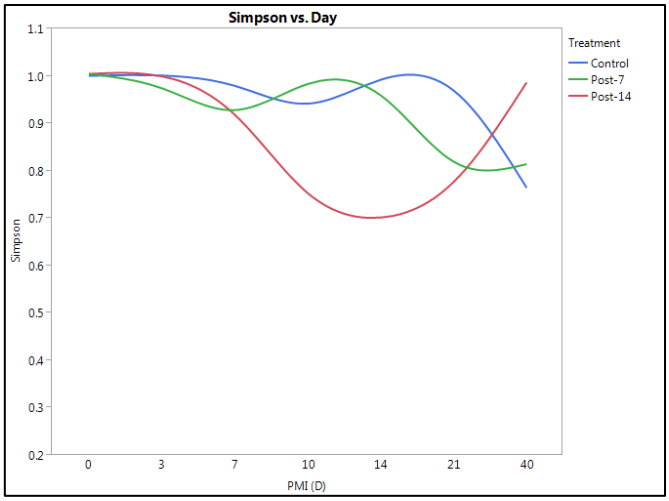

Figure 5.194. Simpson's diversity of the arthropod community (by Order) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas.

Table 5.176. Resilience for Simpson's Diversity of the arthropod community (by Order) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1053	Resistance
Post-7	None	0.5982	Resistance
Post-14	None	0.0743	Resistance

Shannon-Wiener's diversity index

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) was found in Shannon-Wiener's Diversity between treatments in all sampling days (Figure 5.195). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days (Table 5.177).

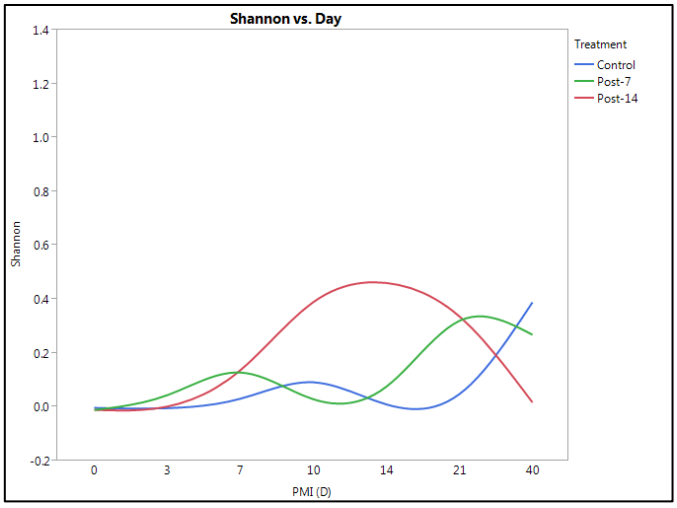

Figure 5.195. Shannon-Wiener's diversity of the arthropod community (by Order) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas.

Table 5.177. Resilience for Shannon-Wiener's Diversity of the arthropod community (by Order) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0647•	Resistance
Post-7	None	0.5786	Resistance
Post-14	None	0.0582•	Resistance

• Marginal significant difference.

Evenness

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) found in evenness between treatments in all sampling days (Figure 5.196). Resilience was tested for all treatments and all of them were resistant throughout all sampling days (Table 5.178).

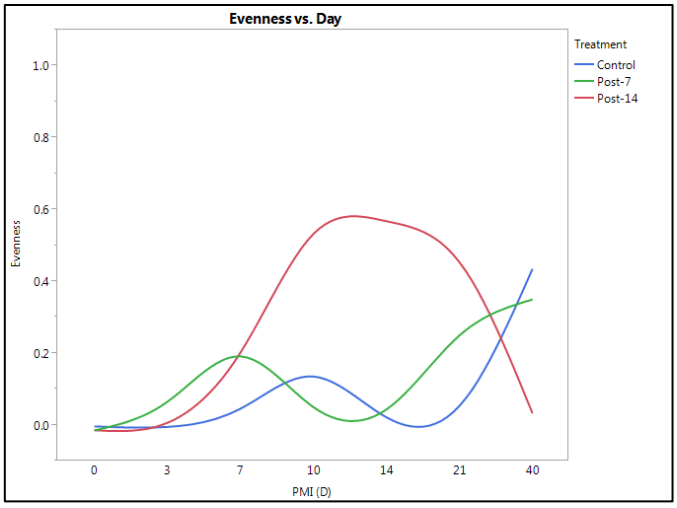

Figure 5.196. Evenness of the arthropod community (by Order) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas.

Table 5.178. Resilience for evenness of the arthropod community (by Order) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1751	Resistance
Post-7	None	0.5470	Resistance
Post-14	None	0.0401*	Resistance

Effective number of species

The full model showed no significant difference ($p > 0.05$) in Day, Treatment or any interaction. There was no significant difference ($p > 0.05$) found in ENS between treatments in all sampling days (Figure 5.197). Resilience was tested for all treatments and all of them were resistant throughout all sampling days (Table 5.179).

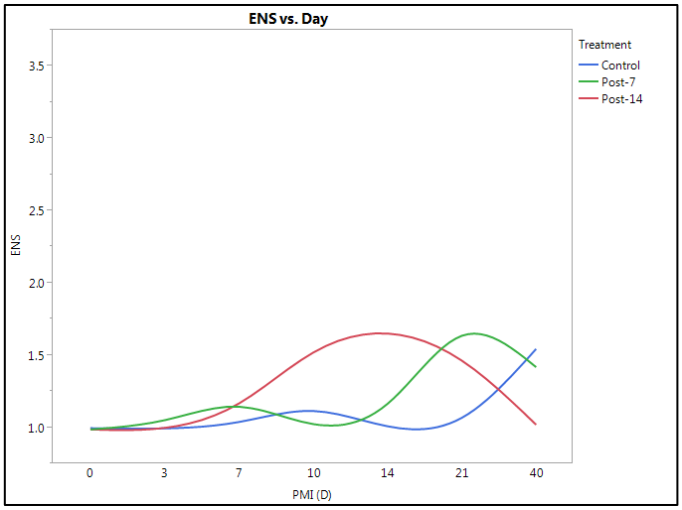

Figure 5.197. Effective Number of Species of the arthropod community (by Order) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas.

Table 5.179. Resilience for ENS of the arthropod community (by Order) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0583•	Resistance
Post-7	None	0.5670	Resistance
Post-14	None	0.0856	Resistance

• Marginal significant difference.

Family in 2014

PERMANOVA was performed on arthropod structural data by Family level. Results showed that there was significant difference in Day ($p = 0.014$), but no significant difference in Treatment, Replicate or any interaction ($p < 0.05$) (Table 5.180).

Table 5.180. Analysis of the arthropod community structure (by Family) collected via sweep nets in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	3.5561	0.014*
Treatment	2	1.3536	0.206
Day x Treatment	2	0.6835	0.731

Since there was significant difference in Day, further analyses were performed. For day of decomposition, most of the day to day comparisons were significantly different, except Day 0 x Day 14, Day 3 x Day 10, Day 14 x Day 21, and Day 14 x Day 40 where there were no significant differences ($p > 0.05$) (Table 5.181). The NMDS plot of stress for arthropod community structure (by Family) (Figure 5.198) and the NMDS ordination for Day was provided for data visualization (Figure 5.199). Minimum stress for given dimensionality was 0.1395 with $r^2 = 0.9000$. The MRPP analysis for day showed A value = 0.2764; Significant of Delta = 0.001 based on 999 permutations).

Table 5.181. Pairwise comparisons of arthropod community structure (by Family) collected via sweep nets between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.001*	0.001*	0.001*	0.084	0.090*	0.003*
3	0.001*	-	0.001*	0.820	0.006*	0.004*	0.001*
7	0.001*	0.001*	-	0.002*	0.001*	0.001*	0.001*
10	0.001*	0.820	0.002*	-	0.020*	0.002*	0.001*
14	0.084	0.006*	0.001*	0.020*	-	0.865	0.078
21	0.090*	0.004*	0.001*	0.002*	0.865	-	0.041*
40	0.003*	0.001*	0.001*	0.001*	0.078	0.041*	-

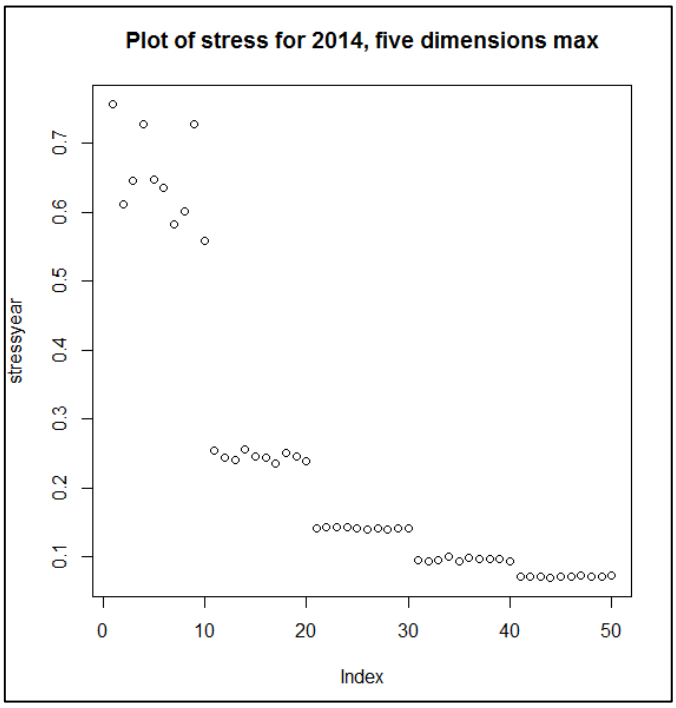

Figure 5.198. NMDS plot of stress for arthropod community structure (by Family) collected via sweep nets in summer 2014 at Snook, Texas (Stress test 0.1395; r^2 = 0.9000).

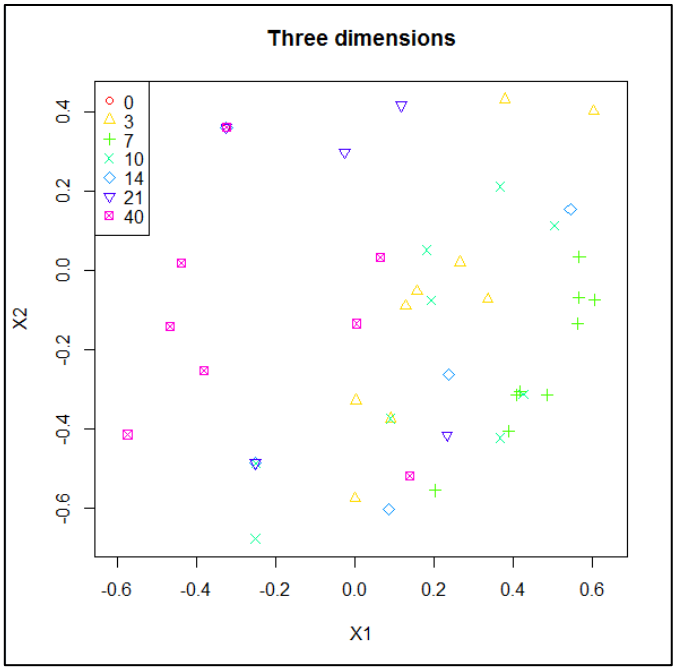

Figure 5.199. NMDS ordinations for arthropod community structure (by Family) by carrion decomposition days collected via sweep nets in summer 2014 at Snook, Texas.

For ISA, results demonstrated that the Family Calliphoridae and Chloropidae were the indicators among arthropods collected via sweep nets in summer 2014 at Snook, Texas (Table 5.182).

Table 5.182. Indicator species analysis by Family for arthropods caught by sweep nets in summer 2014 at Snook, Texas.

Type	Family	Indicator value	P value
Sweep nets	Calliphoridae	0.4713	0.017*
	Chloropidae	0.3494	0.011*

Abundance

The full model showed there was significant difference in Day ($p = 0.0496$), but no significant difference in Treatment or any interaction ($p > 0.05$). There was no significant difference ($p > 0.05$) in arthropod abundance between treatments in all sampling days (Figure 5.200). Resilience was tested and results showed Post-7 carcasses had resilience on Day 10 and then loss the resistance again on Day 40 while Control and Post-14 carcasses were resistant throughout all sampling days (Table 5.183). Average abundance of arthropods according to Family collected at sweep nets in 2014 trial were demonstrated in Figure 5.201. There was no significant difference ($p > 0.05$) in abundance of Calliphoridae or Chloropidae between treatments in all sampling days.

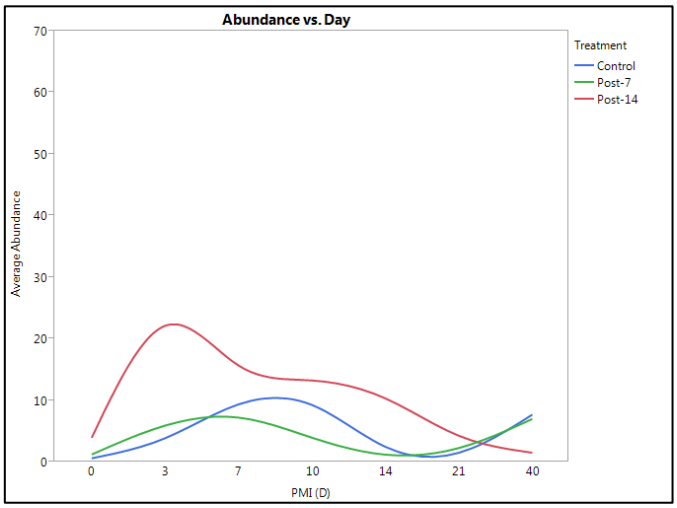

Figure 5.200. Arthropod community abundance (by Family) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas.

Table 5.183. Resilience for arthropod community (by Family) abundance collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.3049	Resistance
Post-7	0 x 7	0.0146*	10
	0 x 40	0.0296*	Loss of resistance on Day 40
Post-14	None	0.1725	Resistance

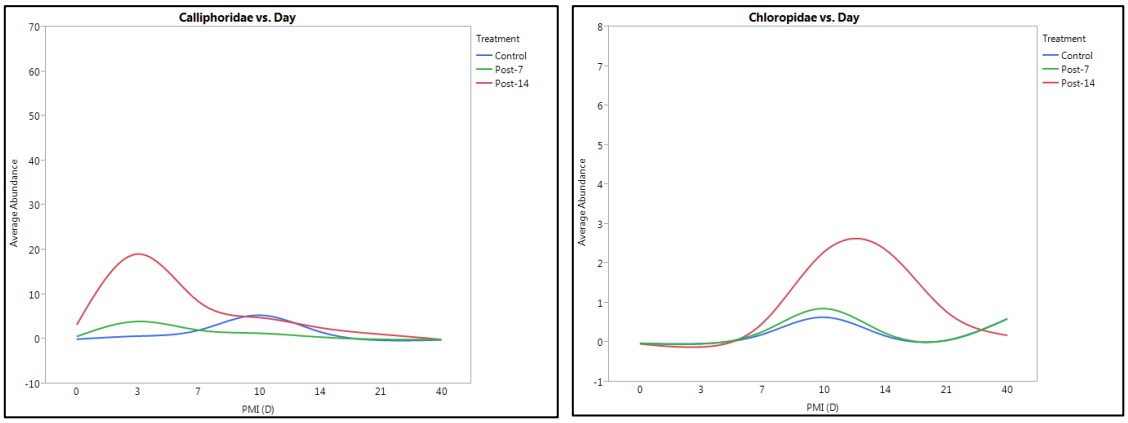

Figure 5.201. Average abundance of arthropods according to Families collected via sweep nets in summer 2014 at Snook, Texas. Left. Abundance of Calliphoridae across Treatments over time. Right. Abundance of Chloropidae across Treatments over time.

Richness

The full model showed a significant difference in Day ($p = 0.0011$), and Treatment ($p = 0.0015$) and a significant interaction Day x Treatment ($p = 0.0046$). There was significant difference ($p > 0.05$) in richness between Control x Post-7 ($p = 0.0297$) and Control x Post-14 ($p = 0.0297$) on Day 7. Also, significant difference was detected on Day 14 between Control x Post-14 ($p = 0.0242$) and Post-7 x Post-14 ($p = 0.0242$) (Figure 5.202). Resilience was tested and results showed Post-7 carcasses had resilience on Day 10 while Post-14 carcasses had resilience on Day 10 and again on Day

21. In contrast, Control carcasses were resistance throughout all sampling days (Table 5.184).

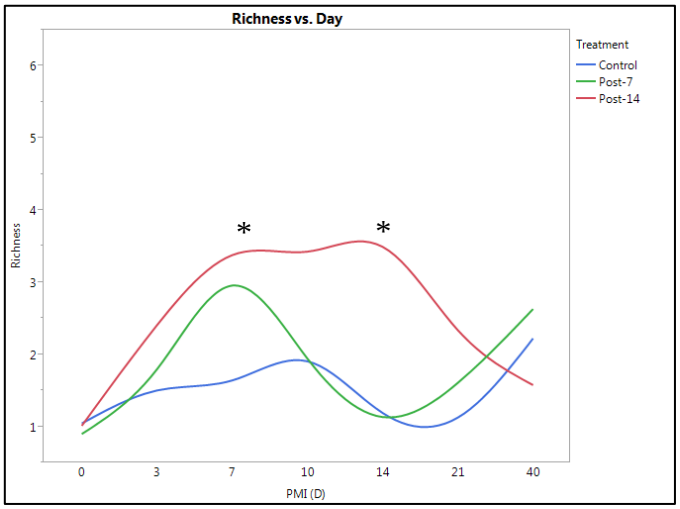

Figure 5.202. Arthropod community richness (by Family) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.184. Resilience for arthropod community (by Family) richness collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.1776	Resistance
Post-7	0 x 7	0.0174*	10
Post-14	0 x 7	0.0409*	10
	0 x 14	0.0184*	21

Simpson's diversity index

The full model showed a significant difference in Day (p = 0.0008), Treatment (p = 0.0050), but without any significant interaction. There was significant difference (p >

0.05) in Simpson's diversity between Control x Post-7 (p = 0.0244) and Control x Post-14 (p = 0.0268) on Day 7. Also, significant difference was detected on Day 14 between Control x Post-14 (p < 0.0001) and Post-7 x Post-14 (p < 0.0001) (Figure 5.203). Resilience was tested and results showed Post-7 carcasses had resilience on Day 10 while Post-14 carcasses had resilience on Day 10 and again on Day 21. In contrast, Control carcasses were resistance throughout all sampling days (Table 5.185).

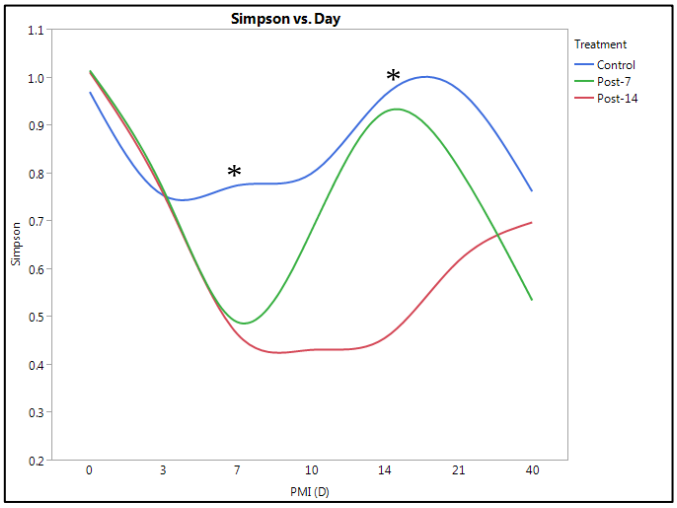

Figure 5.203. Simpson's diversity of the arthropod community (by Family) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.185. Resilience for Simpson's Diversity of the arthropod community (by Family) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2780	Resistance
Post-7	0 x 7	0.0395*	10
Post-14	0 x 7	0.0269*	10
	0 x 14	0.0320*	21

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p = 0.0004$), Treatment ($p = 0.0024$) and an interaction Day x Treatment ($p = 0.0156$). There was significant difference ($p > 0.05$) in Shannon-Wiener's diversity between Control x Post-7 ($p = 0.0163$) and Control x Post-14 ($p = 0.0166$) on Day 7. Also, significant difference was detected on Day 14 between Control x Post-14 ($p = 0.0003$) and Post-7 x Post-14 ($p = 0.0003$) (Figure 5.204). Resilience was tested and results showed Post-7 carcasses had resilience on Day 10 while Post-14 carcasses had resilience on Day 10 and again on Day 21. In contrast, Control carcasses were resistance throughout all sampling days (Table 5.186).

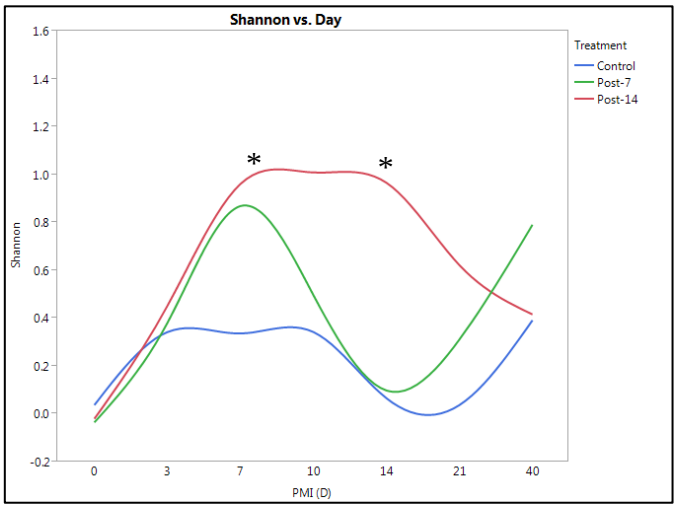

Figure 5.204. Shannon-Wiener's diversity of the arthropod community (by Family) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.186. Resilience for Shannon-Wiener's Diversity of the arthropod community (by Family) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2266	Resistance
Post-7	0 x 7	0.0284*	10
Post-14	0 x 7	0.0171*	10
	0 x 14	0.0194*	21

Evenness

The full model showed a significant difference in Day ($p = 0.0016$) and Treatment ($p = 0.0205$). There was significant difference in evenness on Day 14 between Control x Post-14 ($p < 0.0001$) and Post-7 x Post-14 ($p < 0.0001$) (Figure 5.205). Resilience was tested for all treatments and all of them demonstrated resistance throughout the sampling days, although marginal significant difference was detected on Post-14 carcasses (Table 5.187).

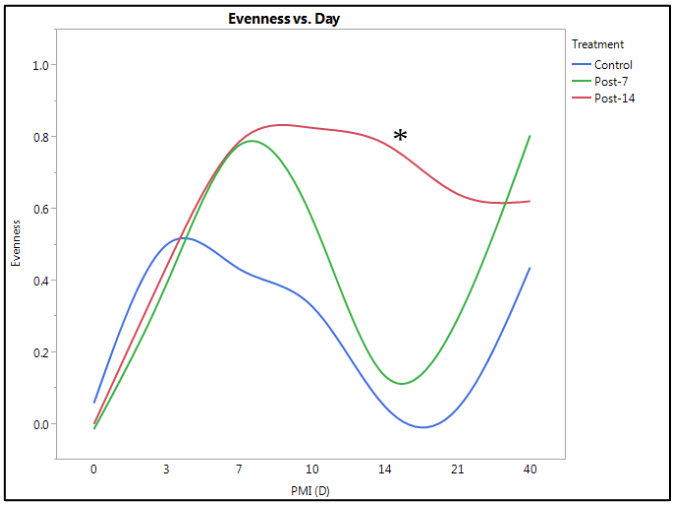

Figure 5.205. Evenness of the arthropod community (by Family) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.187. Resilience for evenness of the arthropod community (by Family) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2766	Resistance
Post-7	None	0.0378*	Resistance
Post-14	None	0.0582•	Resistance

• Marginal significant difference.

Effective number of species

The full model showed a significant difference in Day ($p = 0.0009$), Treatment ($p = 0.0021$) and an interaction Day x Treatment ($p = 0.0127$). There was significant difference ($p > 0.05$) in ENS between Control x Post-7 ($p = 0.0282$) and Control x Post-14 ($p = 0.0294$) on Day 7. Also, significant difference was detected on Day 14 between Control x Post-14 ($p = 0.0041$) and Post-7 x Post-14 ($p = 0.0041$) (Figure 5.206). Resilience was tested and results showed both Post-7 and Post-14 carcasses had resilience on Day 10 and again on Day 21. In contrast, Control carcasses were resistance throughout all sampling days (Table 5.188).

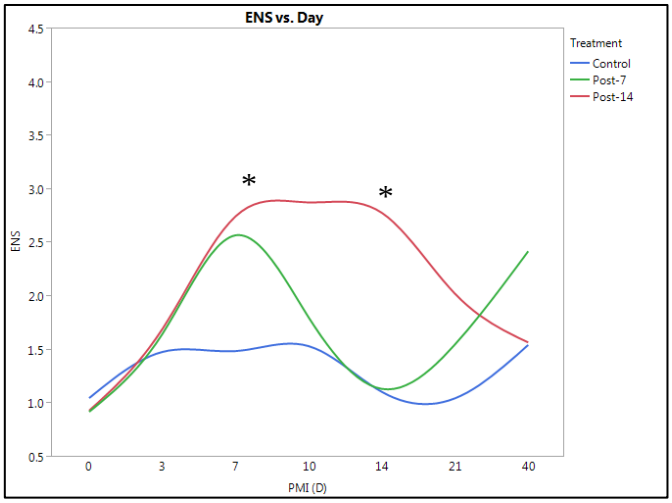

Figure 5.206. Effective Number of Species of the arthropod community (by Family) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.188. Resilience for ENS of the arthropod community (by Family) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.2679	Resistance
Post-7	0 x 7	0.0490*	10
	0 x 14	0.0490*	21
Post-14	0 x 7	0.0318*	10
	0 x 14	0.0354*	21

Genus and species in 2014

PERMANOVA was performed on arthropod structural data by Genus and species level. Results showed that there was significant difference in Day ($p = 0.008$) and Treatment ($p = 0.013$). There was no significant difference in Replicate or any interaction ($p < 0.05$) (Table 5.189).

Table 5.189. Analysis of the arthropod community structure (by Genus and species) collected via sweep nets in summer 2014 at Snook, Texas using Permutational multivariate analysis of variance (PERMANOVA).

Factor	df	F Model	P value
Day	1	4.4486	0.008*
Treatment	2	3.2396	0.013*
Day x Treatment	2	0.7879	0.559

Since there was significant difference in Day and Treatment, further analyses were performed. For days of decomposition, half of the day to day comparisons were significantly different, except Day 0 x Day 14, Day 0 x Day 21, Day 0 x Day 40, Day 3 x Day 7, Day 3 x Day 10, Day 7 x Day 14, Day 7 x Day 21, Day 14 x Day 21, Day 14 x Day 40 and Day 21 x Day 40 where there were no significant differences ($p > 0.05$) (Table 5.190). Comparison between treatments demonstrated that Control x Post-14 was significantly different ($p = 0.002$) (Table 5.191) The NMDS plot of stress for arthropod community structure (Figure 5.207) and NMDS ordinations for Day and Treatment were provided for visualization of data distribution (Figure 5.208 and 5.209, respectively). Minimum stress for given dimensionality was 0.0988 with $r^2 = 0.9650$. The MRPP for day showed A value 0.2674 and Significant of Delta 0.001 while the MRPP for Treatments showed A value 0.0513 and Significant of Delta 0.017 based on 999 permutations.

Table 5.190. Pairwise comparisons of arthropod community structure (by Genus and species) collected via sweep nets between carrion decomposition days in summer 2014 at Snook, Texas after Bonferroni's correction.

Day x Day	0	3	7	10	14	21	40
0	-	0.001*	0.001*	0.001*	0.084	0.207	1.000
3	0.001*	-	0.092	0.446	0.011*	0.006*	0.001*
7	0.001*	0.092	-	0.010*	0.227	0.079	0.003*
10	0.001*	0.446	0.010*	-	0.013*	0.002*	0.001*
14	0.084	0.011*	0.227	0.013*	-	0.823	0.098
21	0.207	0.006*	0.079	0.002*	0.823	-	0.327
40	1.000	0.001*	0.003*	0.001*	0.098	0.327	-

Table 5.191. Pairwise comparisons of arthropod community structure (by Genus and species) collected via sweep nets between treatments in summer 2014at Snook, Texas after Bonferroni's correction.

Treatment	df	SS	MS	F model	R2	P value
Control x Post-7	1	0.4687	0.4687	1.6208	0.0389	0.191
Residual	40	11.5673	0.2891		0.9611	
Total	41	12.0360			1.0000	
Control x Post-14	1	1.7513	1.7512	5.5846	0.1225	0.002*
Residual	40	12.5435	0.3135		0.8775	
Total	41	14.2947			1.0000	
Post-7 x Post-14	1	0.6568	0.6567	1.9902	0.0474	0.098
Residual	40	13.1996	0.3299		0.9526	
Total	41	13.8563			1.0000	

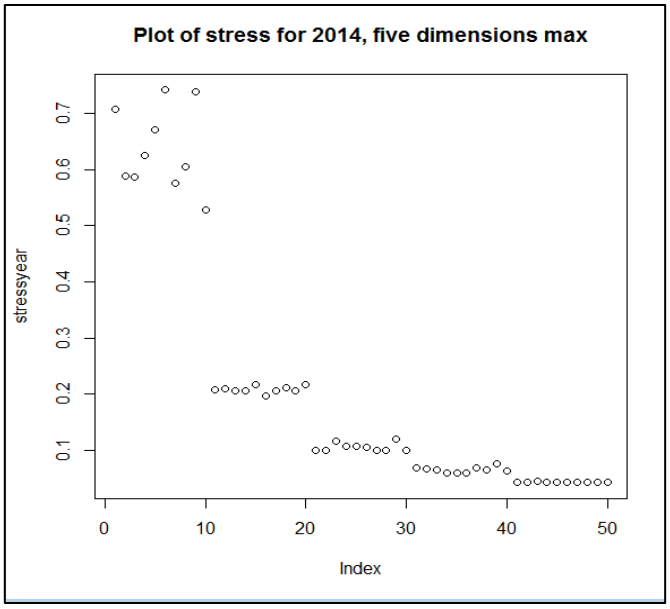

Figure 5.207. NMDS plot of stress for arthropod community structure (by Genus and species) collected via sweep nets in summer 2014 at Snook, Texas (Stress test 0.0988; r^2 = 0.9650).

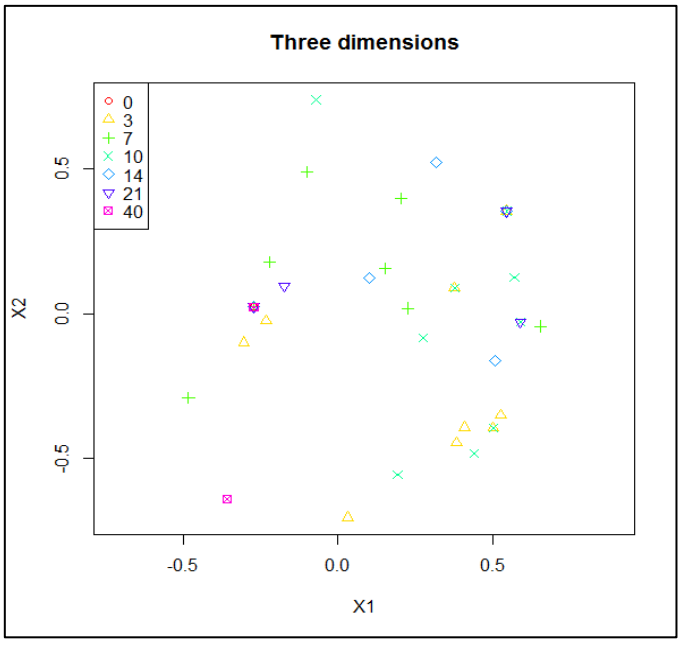

Figure 5.208. NMDS ordinations for arthropod community structure (by Genus and species) by carrion decomposition days collected via sweep nets in summer 2014 at Snook, Texas.

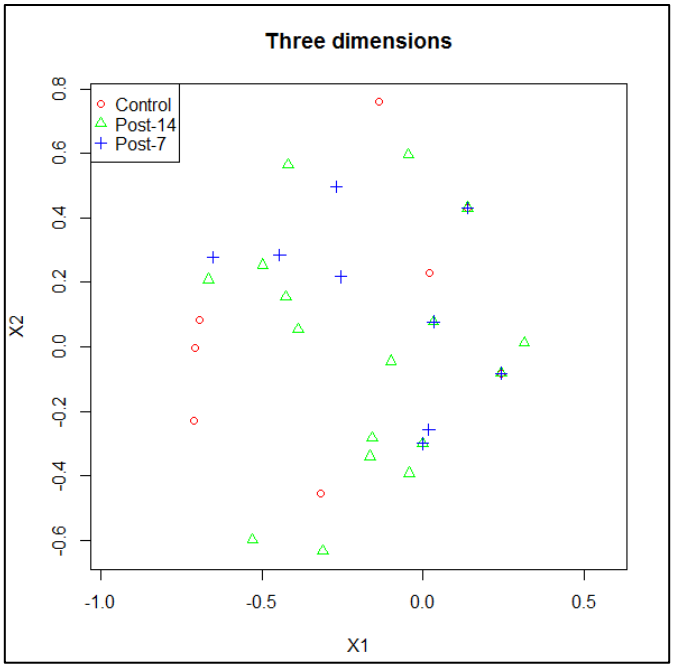

Figure 5.209. NMDS ordinations for arthropod community structure (by Genus and species) by treatments collected via sweep nets in summer 2014 at Snook, Texas.

For ISA, results demonstrated that the adult *Co. macellaria* was the only significant indicator among flying arthropods collected via sweep nets in summer 2014 at Snook, Texas (Figure 5.192).

Table 5.192. Indicator species analysis by Genus and species for arthropods caught by sweep nets in summer 2014 at Snook, Texas.

Type	Genus and species	Indicator value	P value
Sweep nets	*Co. macellaria*	0.4795	0.016*

Abundance

The full model showed a significant difference in Day (p = 0.0228), but not in Treatment (although marginal, p = 0.0576) or any interaction (p = 0.1409). There was

marginal significant difference noted on Day 7 (p = 0.0507). There was significant difference found in abundance on Day 14 between Control x Post-14 (p = 0.0085) and Post-7 x Post-14 (p = 0.0085) (Figure 5.210). Resilience was observed on Day 7 for Post-7 carcasses while Control and Post-14 carcasses were resistant in all sampling days (Table 5.193). The average abundance of several important genera were highlighted in Figure 5.211. For *O. aenescens*, marginal significant difference in abundance was noted on Day 7 (p = 0.0640). There was no significant difference (p > 0.05) detected in abundance between treatments in all sampling days for other genera.

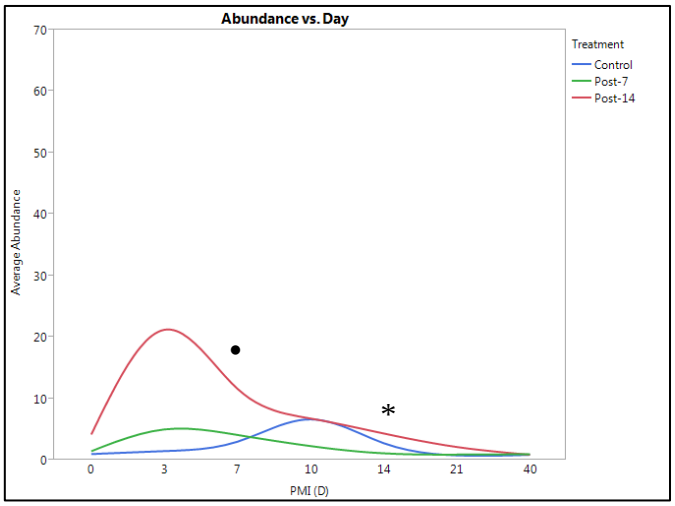

Figure 5.210. Arthropod community abundance (by Genus and species) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (• denotes marginal significant difference; * represents significant difference).

Table 5.193. Resilience for arthropod community (by Genus and species) abundance collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.4141	Resistance
Post-7	0 x 3	0.0012*	7
Post-14	None	0.1208	Resistance

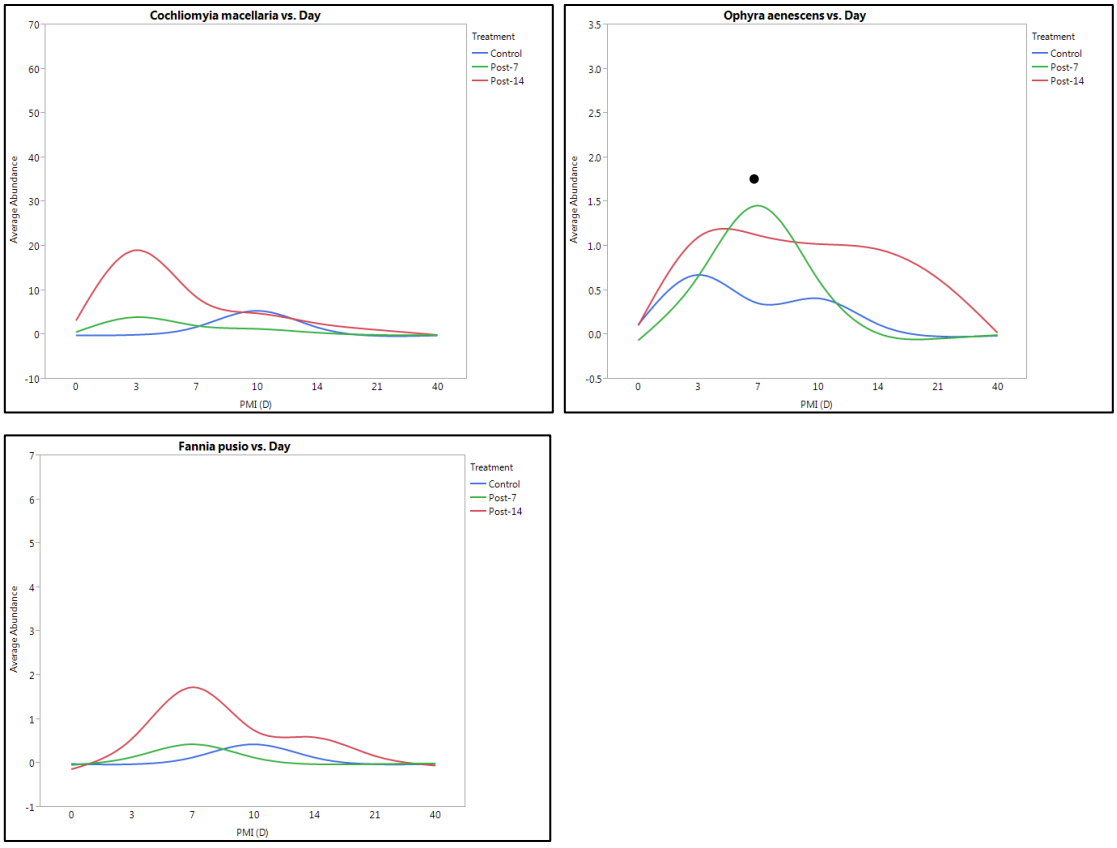

Figure 5.211. Average abundance of arthropods according to Genus and species collected via sweep nets in summer 2014 at Snook, Texas. Upper Left. Abundance of *Co. macellaria* across Treatments over time. Upper Right. Abundance of *O. aenescens* across Treatments over time. Lower Left. Abundance of *F. pusio* across Treatments over time (• denotes marginal significant difference).

Richness

The full model showed a significant difference in Day ($p < 0.0001$), Treatment ($p = 0.0071$) and interaction between Day x Treatment ($p = 0.0046$). There was significant difference found in richness on Day 7 between Control x Post-7 ($p = 0.0310$) and Control x Post-14 ($p = 0.0310$), and on Day 14 (Control x Post-14 ($p = 0.0065$) and Post-7 x Post-14 ($p = 0.0065$)) (Figure 5.212). Resilience was observed on Day 10 for Post-7 carcasses. Post-14 carcasses had two resilience occurrences which were on Day 10 and Day 21, while Control carcasses were resistant in all sampling days (Table 5.194).

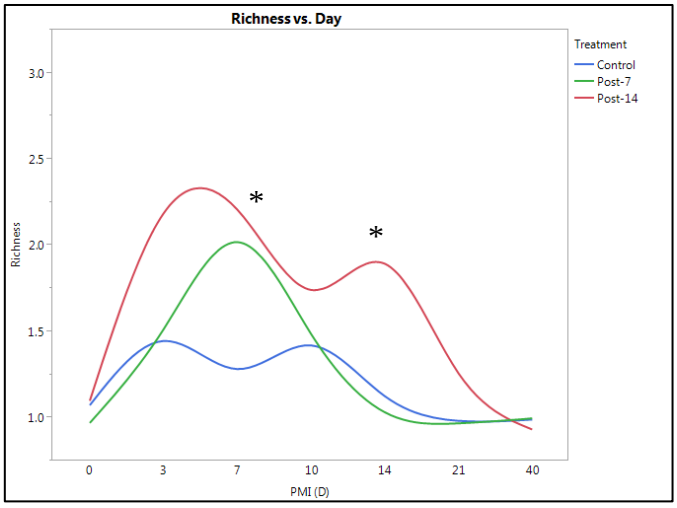

Figure 5.212. Arthropod community richness (by Genus and species) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.194. Resilience for arthropod community (by Genus and species) richness collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.3063	Resistance
Post-7	0 x 7	0.0097*	10
Post-14	0 x 3	0.0278*	10
	0 x 7	0.0278*	21
	0 x 14	0.0278*	

Simpson's diversity index

The full model showed a significant difference in Day ($p < 0.0001$), and Treatment ($p = 0.0109$), and a significant interaction Day x Treatment ($p = 0.0003$). There was significant difference found in Simpson's diversity on Day 7 between Control x Post-7 ($p = 0.0024$) and Control x Post-14 ($p = 0.0018$), and on Day 14 (Control x Post-14 ($p < 0.0001$) and Post-7 x Post-14 ($p < 0.0001$)) (Figure 5.213). Resilience was

observed on Day 10 for Post-7 carcasses while Post-14 carcasses had two events of resilience on Day 10 and Day 21. Control carcasses were resistant in all sampling days (Table 5.195).

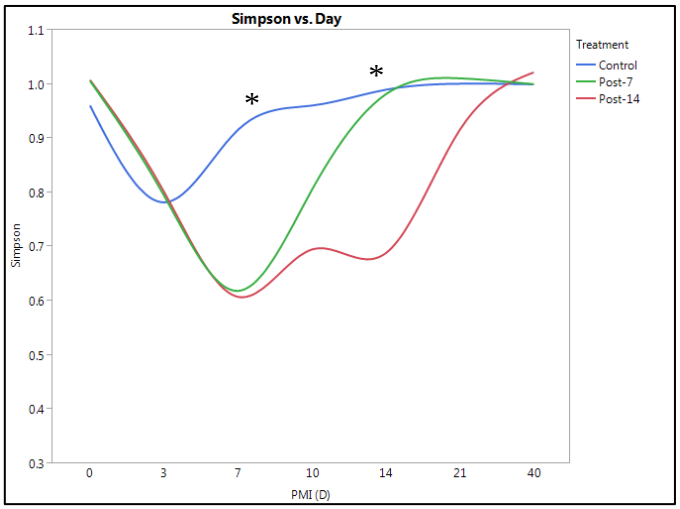

Figure 5.213. Simpson's diversity of the arthropod community (by Genus and species) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.195. Resilience for Simpson's Diversity of the arthropod community (by Genus and species) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0331*	Resistance
Post-7	0 x 7	0.0158*	10
Post-14	0 x 7	0.0021*	10
	0 x 14	0.0075*	21

Shannon-Wiener's diversity index

The full model showed a significant difference in Day ($p < 0.0001$), and Treatment ($p = 0.0076$), but a significant interaction Day x Treatment ($p = 0.0004$). There was significant difference found in Shannon-Wiener's diversity on Day 7 between Control x Post-7 ($p = 0.0099$) and Control x Post-14 ($p = 0.0082$), and on Day 14 (Control x Post-14 ($p = 0.0002$) and Post-7 x Post-14 ($p = 0.0002$)) (Figure 5.214). Resilience was observed on Day 10 for Post-7 carcasses and Day 21 for Post-14 carcasses. Control carcasses were resistant throughout all sampling days (Table 5.196).

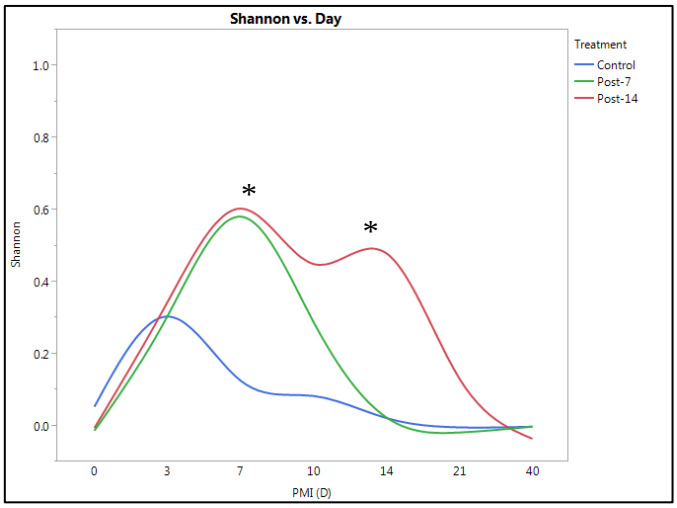

Figure 5.214. Shannon-Wiener's diversity of the arthropod community (by Genus and species) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.196. Resilience for Shannon-Wiener's Diversity of the arthropod community (by Genus and species) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0529•	Resistance
Post-7	0 x 7	0.0127*	10
Post-14	0 x 14	0.0071	21

• Marginal significant difference.

Evenness

The full model showed a significant difference in Day ($p < 0.0001$), and Treatment ($p = 0.0108$), and a significant interaction Day x Treatment ($p = 0.0008$). There was significant difference found in evenness on Day 7 between Control x Post-7 ($p < 0.0001$) and Control x Post-14 ($p < 0.0001$), and on Day 14 (Control x Post-14 ($p < 0.0001$) and Post-7 x Post-14 ($p < 0.0001$)) (Figure 5.215). Resilience was observed on Day 7 for Control carcasses, Day 10 for Post-7 carcasses and two events of resilience were observed on Post-14 carcasses on Day 10 and Day 21 (Table 5.197).

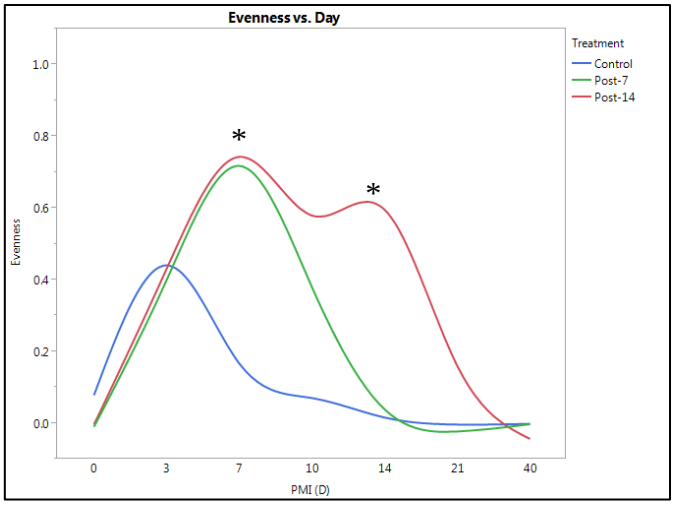

Figure 5.215. Evenness of the arthropod community (by Genus and species) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represents significant difference).

Table 5.197. Resilience for evenness of the arthropod community (by Genus and species) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	0 x 3	0.0465*	7
Post-7	0 x 7	0.0233*	10
Post-14	0 x 7	0.0026*	10
	0 x 14	0.0065*	21

Effective number of species

The full model showed a significant difference in Day ($p < 0.0001$), Treatment ($p = 0.0139$) and a significant interaction Day x Treatment ($p = 0.0010$). There was significant difference found in ENS on Day 7 between Control x Post-14 ($p < 0.0441$), and on Day 14 (Control x Post-14 ($p = 0.0015$) and Post-7 x Post-14 ($p = 0.0015$)) (Figure 5.216). Resilience was observed on Day 10 for Post-7 carcasses, while two events of resilience were observed on Post-14 carcasses on Day 10 and Day 21. Control

carcasses were resistant in all sampling days as pairwise comparisons between days did not reveal any significant pairs ($p > 0.05$) (Table 5.198).

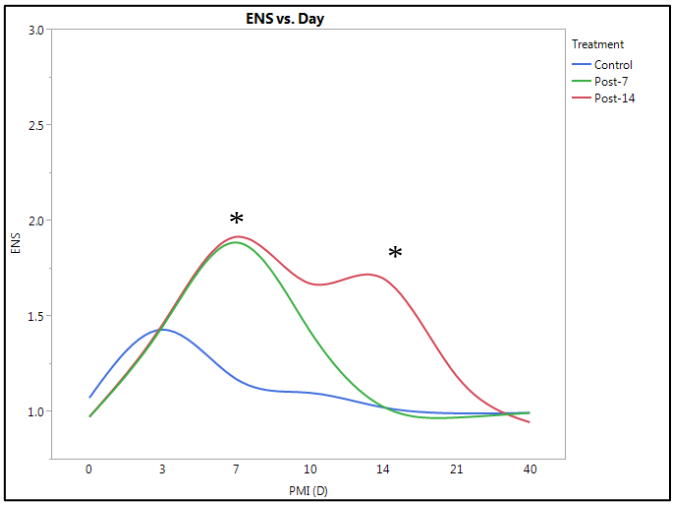

Figure 5.216. Effective Number of Species of the arthropod community (by Genus and species) collected via sweep nets across Treatments over time in summer 2014 at Snook, Texas (* represent significant difference).

Table 5.198. Resilience for ENS of the arthropod community (by Genus and species) collected via sweep nets for each treatment in summer 2014 at Snook, Texas.

Treatment	Significant difference	P value	Resilience on Day
Control	None	0.0445*	Resistance
Post-7	0 x 7	0.0167*	10
Post-14	0 x 7	0.0070*	10
	0 x 14	0.0285*	21

Functions in 2014

Functional groups analyses among arthropods collected through sweep nets in 2014 trial had been performed together with data collected in 2013 trials. It was because there was no significant different ($p > 0.05$) in Replicates between 2013 and 2014 trials. Hence, the two years data (sweep net samples only) was pooled and analyzed (see Page 771).

Comparison of aboveground arthropods between 2013 and 2014 trials

Table 5.199 was provided below to highlight the similarities and differences in the statistical results of aboveground arthropod communities between 2013 and 2014 trials.

Table 5.199. Comparison of significant results for aboveground arthropods collected in 2013 and 2014 trials at Snook, Texas.

General Statistic		
Factor	2013	2014
Temperature*	30.59±7.81°C	29.27±6.49°C
Precipitation	39.116 mm	171.45 mm
ADH (Base 10)*	29209.70	28080.67
Sticky Traps		
Analysis	2013	2014
Year effect	Yes	
Replicate effect	No	
Arthropod Order	14 Orders	14 Orders
Arthropod Family	117 Families	103 Families
Arthropod Genus	48 Genera	112 Genera
Arthropod Function	8 functional groups	8 functional groups

Table 5.199 (Continued).

Analysis	2013	2014
Significant results in community structure analyses:		
Order	Day	Day
	Height	Treatment
	Position	Height
	Day x Height	Position
	Day x Position	Day x Height
	Height x Position	Day x Position
		Treatment x Position
		Height x Position
		Day x Height x Position
		Treatment x Height x Position
Family	Day	Day
	Treatment	Treatment
	Height	Height
	Position	Position
	Day x Height	Day x Treatment
	Day x Position	Day x Height
	Height x Position	Day x Position
		Height x Position
		Day x Height x Position

Table 5.199 (Continued).

Analysis	2013	2014
Significant results in community structure analyses:		
Genus	Day	Day
	Treatment	Treatment
	Height	Height
	Position	Position
	Day x Treatment	Day x Height
		Day x Position
		Height x Position
Function	Day	Day
	Treatment	Treatment
	Height	Height
	Position	Position
	Day x Height	Day x Treatment
	Day x Position	Day x Height
	Height x Position	Day x Position
		Height x Position
Indicator species analysis (Order)	7 Orders	6 Orders
Indicator species analysis (Family)	23 Families	22 Families
Indicator species analysis (Genus)	7 species	14 species
Indicator species analysis (Function)	Herbivores	Herbivores
		Predators/Parasites
		Necrophagous

Table 5.199 (Continued).

Pitfall Traps		
Analysis	2013	2014
Year effect		Yes
Replicate effect		No
Crawling arthropod Order / Suborder	11 Orders	13 Orders
Crawling arthropod Family	23 Families	33 Families
Crawling arthropod Genus	6 Genera	22 Genera
Crawling arthropod Function	5 Functional groups	6 Functional groups
Significant results in community structure analyses:		
Order	Nil	Day
Family	Day	Day
Genus and species	Day Treatment	Day
Function	Day Replicate	Day
Indicator species analysis (Order)	Nil	Diptera (larvae) Coleoptera
Indicator species analysis (Family)	Nil	Calliphoridae (larvae) Histeridae
Indicator species analysis (Genus and species)	Nil	*Co. macellaria* (larvae)

Table 5.199 (Continued).

Analysis	2013	2014
Indicator species analysis (Function)	Nil	Necrophagous
Sweep Nets		
Analysis	2013	2014
Year effect	Yes (community structure); No (functional group)	
Replicate effect	No	
Arthropod Order	6 Orders	8 Orders
Analysis	2013	2014
Arthropod Family	13 Families	20 Families
Arthropod Genus	10 Genera	7 Genera
Arthropod Function	4 Functional groups	5 Functional groups
Significant results in community structure analyses:		
Order	Day x Treatment	Day
Family	Treatment	Day
Genus and species	Treatment	Day Treatment
Function (combined 2013 and 2014 data)	Day Treatment	
Indicator species analysis (Order)	Diptera	Diptera Orthoptera

Table 5.199 (Continued).

Analysis	2013	2014
Indicator species analysis (Family)	Calliphoridae Muscidae	Calliphoridae Chloropidae
Indicator species analysis (Genus and species)	*Co. macellaria* *M. domestica* *O. aenescens*	*C. macellaria*
Indicator species analysis (Function) (Combined 2013 and 2014 data)	Necrophagous	

DISCUSSION

Successional patterns in association with vertebrate carrion decomposition have historically been considered predictable and of deterministic. This model has been applied to a number of associated communities including plant (Clements, 1916), insect (Anderson & VanLaerhoven, 1996; Sharanowski et al. 2008) and microbes (Metcalf et al. 2013; Pechal et al. 2014a; Metcalf et al. 2015; Metcalf et al. 2016). However, results in the current study demonstrate that this assumption is not always correct (see Table 5.199 or Appendix M [Figure M2 – M5]).

In fact, the present study found that insect succession during carrion decomposition is more in line with the Gleasonian model, who proposed that the organism response individually (i.e., individualistic concepts of association) to the environment and therefore succession is less deterministic and should not be stated as a fixed law (Gleason, 1917; Gleason, 1927). Moreover, Clements' successional model has been criticized from both theoretical and practical perspectives by many other ecologists such as R.H. Whittaker, F.E. Egler, J. McCormick, W.H. Drury, I.C.T. Nisbet, J.H.Connell, and R.O. Slatyer (van der Valk, 1981).

Abiotic factors such as weather can influence distribution and abundance of arthropod community structure (Polis, 1994; Campobasso & Introna, 2001). Temperature has been known to affect the time of development and fecundity, as well as the appearance or dynamics of insect populations in the field (Ratte, 1984). And, as recorded in this study, a significant difference in ambient temperatures (ADH) between summers 2013 and 2014 (see Figure 2.18) was determined. Tomberlin & Adler (1998) documented three species of blow flies, *Cynomyopsis cadaverina* (Robineau-Desvoidy), *C. vicina* and *L. illustris* colonized rat (*Rattus rattus* L.) carrion on land during winter in a plowed field in northwestern South Carolina while two different species of blow flies, *Co. macellaria* and *L. sericata*, and a sarcophagid, *S. bullata*, colonized the carrion during summer, thus highlighting seasonal variation in decomposition and colonization patterns of carrion. Precipitation received in summer 2014 trial was in a larger amount compared to the previous trial (see Figure 2.19). This additional ambient humidity could affect aboveground arthropods community structure and population dynamics (Wallner, 1987). For instance, precipitation has been found as the key climatic factor for growth of Norway spruce, *Picea abies* Karst., in Denmark and drought period are often followed by spruce needle miner, *Epinotia tedella* (Clerck) (Lepidoptera: Tortricidae) outbreaks (Münster-Swendsen, 1987). In another example, low winter precipitation (e.g., 18-28 cm from October to May compared to 45 to 65 cm) caused reduced growth of the arroyo willow, *Salix lasiolepis* Benth., with number of shoots per stem initiated and shoot length reduced. This subsequently reduced the resource for the stem-galling sawfly, *Euura lasiolepis* Smith (Hymenoptera: Tenthredinidae), which declined in numbers after the relatively dry winter of 1980-81 (Price & Clancy, 1986).

Arthropod functional groups varied according to sampling methods. Results generated from ISA demonstrated that sticky traps significantly collected more herbivores, predators, parasites and necrophagous guilds, while both pitfall traps and sweep netting significantly collected more necrophagous arthropods (see Table 5.199). These results strengthened that specific collecting technique may target on specific functional groups of arthropods (Missa et al. 2009). However, for the purpose of

studying larger scale of arthropod community assemblage during carrion decomposition, a set of different traps should be employed to provide better picture of arthropod community dynamics over a longer study period.

Interpretation of aboveground arthropod community responses to delayed colonization of swine carrion was dependent on taxonomic resolution implemented (see Table 5.199). The requirement for the optimum taxonomical resolution to detect significant difference in treatment depends on sampling technique used. Based on the results obtained, Order, Family and Genus level of the aboveground arthropod communities collected via sticky traps were able sensitive to detect Treatment effects. However, assessment at the Genus level renders significant results in Treatment for pitfall traps and sweep nets. Thus, when using these techniques (i.e., pitfall traps and sweep nets) in the field, higher taxonomic resolution is required. Marshall et al. (2006) found that very little information (< 6%) was lost by identifying taxa to family (or genus), as oppose to species, and based on a cost/benefit analysis, family level abundance data is recommended as the best resolution for resolving patterns in community assemblages in freshwater environment. Similarly, Olsgard et al. (1997) examined the relationship between taxonomic resolution in analyses of a microbenthic community along an established pollution gradient and they found that higher taxonomic levels are more likely to reflect a contamination gradient than are analyses based on species abundances. Another study conducted in Mid-Atlantic Highlands in U.S. on the effects of macroinvertebrate taxonomic resolution in large landscape bioassessment showed that the identification to the family level is sufficient for many bioassessment purposes (Waite et al. 2004). Nevertheless, the ultimate decision on the taxonomic resolution depends on the objective of the study and the availability of resources (Waite et al. 2004), as well as sampling technique used.

Interpretation of aboveground arthropod community depended on the height and position of the sticky traps (see Table 5.199, Figure 5.7 and Figure 5.113). In this study, results showed consistent arthropod community structure changed according to height (either 0.3 m (bottom) or 1.2 m (above)) and position (either anterior or posterior of the

carrion) of the traps; with the highest caught was the “anterior bottom” traps. These microscales do significantly affect the arthropod community structure, thus highlighting the importance of micro-spatial scale in ecological studies (Levin, 1992). Pechal et al. (2014b) employed sticky traps to quantify community composition, turnover and assembly of adult flying insects attracted to carrion. A single trap was attached to the anti-scavenging cage at the anterior and posterior region of the carcass, each approximately 0.15 m from the carrion. At this scale, Pechal et al. (2014b) found significant shift in necrophagous insect community structure, turnover rates and assembly with overall effects on carrion decomposition. Similarly, Rothschild & Osborn (1988) showed that turbulence in a small spatial scale can have effect on predator-prey contact rates (i.e., zooplankton and phytoplankton), and thus on broader scale dynamics. On terrestrial environment, Antvogel & Bonn (2001) demonstrated that composition of ground beetle assemblages was strongly influenced by microclimatic parameters and vegetation structure within a few meters. Samu et al. (1999) reviewed the micro-scale habitats used by spiders and highlighted several important factors that determined spider abundance and diversity namely microclimate, habitat structure, disturbance, prey availability, predation and territoriality.

Other than year effect and sampling methods, interpretation of results was also dependent on the ecological indices employed. As previously mentioned, richness, Simpson’s diversity index, Shannon-Wiener’s diversity index, evenness and effective number of species (ENS) were studied for each sampling technique. Generally speaking, for arthropod community structure collected by sticky traps in 2014 trial, all ecological indices at the Family level were resistant between treatments (i.e., no divergence or convergence) in all sampling days as compared to control (see Figure 5.136 – 5.140). One of the reasons why there was no significant different between control and treatments was the distant of the sticky traps from the carrion remains (i.e., approximately 1 m away from the carrion) and the duration of collection (i.e., 24 hours, hence included all diurnal and nocturnal arthropods). The distant between sticky traps and carrion was further than in Pechal et al. (2014b), and the collecting duration in Pechal et al. (2014b)

was 12 hours instead of 24 hours as in this study. It is hypothesized that this distant was too far away from the "hot spot" during carrion decomposition, thus collected more incidental taxa from the surrounding environment. This result suggests that the flying arthropod community structure was homogenous at a distant of 1 m away from carrion. In other words, this observation supported the idea that the "carrion zone" is unique and highly patchy (Barton et al. 2013). Second, the collecting duration of 24 hours rendered sufficient time to collect as many incidental taxa as possible from the adjacent environment, including those taxa active diurnally and nocturnally, hence "homogenized" the arthropod community richness, diversity and evenness. Inventories over longer periods will inevitably collect more species than those present at a particular moment (Duelli et al. 1999). Thirdly, incidental taxa were not removed during the analysis as the aim of this study was to assess arthropod community structure at a larger spatial scale during carrion decomposition. Therefore, it is expected that the introduction of incidental taxa may "dilute" the significant effects of necrophagous insects on those ecological indices. Another hypothesis why the ecological indices were resistant between control and the treatments was simply the majority of the aboveground or flying arthropod communities around or on the carrion did not respond neither to the change of the physical state of carrion nor the change in semiochemicals released by microbiome. If this hypothesis is true, then priority effect imposed by the microbiome only impacts certain arthropods but not all species that were present around the carrion.

For pitfall traps, in general, all ecological indices tested at the Genera level in 2013 trial were not significantly different between treatments in all sampling days as compared to control carcasses (see Figure 5.73 - 5.77). These results suggest that the richness, diversity and evenness of crawling arthropod communities around the carrion decomposition site were resistant following perturbation. However, these observations may result from the location of the pitfall traps (i.e., one meter away from the carrion) and the duration of the collection (i.e., 24 hours). As previously mentioned, pitfall traps located a meter away from the decomposition "hotspot" may be too far away to make any significant change to the ecological indices. Furthermore, 24 hours of trapping may

collect more incidental taxa, which eventually neutralized or homogenized the ecological indices (Duelli et al. 1999). It is important to note that the insect exclusion cages used in this study did not exclude crawling arthropods such as ants and beetles (Pechal et al. 2014b) although they were 99% efficient in excluding flying arthropods (see Appendix K). Hence, insect-exclusion cage did not affect the arrival of ground crawling arthropod communities access the carrion.

Sweep netting in 2014 trial demonstrated significant divergence and convergence of the treatments group to control in all ecological indices tested by Genus and species level (see Figure 5.212 – 5.216). In general, arthropod communities collected from treatment carcasses had significantly higher richness, diversity and evenness compared to the control carcasses on certain sampling days. This could be due to the change of carrion physical state and perhaps resource quality as delayed insect colonization on carcasses decreases biomass loss significantly than the immediate insect access carcasses (Pechal et al. 2014b; Anderson, 2011). Hence, the existing carrion resource had supported higher arthropod community richness and diversity for an extended period (Pechal et al. 2014b) while the control carcasses had been largely consumed by necrophagous insects and perhaps left only minimal biomass during that period (approaching dry-remain stage of decomposition), contributing lower arthropod richness and diversity (Payne, 1965).

Aboveground arthropod communities exhibited different degrees of resilience. Resilience is defined as the speed of recovery to the initial level after a perturbation (DeAngelis, 1980). For example, resilience was observed in the arthropod genera richness collected from Post-7 carcasses by pitfall traps in 2014 trial, where significant differences were noted between Day 0 x Day 3 and Day 0 x Day 7 (see Table 5.159). Resilience occurred on Day 10 and onwards as richness in genera had returned to the initial level (i.e., Day 0). For sweep netting method, resilience of ecological indices at the Genus level in 2014 trial was more frequently observed on Post-7 and Post-14 carcasses while ecological indices for Control carcasses were quite resistant in general (see Table 5.194 – 5.196). In other words, richness, diversity and evenness for

immediate insect access carcasses were consistence over time. However, with the impact of delay insect colonization, there was significant change in richness, diversity and evenness over time. Pechal et al. (2014b) did not examine resilience in their setting, however, when insects were allowed to colonize carrion previously excluded from insects, significant increase in insect taxon richness was observed.

While this study was highly informative, weaknesses were determined. Identification of aboveground arthropods was limited. As previously mentioned, identification to Order level alone was most informative for sticky traps. However, for pitfall traps and sweep nets, identification to Genus and species level is required in order to detect treatment effects. Furthermore, sampling frequency was another limiting factor. As decomposition progresses actively and rapidly on insect-access carcasses (i.e., control carcasses), sampling of flying arthropods using sticky traps and sweep nets should be conducted every day to detect any change in arthropod community structure and indices. As for crawling arthropods, such as ants and beetles, pitfall traps should be placed and continued the collection even after 40 days of decomposition. In this study, adults and larvae of *Dermestes* beetles have been observed underneath the carcasses on Day 40. Therefore, sampling days for crawling arthropods should be extended until no more necrophagous arthropod was present on or around the carrion. Furthermore, the sample size in this study was only three (n = 3), which reduce statistical power (Button et al. 2013). Increasing the sample size would enhance the statistical power and confidence interval. However, considerations such as the study objectives, cost, time, and manpower should be taken into account before deciding on the desired sample size for statistical analyses. In the future, similar studies could be conducted in different seasons (e.g., spring or winter), different ecoregions, or different geoclimatic regions. Different arthropod trapping techniques could be employed around the carrion to enhance catching. For instance, yellow pan trapping techniques, malaise traps, or even light traps to collect nocturnal insects at the field. Night collection (10 pm to 2 am) by sweep nets, forceps or handpick are recommended as some insect detritivores were present and consuming the carrion at night (e.g., fulvous wood cockroaches, *P.*

fulvescens, and the black dump fly, *O. aenescens*). There is lack of study regarding insect activities and arthropod community transition from daytime to nighttime on carrion and this arthropod community "circadian rhythm" phenomenon is important from both ecological and forensic perspectives (see Appendix M for more information regarding circadian succession of necrophagous insects on carrion).

For sticky traps, results in both trials demonstrated that there was significant difference in arthropod community structure at different heights of sticky traps (either above or bottom, separated by a distant of 0.9 m) and position from carrion (anterior or posterior, separated by a distant of 3.2 m) (see Table 5.199). This observation highlights the importance of spatial scale of vertical and horizontal axes of the carrion to the community structure of flying arthropods. Future research in carrion ecology should emphasize on the role of spatial scale in affecting arthropod community structure, succession trajectories, ecological indices and functions. By defining and refining the spatial scale during carrion decomposition, collection of arthropods conducted on carrion at a particular spatial scale may render different community results compared to the collection from other spatial scale (*sensu* Levin, 1992). These discrepancies will ultimately affect the interpretation of an ecological system, or possibly will cause errors and mislead insect evidence during forensic investigations.

The potential applications from this study include the advancement in forensic entomology. This study had demonstrated that delayed arthropod colonization on carrion significantly impacted the aboveground arthropod community structure (with their respective optimal taxonomic resolution) and function, as well as successional trajectories. Also, the results from this study highlighted the importance of spatial scale in influencing arthropod community structure and function, which may change the practice or the standard operating procedures by forensic entomologists, where more cautions should be taken about spatial scale (e.g., how high and how near from the remains?) when collecting insect evidence from decomposing remains. Nonetheless, the current study enhances the understanding in carrion ecology about spatial scales, different aboveground arthropod sampling techniques, taxonomic resolution, divergence,

convergence and resilience of ecological indices, community structure and function, as well as biotic factors (e.g., priority effects, absent of competitors and predators), abiotic factors (e.g., temperatures and precipitation) that governed all of these parameters simultaneously.

CONCLUSIONS

Aboveground arthropod community structures were sensitive to the year of trial (could be due to differences in abiotic factors), treatments, and day of decomposition (both depends on year and taxonomic resolution for each sampling technique). For sticky traps, arthropod community structures were affected by height and position of the traps. Hence, based on these results, the null hypothesis was rejected, as there was a shift in aboveground arthropod community structure and function in response to delayed vertebrate decomposition. Likewise, observations on aboveground arthropod community divergence, convergence and resilience were dependent on year, sampling method, taxonomic-scale and specific ecological index.

CHAPTER VI

DISCUSSION AND CONCLUSIONS

DISCUSSION AND CONCLUSIONS

The current study was the first to address the concepts of community divergence, convergence and resilience of ecological indices on carrion experiencing delayed insect colonization over an extended period of insect exclusion (i.e., 14 days of insect exclusion). Furthermore, the current study was the first to examine the impacts of delayed insect colonization on carrion on the three major ecological components during vertebrate carrion decomposition namely the microbial function, the aboveground and belowground arthropod community structure and function, as well as soil chemistry profiles. This study is also the first to examine acari associated with carrion decomposition in Texas in a larger spatial-temporal scale and revealed six new phoretic acari species. Furthermore, this study was the first to examine larger spatial scale (i.e., one meter away from the decomposition remains) of arthropod communities associated with the carrion decomposition process.

The results further confirmed that carrion and its associated "carrion zone" are highly specific, unique and demonstrated a high degree of patchiness. However, this study also demonstrated that the lateral extension of soil nutrients could occur as far as 5 m away from carrion remains, indicating that the spread of cadaver decomposition islands over time. Hence, this study proposes spatial references to define specific regions of the cadaver decomposition island (e.g., *necrozone*), as well as the atmosphere on and around carrion (e.g., *necrosphere,* and *necrotone*), both vertically and horizontally (see Appendix O for graphical introduction of these new terms).

This study also is the first to apply taxonomic resolution in carrion ecology. The results exhibited that different taxonomic scale is needed for each sampling technique to detect significant treatment effects. For instance, Order level for sticky traps, Family

level for Berlese funnels, Genus level for both pitfall traps and sweep nets were the minimum sensitive level to detect treatment effects.

Overall, this study rejected all null hypotheses stated in each objective. I determined specifically that there is a shift in: (i) Microbial metabolic community profiling in response to delayed vertebrate decomposition (ii) Soil chemistry dynamics in response to delayed vertebrate decomposition (iii) Soil arthropod community structure and function in response to delayed vertebrate decomposition, and (iv) Aboveground arthropod community structure and function in response to delayed vertebrate decomposition. This study contributes a new dimension for researchers examining carrion decomposition, namely the impact of delayed insect colonization in relations to abiotic and abiotic elements on arthropod community structure, function, microbial function, soil chemistry as related to nutrient recycling. Furthermore, these results strengthened the tenant that delay insect colonization on carrion is a disturbance event in carrion ecology, as in agreement with Pechal et al. (2014a, 2014b). To further comprehend the impacts of disturbance to carrion decomposition ecosystem and to the adjacent ecosystems, fundamental concepts in ecology such as resistance, divergence, convergence, and resilience should be studied in details, as the recovery or resilience of an ecosystem is vitally important after a perturbation, and the degree of recovery determines the existence or sustainability of the entire biotic communities that are living within the ecosystem (Elmqvist et al. 2003; Walker & Salt, 2012).

Tomberlin et al. (2011b) proposed a framework to link decomposition ecology and applied sciences such as forensics. Such suggestions are important in unifying both fields and facilitate communication and the production of scientific results. I believe that the results generated from this study can be applied in forensic entomology, forensic microbiology, forensic acarology, forensic soil chemistry, mass mortality events assessment or environmental health assessment, disease ecology and biological conservation. Results obtained from this study demonstrated that the impacts of delayed insect colonization on carrion significantly change the arthropod community structure and successional trajectories. This ultimately affects the estimation of time of insect

colonization (TOC or minimum post-mortem interval, mPMI) by forensic entomologists who use arthropod community composition or insect successional sequence in their TOC or mPMI estimation (Matuszewski et al. 2010; Schoenly et al. 1992). As such, estimation of mPMI that did not consider the impact of delayed insect colonization in the case when delayed insect colonization had occurred would pose a serious error to the estimation. The consequence of such misleading mPMI statements could place an innocent suspect in prison and free the offender from criminal charges.

Microbial metabolic community profiles generated from this study might be useful as forensic indicators. Results in this study demonstrated that microbial functions on carrion with immediate insect access were significantly different from carrion with delayed insect colonization, as in agreement with Pechal et al. (2012; 2013). Furthermore, microbial function was differed by day of decomposition. These findings would foster the development of using microbial communities in the determination of mPMI, as well as to determine whether or not the human cadavers have been kept in concealed environment that deterred insect colonization (e.g., by having an unusual higher microbial activities on body).

This study offers the first extensive study on acari community structure, succession, and function during carrion decomposition in Central Texas. Data generated from this study enriches the records of fauna diversity of acari in the North America and documented six new species of phoretic mites associated with necrophagous insects in Texas. These data provide some preliminary results to the development of forensic acarology in this region and could potentially be useful in assisting forensic cases. Delayed insect colonization on carrion did significantly affect the community structure (at the Family level) and function of acari, further strengthened the facts that aboveground carrion decomposition could impact belowground arthropod communities. Hence, linkages between above- and below ground ecosystems are evident and closely connected with each other.

In the events of mass mortality of vertebrate animals (which act as a huge ecosystem disturbance), there could be delayed of insect colonization due to “dilution

effect" (*sensu* Ostfled & Keesing, 2000a) on carrion and subsequently lead to the reduction in the rate of decay. Slower rate of carrion decomposition may or may not contribute negative impacts to the environment (Barton et al. 2013a). As such, post-MMEs assessment to the affected landscape should be studied to determine whether there is undergoing recovery in the ecosystem structure and function. Data generated from this study introduced some ecological concepts such as resistance, community divergence, convergence, and resilience as the parameters to examine when conducting Post-MMEs environmental quality assessment. Samples such as soil microbes, soil arthropods, soil chemistry as well as aboveground arthropods could be collected before, during and after MMEs events for the purpose of environmental health biomonitoring. Some occurrences of mass die-off events can be predicted such as salmon runs, cicada emergence or during hunting seasons (i.e, resource pulses). I propose that environmental assessment should be conducted, studied and documented to access environmental ability in resilience. An ecosystem is considered "healthy" if it is resistant to disturbance or the recovery rate is fast (higher degree of resistance and resilience) (Costanza & Mageau, 1999) whereas an ecosystem with slow recovery rate after a disturbance (low degree of resilience) could indicate an instable ecosystem (Pimm, 1984).

As delayed carrion decomposition prolonged the exposure duration of carrion in the environment, it is possible that the carrion serve as breeding ground to variety of pathogenic bacteria, viruses and insect vectors and consequently contaminate the environment (Smith & Wall, 1997; Russell et al. 1995; Vanderzant & Nickelson, 1969). Once the pathogens have been spread by vectors or animal reservoirs to a greater spatial scale, or contacted by another animals or human hosts, there could be a risk of initiating epidemic of infectious disease (Baker et al. 2007; Wachsmuth et al. 1997). Carrion with delayed insect colonization may also attract animal scavengers such as vultures or coyotes, which could also serve as reservoirs for various pathogenic microorganisms and transmit to other animals or human hosts (Ogada et al. 2012; Jennelle et al. 2009). The results generated from this study indicate microbial activities were more abundant on pig carrion during insect-exclusion period. Hence, this study provides some baseline data to

the development of disease ecology, public health and epidemiology especially when dealing with mass mortality events or carrion with delayed insect colonization.

Carrion with delayed insect colonization promotes biological diversity. Pechal et al. (2014b) observed an increased in taxon richness after the carrion were exposed to the environment following the removal of insect-exclusion cages. Similarly, Barton et al. (2013a) suggested that leaving carrion in ecosystems may be one of the most effective short-term ways of managing biodiversity associated with nutrient cycling process. Likewise, some results from this study demonstrated that richness and diversity were significantly higher in carrion with delayed insect colonization, indicating the importance of carrion in maintaining the richness and diversity of necrophagous guilds in the ecosystem. Perhaps it is time to rethink about the current practice of carrion management system as the current carrion disposal may seriously threatened the survivorship of certain scavengers and necrophagous arthropods (Margalida et al. 2010). For example, the establishment of carcass removal programs all over Spain following the regulations against Bovine Spongiform Encephalopathy (BSE) could seriously affect the stability and future evolution of Griffon vultures, *Gyps fulvus* (Hablitz), due to scarcity of food source (Camiña & Montelío, 2006). Illegal carcass disposal in the past was one of the reasons for the vulture population recovery in Spain (Camiña & Montelío, 2006). In another example, lack of available carrion resource due to over-population of the white-backed vultures (*Gyps africanus* Salvadori) may harm their survivorship as they were fully dependent on the existing carrion biomass to sustain (Kane et al. 2015).

Carrion can be toxic to the environment. For example, data from 2013 and 2014 trials demonstrated that Control carcasses with immediate insect access had significant difference (loss of resistance) in either soil arthropods' family richness, diversity, or evenness at the soil beneath of the carrion during the decomposition process while all ecological indices in carcasses with delayed insect colonization (i.e., Post-7 and Post-14 groups) were resistant in all sampling days. These observations suggest that slower rate in carrion decomposition due to the absent of primary decomposers may result in slower release of nutrient, hence less nutrient toxicity had been built up in the soil. Under this

condition, I hypothesized that this less toxic environment in soil did not significantly impact the soil arthropod community richness, diversity and evenness. From conservation perspective, slower carrion decomposition is actually benefited the soil arthropod community as they are able to resist and recover from the detrimental toxicity effects (e.g., ammonium-N) as shown in soil beneath the Control carcasses.

There were several limitations in this study. Sample size was one of the major drawbacks as there were only three replicates in each sample (n = 3). It will be ideal if sample size can be increased, and so do the statistical power (MacCallum et al. 1996). To avoid pseudoreplication, this study was conducted in two consecutive summers (two trials in total). However, only one season (i.e., summer) was studied. Hence, the data generated from this study could only explain the ecological parameters under summer condition in Central Texas. Another major concern was the invasion of blow flies into the insect-exclusion cages during the exclusion period. Although we managed to reduce blow fly colonization by overall 99% (see Appendix K), there were some adult calliphorids, sarcophagids and muscids squeezed into the cages (through the openings between the cage and the soil surface) and oviposited on the carrion. Prevention steps have been taken seriously by fixing the opening between insect-exclusion cages and the soil surface and rescue efforts such as immediate eggs and larvae removal have been performed twice on every single insect-exclusion day to assure that no other eggs or larvae remains on the pig carrion. Still, under this situation, pig carrion free from blow flies had been "contaminated" by microbes brought by insects, and some of the larvae activities had changed the carrion physical state, as well as contributed to the soil chemistry by rupturing the skin, which allowed more decomposition fluid seeped into the soil ecosystem. Other than that, the rescue efforts were causing aeration to the soil beneath the carrion. It is because when the pig carrion was examined for fly eggs or larvae, the carrion was lifted or rolled to the side, such actions allowed oxygen to permeate the space between carrion and the surface of soil beneath, and this "re-oxygenation" process may impact the normal microbial succession by reversing the sequence from anaerobic to aerobic microbial communities. Consequently, microbial

community structure and function may be seriously affected, as well as influencing the nitrogen transformation process such as ammonification (Broadbent & Stojanovic, 1952). It must be noted that insect-exclusion cages used in this study did not exclude all insects and arthropods, especially the crawling arthropods such as ants, beetles, collembolans, and mites (Pechal et al. 2014b). Those arthropods inevitably contributed microbes to the carrion and change the carrion physical state as well as soil chemistry profiles. During the insect exposure period, all carrion was protected by anti-scavenging cage. However, under rare occasions, there was opossum (*Didelphis virginiana* (Kerr)) scavenged the remains of pig carrion at night time, as noted in this study. These scavenging activities may introduce microbes to the remains (Lauber et al. 2014), disturbed the distribution of microbes on carrion and also changed the soil chemistry dynamics underneath the carrion (Aitkenhead-Peterson et al. 2012). Furthermore, animal scavengers may defecate right after they have visited the carrion and eventually contribute more microbes to the carrion and alter the soil chemistry by contributing nutrients from its feces.

As the decomposition rate may increase under warmer temperature (i.e., during summer season) (Archer, 2004), the bloated and active decay stages were the most attractive stages to insects and intense insect activities such as oviposition can be observed during these stages. However, the interval of sampling regimes was large between days for all collection and trapping methods employed in this study such as swab collections, sticky traps, pitfall traps, soil arthropod collections and sweep netting. It is known that many biotic parameters changes occurred during the first five days of decomposition (e.g., arthropod and microbial activities on carrion) (Pechal et al. 2014a, 2014b). By having a large gap between sampling intervals, we may lost a lot of useful and valuable data. Hence, it is suggested that the sampling interval regimes should be performed on daily basis at least for the first seven days when the microbes and insect activities were the most abundant. Furthermore, we observed insect activities underneath the carrion on Day 90 of decomposition (i.e., *Dermestes* larvae). Due to this reason, pitfall traps should be placed in the field for an extended period (e.g., Day 90 and Day 180) to collect those crawling arthropods.

In terms of taxonomy, one of the limitations is the ability to identify the arthropod specimens to the lowest taxonomical rank (i.e., genus and species), as some of the arthropod taxonomical groups are complicated and require experts assistance and confirmation (e.g., mites, collembolans, beetles, parasitic hymenopterans etc.), and some of the arthropod groups are cryptic and required identification by molecular techniques (e.g., strepsiterans, sarcophagids). Molecular identification of microbial community structures on the pig carrion as well as the soil of CDIs were not able to conduct due to time and manpower constraints. Although there were some other minor limitations in the experimental designs, such as the sub-sampling method for mite mounting purpose. The sub-sampling method may tend to over- or underestimate of the actual mite diversity in the soil samples. Besides, the effects of putting insect exclusion cages in the field allow insects to rest on the cages. As a result, these insect aggregations may increase the efficiency of sweep netting or sticky traps and resulted in higher numbers of catching. Nevertheless, the major but also universal limitations of this research were the time, financial and manpower limitations. The results obtained could be more solid and replicable if we have larger sample size, more replicates, longer sampling periods, and higher taxonomic resolution. Sophisticated statistical analyses could also be employed to analyze more ecological parameters (e.g., species turnover, network analysis) or to build mathematical models for carrion decomposition process (see Appendix P for ecological network). Furthermore, this research is in need of validation before putting the results in real applications.

There are many future studies that can be extended from the current research. Future studies should consider repeating the fieldworks and testing the ecological parameters (for microbe and arthropod communities) as well as soil chemistry dynamics in different seasons in Texas such as spring or winter. Differences in seasonality should be compared to better understand how carrion ecosystem works under different seasonality. Other than seasons, repeating the fieldworks in different ecoregions (e.g., Piney woods, Gulf prairie, Post oak savannah, high plains), different geoclimatic areas (e.g., tropical, monsoon, arid, semiarid, Mediterranean, humid subtropical, continental or

subarctic) or on different soil types (e.g., sandy, silty, peaty, saline, and loamy soils) should be considered. By comparing the mechanisms of carrion ecosystems in different types of seasons, ecoregion, climatic zones, and soil types across the Earth longitudes and latitudes will allow researchers to arrive at a core concept of carrion ecology at the global scale.

Longer insect exclusion period (> 14 days or weeks, months, or even years) could be one of the interesting research questions. Furthermore, complete insect exclusion, including ground arthropods, has not been tested for its impacts to the ecosystems. Complete insect exclusion can be achieved, for example, by placing the carcass into a sterile, well fitted, completely tight plastic box, and then re-introduce the carcass to the environment after the desired exclusion period. Under this condition, the carcass is assumed to be dominated by microbes and when insects are allowed to colonize the carrion, researchers are able to examine how microbes on the carrion interact with the insects.

Microbial community structures on carrion with delayed insect colonization, as well as the associated soil microbiome should be studied using the Next-Generation Sequencing (NGS) to determine bacterial or other eukaryotes species that are present during carrion decomposition. Although there are recent literatures regarding epinecrotic microbial community succession using metagenomic sequencing (Pechal et al. 2014a; Benbow et al. 2015; Iancu et al. 2015; Hyde et al. 2015), however, microbiome associated with carrion experiencing delayed insect colonization for an extended period (e.g., >14 days) has not been available to the scientific communities. Moreover, microbial volatile organic compounds (MVOCs) released from necrobiome of carrion with delayed insect colonization should be studied and compared with MVOCs released from carrion with immediate insect access. Once the MVOCs have been identified, the mechanisms of interkingdom communications between bacteria and arthropods, or vertebrate animals can be studied in details (Davis et al. 2013; Tomberlin et al. 2012; Ezenwa et al. 2012), including the behaviors of arthropods or animals responding to the MVOCs.

Soil arthropods, including the acari, associated with carrion decomposition are understudied (Perotti et al. 2009; Bornemissza, 1957). Future studies should focus on the diversity, ecology, biology and evolution of acari associated with carrion decomposition, as well as the phoretic acari on necrophagous insects (e.g., blow flies and beetles). Many new species of acari associated with ephemeral resources are undocumented and the opportunity for new discoveries are promising (OConnor, 2009a). The anaerobic environment in the soil beneath the carrion could be highly anoxic and chemically toxic (Anderson et al. 2013; Carter et al. 2007) and how the soil arthropods and mite survive and thrive in such environment remains enigmatic. Furthermore, future studies should focus on the relationship between mite population dynamics and soil chemistry change (e.g., pH, conductivity, NO_3^-, NH_4^-, PO_4^-) associated with the cadaver decomposition islands (CDIs) (see Appendix Q for correlations between variables and Appendix R for an example of a model predicting ADH). In addition, ecological relationships (competition, predation, parasitism, mutualism etc.) between soil mites and aboveground arthropods, the phoretic hosts, or soil microbial community structure should be determined. The basic ecological studies on acari associated with carrion decomposition will nurture healthy development of forensic acarology, and the future application of forensically importance mites in criminal cases is promising.

Soil chemistry profiles associated with carrion decomposition is another major developing discipline to be explored, either for environmental indicator assessment or forensic use (Tibbett & Carter, 2008). Lateral extent of soil chemistry or the CDIs had been observed in this study and it is therefore recommended that the movement of soil nutrients following carrion decomposition should be studied for the purpose of mPMI determination. Furthermore, location of death or the presence of scavenger activities on carrion could be determined based on the pattern of lateral extent of CDIs with soil chemistry (Aitkenhead-Peterson et al. 2012). Note that the speed of lateral or vertical extent of soil nutrients can be affected by the degree of slope, or type of soils (Aitkenhead-Peterson et al. 2012; Jobbágy & Jackson, 2001), which is another area of interest to be examined in detail. On top of that, vertical movement of CDIs is crucial

during carrion decomposition. Thus far, no literature has address the issue on the depth of CDIs movement and how the mass mortality events can affect nutrient recycling processes and underground water system. Also, many other soil minerals and elements can be examined during carrion decomposition such as the concentrations of Na, K, Ca, Mg, and S. However, the dynamics of these nutrients have not been examined on carrion impacted with delay insect colonization.

It is still debatable whether arthropod assemblage and succession are deterministic or stochastic process (Ellwood et al. 2009). Hence, it is an opportunity to examine the core concept of arthropod succession following disturbances (e.g., burnt, submerged, or buried carcasses) in carrion ecology (see Appendix M and Appendix S). As spatiotemporal scale is very relevant when explaining succession phenomenon, future studies should examine multilevel spatial (e.g., mm, cm, m, km) and temporal scales (s, min, hour, day, week, month, year), as well as multi-taxonomic resolutions (Order, Family, Genus, species) simultaneously when examining community structure, abundance, succession and ecological indices. Furthermore, most of the arthropod succession studies were conducted during day time (Anderson, 2001). We suggest that arthropod successional study should be continued for night observations to determine the transition dynamics of necrophagous insects through different phases. It is known that certain necrophagous species (e.g., some species of blow flies and cockroaches) are active nocturnally (Amendt et al. 2008; Denic et al. 1997; Greenberg, 1990). However, not many studies have documented this pattern in detail. These necrophagous community transitions between diurnal and nocturnal phases, or so called "circadian assemblage" or "circadian succession" should be determined as one of the important aspect in carrion ecology (see Appendix M).

Carrion decomposition should be studied within the framework of ecosystem ecology. In this study, arthropod or microbial communities were investigated based on locations, namely aboveground and belowground communities, and hence more towards community ecology. However, it is time to link both ecosystems and examine how the aboveground communities interact and affect the belowground communities or vice

versa. For example, during delayed insect colonization on carrion, flying insects such as blow flies were prevented from access to carcasses. However, the insect exclusion cages did not prevent crawling arthropods from the carrion. Hypothetically, phoretic mites that arrived with the beetles may reproduce and dominate the soil beneath the carcasses due to the absence of interspecific competition from phoretic mites that are transported by Diptera. Another hypothesis where the absence of dipteran larvae on carrion during insect exclusion period may slow down the rate of decomposition, and thus provide more resource (e.g., blood or decomposition fluids) to the belowground fauna, subsequently enrich the biodiversity of soil dwelling arthropods and increase the soil microbiota metabolic activities. However, these linkages between causal and effects should be confirmed by future studies.

Does delay insect colonization change the carrion quality? What are the differences in term of nutritional aspects of the carrion first colonized by microbes and carrion first colonized by insects? Carrion with delayed insect colonization may favor microbial proliferation and colonization on carrion (see Chapter 2). These microbial communities produce MVOCs that could interact with other organisms (i.e., interkingdom communication) (Davis et al. 2013; Tomberlin et al. 2012). Similarly, bacterial metabolism on carrion may produce waste products or secondary compounds that may serve as the medium of attraction or repellent to certain organisms. As a result, these microbial community may impose priority effect to other arthropods through facilitation or inhibition effects (Connell & Slatyer, 1977), thus changing the sequence of arthropod succession. Similarly, carrion with immediate insect access will be colonized mainly by dipteran larvae. The feeding activities by fly larvae create spaces on carrion, along with the secretions and excretions by fly larvae (which release digestive enzymes or frass toxins), could initiate or inhibit the subsequent arrival of other organisms. Whether the microbial metabolic products, digestive enzyme and frass toxin significantly change the carrion quality and then alter the subsequent succession trajectories of other organisms still remains unknown. Bukovinszky et al. (2008) showed that variation in plant quality has cascading effects across trophic levels as mediated by

changes in the abundance and size of resource items. The bottom of the food chain (i.e., the plant type, which are the feral and domesticated *Brassica*), affects the herbivorous aphids (*Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulzer)), which in turn controls the quality and abundance of primary parasitoids (*Diaeretiella rapae* (M'intosh)), and eventually affects the top of the food web, the secondary parasitoids (*Alloxysta fuscicornis* (Hartig), *Asaphes vulgaris* Walker, *Asaphes suspensus* (Nees)). The results suggested that the increased in size and fitness in both aphids and the primary and secondary parasitoids was the results of differences in plant qualities (e.g., plant metabolites, defense chemicals and architecture). They concluded that the change of resource quality could alter species interaction by limiting energy transfer to consumers and their predators, thus affecting life history and morphological traits (Bukovinszky et al. 2008).

Future studies may also examine whether carrion quality could be the driver for the evolution of necrophagous insects, the hypothesis is that the better quality of carrion initiates more fierce competition among necrophagous arthropods and selects for the best necrophagous species over time. In theory, by comparing arthropod fitness breed on carrion with high nutritional status and one with low nutritional status, one will produce offspring with different fitness according to the resource quality that they fed on. Although it is unknown whether carrion quality directly or indirectly shapes the evolution of necrophagous species, yet, it is an important aspect in carrion ecology to understand how detritivores and decomposers evolved into a new species, and most importantly, contribute to our holistic understanding about the food webs and nutrient cycling.

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APPENDIX A

COMMON VEGETATION AT THE STUDY SITE

Figure A1. The vegetation found at the study site at Snook, Texas. This vegetation represents the surrounding environment where the pig (*S. scrofa* L.) carrion placed during the summers of 2013 and 2014. This study site belongs to Texas A&M Field Laboratory, approximately 8 miles from College Station towards the direction to Snook, Texas via Raymond Stotzer Parkway (scale bar = 30 cm).

APPENDIX B

PROTOCOLS FOR DETERMINING MICROBIAL FUNCTION

PROTOCOLS FOR PREPARING RINGER SOLUTION

¼ strength Ringer solution is the following in 1 L of DI water

- NaCl: 1.8 g
- CaCl: 0.0425 g
- KCl: 0.0925 g

Example: To prepare a **5 Liter of Ringer solution**, mix **5 L of DI water** with **9 g NaCl**, **0.2125 g $CaCl_2$** and **0.4625 KCl**.

PROTOCOLS FOR SAMPLE PROECESSING FOR BIOLOG ECOPLATES

Biolog Ecoplate™ Plating Procedure

1. First, fill up the 50 ml centrifuge tubes with **40 ml of the sterilized Ringer solution**.
2. Add **15 sterilized glass beads** to each tube.

3. **For swab:** Add the **swab** to centrifuge tubes **and label tubes** accordingly.
   For soil: Take **1 gm of soil** to centrifuge tubes **and label tubes** accordingly.

4. Place the tube at the vortex and allow it to shake for 2 minutes at the power ranking 6-9.
5. Then, **centrifuge** the tubes for **2 minutes** at **800 g or 2000 rpm at Dr Tarone's lab.**

6. **For swab:** Pour **20 ml of the supernatant** into a **petri dish**.
   For soil: Take **2 ml of sample** and dilute with **18 ml of additional Ringer solution** in a **petri dish.**

7. Using the **8 channel pipette**, aliquot **100 µl of the supernatant** into each well of the 96 well Biolog ECOplates.
8. Make sure the **plates are labeled** the same as the sample in which it contains.
9. Place in box at **room temperature** (22°C) and without light.

PROTOCOL FOR BIOLOG ECOPLATE READING

TECAN Protocol for Plate Reading

1. Open Magellan on the Desktop. Make sure the serial port is plugged in, and the switch on the back of the TECAN is turned on, otherwise the menu will not appear correctly.

    a. If screen comes up, asking "What do you want to DO?" Select **"Start Measurement".**

2. Put on **one glove.**

3. Place Plate in the machine with cover on with the **Biolog words to the right side**.

4. Select **"EcoPlate1"** from the favorites menu, then click "make selection" on bottom right corner. **Hit start.**

5. On the "Evaluate Results" page, it will show you absorbance of the plate, hit **"next".**

6. An excel sheet will pop up, double check that the entire plate was read.

7. **Name** the **ENTIRE EXCEL FILE** as the time it read, including the time point (i.e.D0_R1_SB_1).

-This file will go into the folder on the desktop named (Chin Summer Study).

-In this folder select the correct **treatment of plating (CR or CM or CT).**

-In this folder select the correct **date sampled (D0 or D3 or D5 or D14)**.

-Once the result is out, please calculate the mean, place your cursor at box D2, then type **=AVERAGE (B2: B97)**

a. Rename each **TAB of the excel sheet** according to the **sampling site** being read **(i.e SB, SL, 5m, O, SK, A) – click SAVE.**

SB= soil beneath
SL= soil lateral
5m= 5 meter away (served as the control)
O= oral region
SK= skin region
A= anal region

b. Each **subsequent plate** will open on **a new tab on the SAME excel sheet**. **Make one excel sheet per reading time point**, this reduces the number of files saved and the time it takes to save.

c. **When you come to a different sampling date, make a new excel sheet!** This way for each date we will have 1 sheet per time points that the plates were read.

8. Hit **"finish"** in the Magellan window. Click **"Yes"** when it asks if you want to save the data. Remember, all we want are the **excel sheets**. Continue reading the rest of the plates.

9. Place Biolog EcoPlate™ **in dark box** at ~22^{o}C

10. Read Biolog EcoPlate™ activity every 12 h at 590 nm.

Read plates for a total of 5 d or until both the average **optical density (OD)** has reached average **0.7** for both the whole plate and the whole plate subtracting the average water wells OD, if possible.

11. After every reading, all data are saved to both a **flash drive** and to a **computer.**

APPENDIX C

BIOLOG ECOPLATE™ CARBON SOURCES

BIOLOG Microbial Community Analysis

EcoPlate™

A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

FIGURE 1. Carbon Sources in EcoPlate

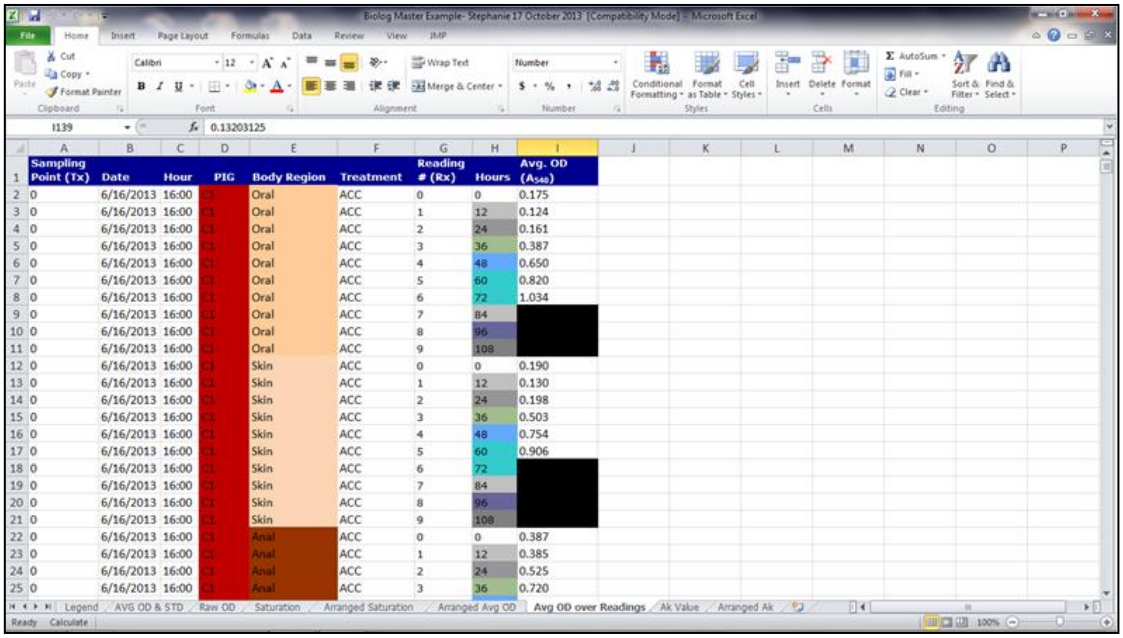
Figure C1. Biolog EcoPlate™ with 31 carbon sources and a water well (serve as Control) impregnated in a single plate with triplicates.
(Image downloaded from http://openwetware.org/wiki/M465:Biolog_Ecoplates).

APPENDIX D

DATA ENTRY AND DATA ANALYSIS FOR MICROBIAL FUNCTION

PROTOCOLS ON DATA ENTRY FOR MMCPs ANALYSIS

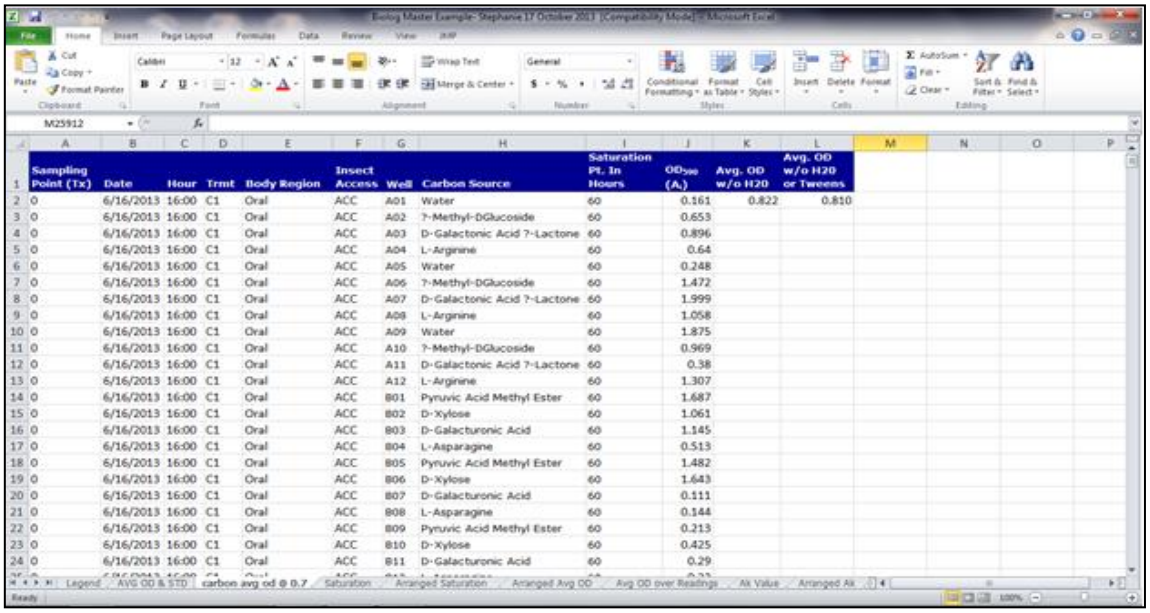
The following protocols were compiled by Heo CC & Thornton SN (2014).



	Sampling Point (Tx)	Date	Hour	PIG	Body Region	Treatment	Reading # (Rx)	Hours	Avg. OD (A_{540})
2	0	6/16/2013	16:00		Oral	ACC	0	0	0.175
3	0	6/16/2013	16:00		Oral	ACC	1	12	0.124
4	0	6/16/2013	16:00		Oral	ACC	2	24	0.161
5	0	6/16/2013	16:00		Oral	ACC	3	36	0.387
6	0	6/16/2013	16:00		Oral	ACC	4	48	0.650
7	0	6/16/2013	16:00		Oral	ACC	5	60	0.820
8	0	6/16/2013	16:00		Oral	ACC	6	72	1.034
9	0	6/16/2013	16:00		Oral	ACC	7	84	
10	0	6/16/2013	16:00		Oral	ACC	8	96	
11	0	6/16/2013	16:00		Oral	ACC	9	108	
12	0	6/16/2013	16:00		Skin	ACC	0	0	0.190
13	0	6/16/2013	16:00		Skin	ACC	1	12	0.130
14	0	6/16/2013	16:00		Skin	ACC	2	24	0.198
15	0	6/16/2013	16:00		Skin	ACC	3	36	0.503
16	0	6/16/2013	16:00		Skin	ACC	4	48	0.754
17	0	6/16/2013	16:00		Skin	ACC	5	60	0.906
18	0	6/16/2013	16:00		Skin	ACC	6	72	
19	0	6/16/2013	16:00		Skin	ACC	7	84	
20	0	6/16/2013	16:00		Skin	ACC	8	96	
21	0	6/16/2013	16:00		Skin	ACC	9	108	
22	0	6/16/2013	16:00		Anal	ACC	0	0	0.387
23	0	6/16/2013	16:00		Anal	ACC	1	12	0.385
24	0	6/16/2013	16:00		Anal	ACC	2	24	0.525
25	0	6/16/2013	16:00		Anal	ACC	3	36	0.720

Figure D1. Excel sheet arrangement of the mean value of average OD for each sample at each time point.

1. Figure D1 is a table used to organize all of the mean values generated from the Tecan machine.
2. Copy the mean OD from each reading from the spreadsheet generated by the Tecan machine, and paste into 'AVG OD' column (column I in this case) in 'AVG OD Over Readings Sheet'.
3. Copy all values until 0.7 has been reached or 10 readings (When readings reached 0.7 before the 10th readings, unused spaces were filled black as a visual marker).



	Sampling Point (Tx)	Date	Hour	Trmt	Body Region	Insect Access	Well	Carbon Source	Saturation Pt. In Hours	OD_{590} (A)	Avg. OD w/o H20	Avg. OD w/o H20 or Tweens
2	0	6/16/2013	16:00	C1	Oral	ACC	A01	Water	60	0.161	0.822	0.810
3	0	6/16/2013	16:00	C1	Oral	ACC	A02	?-Methyl-DGlucoside	60	0.653		
4	0	6/16/2013	16:00	C1	Oral	ACC	A03	D-Galactonic Acid ?-Lactone	60	0.896		
5	0	6/16/2013	16:00	C1	Oral	ACC	A04	L-Arginine	60	0.64		
6	0	6/16/2013	16:00	C1	Oral	ACC	A05	Water	60	0.248		
7	0	6/16/2013	16:00	C1	Oral	ACC	A06	?-Methyl-DGlucoside	60	1.472		
8	0	6/16/2013	16:00	C1	Oral	ACC	A07	D-Galactonic Acid ?-Lactone	60	1.999		
9	0	6/16/2013	16:00	C1	Oral	ACC	A08	L-Arginine	60	1.058		
10	0	6/16/2013	16:00	C1	Oral	ACC	A09	Water	60	1.875		
11	0	6/16/2013	16:00	C1	Oral	ACC	A10	?-Methyl-DGlucoside	60	0.969		
12	0	6/16/2013	16:00	C1	Oral	ACC	A11	D-Galactonic Acid ?-Lactone	60	0.38		
13	0	6/16/2013	16:00	C1	Oral	ACC	A12	L-Arginine	60	1.307		
14	0	6/16/2013	16:00	C1	Oral	ACC	B01	Pyruvic Acid Methyl Ester	60	1.687		
15	0	6/16/2013	16:00	C1	Oral	ACC	B02	D-Xylose	60	1.061		
16	0	6/16/2013	16:00	C1	Oral	ACC	B03	D-Galacturonic Acid	60	1.145		
17	0	6/16/2013	16:00	C1	Oral	ACC	B04	L-Asparagine	60	0.513		
18	0	6/16/2013	16:00	C1	Oral	ACC	B05	Pyruvic Acid Methyl Ester	60	1.482		
19	0	6/16/2013	16:00	C1	Oral	ACC	B06	D-Xylose	60	1.643		
20	0	6/16/2013	16:00	C1	Oral	ACC	B07	D-Galacturonic Acid	60	0.111		
21	0	6/16/2013	16:00	C1	Oral	ACC	B08	L-Asparagine	60	0.144		
22	0	6/16/2013	16:00	C1	Oral	ACC	B09	Pyruvic Acid Methyl Ester	60	0.213		
23	0	6/16/2013	16:00	C1	Oral	ACC	B10	D-Xylose	60	0.425		
24	0	6/16/2013	16:00	C1	Oral	ACC	B11	D-Galacturonic Acid	60	0.29		

Figure D2. Excel sheet arrangement of the mean value of average OD for each sample at each time point.

1. Look at the AVG OD for each Reading sheet, and determine when each sample reached 0.7.
2. Copy the carbon source data from the original Biolog Excel that contains the data for the 0.7 reading or the 10th reading.
3. Paste into column ‘OD 590’ at “Carbon Average OD @ 0.7” (Figure D2).
4. Use the formula =average(J3:J5,J7:J9,J11:J97) in the column headed ‘Avg. OD w/o H2O. This formula is **excluding water** since it is a baseline. Copy and paste this formula for each new sample that is input. The formula should change to resemble the new rows. (A colon means inclusion, and a comma means exclusion. Example above: J3:J5 contains J3, J4, and J5, while J5,J7 excludes J6).
5. It is now time to make a Pivot table. It may be necessary to create a new Excel file, for the pivot table may be too large to fit into the compiled excel sheet created thus far.
6. Copy the ‘Carbon Source AVG OD at 0.7’ sheet and paste into the new excel file.
7. Go to the ‘Insert’ tab and click on ‘Pivot Table’ located on the far left of the screen (Figure D3).
8. A ‘Create Pivot Table’ box will appear (Figure D4). In the ‘Table/Range’ blank, CTRL A (select all) the ‘Carbon Source AVG OD at 0.7’ sheet. The data will now appear in the blank. Press ‘Ok’ and continue.

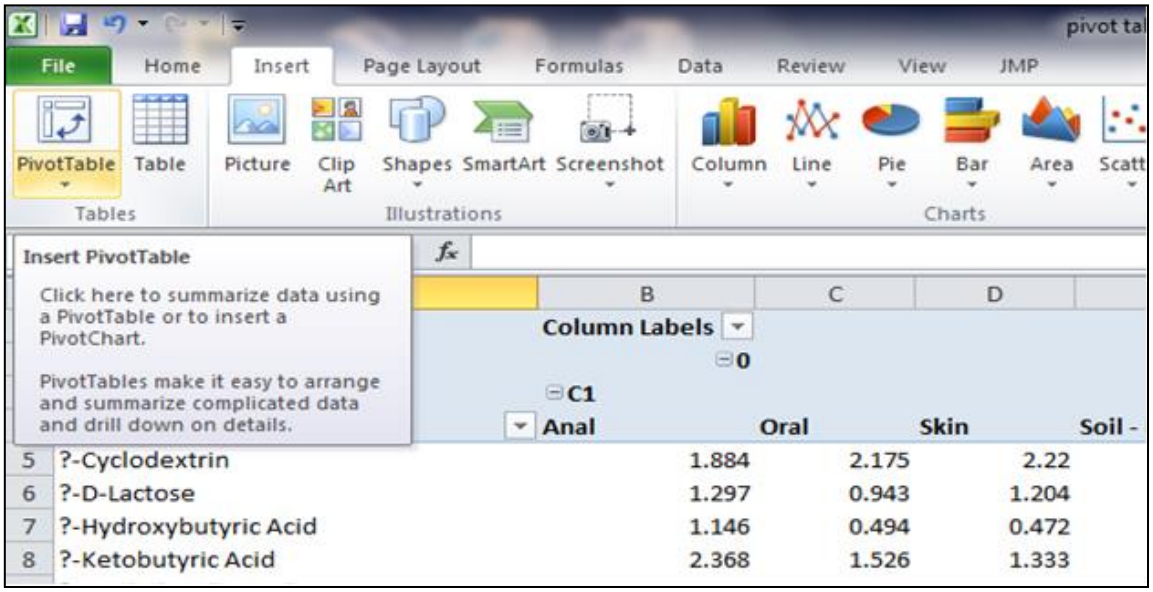


Figure D3. Location of "Pivot Table" as shown under the tab "Insert" in Microsoft Excel 2010.

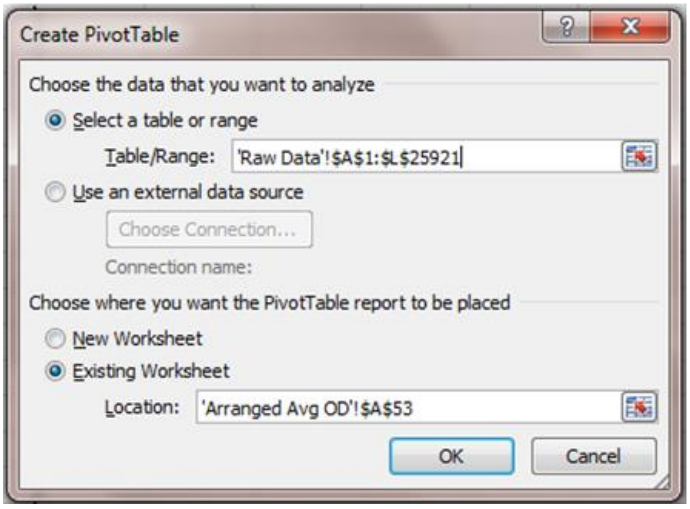


Figure D4. Dialog box of Pivot Table.

Figure D5. Dialog box of Pivot Table Builder. Check Sampling Point, Treatment, Body Region, Carbon Source, and Average OD590 (Image courtesy of Dr. Jonathan Cammack).

9. The column labels, row labels, and values must be defined. Put a check next to the variables that are wanted for comparison. In this example, Sampling Point, Treatment, Body Region, Carbon Source, and OD590 are checked. The AVERAGE of OD590 is placed in the 'Values' box, Carbon Source is placed in the 'Row Labels' box, and Sampling Point, Treatment, and Body Region are placed in the 'Column Labels' box (Figure D5).
10. **IMPORTANT:** In the 'Values' box, click the down arrow, and then proceed to click the 'field settings' tab. Change the selection field to **'AVERAGE'** (Figure D6).

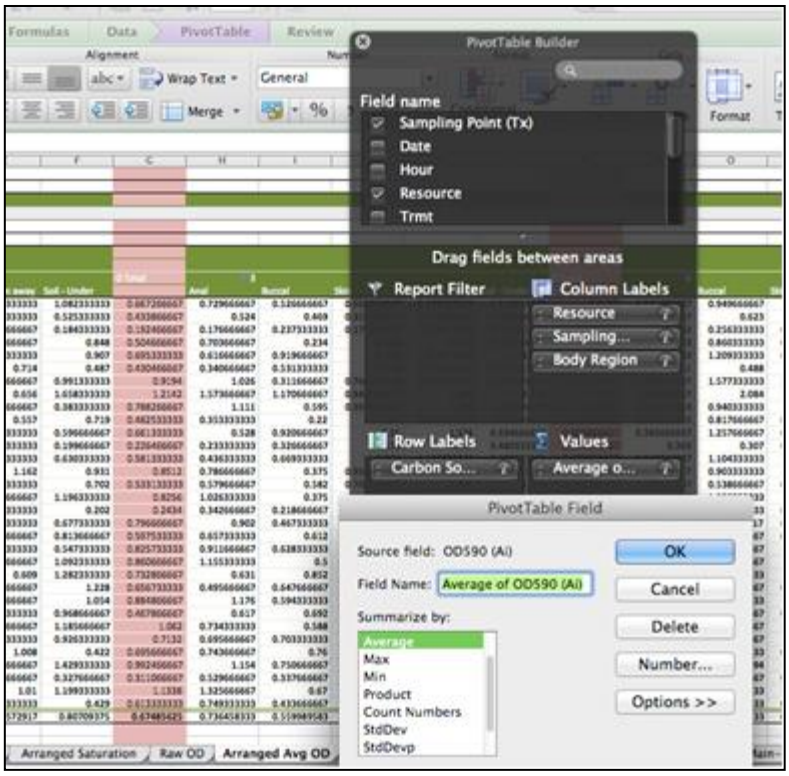
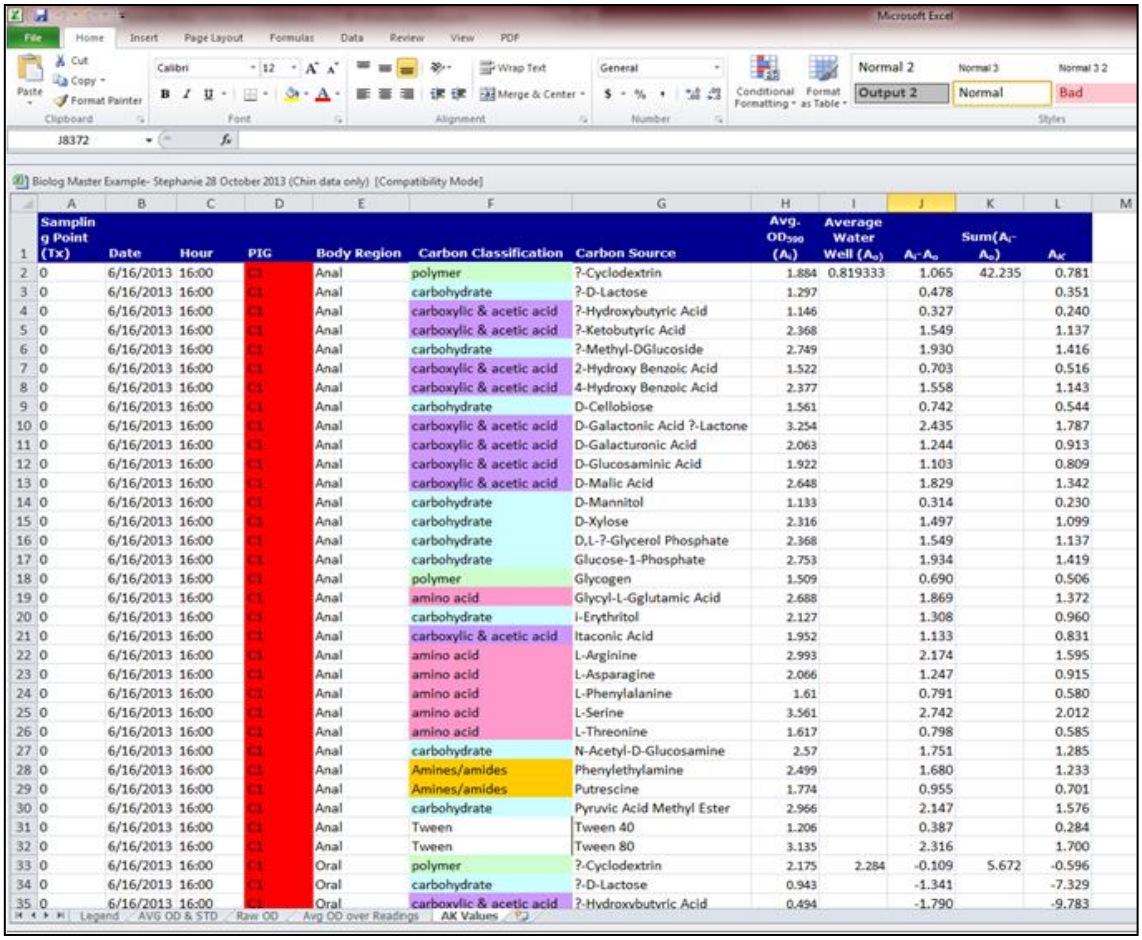


Figure D6. Pivot Table Field Dialog box. Change the selection field to 'AVERAGE' (Image courtesy of Dr. Jonathan Cammack).

CALCULATING AK VALUES



	A	B	C	D	E	F	G	H	I	J	K	L
1	Sampling Point (Tx)	Date	Hour	PIG	Body Region	Carbon Classification	Carbon Source	Avg. OD_{590} (A_i)	Average Water Well (A_0)	A_i-A_0	Sum(A_i-A_0)	A_K
2	0	6/16/2013	16:00	C1	Anal	polymer	?-Cyclodextrin	1.884	0.819333	1.065	42.235	0.781
3	0	6/16/2013	16:00	C1	Anal	carbohydrate	?-D-Lactose	1.297		0.478		0.351
4	0	6/16/2013	16:00	C1	Anal	carboxylic & acetic acid	?-Hydroxybutyric Acid	1.146		0.327		0.240
5	0	6/16/2013	16:00	C1	Anal	carboxylic & acetic acid	?-Ketobutyric Acid	2.368		1.549		1.137
6	0	6/16/2013	16:00	C1	Anal	carbohydrate	?-Methyl-DGlucoside	2.749		1.930		1.416
7	0	6/16/2013	16:00	C1	Anal	carboxylic & acetic acid	2-Hydroxy Benzoic Acid	1.522		0.703		0.516
8	0	6/16/2013	16:00	C1	Anal	carboxylic & acetic acid	4-Hydroxy Benzoic Acid	2.377		1.558		1.143
9	0	6/16/2013	16:00	C1	Anal	carbohydrate	D-Cellobiose	1.561		0.742		0.544
10	0	6/16/2013	16:00	C1	Anal	carboxylic & acetic acid	D-Galactonic Acid ?-Lactone	3.254		2.435		1.787
11	0	6/16/2013	16:00	C1	Anal	carboxylic & acetic acid	D-Galacturonic Acid	2.063		1.244		0.913
12	0	6/16/2013	16:00	C1	Anal	carboxylic & acetic acid	D-Glucosaminic Acid	1.922		1.103		0.809
13	0	6/16/2013	16:00	C1	Anal	carboxylic & acetic acid	D-Malic Acid	2.648		1.829		1.342
14	0	6/16/2013	16:00	C1	Anal	carbohydrate	D-Mannitol	1.133		0.314		0.230
15	0	6/16/2013	16:00	C1	Anal	carbohydrate	D-Xylose	2.316		1.497		1.099
16	0	6/16/2013	16:00	C1	Anal	carbohydrate	D,L-?-Glycerol Phosphate	2.368		1.549		1.137
17	0	6/16/2013	16:00	C1	Anal	carbohydrate	Glucose-1-Phosphate	2.753		1.934		1.419
18	0	6/16/2013	16:00	C1	Anal	polymer	Glycogen	1.509		0.690		0.506
19	0	6/16/2013	16:00	C1	Anal	amino acid	Glycyl-L-Gglutamic Acid	2.688		1.869		1.372
20	0	6/16/2013	16:00	C1	Anal	carbohydrate	I-Erythritol	2.127		1.308		0.960
21	0	6/16/2013	16:00	C1	Anal	carboxylic & acetic acid	Itaconic Acid	1.952		1.133		0.831
22	0	6/16/2013	16:00	C1	Anal	amino acid	L-Arginine	2.993		2.174		1.595
23	0	6/16/2013	16:00	C1	Anal	amino acid	L-Asparagine	2.066		1.247		0.915
24	0	6/16/2013	16:00	C1	Anal	amino acid	L-Phenylalanine	1.61		0.791		0.580
25	0	6/16/2013	16:00	C1	Anal	amino acid	L-Serine	3.561		2.742		2.012
26	0	6/16/2013	16:00	C1	Anal	amino acid	L-Threonine	1.617		0.798		0.585
27	0	6/16/2013	16:00	C1	Anal	carbohydrate	N-Acetyl-D-Glucosamine	2.57		1.751		1.285
28	0	6/16/2013	16:00	C1	Anal	Amines/amides	Phenylethylamine	2.499		1.680		1.233
29	0	6/16/2013	16:00	C1	Anal	Amines/amides	Putrescine	1.774		0.955		0.701
30	0	6/16/2013	16:00	C1	Anal	carbohydrate	Pyruvic Acid Methyl Ester	2.966		2.147		1.576
31	0	6/16/2013	16:00	C1	Anal	Tween	Tween 40	1.206		0.387		0.284
32	0	6/16/2013	16:00	C1	Anal	Tween	Tween 80	3.135		2.316		1.700
33	0	6/16/2013	16:00	C1	Oral	polymer	?-Cyclodextrin	2.175	2.284	-0.109	5.672	-0.596
34	0	6/16/2013	16:00	C1	Oral	carbohydrate	?-D-Lactose	0.943		-1.341		-7.329
35	0	6/16/2013	16:00	C1	Oral	carboxylic & acetic acid	?-Hydroxybutyric Acid	0.494		-1.790		-9.783

Figure D7. Data arrangement in "AK Values" tab in Microsoft Excel 2010.

1. From the pivot table created, manually copy rows 5 through 35,being cautious to not copy the 'water' row, and paste the data into the 'AK Values' sheet in the column labeled 'Avg. OD590 (column H in this case) (Figure D7).
   IMPORTANT NOTE: Be sure to triple check the data you are inputting into the 'AK Values' sheet from the pivot table. For example, be sure to make sure the data you are pasting is for C1, region: anal, carbon source: cyclodextrin. Any mistakes during this process can have a detrimental or skewed effect on your data.
2. Return to the Pivot table and now only copy the single value in the row labeled 'water'. Paste this value into the column labeled 'Average Water Well.' You

may paste this once next to the first carbon source. In the column labeled Ai-Ao subtract the AVG OD of the carbon source from the baseline, water. You can do this quickly with the formula, =H2-I2. H2 is the carbon source you are looking at and I2 is the baseline for that region. The '$' holds that value constant. This allows you to drag the formula downward for all the carbon sources in that region, subtracting each carbon source from the single baseline value.

3. The next step is to create a sum of the 'Ai-Ao' column. Remember you must create a sum for each new region. (One sum value per region). Place this value into a separate column labeled 'Sum Ai-Ao'.
4. The AK value (Figure D8) can now be created using the formula, **=J2/ ((1/31)*K2).** Let us break this formula down:
   - J2 is the average optical density of a single carbon source minus the baseline, water.
   - The fraction 1/31 shows that you are looking at one carbon source, or one variable, out of 31 different variables.
   - K2 is the sum of all the carbon sources minus the baseline, water. Remember, '$' holds the sum value constant so that you can easily drag this formula downward for each region, preventing you from having to continuously type out the formula. For each new region, you must check that you have changed your 'Sum Ai-Ao' value.

$$\bar{A}_k = \frac{A_k - A_o}{\frac{1}{31}\sum_{i=1}^{31}(A_i - A_o)}$$

Figure D8. Formula to calculate AK value (as normalized microbial metabolic function) from OD data obtained from Biolog EcoPlate reading.

CREATING A DATA TABLE FOR USE IN R

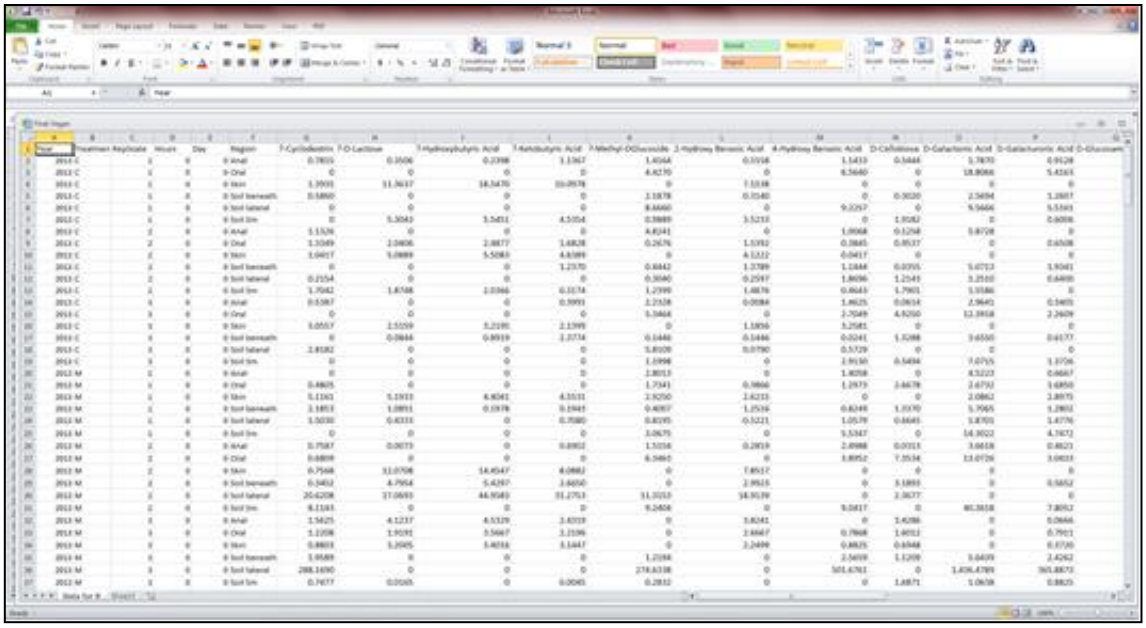


Figure D9. Data arrangement for analysis in R.

1. Create a new Excel file. This file will be used for R, therefore it is important the only data present in this file is the data to be analyzed (Figure D9).
2. Each covariate must contain its own column. Every space below the column must be utilized. In order for R to provide accurate analysis, every data point must be fully characterized. As seen in figure every row is filled with the appropriate data.
3. **IMPORTANT:** If one is using real world data, negative values are virtually meaningless. When calculating Ak values it is possible to obtain a multitude of negative values, therefore one must change these negative values to **zeros**. One can quickly do this in excel by selecting all numerical data, right clicking the selection, click 'Format Cells', click 'Number' tab, click 'Custom' category, and type #,##0.000;"0" in the dialogue box. There is another way by using the formula **=IF(A1<0,0,A1).** The later formula is the better one because it changes the negative value to true zero. Copy the whole data (CTRL A), then paste it in a new Excel sheet. Place the formula at a blank cell, and drag it to the left and to the bottom until all data has been covered.
4. All of your hard work is now ready to be used in R. Save your excel file that contains only the data to be analyzed. Use the 'Save as' function to save the data as a tab delimited .txt file. Upload this text file into the R statistical program, and begin your analysis! It is possible to upload Excel file in R using the library "xlsx".

BASIC R CODES USED FOR DATA ANALYSIS

```
#Upload the Excel file into R
library(xlsx)
op<- read.xlsx("C:/Users/Chong Chin/Desktop/Redo2013forRusethisforanalysis.xlsx",1)

#Upload packages into R
library(vegan)
library(ellipse)
library(ecodist)
library(BiodiversityR)

#Check Replicate Effect
year.com<-(op[,7:37])
year.env<-(op[,1:6])
year.M1<-adonis(year.com~Replicate, data=year.env, permutations = 999)
year.M1

#Run PERMANOVA analysis
year.com<-(op[,7:37])
year.env<-(op[,1:6])
year.com.dist<-vegdist(year.com, method="bray")
year.M1<-adonis(year.com~Hours*Treatment*Region, data=year.env, permutations = 999)
year.M1

##NMDS plot of Stress
yeartest<-nmds(year.com.dist, mindim=1, maxdim=5, nits=10)
stressyear<-yeartest$stress
plot(stressyear, main="Plot of stress for 2013, five dimensions max")

##NMDS Ordination Plot for Treatment
year.day=year.env[,2]
year.stress<-nmds(year.com.dist, mindim=3, maxdim=3, nits=100)
year.lowest.stress <- nmds.min(year.stress, dims=3)
year.frame.day=data.frame(year.day)
year.nmds.plot <- ordiplot(year.lowest.stress, type ="n", main="Three dimensions")
ordisymbol(year.nmds.plot, y = year.frame.day, factor="year.day", rainbow=T, col=env, legend=T)

#MRPP Analysis for Treatment
treatment.mrpp <- mrpp(year.com, op[,2], distance="bray")
treatment.mrpp

#ISA Analysis
library(labdsv)
year.com.m=as.matrix(year.com)
year.env.m=as.matrix(year.env)
year.is<-indval(year.com.m,year.env.m)
summary(year.is)
```

APPENDIX E

STAGES OF PIG CARRION DECOMPOSITION IN SUMMERS 2013 AND 2014

STAGES OF DECOMPOSITION (2013)

Figure E1. Stages of decomposition of pig carrion over time (Day 7, Day 21, and Day 90) according to treatments in summer 2013at Snook, Texas.

STAGES OF DECOMPOSITION (2014)

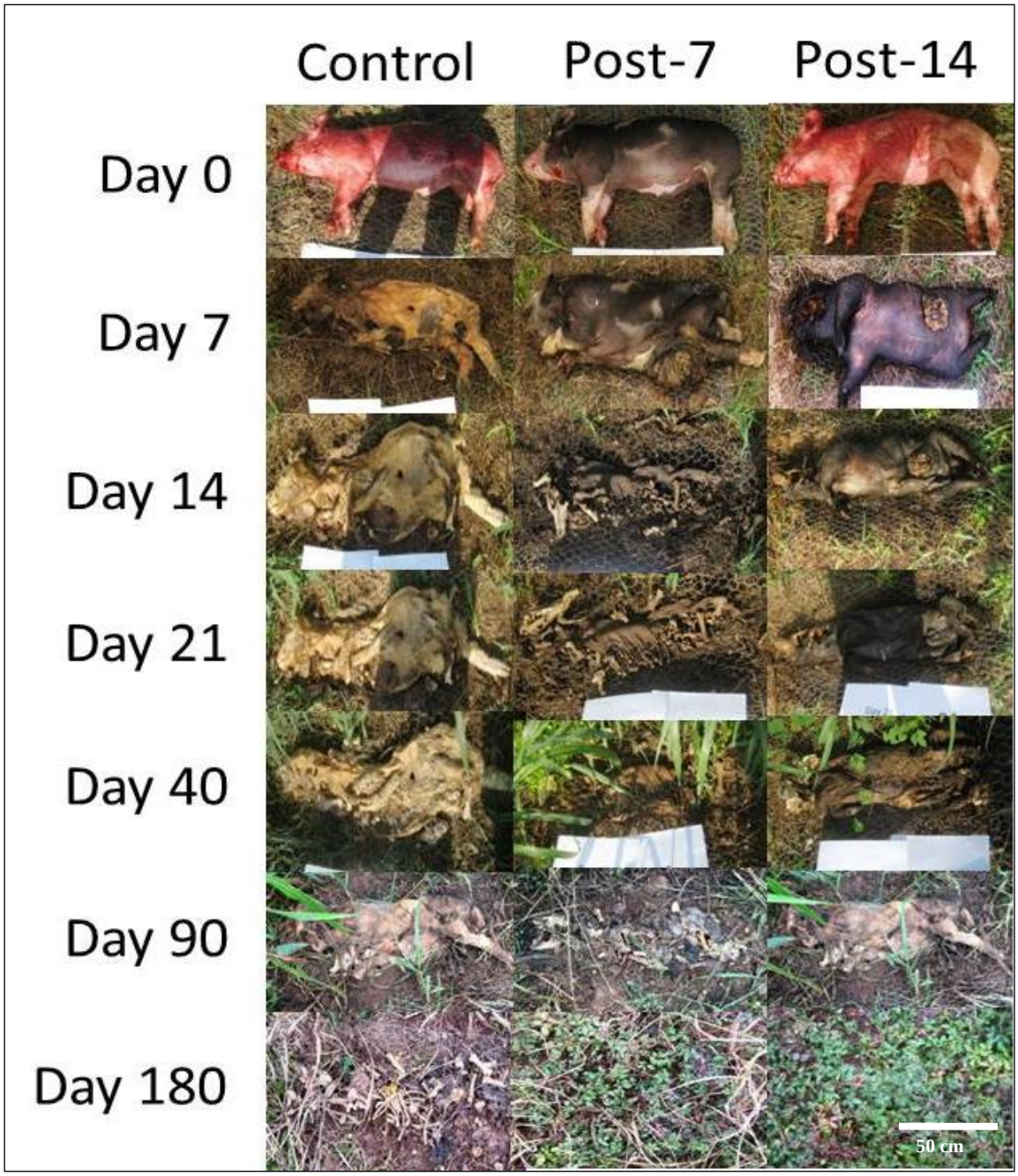


Figure E2. Stages of decomposition of pig carrion over time (Day 0, 7, 14, 21, 40, 90 and 180) according to treatments in summer 2014 at Snook, Texas.

APPENDIX F

MEAN CONCENTRATION OF SOIL NUTRIENTS ASSOCIATED WITH PIG CARRION IN SUMMER 2013 AND 2014

MEANS AND STANDARD DEVIATIONS OF SOIL NUTRIENTS IN 2013

Table F1. Soil chemistry at soil beneath the pig carcasses according to treatments in summer 2013 at Snook, Texas.

Soil Beneath												
Control												
Day	**pH**	**SD**	**Conductivity**	**SD**	**NO3-N**	**SD**	**NH4-N**	**SD**	**PO4-P**	**SD**	**H2O**	**SD**
-5	9.25	0.11	173.33	28.87	5.97	4.47	1.61	0.09	4.95	2.51	9	0
0	8.95	0.05	143.33	25.17	20.04	9.01	1.39	0.05	4.03	0.85	5.67	1.53
7	8.66	0.24	670.00	348.28	7.68	0.88	990.60	749.26	130.03	176.72	13.67	2.08
14	7.9	0.08	650.00	199.75	2.83	1.13	591.02	174.82	122.14	105.35	7.67	0.58
21	7.55	0.03	900.00	117.90	5.09	4.25	47541.78	6182.33	251.75	53.56	8.33	0.58
40	8.3	0.36	840.00	135.28	58.00	93.15	1189.58	265.90	497.38	238.79	22.33	1.53
90	6.74	0.18	886.67	200.33	711.91	121.62	483.63	167.18	136.05	40.12	8.67	1.53
180	7.47	0.25	193.33	102.14	135.76	176.33	4.11	4.74	57.84	36.10	22.33	2.52
Post-7												
Day	**pH**	**SD**	**Conductivity**	**SD**	**NO3-N**	**SD**	**NH4-N**	**SD**	**PO4-P**	**SD**	**H2O**	**SD**
0	10.02	0.74	163.33	23.09	11.32	1.65	2.06	0.08	2.7	0.88	5.33	1.53
7	8.19	0.31	760	365.92	2.84	1.42	1336.12	1096.12	145.19	98.63	22	7.21
14	8.72	0.29	1346.67	422.53	7.81	2.53	2831.05	1430.06	242.81	91.25	21	8.54
21	8.74	0.35	1200	420	7.08	1.35	2418.6	1386.76	333.82	70.84	14	6.08
40	8.64	0.43	1123.33	420.04	58.64	89.65	2421.02	1327.5	415.97	70.59	24.67	1.53
90	6.74	0.18	1166.67	297.04	751.68	494.06	1127.58	419.71	192.93	65.81	8	1
180	7.46	0.16	156.67	20.82	61.73	8.62	1.83	0.57	86.45	21.88	21.67	2.08
Post-14												
Day	**pH**	**SD**	**Conductivity**	**SD**	**NO3-N**	**SD**	**NH4-N**	**SD**	**PO4-P**	**SD**	**H2O**	**SD**
0	9.54	0.66	123.33	28.87	12.17	5.09	1.66	0.3	5.22	0.49	7.33	1.15
7	8.58	0.39	886.67	432.47	3.27	1.49	1924.51	1327.31	141.28	42.77	25	1
14	8.75	0.37	1823.33	468.76	4.4	1.49	103409.8	21521.02	495.54	130.33	25.33	6.43
21	8.78	0.17	2116.67	813.65	5.83	1.77	4625.13	2260.84	401.59	26.89	20.00	6.08
40	7.95	0.23	1023.33	269.51	4.50	0.82	1914.63	548.21	576.28	167.78	20.67	1.53
90	7.04	0.12	1116.67	566.07	330.93	133.81	1271.39	1082.10	370.07	366.64	8.33	2.08
180	7.7	0.19	143.33	49.33	80.42	49.78	2.28	1.75	43.77	39.25	18.33	2.52

Table F2. Soil chemistry at soil lateral of the pig carcasses according to treatments in summer 2013 at Snook, Texas.

Soil Lateral												
Control												
Day	**pH**	**SD**	**Conductivity**	**SD**	**NO3-N**	**SD**	**NH4-N**	**SD**	**PO4-P**	**SD**	**H2O**	**SD**
-5	9.25	0.11	173.33	28.87	5.97	4.47	1.61	0.09	4.95	2.51	9	0
0	9.20	0.33	116.67	5.77	15.71	6.42	1.45	0.16	3.31	0.25	5.67	0.58
7	8.68	0.14	220.00	43.59	8.20	2.71	108.65	51.86	11.38	10.80	8.00	2.00
14	7.77	0.04	326.67	86.22	3.27	1.24	175.95	81.20	14.37	9.17	5.67	1.53
21	7.56	0.12	273.33	75.06	10.65	5.73	136.40	84.97	11.39	5.18	5.33	0.58
40	7.91	0.17	190.00	95.39	122.04	122.25	113.80	97.60	51.68	52.61	13.33	2.89
90	7.02	0.10	250.00	85.44	182.63	72.18	65.25	23.37	18.98	3.28	7.33	1.15
180	7.63	0.19	100.00	10.00	40.92	36.63	1.75	0.53	14.07	6.46	21.33	3.06
Post-7												
Day	**pH**	**SD**	**Conductivity**	**SD**	**NO3-N**	**SD**	**NH4-N**	**SD**	**PO4-P**	**SD**	**H2O**	**SD**
0	9.82	0.62	200.00	45.83	10.81	1.86	2.31	0.55	2.61	0.75	7.67	1.15
7	8.21	0.08	623.33	889.12	8.00	5.22	1323.12	2151.18	83.53	128.07	14.33	9.29
14	8.30	0.10	140.00	0.00	14.30	4.44	106.44	66.07	8.91	3.61	11.00	7.21
21	8.06	0.10	273.33	111.50	12.10	9.62	153.68	70.90	39.28	50.81	6.00	1.00
40	7.97	0.15	293.33	145.03	235.80	160.19	236.25	92.86	51.35	38.48	15.67	1.53
90	6.72	0.04	246.67	70.95	158.79	88.25	88.00	62.89	17.54	10.73	7.00	1.00
180	7.59	0.08	90.00	10.00	24.75	4.62	1.48	0.27	21.39	5.35	19.33	2.31
Post-14												
Day	**pH**	**SD**	**Conductivity**	**SD**	**NO3-N**	**SD**	**NH4-N**	**SD**	**PO4-P**	**SD**	**H2O**	**SD**
0	9.38	0.38	113.33	15.28	12.85	4.82	1.63	0.21	4.84	0.89	6.67	1.53
7	8.44	0.39	266.67	202.32	10.59	9.56	210.33	262.18	67.87	108.92	12.00	3.00
14	7.96	0.21	340.00	100.00	3.93	2.72	343.19	113.56	73.01	48.83	9.00	1.73
21	8.12	0.03	300.00	141.07	46.83	48.58	525.83	250.35	48.87	18.57	7.00	1.00
40	7.24	0.88	280.00	65.57	111.83	64.12	228.76	163.39	79.03	91.26	13.00	3.61
90	6.99	0.26	246.67	141.54	191.44	197.81	136.60	64.89	22.69	9.47	9.33	2.52
180	7.77	0.13	106.67	20.82	48.48	48.83	1.52	0.57	19.09	6.70	17.67	3.06

Table F3. Soil chemistry at soil 5 m from the pig carcasses according to treatments in summer 2013 at Snook, Texas.

Soil 5 m												
Control												
Day	**pH**	**SD**	**Conductivity**	**SD**	**NO3-N**	**SD**	**NH4-N**	**SD**	**PO4-P**	**SD**	**H2O**	**SD**
-5	9.25	0.11	173.33	28.87	5.97	4.47	1.61	0.09	4.95	2.51	9	0
0	9.13	0.26	156.67	37.86	22.22	9.23	1.61	0.37	2.73	1.29	3.67	0.58
7	8.76	0.25	183.33	50.33	24.16	21.10	11.11	4.04	2.36	0.60	6.00	1.00
14	7.93	0.17	150.00	20.00	13.43	3.41	26.34	10.00	3.82	1.57	3.00	1.00
21	7.65	0.08	156.67	15.28	24.53	24.87	15.52	5.53	2.25	0.24	3.33	0.58
40	8.00	0.01	96.67	5.77	24.35	17.83	7.17	3.86	4.91	1.91	6.00	3.00
90	7.09	0.22	113.33	5.77	32.68	16.14	15.86	16.14	7.05	4.15	5.33	1.15
180	7.62	0.08	76.67	11.55	12.08	2.68	1.42	0.15	4.86	1.17	15.67	3.06
Post-7												
Day	**pH**	**SD**	**Conductivity**	**SD**	**NO3-N**	**SD**	**NH4-N**	**SD**	**PO4-P**	**SD**	**H2O**	**SD**
0	9.66	0.49	180.00	50.00	13.26	1.67	2.30	0.51	1.92	0.52	5.33	2.31
7	8.00	0.15	146.67	55.08	21.42	14.13	35.61	44.88	4.86	3.56	6.33	0.58
14	8.17	0.05	153.33	35.12	20.44	4.08	12.26	4.84	2.66	0.31	3.33	0.58
21	8.24	0.14	156.67	5.77	15.61	1.53	91.09	136.45	10.94	14.35	2.67	0.58
40	8.05	0.08	106.67	20.82	40.41	24.74	27.78	23.22	7.47	3.93	7.33	0.58
90	6.83	0.18	163.33	30.55	25.94	9.46	8.41	4.43	5.46	0.36	6.67	2.31
180	7.69	0.21	83.33	5.77	10.01	4.23	1.26	0.02	5.62	0.93	19.00	6.24
Post-14												
Day	**pH**	**SD**	**Conductivity**	**SD**	**NO3-N**	**SD**	**NH4-N**	**SD**	**PO4-P**	**SD**	**H2O**	**SD**
0	9.28	0.40	133.33	5.77	11.99	1.91	1.50	0.05	4.04	1.24	4.67	1.15
7	8.41	0.15	126.67	15.28	13.32	3.36	33.02	42.29	11.37	15.58	5.33	0.58
14	7.96	0.23	136.67	25.17	11.40	4.72	48.85	16.70	10.11	5.08	3.67	1.15
21	8.08	0.07	156.67	25.17	14.34	9.75	57.45	26.31	5.96	1.24	3.67	0.58
40	6.64	0.49	110.00	17.32	18.50	4.99	32.39	34.18	11.38	8.17	9.00	3.00
90	7.23	0.11	140.00	17.32	29.22	6.45	39.02	49.39	13.81	15.31	6.33	1.53
180	7.77	0.16	93.33	15.28	12.59	6.88	1.58	0.39	8.83	4.60	19.00	3.61

MEANS AND STANDARD DEVIATIONS OF SOIL NUTRIENTS IN 2014

Table F4. Soil chemistry at soil beneath the pig carcasses according to treatments in summer 2014 at Snook, Texas.

Soil Beneath																
Control																
Day	**pH**	**SD**	**Cond**	**SD**	**NO3**	**SD**	**NH4**	**SD**	**PO4**	**SD**	**NPOC**	**SD**	**TN**	**SD**	**H2O**	**SD**
-5	7.06	0.06	140.00	43.59	9.87	2.62	2.98	0.41	7.81	3.12	125.70	48.08	14.69	5.16	23.00	2.65
0	7.09	0.26	140.00	10.00	13.88	5.35	2.05	0.41	9.76	3.16	116.01	10.47	19.11	5.06	19.00	2.00
7	7.75	0.35	966.67	292.80	12.02	8.29	85.73	18.25	7.16	4.14	4695.35	2208.56	1763.05	596.16	18.00	0.00
14	8.53	0.23	640.00	170.88	53.98	41.95	701.65	144.67	421.61	438.78	721.86	501.41	744.20	222.34	21.67	2.52
21	8.23	0.32	673.33	104.08	271.86	70.27	693.23	357.38	249.71	193.71	822.20	325.69	948.95	346.09	21.00	3.00
40	7.36	0.13	503.33	124.23	425.91	163.08	198.78	169.24	87.73	29.76	315.38	84.45	562.80	265.13	20.00	1.00
90	7.86	0.29	350.00	157.16	290.25	177.36	98.59	137.90	37.50	22.46	269.18	115.15	395.50	235.47	24.00	2.00
180	8.21	0.05	160.00	43.59	90.54	54.95	5.10	0.04	29.12	8.15	160.70	36.76	109.45	64.46	25.00	1.00
Post-7																
Day	**pH**	**SD**	**Cond**	**SD**	**NO3**	**SD**	**NH4**	**SD**	**PO4**	**SD**	**NPOC**	**SD**	**TN**	**SD**	**H2O**	**SD**
0	7.09	0.26	140.00	10.00	13.88	5.35	2.05	0.41	9.76	3.16	116.01	10.47	19.11	5.06	19.00	2.00
7	7.75	0.35	966.67	292.80	12.02	8.29	85.73	18.25	7.16	4.14	4695.35	2208.56	1763.05	596.16	18.00	0.00
14	8.53	0.23	640.00	170.88	53.98	41.95	701.65	144.67	421.61	438.78	721.86	501.41	744.20	222.34	21.67	2.52
21	8.23	0.32	673.33	104.08	271.86	70.27	693.23	357.38	249.71	193.71	822.20	325.69	948.95	346.09	21.00	3.00
40	7.36	0.13	503.33	124.23	425.91	163.08	198.78	169.24	87.73	29.76	315.38	84.45	562.80	265.13	20.00	1.00
90	7.86	0.29	350.00	157.16	290.25	177.36	98.59	137.90	37.50	22.46	269.18	115.15	395.50	235.47	24.00	2.00
180	8.21	0.05	160.00	43.59	90.54	54.95	5.10	0.04	29.12	8.15	160.70	36.76	109.45	64.46	25.00	1.00
Post-14																
Day	**pH**	**SD**	**Cond**	**SD**	**NO3**	**SD**	**NH4**	**SD**	**PO4**	**SD**	**NPOC**	**SD**	**TN**	**SD**	**H2O**	**SD**
0	7.09	0.26	140.00	10.00	13.88	5.35	2.05	0.41	9.76	3.16	116.01	10.47	19.11	5.06	19.00	2.00
7	7.75	0.35	966.67	292.80	12.02	8.29	85.73	18.25	7.16	4.14	4695.35	2208.56	1763.05	596.16	18.00	0.00
14	8.53	0.23	640.00	170.88	53.98	41.95	701.65	144.67	421.61	438.78	721.86	501.41	744.20	222.34	21.67	2.52
21	8.23	0.32	673.33	104.08	271.86	70.27	693.23	357.38	249.71	193.71	822.20	325.69	948.95	346.09	21.00	3.00
40	7.36	0.13	503.33	124.23	425.91	163.08	198.78	169.24	87.73	29.76	315.38	84.45	562.80	265.13	20.00	1.00
90	7.86	0.29	350.00	157.16	290.25	177.36	98.59	137.90	37.50	22.46	269.18	115.15	395.50	235.47	24.00	2.00
180	8.21	0.05	160.00	43.59	90.54	54.95	5.10	0.04	29.12	8.15	160.70	36.76	109.45	64.46	25.00	1.00

Table F5. Soil chemistry at soil lateral of the pig carcasses according to treatments in summer 2014 at Snook, Texas.

Soil Lateral																
Control																
Day	**pH**	**SD**	**Cond**	**SD**	**NO3**	**SD**	**NH4**	**SD**	**PO4**	**SD**	**NPOC**	**SD**	**TN**	**SD**	**H2O**	**SD**
-5	7.06	0.06	140.00	43.59	9.87	2.62	2.98	0.41	7.81	3.12	125.70	48.08	14.69	5.16	23.00	2.65
0	7.16	0.18	146.67	5.77	13.34	3.80	2.51	0.41	10.66	3.34	133.74	6.16	20.36	3.94	19.33	2.08
7	7.37	0.10	696.67	145.72	9.94	12.94	42.83	27.96	3.41	0.88	3467.30	963.97	1222.38	405.47	13.33	2.89
14	8.10	0.30	320.00	60.00	108.35	49.87	217.83	102.13	41.53	19.10	245.37	39.83	318.32	118.93	22.33	1.53
21	8.10	0.00	190.00	60.00	137.04	65.71	44.90	22.67	24.77	12.08	173.16	19.00	178.62	83.60	20.33	1.53
40	7.52	0.39	526.67	427.12	356.43	314.25	157.41	227.11	94.95	121.28	355.30	344.76	470.72	479.59	17.33	2.31
90	7.97	0.37	206.67	72.34	124.94	67.00	13.39	5.75	25.23	16.67	189.48	81.71	148.80	81.29	26.00	2.00
180	8.36	0.08	123.33	32.15	44.08	43.19	5.20	0.56	17.31	2.78	119.47	13.92	45.86	38.43	22.67	0.58
Post-7																
Day	**pH**	**SD**	**Cond**	**SD**	**NO3**	**SD**	**NH4**	**SD**	**PO4**	**SD**	**NPOC**	**SD**	**TN**	**SD**	**H2O**	**SD**
0	7.33	0.20	123.33	20.82	11.49	1.99	2.36	0.76	5.55	0.58	110.24	15.79	14.75	1.42	18.33	3.51
7	7.71	0.37	150.00	17.32	18.60	1.47	69.72	34.07	9.71	2.66	251.98	92.27	92.35	30.51	17.33	2.89
14	7.90	0.10	336.67	132.04	25.78	20.71	423.60	234.85	53.57	44.85	449.74	159.96	416.61	216.94	20.67	1.15
21	7.84	0.37	346.67	85.05	112.54	57.81	189.38	156.32	30.09	18.60	306.46	175.95	273.02	101.00	17.00	0.00
40	7.71	0.05	300.00	55.68	181.64	48.80	47.53	32.58	30.20	12.86	189.55	60.54	221.06	66.70	16.67	0.58
90	7.87	0.33	383.33	289.19	340.84	314.47	213.67	301.42	49.06	55.91	271.25	192.26	526.81	563.55	23.33	0.58
180	8.21	0.32	140.00	69.28	36.10	29.01	5.43	0.40	16.85	13.69	121.62	44.03	42.13	34.51	22.00	3.00
Post-14																
Day	**pH**	**SD**	**Cond**	**SD**	**NO3**	**SD**	**NH4**	**SD**	**PO4**	**SD**	**NPOC**	**SD**	**TN**	**SD**	**H2O**	**SD**
0	7.64	0.05	133.33	5.77	16.88	0.94	3.83	1.60	9.13	3.01	121.99	17.74	21.82	2.16	22.67	1.15
7	7.85	0.25	246.67	126.62	29.58	12.44	320.34	125.18	35.33	14.45	495.01	179.55	316.62	126.97	17.67	1.53
14	8.03	0.12	153.33	11.55	25.02	18.08	102.67	26.11	27.93	4.10	350.57	128.76	153.64	34.44	22.33	1.53
21	7.65	0.35	363.33	113.72	149.68	78.39	92.65	97.53	20.63	6.52	200.26	61.47	213.99	139.10	19.00	0.00
40	7.69	0.21	273.33	101.16	146.70	82.10	22.29	9.45	19.20	4.83	172.27	30.04	174.41	87.04	16.00	1.00
90	7.91	0.26	340.00	168.23	316.20	243.07	129.89	139.75	37.37	42.84	262.93	133.98	425.15	312.54	27.33	4.04
180	8.15	0.36	166.67	70.24	60.68	41.29	6.71	2.34	36.54	23.24	153.06	50.74	75.24	50.57	26.33	2.52

Table F6. Soil chemistry at soil 5 m from the pig carcasses according to treatments in summer 2014 at Snook, Texas.

Soil 5 M																
Control																
Day	**pH**	**SD**	**Cond**	**SD**	**NO3**	**SD**	**NH4**	**SD**	**PO4**	**SD**	**NPOC**	**SD**	**TN**	**SD**	**H2O**	**SD**
-5	7.06	0.06	140.00	43.59	9.87	2.62	2.98	0.41	7.81	3.12	125.70	48.08	14.69	5.16	23.00	2.65
0	7.26	0.11	136.67	37.86	17.22	7.69	2.15	0.97	11.72	7.06	122.34	31.07	23.69	11.24	17.67	3.51
7	7.34	0.09	166.67	15.28	24.92	6.78	30.08	4.62	14.32	4.40	241.62	43.65	78.84	16.54	9.00	1.73
14	7.80	0.30	120.00	17.32	14.66	4.13	15.75	7.02	15.62	5.97	158.53	21.79	38.67	4.15	19.00	2.00
21	8.23	0.12	120.00	10.00	20.74	5.91	7.26	1.87	16.14	6.30	170.44	50.84	33.00	7.27	20.33	3.21
40	7.88	0.08	180.00	62.45	25.70	8.73	13.34	2.45	16.41	8.51	147.84	41.82	44.66	11.07	15.67	4.04
90	8.30	0.22	106.67	5.77	6.78	3.93	3.63	0.73	8.12	1.93	110.95	9.54	13.93	5.83	26.67	1.53
180	8.28	0.27	136.67	63.51	10.80	6.30	2.21	0.09	14.75	10.42	116.62	2.98	16.90	7.34	26.67	2.08
Post-7																
Day	**pH**	**SD**	**Cond**	**SD**	**NO3**	**SD**	**NH4**	**SD**	**PO4**	**SD**	**NPOC**	**SD**	**TN**	**SD**	**H2O**	**SD**
0	7.35	0.20	120.00	0.00	12.17	3.37	2.03	0.23	4.66	2.24	102.49	8.26	15.65	3.08	13.67	1.53
7	7.50	0.29	170.00	26.46	14.50	6.46	10.48	3.84	8.32	6.65	141.22	32.41	28.43	6.80	9.00	1.00
14	8.13	0.06	113.33	11.55	13.79	5.74	26.24	10.57	8.23	2.92	125.29	6.25	43.19	14.54	16.33	2.52
21	7.88	0.12	166.67	37.86	13.30	4.17	19.86	16.21	9.32	2.17	146.17	36.45	35.87	19.36	17.67	1.15
40	8.15	0.11	110.00	0.00	16.08	5.06	13.57	10.30	8.37	3.31	116.82	21.55	34.07	17.15	13.33	0.58
90	8.29	0.07	93.33	5.77	14.87	7.21	9.57	8.23	7.37	5.45	122.32	8.12	29.17	15.50	25.33	0.58
180	8.37	0.12	106.67	37.86	8.13	1.66	3.12	1.48	8.14	4.85	102.21	13.58	12.45	3.44	23.67	1.53
Post-14																
Day	**pH**	**SD**	**Cond**	**SD**	**NO3**	**SD**	**NH4**	**SD**	**PO4**	**SD**	**NPOC**	**SD**	**TN**	**SD**	**H2O**	**SD**
0	7.67	0.07	133.33	5.77	13.65	3.46	2.47	0.26	7.35	1.58	97.83	8.70	17.21	3.78	13.00	5.57
7	7.62	0.19	163.33	5.77	19.00	6.44	21.80	7.69	10.82	3.23	159.22	16.94	46.77	12.27	10.00	2.65
14	8.17	0.06	130.00	10.00	12.36	3.22	12.73	2.24	9.95	1.19	124.85	15.72	29.10	6.60	19.00	2.00
21	7.75	0.13	190.00	90.00	19.69	6.90	10.68	9.70	11.37	4.98	146.60	31.40	32.82	6.56	18.00	0.00
40	8.04	0.08	126.67	5.77	9.37	2.54	16.07	21.50	11.97	6.96	132.20	51.70	32.92	26.25	15.00	1.00
90	8.24	0.11	106.67	5.77	9.88	3.39	9.61	4.53	8.58	3.49	120.62	12.69	27.51	13.27	26.67	2.08
180	8.40	0.31	110.00	43.59	11.98	5.25	4.20	3.66	14.90	11.01	104.06	6.46	16.47	9.20	26.33	2.31

Table F6. Soil chemistry at the upper slope of the field site according to sampling day in summer 2014 at Snook, Texas.

Upper Slope Soil																
Upper slope																
Day	pH	SD	Cond	SD	NO3	SD	NH4	SD	PO4	SD	NPOC	SD	TN	SD	H2O	SD
-5	8.03	0.13	100.00	10.00	13.35	5.89	1.80	0.30	2.33	0.34	102.40	10.98	16.44	5.90	18.40	2.70
0	7.98	0.02	110.00	12.25	16.98	8.09	2.01	0.38	2.63	0.63	104.57	3.00	20.81	8.05	16.80	1.30
7	7.99	0.05	114.00	20.74	16.65	6.77	1.74	0.39	2.12	0.22	117.82	34.23	20.00	6.82	14.00	3.39
14	8.07	0.07	110.00	15.81	18.46	10.98	1.65	0.35	2.59	0.47	119.54	11.85	22.69	10.51	15.80	2.77
21	8.06	0.03	112.00	10.95	24.40	14.87	1.59	0.40	2.92	0.72	127.47	19.49	28.53	14.85	15.20	2.77
40	8.22	0.04	86.00	15.17	13.52	7.76	1.90	0.33	2.37	0.22	116.33	18.62	18.91	8.49	14.40	1.95
90	6.50	0.37	96.00	8.94	9.67	2.46	1.38	0.05	3.60	1.14	112.17	32.78	12.95	2.57	27.00	2.92
180	8.29	0.07	108.00	19.24	10.32	2.88	2.13	0.19	2.93	0.41	118.98	20.33	17.13	3.80	23.00	1.73

APPENDIX G

LIST OF ARTHROPOD FUNCTIONS ACCORDING TO ORDER/FAMILY/GENUS

Order/Family/Genus	**Function**
Acrididae	Herbivore
Agelenidae	Predator/Parasite
Agromyzidae	Herbivore
Aleocharinae	Predator/Parasite or Fungivore
Andrenidae	Nectarivores
Anotylus sp.	Detritivore
Anthicidae	Predator/Parasite
Anthocoridae	Predator/Parasite
Aphididae	Herbivore
Araneidae	Predator/Parasite
Armadillidiidae	Detritivore
Asilidae	Predator/Parasite
Asteiidae	Fungivore
Ataeneus	Detritivore
Baetidae	Do not feed
Berytidae	Nectarivores
Blattellidae	Detritivore
Blattidae	Detritivore
Bourlettilliedae	Detritivore
Braconidae	Predator/Parasite
Bostrichidae	Herbivore
Calliphoridae	Necrophagous
Cantharidae	Nectarivores
Carabidae	Predator/Parasite
Cecidomyiidae	Herbivore
Cerambycidae	Herbivore
Ceraphronidae	Predator/Parasite
Ceratopogonidae	**Hematophagous** or Predator/Parasite or Nectarivores
Cercopidae	Herbivore
Chironomidae	Do not feed
Chloropidae	**Nectarivores** or Herbivore
Chrysididae	Predator/Parasite

Order/Family/Genus	Function
Chrysomelidae	Herbivore
Chrysopidae	Nectarivores
Cicadellidae	Herbivore
Cleridae	**Predator/Parasite** or Detritivore
Coccidoidae	Herbivore
Coccinellidae	Predator/Parasite
Collectidae	Nectarivores
Coniopterygidae	Predator/Parasite
Corioxenidae	Predator/Parasite
Corylophidae	Fungivore
Crytolestes	Herbivore
Culicidae	Female= **Hematophagous**, Male= Nectarivores
Curculionidae	Herbivore
Curculionidae: Baridinae	Herbivore
Cydnidae	Herbivore
Cynipidae	Predator/Parasite (for plant)
Dermestidae	Necrophagous
Diapriidae	Predator/Parasite
Dolichopodidae	Predator/Parasite
Drosophilidae	Nectarivores
Dryinidae	Predator/Parasite
Elateridae	Detritivore
Elateridae larvae	Predator/Parasites
Entomobryidae	Detritivore
Eosentomidae	Fungivore
Ephydridae	Herbivore
Erotylidae	Fungivore
Galumnidae	Detritivore
Fanniidae	Necrophagous
Formicidae: *Strumigenys* sp.	Predator/Parasite
Formicidae	**Predator/Parasite**, Alate (male) = Nectarivores
Hemiptera nymph	Predator/Parasite
Halictidae	Herbivore
Histeridae	Predator/Parasite
Hybotidae	Predator/Parasite

Order/Family/Genus	**Function**
Hypogastruridae	Detritivore
Ichneumonidae	Predator/Parasite
Isotomidae	Detritivore
Japygidae	Predator/Parasite
Lachesillidae	Detritivore
Laemophloeidae	Fungivore
Lasiochilidae	Predator/Parasite
Latridiidae	Fungivore
Lauxaniidae	Fungivore
Liposcelididae	Detritivore
Megachilidae	Nectarivores
Megaspilidae	Predator/Parasite
Meloidae	Herbivore
Membracidae	Herbivore
Milichiidae	Predator/Parasite
Miridae	Herbivore
Monomorium	Predator/Parasite
Monotomidae	Herbivore
Mordellidae	Herbivore
Muscidae	Detritivore
Musca domestica	Detritivore
Mymaridae	Predator/Parasite
Noctuidae	Nectarivores
Nitidulidae	**Necrophagous** or Detritivore
Oedemeridae	Herbivore
Hydrotaea sp.	Necrophagous
Oxyopidae	Predator/Parasite
Paederinae	Predator/Parasite
Pentatomidae	Herbivore
Perilampidae	Predator/Parasite
Phalacridae	Fungivore
Philodromidae	Predator/Parasite
Phlaeothripidae	Herbivore
Phoridae	Necrophagous
Phyllophaga	Herbivore

Order/Family/Genus	Function
Phylloxeridae	Herbivore
Piophilidae	Necrophagous
Pipunculidae	**Nectarivores**, Larva = Predator/Parasite
Platygastridae	Predator/Parasite
Pogonomyrmex	Herbivore
Psocidae	Detritivore
Psocoptera	Detritivore
Psychodidae	Detritivore
Psyllidae	Herbivore
Ptiliidae	Detritivore
Pyralidae	Herbivore
Reduviidae	Predator/Parasite
Rhyparochromidae	Herbivore
Rophalidae	Herbivore
Salticidae	Predator/Parasite
Sarcophagidae	Necrophagous
Scarabaeidae	Detritivore
Scatopsidae	**Detritivore** or Nectarivores
Scelionidae	Predator/Parasite
Sciaridae	Fungivore
Sepsidae	Detritivore
Silphidae	Necrophagous
Silvanidae	Fungivore
Simuliidae	Female= **Hematophagous**, Male= Nectarivores
Sminthuridae	Detritivore
Solenopsis invicta	Predator/Parasite
Sphaeroceridae	Detritivore
Sphecidae	Predator/Parasite
Staphylinidae	Predator/Parasite
Stenopsocidae	Detritivore
Stratiomyidae	Detritivore
Syrphidae	Nectarivores
Tabanidae	Hematophagous
Tachinidae	Predator/Parasite
Tenebrionidae	Detritivore

Order/Family/Genus	Function
Tenthredinidae	**Nectarivores** or Herbivore
Tephritidae	Herbivore
Tettigoniidae	Herbivore
Tetrachynidae	Herbivore
Tetragnathidae	Predator/Parasite
Tineidae	Herbivore
Tingidae	Herbivore
Therevidae	Nectarivores; Predator/Parasite (Larva)
Thomisidae	Predator/Parasite
Thripidae	Herbivore
Trichogrammatidae	Predator/Parasite
Trogidae	Necrophagous
Uloboridae	Predator/Parasite
Ulidiidae	**Herbivore** or Nectarivores
Vespidae	Predator/Parasite

Bold characters = Primary arthropod function to be considered during data entry.

Arthropod functional group classifications and descriptions used in the present study:

Herbivore= Plant feeder, stem borer, sap feeder, xylem feeder, phloem feeder.
Predator/Parasite= Hunts for other insects or arthropods, kill the host, parasitize either outside or inside the host, or infects plant to produce galls.
Nectarivores= Feeds on flower nectar, plant juice that flows from the plant.
Detritivore= Feeds on decaying organic plant or wood matter.
Necrophagous = Feeds on necrotic tissues or decompositional fluid on carrion.
Fungivore = Feeds on fungi, yeast, and spore.
Haematophagous= Feeds on blood by sucking or piercing.
Do not feed= Do not possess functional mouthpart to feed on anything.

APPENDIX H

LIST OF MITE SPECIES RECOVERED FROM SOIL SAMPLES IN SUMMERS 2013 AND 2014 AT SNOOK, TEXAS (EXCLUDING PHORETIC MITE SPECIES)

No.	**Family**	**Genus and species**
1	Nanorchestidae	*Speleochestes* sp.
2	Blattisociidae	*Blattisocius* sp.
		Lasioseius sp.
3	Laelapidae	*Euandrolaelaps* sp.
		Ololaelaps sp.
		Holastaspis sp.
		Laelaspis sp.
		Pseudoparasitus sp.
		Gaeolaelaps sp.
4	Ameroseiidae	*Ameroseius* sp.
		Ameroseiella sp.
5	Acaridae	***Sancassania* n. sp. A**
		Sancassania sp. B
6	Histiostomatidae	*Hexanoetus* n. sp.
7	Melicharidae	*Proctolaelaps* sp.
8	Uropodidae	*Discourella* sp.
9	Parasitidae	*Parasitus* sp.
10	Eviphididae	*Alliphis* sp.
11	Cunaxidae	*Bonzia* sp.
12	Euphthiracaridae	*Rhysotritia* sp.
13	Erythraeidae	*Balaustium* sp.
14	Cosmochthoniidae	*Cosmochthonius* sp.
15	Epilohmanniidae	*Epilohmannia* sp.
16	Hypochthoniidae	*Eohypochthonius* sp.
17	Tetranychidae	*Tetranychus* sp.
18	Ascidae	*Gammaselloides* sp.
		Asca sp.
19	Teneriffiidae	*Parateneriffia* sp.
20	Scutacaridae	*Scutacarus* sp.
		Imparipes sp.
21	Cryptognathidae	*Cryptognathus* sp.

Bold characters = possibly species new to science (Dr. Barry O'Connor, personal communication).

APPENDIX I

LIST OF ACARI FAMILIES WITH ECOLOGICAL FUNCTIONS

No	Family of Acari	Function
1	Acaridae	Detritivore
2	Adamystidae	Predator/Parasite
3	Ameroseiidae	Fungivore
4	Anystidae	Predator/Parasite
5	Ascidae	Predator/Parasite
6	Bdellidae	Predator/Parasite
7	Caligonellidae	Predator/Parasite
8	Cunaxidae	Predator/Parasite
9	Cryptognathidae	Herbivore
10	Digamasellidae	Predator/Parasite
11	Ereynetidae	Predator/Parasite
12	Erythraeoidea	Predator/Parasite
13	Eupodoidea	Predator/Parasite
14	Eviphididae	Predator/Parasite
15	Histiostomatidae	Detritivore
16	Laelapidae	Predator/Parasite
17	Macrochelidae	Predator/Parasite
18	Melicharidae	Predator/Parasite
19	Nanorchestidae	Fungivore / Herbivore
20	Oribatida	Detritivore
21	Parasitidae	Predator/Parasite
22	Phytoseiidae	Predator/Parasite
23	Pygmephoridae	Fungivore
24	Pygmephoroidea	Fungivore
25	Rhagidiidae	Predator/Parasite
26	Scutacaridae	Fungivore
27	Smaridiidae	Predator/Parasite
28	Tenerifiidae	Predator/Parasite
29	Tetranychoidea	Herbivore
30	Uropodidae	Detritivore

APPENDIX J

PICTORIAL KEY OF SARCOPHAGIDAE ASSOCIATED WITH PIG CARRION

COLLECTED AT SNOOK, TEXAS IN SUMMERS 2013 AND 2014

(Sarcophagid specimens and pictorial keys have been examined by Dr. Gregory Dahlem, Northern Kentucky University)

1- Rows of frontal bristles parallel or slightly diverging towards lunule (Fig. 1), with orangish tegula……...…….2

- Rows of frontal bristles strongly diverging at level of lunule (Fig. 2)……..……4

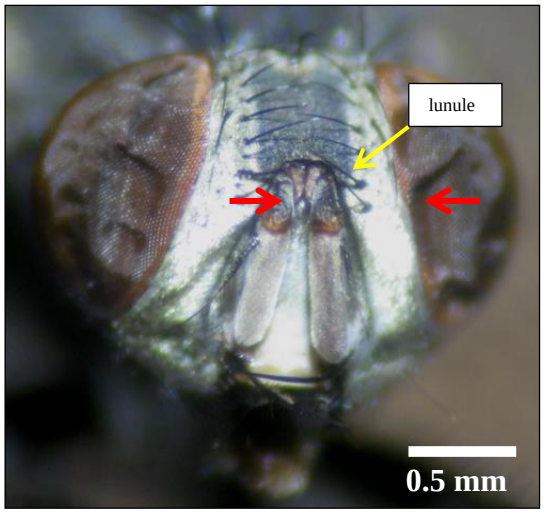


Fig. 1. Frontal bristles parallel towards lunule

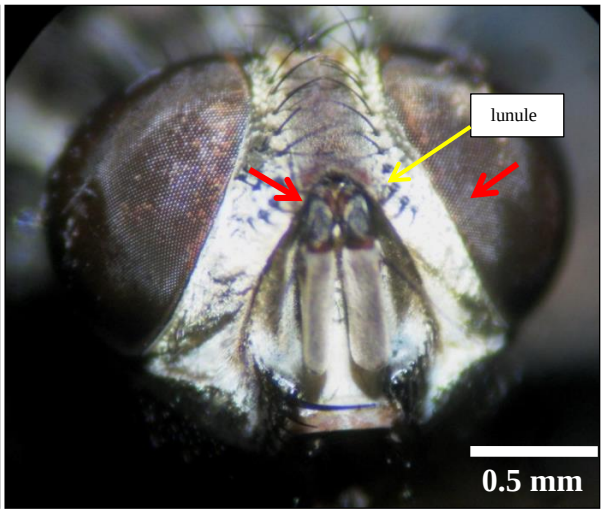


Fig. 2. Frontal bristles strongly diverging at lunule

2- Setae on R1 present (Fig 3)…….*Ravinia derelicta* (Walker) or *Ravinia stimulans* Walker, 1849

- Setae on R1 absent* (Fig 4)……...3

(*may be some other species such as *Ravinia anxia* and *Ravinia sueta*)

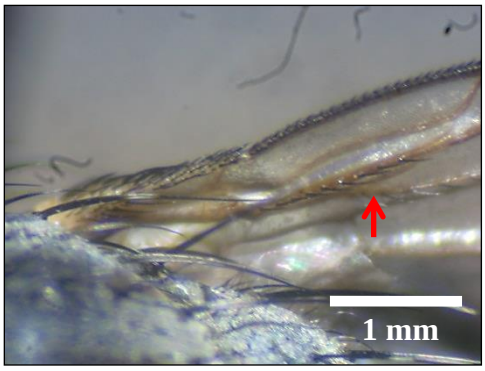


Fig. 3. Setae on R1 present

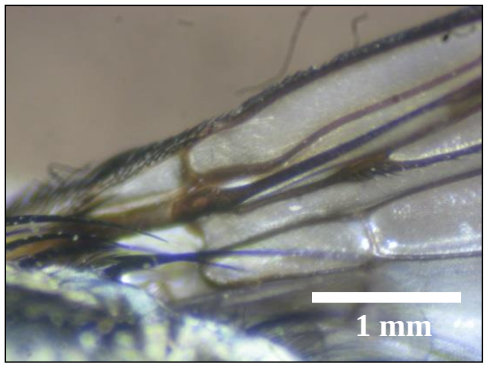


Fig. 4. Absent of seta on R1

3- Golden pruinescene at the apical segment of abdomen (Fig. 5)……..*Ravinia lherminieri* (Robineau-Desvoidy)

- Gray pruinescene at the apical segment of abdomen (Fig. 6).....*Ravinia querula* (Walker)

Fig. 5. Golden dusting

Fig. 6. Gray dusting

4- 1 av and 1 pv on apical tip of hind tibia (Fig. 7)……..*Sarcophaga* (5)

- Only 1 strong bristle at the apical tip of hind tibia (Fig. 8)……*Blaesoxipha* (6)

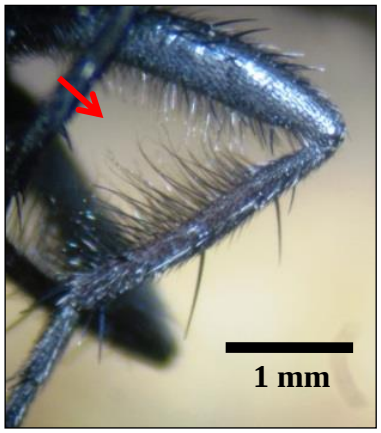
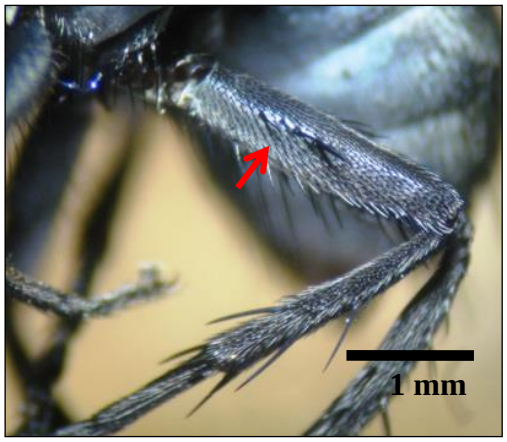
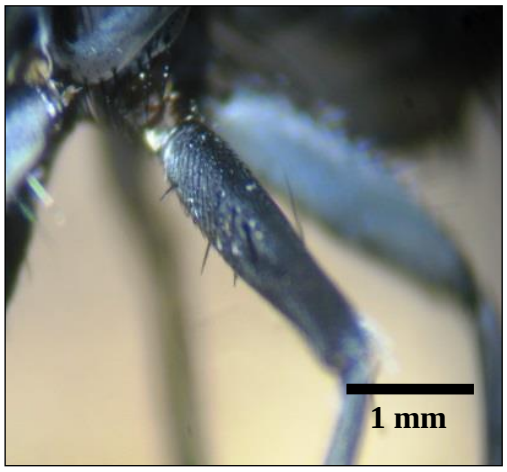


Fig. 7. Male hind tibia with fringe of hairs



Female mid femur with row of bristles

Fig. 8. Male hind tibia without fringe of hairs



Female mid femur without row of bristle

5- Gena largely covered by black setae; less hairs on parafacialis (Fig. 9)…..*Sarcophaga (Liosarcophaga) sarracenioides* (Aldrich)

- Anterior half of gena covered by black setae; posterior half of gena and postgena covered by white setae•; frontal-orbital bristles 10 pairs or more; more hairs on parafacialis; reddish terminalia (Fig. 10)…….*Sarcophaga (Neobellieria) bullata* Parker

•Could be some other species such as *Sarcophaga crassipalpis* and *Sarcophaga africa*.

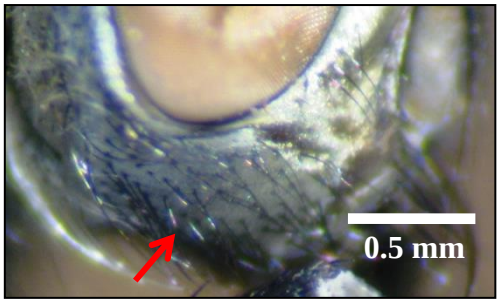


Fig. 9. Gena largely covered by black setae

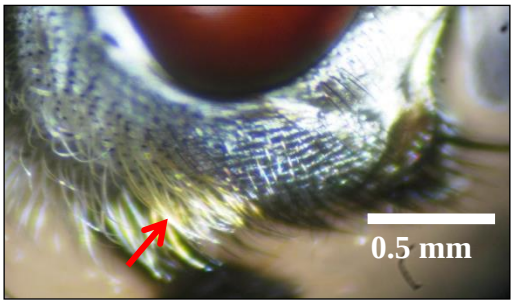


Fig. 10. Anterior half of gena covered by black setae, posterior half of gena and postgena covered by white setae

6- Sternopleural bristles 1+1; postgena with whitish setae, reddish terminalia (Fig. 11)……..*Blaesoxipha plinthopyga* (Wiedemann)

- Sternoplueral bristles 1+1+1 (Fig 12)……..*Blaesoxipha impar* (Aldrich)

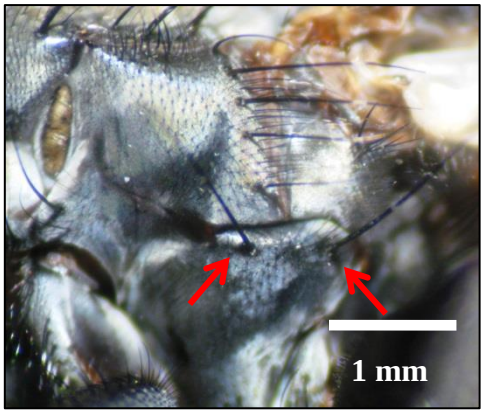


Fig. 11. Sternopleural bristles 1+1

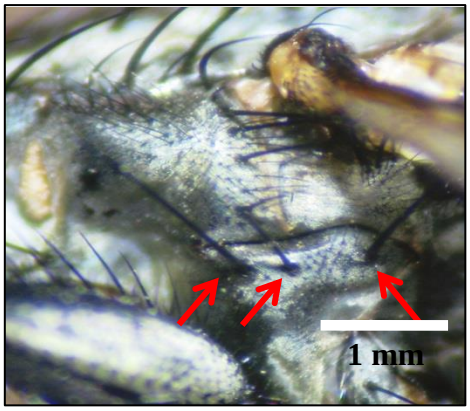
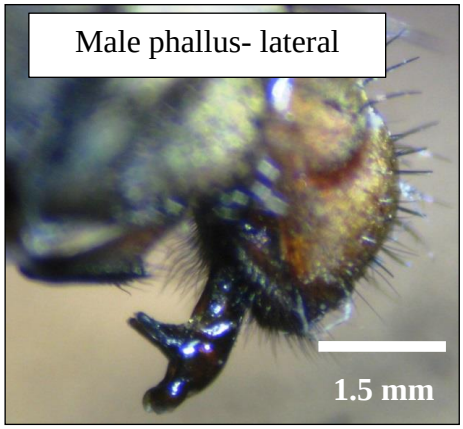
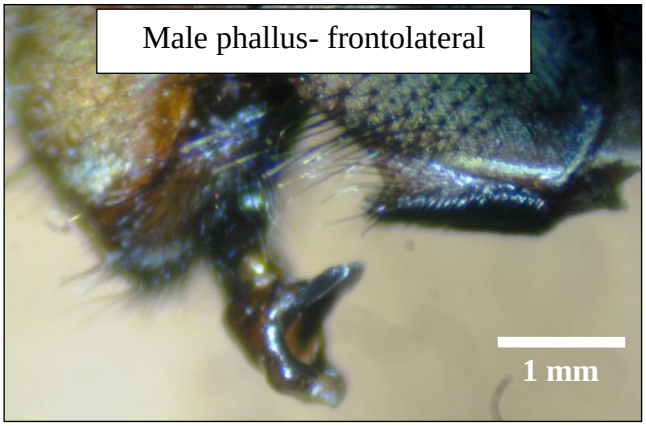


Fig 12. Sternopleural bristles 1+1+1

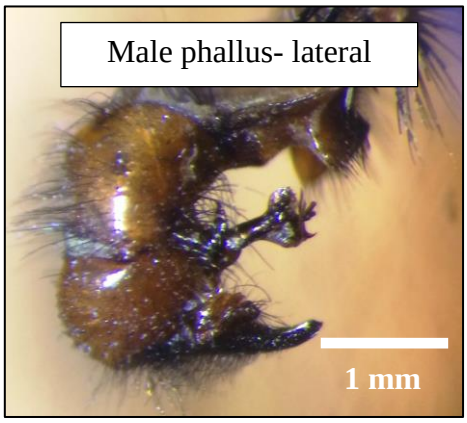
CONFORMATION DIAGNOSIS BY MALE PHALLUS / FEMALE GENITAL IDENTIFICATION

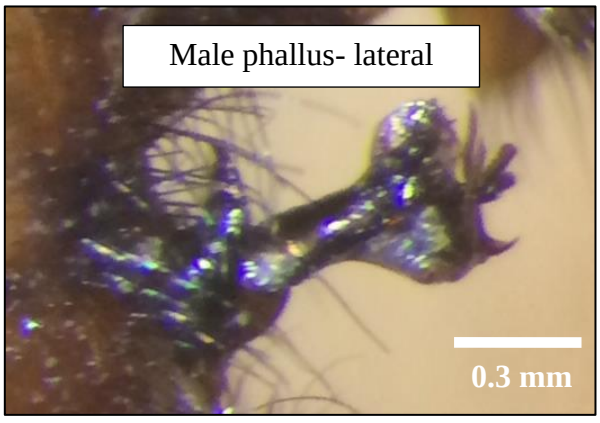
Ravinia iherminieri (Robineau-Desvoidy)



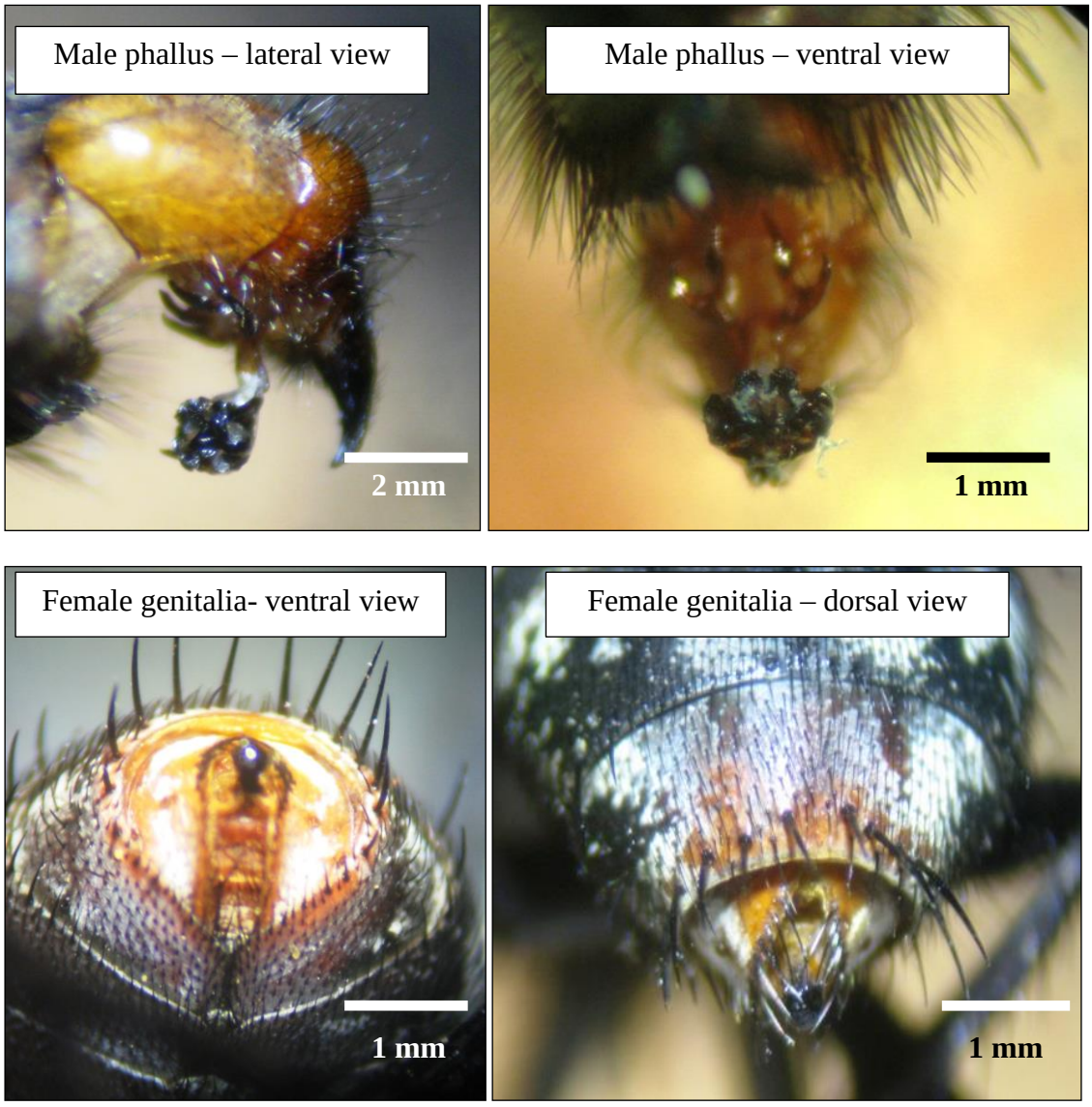


Sarcophaga (Liosarcophaga) sarracenioides (Aldrich)

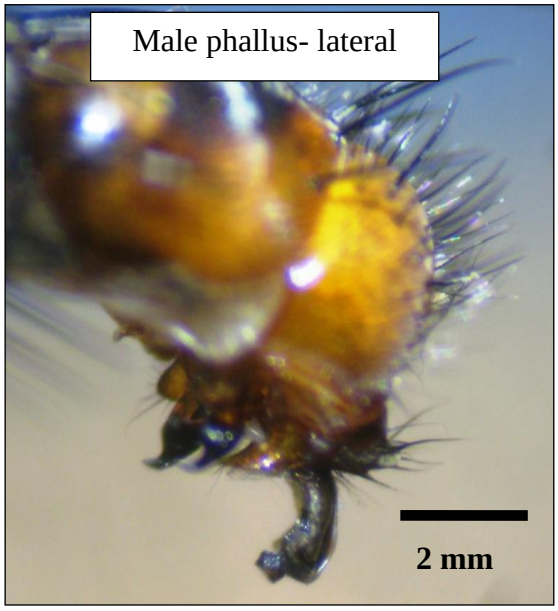


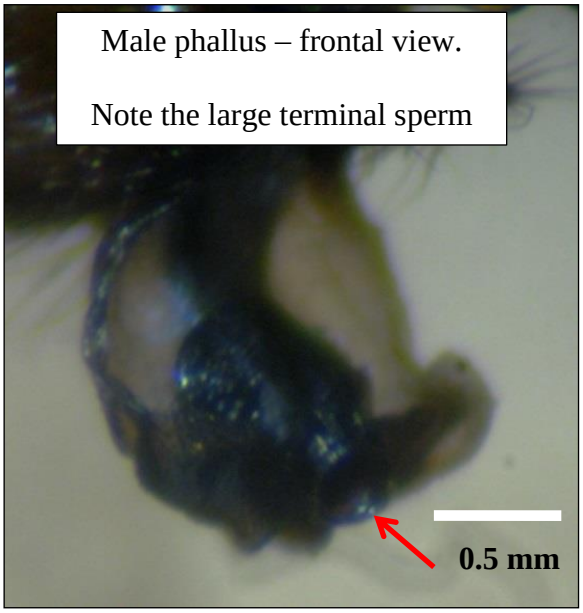


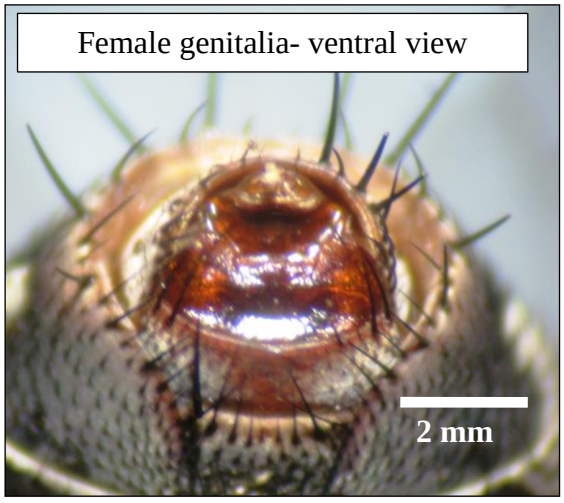
Sarcophaga bullata Parker

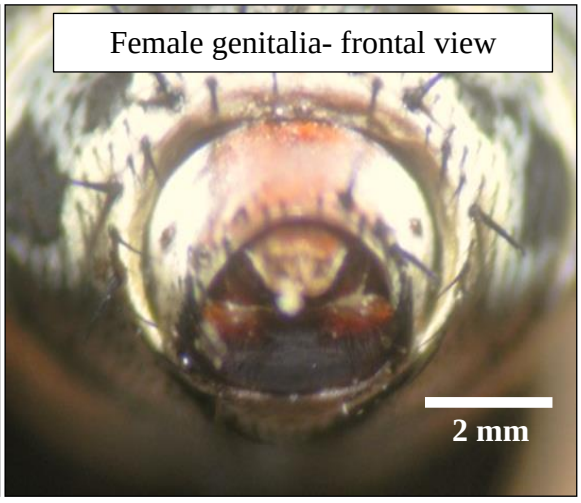


Blaesoxipha plinthopyga (Wiedemann)









THIRD INSTAR OF SARCOPHAGIDAE COLLECTED FROM PIG CARRION IN SUMMERS 2013 AND 2014 AT SNOOK, TEXAS

Sarcophaga bullata Parker

Figure J1. Third instar *Sarcophaga bullata*. Cephalopharyngeal skeleton (x5).

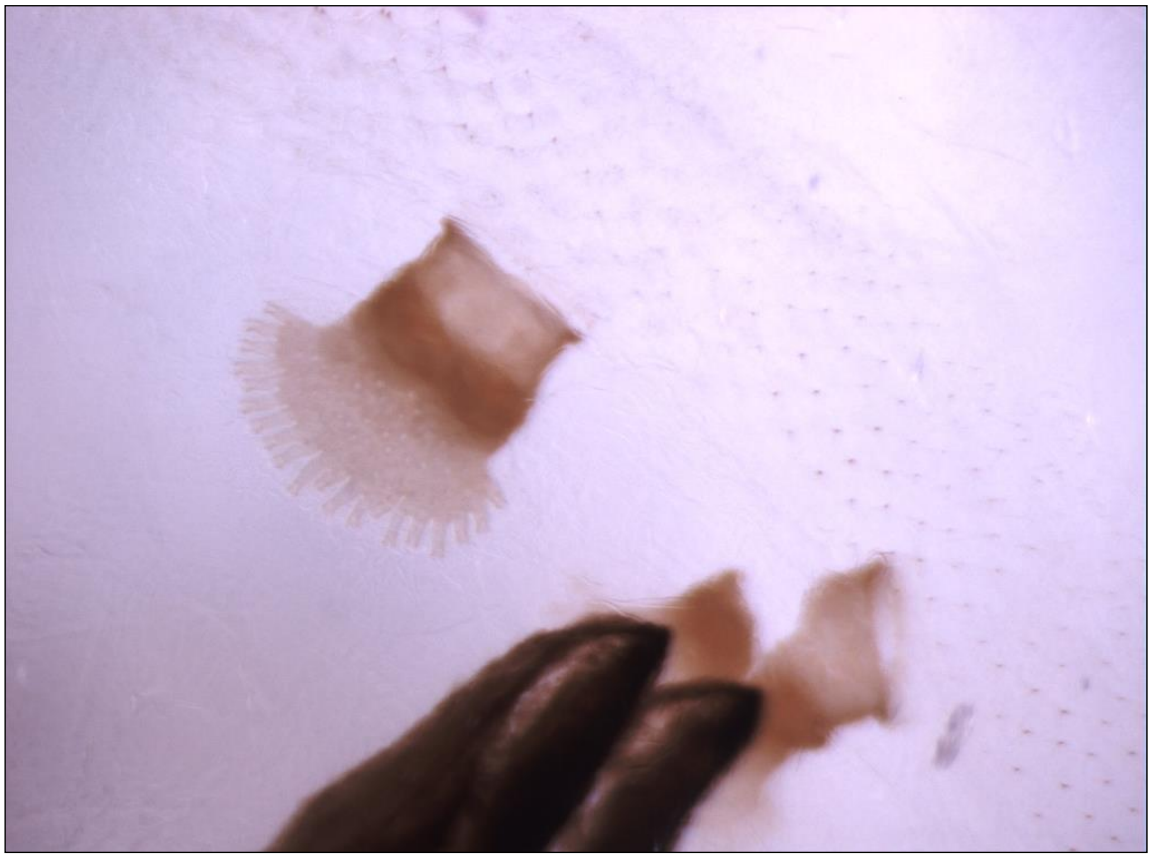


Figure J2. Third instar *Sarcophaga bullata*. Anterior spiracle with 20 papillae (x16).

Figure J3. Third instar *Sarcophaga bullata*. Posterior spiracles (x10).

Blaesoxipha plinthopyga (Wiedemann) – This larva was not recovered from pig carrion in the field of the current study. The images below were obtained from the specimens reared from laboratory colony maintained by Meaghan Pimsler at F.L.I.E.S. Facility. These *B. plinthopyga* images here are served as a comparison to *S. bullata* larvae.

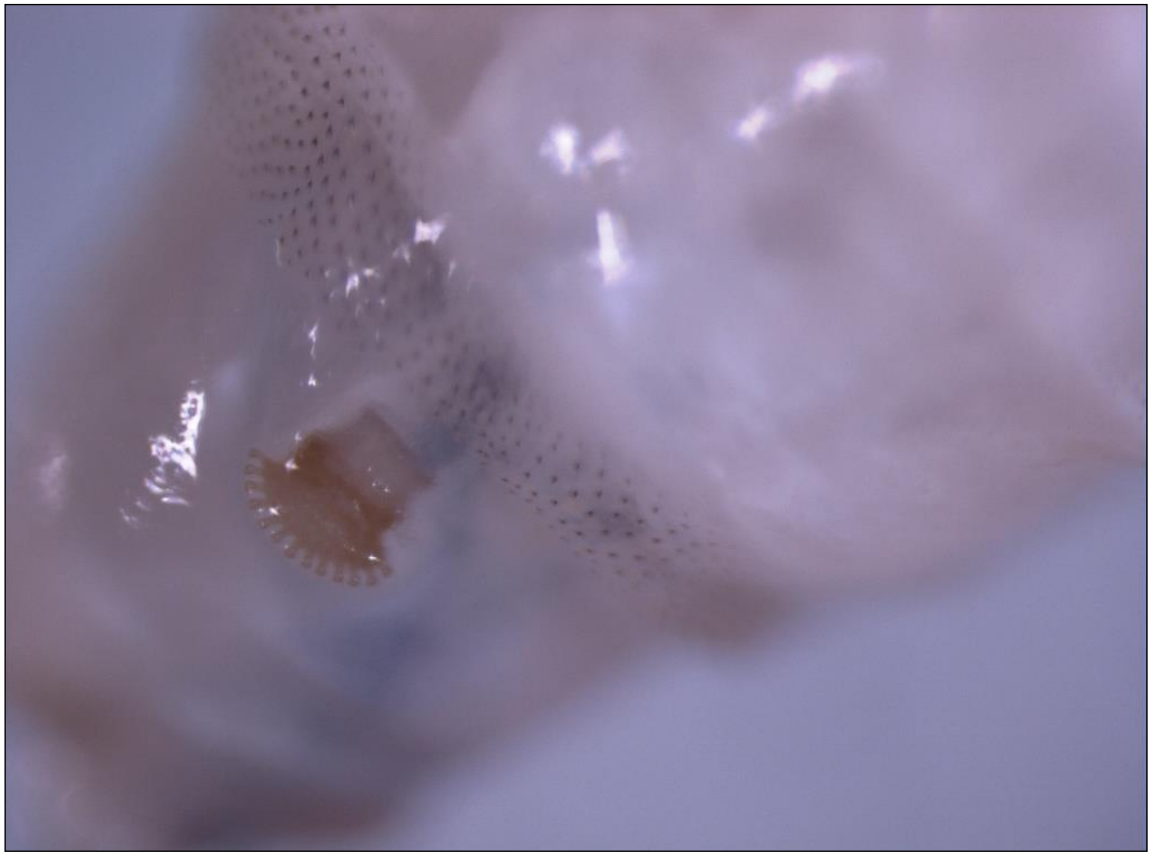


Figure J4. Third instar *Blaesoxipha plinthopyga*. Anterior spiracle with 13 papillae (x 12.5).

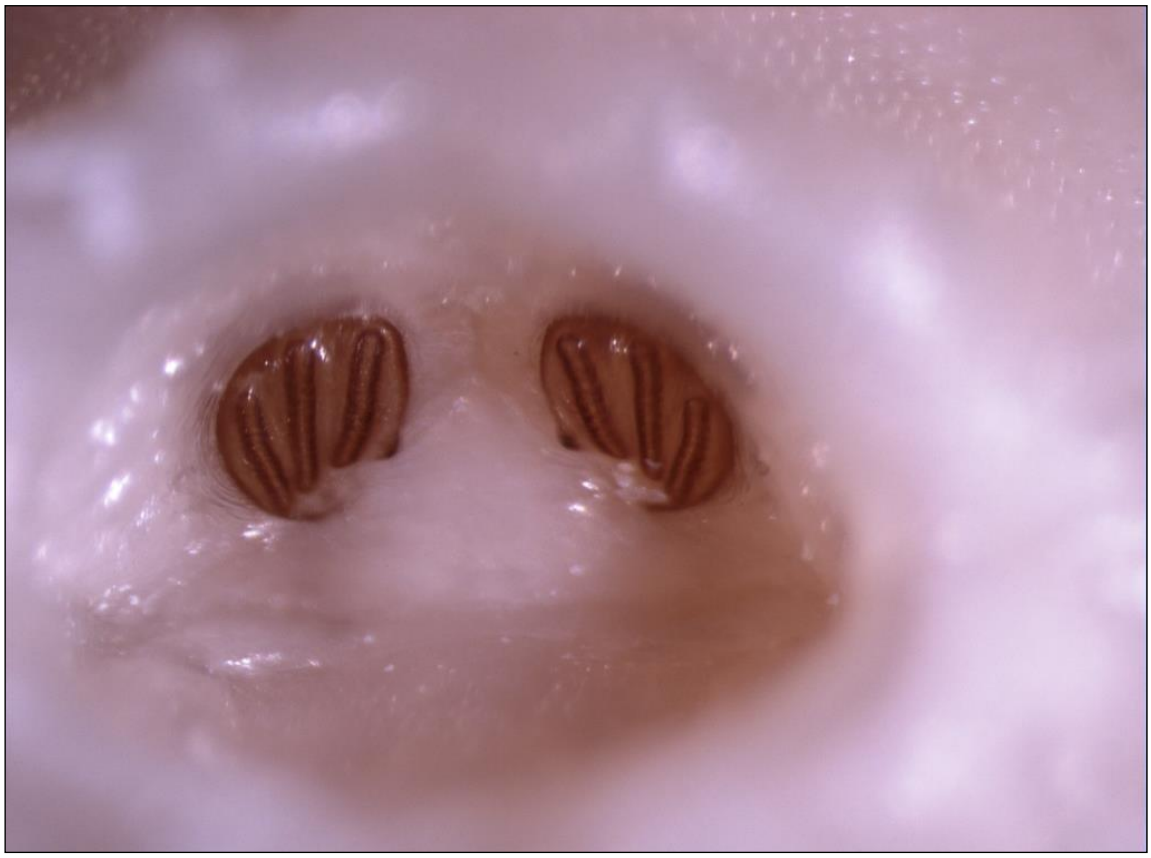


Figure J5. Third instar *Blaesoxipha plinthopyga*. Posterior spiracles (x 12).

APPENDIX K

IMPORTANT EVENTS DURING FIELD OBSERVATION IN 2013 AND 2014

2013

DAY 0 (Initial day of experiment)

- *Cochliomyia macellaria* and *Musca domestica* adults first seen on carrion

DAY 1

- *Chrysomya rufifacies, Hydrotaea,* and *Sarcophaga* adults first seen on carrion
- Ant nests formed on 2 pig carrion
- *Nicrophorus marginatus* (Silphidae) with phoretic mites first seen and collected from pig carrion

DAY 2

- Vultures seen hovering above pig carrion
- Scavenger activity observed (suspected opossum)

DAY 3

- Staphylinidae and Blattodea first sighted on Control pig

DAY 4

- Sudden increased in Odonata population in the field

DAY 5

- *Hermetia illucens* adults first seen on carrion

DAY 7

- Control pig (C2) was disturbed by scavenger activity (suspected opossum).
- When Post-7 (M) cages were removed, oviposition by *C. macellaria* observed.

DAY 9- DAY 10

- *Cochliomyia macellaria* adults emerged from pupae (Control pigs)
- Newly emerged flies resting on cage for > 6 hours in the field
- First *Hermetia illucens* maggot was collected from Post-14 Exclusion Cage (R3)

DAY 12- DAY 15

- *Chrysomya rufifacies* adults emerged from pupae (Control pigs). In summary, *C. rufifacies* emerged later than *C. macellaria*.
- First *Dermestes* larvae collected from exclusion cages (R pigs)

DAY 17- DAY 19

- Newly emerged *C. macellaria* from M pigs (fly developmental time was similar with Control pigs, which was about 10 days. In other words, fly development was not affected by un-fresh carcasses which have been delayed by insect colonization for 7 days)

- On Day 19, *Chrysomya rufifacies* adults emerged from M3 pig. Suspect oviposition on Day 7 after exposure to environment.

DAY 23 – DAY 40

- All pigs (C, M, and R) have *Dermestes* larvae underneath and lasted until Day 40 and Day 90.

DAY 25

- Newly emerged *Sarcophaga* sp. observed on pig (M3).
- Two newly emerged *C. macellaria* observed on pig (C3) together with 25 newly emerged *Sarcophaga*. This demonstrated that *C. macellaria* continued to oviposit perhaps on Day 15, then the adult emerged on Day 25 (assumed that the development needed 10 days as usual).

DAY 26- DAY 28

- Newly emerged *C. macellaria* observed on pig (R3)- Again, *C. macellaria* development is not affected by un-fresh carcasses. Duration of development was about 10 days.

DAY 31

- After rain, fungus started to grow under the soil of the pig carrion

DAY 33

- ALL Control pigs had fungus, but only 1 M pig had fungus and none of the R pig infested with fungus.

DAY 34- DAY 39

- *Sarcophaga* 3rd instar larvae were seen on Day 34 (suspect larviposition on Day 32) on pig carrion (M1), Day 36 on M2 pig, Day 35 on M3 pig, Day 37 on R1 pig and Day 39 on R2 pig. Again, this demonstrated repeated larviposition on pig carrion regardless of successional sequence. Female flesh flies did come back to old carrion resource for larviposition as long as the resources for larval feeding are still available.

DAY 36-DAY 37

- *Hermetia illucens* larvae were recovered on Day 36 (pig R3) and Day 37 (pig M1)

DAY 39

- 100 *Dermestes* larvae; *Sarcophaga* larvae; 5 *Hydrotaea* larvae; 2 *Hermetia illucens* larvae were recovered from the soil under the pig carrion (R2) (4 species infestation on Day 39!). This was 25 days after the R pigs exposure to environment. Compared to Control pigs on 25 days post-exposure, all of them only have *Dermestes* larvae.

DAY 40

- 10 *Hermetia illucens* larvae; 20 *Hydrotaea* larvae; 1 *Sarocophaga* larvae; 20 *Dermestes l*arvae were recovered from pig R3.
- In conclusion, R2 and R3 have 4 species infestation on Day 40.

DAY 90

- *Dermestes* larvae and adults still can be recovered from pig carrion (C, M, and R).

INTERESTING INSECT BEHAVIOR OBVSERVATIONS

1. Larval succession on carrion – from *Cochliomyia macellaria* larvae to *Chrysomya rufifacies* larvae first noted on Day 5.

2. On Day 6, full of *C. rufifacies* maggots underneath the body, but not on the skin (*C. rufifacies* maggots preferred hiding in the soil). *C. rufifacies* maggots came out at 7 pm and spread over all the pig body. Pig skin was still intact, may serve as protective layer to maggots from direct sunlight or potential predators.

3. Strange phenomenon (in two occasions)- Majority of adult flies swarmed on top of the R cages (on Day 12, and on Day 13)

4. Early colonizer can become late colonizer as demonstrated by Control pig (C1). 3rd instar of *C. macellaria* can be seen on C1 on Day 13 whereas the first generation of *C. macellaria* and *C. rufifacies* were emerged on Day 9 and Day 12, respectively. In other words, repeated oviposition on carrion by *C. macellaria* can occur someday between Day 0- Day 10.

5. *Dermestes* adult beetles can be found in the oral cavity of swine carrion. Perhaps looking for oviposition site.

6. *Dermestes* adults have been observed mating on pig carrion.

7. At night, the *Dermestes* larvae surfaced to the body (outer skin). During daytime, they were hiding under the carrion.

8. *Hydrotaea aenescens* were active throughout the day and night. At night (~10 pm), they can be seen frequenting the pig carcasses in the field, together with other nocturnal insects such as cockroaches, moth, and katydids, while other necrophagous insects such as calliphorids and sarcophagids were not seen (they were diurnal species).

9. Insect oviposition is resource oriented regardless of "typical insect successional sequence". Delayed carrion resource is still attractive to insect colonizers. Repeated oviposition can still occur inside the cage (Example, *Hydrotaea* sp., *C. macellaria*, *S. bullata*).

10. *Cochliomyia macellaria* larvae were actively feeding on carrion even at 3 am in the field. This suggests that the maggots feed actively regardless of the time phases in order to achieve pupation stage as quick as possible. Perhaps circadian rhythm did not apply on active feeding larval stages.

11. Adult fly emergence time can be varied. Both *C. macellaria* and *C. rufifacies* had been observed emerged during daytime, while *C. rufifacies* had been observed to emerge during night time.

12. Newly emerged *C. rufifacies* rested on vegetation about 10 meters away from pig carrion.

13. When M pigs were exposed to environment on Day 7, fly oviposition was noted on the same day (on the first night of Day 7 (by *C. macellaria*)). Note that oviposition can still take place around 6.30 pm in the field. However, when R pig exposed to environment on Day 14, no fly eggs were observed during the same day (the first night of Day 14). The fly oviposited on R pigs in the evening of Day 15 and Day 16 (by *C. macellaria*). Perhaps it is due to change of resource quality and attractiveness of the carcasses to female blow flies.

14. Male cockroaches, *Parcoblatta fluvescens* (Ectobiidae), waited on the anti-scavenging cage while female cockroaches were feeding on pig carrion to obtain protein sources.

15. Fire ants built a nest on Pig R3 on Day 20.

16. One *Chrysomya megacephala* was collected from pig carrion on Day 21 (Pig R2).

17. When rain, more male cockroaches were trapped on sticky trap.

18. When rain, no flying insect activity was observed, but adult cockroaches and *Dermestes* larvae are still active and can be seen on the carrion.

19. *Dermestes* larvae and *Hydrotaea* larvae co-existed under the pig carrion. In some cases, *Sarcophaga* larvae and *Hermetia illucens* larvae can occur together under the soil of the same carrion (4 species infestation together with *Dermestes* and *Hydrotaea*). While larval succession was observed in the case of *C. macellaria* and *C. rufifacies*, where *C. macellaria* colonize the pig carrion first, then succeeded by *C. rufifacies* larvae, they rarely co-occurred at the same spot on the carrion.

20. Most importantly, we did not see and collect any *C. rufifacies* larva from M pigs and R pigs (after exposure to the environment). The food selection range by *C. rufifacies* maybe narrower compared to *C. macellaria*, which is more broad and flexible in food selection. However, *C. rufifacies* larva was seen on M3 pig and 20 new adults emerged on Day 19. This suggests *C. rufifacies* did come back to colonize the un-fresh carcass, but only 16.67% of all excluded pig carrion.

GROWTH OF SUNFLOWER PLANT (*Helianthus annus*) AT THE SIDE OF PIG CARRION: POSSIBLE NUTRIENT TRANSFER FROM CARRION TO PLANT

Date and Plant height:
6 July- 71 cm
8 July- 83 cm (+12 cm; growth rate = 6 cm/day)
11 July- 97 cm (+14 cm; growth rate = 4.6 cm/ day)
15 July- 130 cm (+33 cm; growth rate= 8.25 cm/ day)
24 July- 180 cm (+50 cm; growth rate= 5.5 cm / day)
26 July- 197 cm (+17 cm; growth rate= 8.5 cm / day)
14 Sept- ~250 cm (+50 cm; growth rate= 1 cm / day)

EXCLUSION CAGE CONTAMINATION (Day 0 to Day 6)

Exclusion cages infested by:
M1- *C. macellaria, C. rufifacies*
M2- *C. macellaria, C. rufifacies*
M3- C. *macellaria, Hydrotaea, Sarcophaga*
R1- *C. macellaria, Hydrotaea*
R2- *C. macellaria, C. rufifacies, Sarcophaga, Hydrotaea*
R3- *C. macellaria, Sarcophaga*

Summary:
1. *C. macellaria* infested all exclusion cages
2. *C. rufifacies* infested three cages- M1, M2, R2
3. *Sarcophaga* infested three cages- M3, R2, R3
4. *Hydrotaea* infested three cages- M3, R1, R2
5. Double species infestation- 4 cages
6. Triple species infestation- 2 cages (M3, R2)

Table K1. Total number of maggots and adults collected inside the insect exclusion cages during the insect exclusion period in summer 2013 (From Day 4-Day 6) at Snook, Texas. Note that there were seven days of insect exclusion for M cages and 14 days of insect exclusion for R cages.

	Maggot collected				**Adult collected**			
Pig	**Day 4**	**Day 5**	**Day 6**	**Total**	**Day 4**	**Day 5**	**Day 6**	**Total**
M1	100	35	21	**156**	0	0	0	**0**
M2	20	65	40	**125**	48	15	15	**78**
M3	3	45	36	**84**	2	7	16	**25**
R1	23	30	10	**63**	4	3	4	**11**
R2	50	160	45	**255**	0	0	4	**4**
R3	9	70	16	**95**	1	8	4	**13**
Total	**205**	**405**	**168**	**778**	**55**	**33**	**43**	**131**
Average/Pig	**34**	**67**	**28**		**9**	**5**	**7**	

EXCLUSION CAGES CONTAMINATION – For R cages only (Day 7-Day 14)
Exclusion cages infested by:
R1- *C. macellaria,* ***C. rufifacies******,** *Hydrotaea,* **Dermestid larva***
R2- *C. macellaria, C. rufifacies, Sarcophaga,* ***Hermetia illucens*, Hydrotaea****
R3- *C. macellaria, Sarcophaga, Hydrotaea ,* ***Hermetia illucens****
***New species arrived**

Summary:
1. *Hydrotaea* infested all R exclusion cages
2. *Hermetia illucens* infested two cages – R2 and R3.
3. Quadruple species infestation- 2 cages (R1, R3)
4. Quintuple species infestation: 1 cage (R2)

Table K2. Total number of maggots and adults collected inside the R insect exclusion cages during the insect exclusion period in summer 2013 (from Day 7-Day 14) at Snook, Texas.

Maggot collected										**Adult collected**								
Pig	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**T**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**T**
R1	6	11	55	17	4	29	16	11	**149**	1	0	2	3	2	1	2	1	**12**
R2	30	48	55	75	48	88	19	18	**381**	2	7	8	2	33	14	4	1	**71**
R3	22	43	75	177	80	32	37	15	**481**	1	1	5	0	1	7	1	1	**17**
T	**58**	**102**	**185**	**269**	**132**	**149**	**72**	**44**	**1011**	**4**	**8**	**15**	**5**	**36**	**22**	**7**	**3**	**100**
A	**19.3**	**34**	**61.6**	**89.6**	**44**	**49.6**	**24**	**14.6**	**337**	**1.3**	**2.6**	**5**	**1.6**	**12**	**7.3**	**4.3**	**4.6**	**33.3**

T= Total number collected
A= Average per pig

Total Maggots Collected inside the cage (Day 0- Day 14) in 2013:
M1- 156
M2- 125
M3- 84
R1- 63+149=**212**
R2- 255+381=**636**
R3- 95+481=**576**
Grand total: 1789 maggots

Total adult insect collected inside the cages (Day 0- Day 14) in 2013:
M1- 0
M2- 78
M3- 25
R1-23
R2- 75
R3- 30
Grand total: 231 adult insects

Disturbance by scavengers (4 times) in 2013 trial on:
Day 2, Day 14, Day 15, Day 16.

Table K3. Efficiency of insect exclusion cages in excluding insect colonization on pig carrion in summer 2013 at Snook, Texas.

Exclusion Pig	**Weight of the pig (kg)**	**Total maggots estimation (Based on the assumption that 550 gm rat cadaver produces 1500 maggots) (Banfield et al. Unpublished data)**	**Total maggot collected in the cage (contamination)**	**Percentage of exclusion (%)**
M1	28	76364	156	99.80
M2	30	81818	125	99.84
M3	28	76364	84	99.89
R1	18	49090	212	99.56
R2	30	81818	636	99.22
R3	28	76364	576	99.25
Average	**27**	**73636**	**1789**	**99.59**

IMPORTANT EVENTS DURING FIELD OBSERVATION IN SUMMER 2014

2014

DAY 0

- Oviposition on pig carrion took place less than 1 hour (by *C. macellaria*).
- Mouthpart was the first oviposition site by blow flies.
- Other than flies, fire ants (*Solenopsis invicta*) were among the first insects to arrive on carcasses. Ants build nests in mouth within several hours of death.
- At night, all C pigs had been colonized by blow fly eggs.
- On first night, cockroaches (*P. fluvescens*) appear on pig carcasses.
- Scarabaeidae (June bugs) are nocturnal and were attracted to the pig carrion odor.

DAY 1

- All exclusion pigs have fire ants in their mouths.
- *C. rufifacies* adults first appeared on Day 1 (10 am); but the population number was lower than *C. macellaria* adults.

DAY 2

- Fire ants (*S. invicta*) have been observed predating the fly eggs.
- June bug (*Phylophaga* sp.) have been observed to get into the exclusion cages.

DAY 3

- Libellulidae (Odonata) had been observed to predate on *C. macellaria* adults.
- Asilidae (Diptera) had been observed to predate on *C. macellaria* adults
- Female flies laid eggs on the zipper on cage R2.

DAY 5

- *Chrysomya rufifacies* larvae and *Hydrotaea* larvae were observe to co-exist on the same pig carcass.

DAY 6

- Fire ants were observed to predate on blow fly larvae
- Possible beetle succession observed: Silphidae- Staphylinidae- Dermestidae- Trogidae- Cleridae- Histeridae.
- *Dermestes caninus* appears earlier than *Dermestes marmolatus.*
- *Sarcophaga bullata* larvae infested exclusion pig (R1).

DAY 7

- Scavenger activity noted on day 7 on C3 (jaw detached)

DAY 8

- All M pigs had egg masses and first instar larvae on Day 8. The present of the first instar suggested oviposition were initiated on Day 7 evening.
- *Hydrotaea* adults were found underneath the body (oviposition may initiated beneath the body).
- First instar *C. macellaria* larvae (repeated oviposition) were found on R2 pig.

DAY 9

- Same as last year, new adults of *C. macellaria* emerged on Day 9.

- When humidity is high, June beetle population increased in the field.

DAY 10

- Repeated oviposition by *C. macellaria* was observed on C3 pig on Day 10.

- *Chrysomya rufifacies* larvae were first discovered on M2 pig. This suggest *C. rufifacies* did come back to colonize un-fresh carcasses.

DAY 11

- *C. macellaria* larvae are recovered on C2 on Day 11.

- *Hermetia illucens* adult first seen on Day 11 on C3 pig.

- *Sarcophaga* can still larviposit on C pig (C1) and M pig (M2) and its larvae were recovered on Day 11. Thus, ***C. macellaria*** **larvae and** ***Sarcophaga bullata*** **larvae** can be misleading in mPMI estimation **(Due to too many events of repeated oviposition occurred).**

- *C. rufifacies* larvae colonized M3. Again, this suggest *C. rufifacies* did come back to colonize un-fresh carcasses. However, *C. rufifacies* did not come back to colonize M1 pig. Note that M1 pig has been contaminated by *C. rufifacies* larvae during the exclusion period.

- *C. macellaria* larval migration was observed to migrate to North or East side from carrion, rarely South, and never migrate to West.

- On Day 11, all Control pigs have been infested by *C. macellaria* larvae again (we collected the larvae). Note that first generation of *C. macellaria* has been emerged between Day 9- Day 10.

DAY 12

- Dermestidae beetles can actually fly around the cages.
- *C. macellaria* maggots migrated to North and East. Maggots built tunnel under sand (M1).
- 7 adult Hister beetles were seen in the maggot mass (M1).
- 1 *Sarcophaga* larva and *Hydrotaea* larvae were seen underneath the pig (M1). They can co-exist underneath the carrion but not at the same spot.
- 1 Staphylinidae beetle had been observed to attack fly larvae; 1 *Sarcophaga* larva was attacked by ants (M3).
- White fungus grew on R3 pig.
- Newly emerged *C. rufifacies* adults were observed on Day 12 evening on C3.

DAY 13

- On Day 13, newly emerged *C. macellaria* adults still can be observed. *Dermestes* larvae first appeared on C1 and C3 pigs.
- Cockroaches were observed during daytime underneath the pig carcasses.
- 3rd instar *C. macellaria* found in pitfall trap (North side); maggots migrating up to 4 meters away to the East (M1).

DAY 14

- Day 14, all Control pigs have *Dermestes* beetle larvae.
-
- *Lucilia* adults were very rare in observation. None of the *Lucilia* larvae had been collected.
- No eggs were observed on R pigs when the exclusion cages were removed, similar observations as in 2013.

DAY 15

- Fly eggs observed on Day 15 on R1 pig only. 1st instar seen on R3 pig in the afternoon. Similar observation as in 2013 trial.
- Among adults, *C. macellaria* was dominant, and *C. rufifacies* was fewer than *C. macellaria* population.

DAY 16

- New *C. macellaria* adults emerged on Day 16 from M pigs. In 2013, *C. macellaria* adult emerged on Day 17.

- Emergence time for *C. macellaria* was different between M1, M2 and M3 pigs. There are variations in developmental time of *C. macellaria* among M pigs.

DAY 17

- *C. macellaria* and *Sarcophaga* larvae can co-exist, and had demonstrated flexibility to oviposit/larviposit on un-fresh carcasses.

- All R pigs had *Sarcophaga* larvae on Day 16.

DAY 18

- *Hydrotaea* larvae and *Sarcophaga* larvae were persisted longer than *C. macellaria* larvae on pig carrion.

- All R pigs have *C. macellaria* and *Sarcophaga bullata* larvae.

DAY 19

- Newly emerged *C. rufifacies* were seen on M2 pig on Day 19.

- New *C. macellaria* adults emerged from M pigs between Day 16-19.

- *Hydrotaea* larvae can be seen from M1, M2, M3, R1 and R3 pigs. *Hydrotaea* larvae were later detected on R2 on Day 25.

- All *Sarcophaga* larvae were identified as *Sarcophaga bullata*.

- Ants had been observed to carry away *Dermestes* larvae.

DAY 20

- *Dermestes* larvae first seen on M1, M3 and R2 pigs on Day 20.

DAY 21

- *Sarcophaga* and *C. macellaria* larvae colonized different areas on pig carcass.

- Fungus grew at the beneath of pig carrion.

- *Sarcophaga* larvae highest record= approximately 70 larvae (Day 21 on R2). *Sarcophaga* larvae usually appear in small number ranging from 1-5, rarely exceed 10 larvae in a group. In 2013, maximum number of *Sarcophaga* larvae was approximately 100 larvae.

DAY 22

- Wasp, *Polistes* sp. (Vespidae), is building nest under the cage.

DAY 25

- New *C. macellaria* emerged from R pig, total development was 11 days, which was one day longer than Control and M pigs, which were 9 days. In 2013, fly development was not affected by un-fresh carcasses (it was 10 days for 2013 trial). However, the number of new *C. macellaria* adults was much lower (<1000) compared to Control and M group. No new *C. macellaria* adults were seen on R3 pig.

- *Hydrotaea* larvae have been observed on R2 pig.

DAY 26

- *Sarcophaga* and *C. macellaria* adults emerged from M3 pig.

- New *C. macellaria* emerged from R3 pig, which took 12 days (2 days longer than normal), total number of *C. macellaria* emerged was much lower, and emergence time was at night. These discrepancies in fly developmental time may be related to the change of resource quality.

DAY 27

- *Hydrotaea* larvae persisted longer than *Sarcophaga* larvae under pig carcasses.

DAY 28

- 100 *Dermestes* adults were found underneath the R3 pig.

DAY 31

- Possible scavenger activities observed on Day 31

DAY 32

- More fungus grew under pig carcasses after rain.

DAY 33

- More cockroaches can be seen when the pig carcasses are wet after rain.

DAY 34

- *Hermetia illucens* larvae first appeared on Day 34 (R1)

DAY 35

- *Sarcophaga* could larviposit repeatedly on old carcass (C2 pig) even on Day 36

DAY 37

- *Hermetia larvae* can co-exist with *Dermestes* larvae and *Hydrotaea* larvae

DAY 39

- R1, R2 and R3 have *Hermetia illucens* larvae

DAY 40

- On Day 40, M1, and M3 pig are the only pigs with *Dermestes* larvae, while Control pigs, M2 pigs are left with fire ants. R1 and R2 have double infestations: *Hermetia illucens* and *Dermestes* larvae; R3 pig has triple infestation: *Hermetia illucens* larvae, *Hydrotaea* larvae and *Dermestes* larvae.

- Most importantly, of all exclusion pigs, only M2 and M3 pigs had been colonized by *C. rufifacies* larvae (33.34% infestation by *C. rufifacies* after exposure). The other insect-excluded pig carcasses (M1, R1, R2, R3) did not infested by *C. rufifacies*.

Disturbance by Scavengers (3 times) in 2014 trial on:
Day 7, Day 18, Day 31.

Scale of contamination
M1- 4/10
M2- 10/10
M3- 10/10
R1- 6/10
R2- 8/10
R3- 10/10
Scale=
1 – Excessively infested by fly larvae; 10- No infestation by fly larvae

Table K4. Total maggots and adult flies collected inside the insect exclusion cages (M and R cages) in summer 2014 (from Day 0-Day 6) at Snook, Texas.

Maggot collected									**Adult collected**							
Pig	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**T**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**T**
M1	0	0	0	50	100	1	50	**201**	0	28	91	81	10	8	0	**218**
M2	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0	0	**0**
M3	0	0	0	0	0	0	0	**0**	0	1	0	0	0	0	0	**1**
R1	0	0	0	100	100	125	100	**425**	0	31	300	50	202	30	0	**613**
R2	0	0	0	0	0	36	10	**46**	0	3	25	0	0	0	0	**28**
R3	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0	0	**0**
T	**0**	**0**	**0**	**150**	**200**	**162**	**160**	**672**	**0**	**63**	**416**	**131**	**212**	**38**	**0**	**860**
A	**0**	**0**	**0**	**25**	**33.3**	**27**	**26.6**	**112**	**0**	**10.5**	**69.3**	**21.8**	**35.3**	**6.3**	**0**	**143.3**

T = Total number collected
A = Average number per pig
Green= pig carcasses with complete exclusion from blow fly colonization
No larvae infestation at all = M2, M3, R3
No adults infestation at all= M2, R3

Exclusion cages infested by:
M1- *C. macellaria* larvae, *Hydrotaea* larvae, ***Chrysomya rufifacies*** larvae
M2- None (free from blow fly colonization)
M3- None (free from blow fly colonization)
R1- *C. macellaria* larvae, *Hydrotaea* larvae, *Sarcophaga* larvae
R2- *C. macellaria* larvae
R3- None (free from blow fly colonization)

Summary:
1. *C. macellaria* infested 3 cages- M1, R1 and R2
2. Triple infestation in two cages namely M1 and R1
2. R2 has a single infestation- *C. macellaria*.

EXCLUSION CAGES CONTAMINATION – For R cages only (Day 7-Day 14) in 2014

Exclusion cages infested by:
R1- *C. macellaria* larvae, *Hydrotaea* larvae, *Sarcophaga* larvae
R2- *C. macellaria*
R3- ***C. macellaria****
*** New arrival on Day 10**

Table K5. Total maggots and adult flies collected inside the R insect exclusion cages in summer 2014 (from Day 7-Day 14) at Snook, Texas.

Maggot collected										**Adult collected**								
Pig	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**T**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**T**
R1	31	15	4	0	51	0	0	0	**101**	0	28	1	1	0	13	14	2	**59**
R2	0	23	24	2	0	0	0	0	**49**	0	0	0	3	3	14	2	0	**22**
R3	0	0	0	7	0	1	0	0	**8**	0	0	0	0	0	2	0	0	**2**
T	**31**	**38**	**28**	**9**	**51**	**1**	**0**	**0**	**158**	**0**	**28**	**1**	**4**	**3**	**29**	**16**	**2**	**83**
A	**10.3**	**12.6**	**9.3**	**3**	**17**	**0.3**	**0**	**0**	**52.6**	**0**	**9.3**	**0.3**	**1.3**	**1**	**9.6**	**5.3**	**0.6**	**27.6**

Total Maggots Collected inside the insect exclusion cages (Day 0- Day 14) in 2014:

M1- 201
M2- 0
M3- 0
R1-425+101= 526
R2- 46+49= 95
R3- 0+8= 8
Grand total: 830 maggots

Total adult insect collected inside the insect exclusion cages (Day 0- Day 14) in 2014:

M1- 218
M2- 0
M3- 1
R1- 613+59= 672
R2- 28+22=50
R3- 0+2=2
Grand total: 943 adult insects

Table K6. Efficiency of insect exclusion cages in excluding insect colonization on pig carrion in summer 2014 at Snook, Texas.

Exclusion Pig	**Weight of the pig (kg)**	**Total maggots estimation (Based on the assumption 550 gm of rat cadaver produces 1500 maggots (Banfield et al. Unpublished data)**	**Total maggot collected in the cage (contamination)**	**Percentage of exclusion (%)**
M1	25	68181	201	99.70
M2	27.5	73636	0	100
M3	29.5	80455	0	100
R1	17.5	47727	526	98.89
R2	15.5	42273	95	99.77
R3	21	57273	8	99.98
Average	**22.67**	**61591**	**830**	**99.72**

Table K7. Comparison of efficiency of the insect exclusion cages between summers 2013 and 2014 at Snook, Texas.

Year	**2013**	**2014**
Total maggots collected	1626	830 (more eggs were removed)
Total adults collected	231	943
Percentage of exclusion (%)	99.62	99.72

APPENDIX L

LIST OF ARTHROPODS COLLECTED FROM THE FIELD IN SUMMER 2013 (BY FORCEPS) AND PRESERVED IN 70% ETHANOL

Label	**Identification**	**Quantity**
D-5 (PT)	Lycosidae	1
D-5 (PT)	*Apis* sp.	1
D-5 (PT)	*Solenopsis invicta*	5
D-5 (PT)	Carabidae: *Scarite* sp.	1
D0-C2-PT	Formicidae	1
D0-C2-PT	Armadillodiidae	1
	Carabidae	1
D0-C1-PT	Geocoridae: *Geocoris* sp.	1
D0-M2-PT	Carabidae: *Scarite* sp.	1
D0-M3-PT	Diplopoda	1
D0-R2-PT	Araneidae	2
D3-C1-PT	Silphidae: *Necrodes surinamensis*	1
D3-M2-PT	Tenebrionidae	3
D7-M2-SL (Mite jar)	Diptera larvae (first and second instar)	7
D7-M2-SL (Mite jar)	*Hydrotaea* 3rd instar	1
D7-M2-SL (Mite jar)	*Hydrotaea* 3rd instar	3
D14-M2-SB (Mite jar)	*Hydrotaea* 3rd instar	2
D14-R1-SB (Mite jar)	*Hydrotaea* 3rd instar	1
D10-C2-PT	Tenebrionidae	1
D14-M2-PT	*Dermestes* adult	1
D14-M1-PT	Curculionidae: *Sphenophorus* sp.	1
D14-C3-PT	Phoridae: *Megaselia scalaris*	2
D40-C2-PT	Araneidae	1
D40-M3-PT	Staphylinidae: Aleocharinae	1
D40-M3-PT	n/a	
D10-R1-PT	Geocoridae: *Geocoris* sp.	1

Label	Identification	Quantity
D7-M3-PT	Lepidoptera larva	1
D7-R1-PT	Tenebrionidae	1
D7-C2-PT	*Solenopsis* sp.	1
D3-C3-PT	Araneidae	1
	Lycosidae	1
	Salticidae	1
D3-C3-PT	Histeridae: *Xerosaprinus* sp.	1
D14-M1-SB	*Hydrotaea* larva infected with fungi	1
D40-R2-PT	Anthicidae	1
D40-C1-PT	Scarabaeidae: *Ataneus* sp. with hypopi (Acaridae: *Sancassania* sp.)	1
	Cocinellidae: *Hippodamia convergens*	1
D10-M3-PT	Buthidae	1
D14-C2-SB (Mite jar)	Lasiochilidae	2
D14-C2-SB (Mite jar)	*Dermestes* larva	1
	Beetle larva	1
D3-R1-PT	Trogidae: *Omorgus* sp.	1
	Armadillodiidae	1
	Tenebrionidae	1
	Mordellidae	1
R2 (28.vi.2013)	Sarcophagidae 3rd instar	3
6.vii.2013	*Omorgus* sp.	1
	Dermestes larva	1
	Histeridae: Saprininae	3
D7-M3-SB (Mite jar)	Phoridae larvae	3
D0-M3-UP-A (sticky trap)	*Promochus bastardii* adult	1
7.vii.2013	Tettigoniidae: *Conocephalus* sp.	1
M3- June 2013	Sarcophagidae 3rd larvae	9
R2- 7.vii.2013	*Sarcophaga bullata* adult	1
R3- 26.vi.2013	*Hermetia illucens* larvae	13
	Hydrotaea larva	1

Label	Identification	Quantity
23.vii.2013	*Hermetia illucens* larva	1
R3-5.vii.2013	Siphidae: *Necrodes surinamensis*	1
	Ectobiidae: *Parcoblatta fulvescens* female	1
6.vii.2013	*Fannia* sp. adult	1
R2	*Hydrotaea* larvae	4
	Dermestes larvae	5
24.vii.2013	Histeridae: *Hister* sp.	4
R3	Sarcophagidae 3rd instar (*Sarcophaga bullata*)	4
M1 (Day 40)	Coccinellidae: *Cryptolaemus montrouzieri* (Mealybug Destroyer)	1
C3- 25.vii.2013	Pentatomidae	1
	Tenebrionidae	1
	Acari	30
18.vi.2013	Acari collected from Sihphidae	9
18.vi.2013	Cochliomyia macellaria females (for dissection). Results: All are non-gravid flies.	4
21.vii.2013	Acrididae	1
25.vii.2013	Sarcophagidae 3rd instar	2
7.vii.2013	Cleridae: *Necrobia rufipes*	1
R3	Scarabaeidae: *Ataneus* sp.	1
R3- 25.vii.2013	*Hydrotaea* larvae	2
M3- 29.vii.2013	Sarcophagidae larvae (*Sarcophaga bullata*)	3
R3- 8.vii.2013	*Nicrophorus marginatus* with acari	1 Acari x 15
M2- 21.vii.2013	*Dermestes* larva	1
	Omorgus sp. adult	1
	Necrobia rufipes adult	1
	Sarcophaga larva	1
6.vii.2013	*Dermestes marmolatus* adult	1
	Dermestes larva	1
7.vii.2013 (PT)	Lycosidae	1
R1- 30.vi.2013	*Dermestes caninus* with hypopi	1

Label	Identification	Quantity
	Dermestes larva	2
M1- 21.vii.2013	*Necrobia rufipes*	3
	Dermestes larva	1
	Histeridae: Saprininae	1
	Dermestes maculatus (with 3 acari)	1
M2- 5.vii.2013	*Hydrotaea* 3rd instar	5
M2- 25.vii.2013	*Sarcophaga* 3rd instar	1
	Hermetia illucens larva	1
R3- 13.vii.2013	Reduviidae: *Apiomerus* sp.	1
R2- 25.vii.2013	*Hermetia illucens* larvae	2
	Hydrotaea 3rd instar	2
	Dermestes larva	1
	Sarcophaga 3rd instar	1
C1 (D40)	Tenebrionidae adult with hypopi	1
M1- 25.vii.2013	*Hydrotaea* 3rd instar	1
M1- 24.vii.2013	*Hermetia illucens* larva	15
C3 – 30.vi.2013	*Chrysomya rufifacies* adult (newly emerged)	1
R3- 5.vi.2013	*Sarcophaga* 3rd instar	1
M1- 13.vii.2013	*Dermestes maculatus* with acari	1
R3- 12.vii.2013	Salticidae: *Phidippus audax*	1
M1- 5.vii.2013	*Necrobia rufipes* with acari	1
R2- 5.vii.2013	*Dermestes caninus* with acari	1
	Necrobia rufipes	1
7.vii.2013	*Sarcophaga bullata* adult	1
R1- 24.vii.2013	*Dermestes l*arvae	10
	Dermestes caninus adult	1
	Acari floating in 70% ethanol solution	Multiple individuals
C1- 5.vii.2013	*Dermestes* larva3	3
R1 (D40)	Exuvia of *Dermestes* sp. with acari	3

Label	Identification	Quantity
M3 (D40)	*Sarcophaga* 3rd instar	1
	(*Sarcophaga bullata*)	1
	Dermestes caninus with acari under elytra	
R1- 14.vii.2013	Chironomidae: Chironominae	1
	Chloropidae	11
	Scythrididae: *Scythris trivinctella*	1
M2- 24.vii.2013	*Sarcophaga* larvae (*Sarcophaga bullata*)	2
C2- 5.vii.2013	*Dermestes* larvae	2
R2 (D40)	*Hermetia illucens* larvae	2
R1- 7.vii.2013	*Sarcophaga* 3rd instar	2
M1- 16.vii.2013	*Omorgus* sp. with acari	1
R2- 29.vii.2013	*Necrobia rufipes*	1
	Histeridae (*Hister* sp.) with acari	1
R2- 21.vii.2013	*Dermestes caninus* adult with 2 acari	2
	Necrobia rufipes	3

LIST OF ARTHROPODS COLLECTED FROM THE FIELD IN SUMMER 2014 (BY FORCEPS) AND PRESERVED IN 70% ETHANOL

Label	Identification	Quantity
R2- 6.vii.2014	*Sarcophaga* 3rd lnstar	3
27.vi.2014	Histeridae: Saprininae	1
D21-R3-SB (Mite jar)	*Cochliomyia macellaria* 3rd	2
	instar	1
	Sarcophaga larvae	
M3- 30.vi.2014	Histeridae: *Hister* sp.	1
	Histeridae: *Xerosaprinus* sp.	1
	with acari	
R2- 1.vii.2014	Histeridae: *Hister* sp.	1
9.vii.2014	Chrysomelidae: *Chaetocnema* sp.	1
D21-M2-5M (Mite jar)	Tenebrionidae: *Opatrinus* sp.	1
20.vi.2014	Chrysomelidae: *Chaetocnema* sp.	1
R1- 21.vi.2014	*Sarcophaga* 3rd instar	2
C3 (D41)	Fungus grew on bone	1

Label	Identification	Quantity
M2 (D13)	Histeridae: *Hister* sp.	2
	Cochliomyia macellaria 3rd instar	1
	Chrysomya rufifacies 3rd instar	1
	Dermestes caninus adults	2
R3- 25.vi.2014	*Cochliomyia macellaria* 1st instar	5
19.vi.2014	Reduviidae: *Sinea* sp.	1
20.vi.2014	Tenebrionidae: *Opatrinus* sp.	1
M1- 20.vi.2014 (inside cage)	*Fannia pusio* adults	7
R1- 22.vii.2014	*Hermetia illucens* larvae	4
C1- 1.vii.2014	*Dermestes* larvae	1
R3- 6.vii.2014	*Sarcophaga* 3rd instar (*Sarcophaga bullata*)	5
M3- 24.vii.2014	Scarabaeidea larva (grub)	1
R1- 23.vii.2014	Coccinellidae larva	1
R1- 6.vii.2014	*Sarcophaga* 3rd instar	1
20.vi.2014	*Dermestes caninus* adult	1
C2- 11.vii.2014 (D22)	*Polietes* sp. nest	1
27.vi.2014	Scarabaeidae adult	1

LIST OF ARTHROPODS COLLECTED FROM THE FIELD IN SUMMER 2013 (BY SWEEP NETS OR FORCEPS) AND WERE PRESERVED IN INSECT BOX

Table L5. Arthropods collected from the field and preserved in insect box in 2013.

Label	Identification	Quantity
6.vii.2013 (sweep)	Sarcophagidae: *Ravinia lherminieri* (det. G.A. Dahlem)	1
6.vii.2013 (sweep)	Sarcophagidae: *Sarcophaga (Liosarcophaga) sarracenioides* (det. G.A. Dahlem)	1
M3- 7.vii.2013 (sweep)	Sarcophagidae: *Blaesoxipha (Giganthotheca) impar* (det. G.A. Dahlem)	1
6.vii.2013 (sweep)	Sarcophagidae: *Ravinia querula* (det. G.A. Dahlem)	1
11.vii.2013 (sweep)	Sarcophagidae: *Sarcophaga (Neobellieria) bullata* (det. G.A. Dahlem)	1
R1- 7.vii.2013 (sweep)	Sarcophagidae: *Blaesoxipha (Giganthotheca) plinthopyga* (det. G.A. Dahlem)	1

Label	Identification	Quantity
C1- 7.vii.2013 (sweep)	Sarcophagidae: *Ravinia derelicta* (det. G.A. Dahlem)	1
D0-M2-Sweep	Ichneumonidae: *Compsocryptus* sp.	1
D0-C3-Sweep	Chrysomelidae: *Chaetonecma* sp.	1
D3-C3-Sweep	Hymenoptera	1
D3-M1-Sweep	Diptera: Acalyptrate	1
D3-R1-Sweep	Cicadellidae: *Xyphon flaviceps*	1
D3-R1-Sweep	Neuroptera: *Chrysoperla* sp.	1
D14-R2-Sweep	Diptera (damaged)	1
D3-R1-Sweep	Fanniidae: *Fannia pusio*	1
D7-M2-Sweep	Diptera	1
D7-M3-Sweep	Fanniidae: *Fannia pusio*	1
D3-R1-Sweep	Fanniidae: *Fannia pusio*	2
D7-R3-Sweep	Fanniidae: *Fannia pusio*	2
D21-R1-Sweep	Fanniidae: *Fannia pusio*	1
D14-C3-Sweep	Calliphoridae: *Chrysomya rufifacies*	1
D10-R3-Sweep	Calliphoridae: *Cochliomyia macellaria*	2
D10-R3-Sweep	Muscidae: *Hydrotaea aenescens*	1
D0-R1-Sweep	Sarcophagidae	1
D7-R3-Sweep	Cicadellidae	1
D3-M1-Sweep	Calliphoridae: *Chrysomya rufifacies*	1
D3-R2-Sweep	Muscidae: *Musca domestica*	1
D3-M2-Sweep	Silphidae: *Nicrophorus marginatus*	1
D3-R3-Sweep	Muscidae: *Hydrotaea aenescens*	1
D3-R3-Sweep	Calliphoridae: *Cochliomyia macellaria*	1
D0-R1-Sweep	Tettigoniidae: *Conocephalus* sp.	1
D0-R3-Sweep	Cercopidae	1
D0-C1-Sweep	Cicadellidae	1
D0-R3-Sweep	Acrididae	1
D14-C3-Sweep	Calliphoridae: *Chrysomya rufifacies*	1
D21-M2-Sweep	Sarcophagidae: *Sarcophaga bullata*	1
D21-M2-Sweep	Asilidae	1
D21-R2-Sweep	Muscidae: *Hydrotaea aenescens* male	2
D21-R1-Sweep	Calliphoridae: *Chrysomya megacephala* male	1
D21-R3-Sweep	Sarcophagidae: *Ravinia lherminieri* (female)	1
D40-R3-Sweep	Halictidae	1
D40-R3-Sweep	Muscidae: *Hydrotaea aenescens*	1
14.vii.2013 (D28)	Sarcophagidae: *Sarcophaga bullata* male	1

Label	Identification	Quantity
D21-M2-Sweep	Sarcophagidae	3
R1- 7.vii.2013	Sarcophagidae	1
6.vii.2013	Fanniidae: *Fannia* sp.	1
R1 & R3 Collected: 7.vii.2013 (third instar) Emerged: 20- 21.vii.2013 Pupation: 13-14 days	Sarcophagidae: *Sarcophaga bullata*	10
M1 Collected: 23.vii.2013 Emerged: 26.vii.2013 Pupation: 34 days	Stratiomyiidae: *Hermetia illucens*	5
R3- 25.vi.2013 (D39)	Stratiomyiidae: *Hermetia illucens* with an acari	1
R1 Collected: 7.vii.2013 (third instar) Emerged: 20.vii.2013 Pupation: 13 days	Sarcophagidae: *Sarcophaga bullata*	12
R2 Collected: 7.vii.2013 (third instar) Emerged: 22.vii.2013 Pupation: 15 days	Sarcophagidae: *Sarcophaga bullata*	3
R3 Collected: 7.vii.2013 (third instar) Emerged:20- 21.vii.2013 Pupation: 13-14 days	Sarcophagidae: *Sarcophaga bullata*	4

LIST OF ARTHROPODS COLLECTED FROM THE FIELD IN SUMMER 2014 (BY SWEEP NETS OR FORCEPS) AND WERE PRESERVED IN INSECT BOX

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Label	Identification	Quantity
26.vi.2014	Libellulidae: *Tramea* sp.	1
29.vi.2014	Libellulidae: *Tramea* sp.	1

Label	Identification	Quantity
18.vi.2014	Libellulidae: *Erythemis simplicicollis*	1
22.vi.2014	Nymphalidae:	1
22.vi.2014	Nymphalidae:	1
28.vi.2014	Acrididae	1
28.vi.2014	Nymphalidae: *Agraulis vanillae*	1
28.vi.2014	Dermestidae: *Dermestes marmolatus*	1
20.vi.2014	Hymenoptera	1
24.vi.2014	Sarcophagidae: *Ravinia* sp.	1
24.vi.2014	Hemiptera	1
21.vi.2014	Sarcophagidae: *Ravinia* sp. male	2
17.vi.2014 (R1)	Sarcophagidae: *Ravinia lherminieri*	1
19.vi.2014	Staphylinidae: *Creophilus maxillosus*	1
22.vi.2014	Sarcophagidae: *Sarcophaga bullata* male	1
19.vi.2014	Sarcophagidae: *Ravinia lherminieri* male	1
22.vi.2014	Sarcophagidae: *Ravinia* sp. male	1
26.vi.2014	Staphylinidae: *Creophilus maxillosus*	1
28.vi.2014	Coenagrionidae: *Argia apicalis*	1
19.vi.2014	Coenagrionidae: *Argia apicalis*	2
24.vi.2014 (R2)	Muscidae: *Musca domestica*	2
24.vi.2014 (R2)	Sarcophagidae: *Sarcophaga bullata* male	1
26.vi.2014	Sarcophagidae: *Sarcophaga bullata* female	1
27.vi.2014	Histeridae	1
18.vi.2014	Sarcophagidae: *Ravinia lherminieri* female	1
20.vi.2014	Diptera	1
27.vi.2014	Histeridae	1
26.vi.2014	Staphylinidae: *Creophilus maxillosus*	1
27.vi.2014	Sarcophagidae: *Ravinia* sp. male	1
26.vi.2014	Sarcophagidae: *Sarcophaga bullata* male	1
22.vi.2014	Sarcophagidae: *Sarcophaga bullata* male	1
27.vi.2014	Silphidae: *Nicrophorus marginatus*	1
21.vi.2014	Dermestidae: *Dermestes caninus*	1
28.vi.2014	Muscidae: *Musca domestica*	1
27.vi.2014	Sarcophagidae: *Sarcophaga bullata* male	1
28.vi.2014	Sarcophagidae	1
28.vi.2014	Sarcophagidae: *Sarcophaga bullata* male	1
27.vi.2014	Formicidae: Myrmecinae	1
27.vi.2014	Sarcophagidae: *Ravinia* sp. male	1
19.vi.2014	Histeridae	1

Label	Identification	Quantity
20.vi.2014	Sarcophagidae: *Ravinia lherminieri* male	1
28.vi.2014	Sarcophagidae: *Sarcophaga bullata* male	1
29.vi.2014	Sarcophagidae: *Ravinia* sp. male	1
29.vi.2014	Sarcophagidae: *Blaesoxipha plinthopyga* male	1
30.vi.2014	Sarcophagidae: *Ravinia* sp. male	1
30.vi.2014	Sarcophagidae: *Ravinia* sp. male	1
30.vi.2014	Sarcophagidae: *Blaesoxipha plinthopyga* male	1
30.vi.2014	Sarcophagidae: *Ravinia* sp. female	1
30.vi.2014	Sarcophagidae: *Blaesoxipha plinthopyga* female	1
1.vii.2014	Coenagrionidae: *Ischnura hastata*	1
1.vii.2014	Chrysomelidae: *Deloyala quttata*	1
1.vii.2014	Asilidae	1
30.vi.2014	Sarcophagidae: *Ravinia* sp. male	1
3.vii.2014	Apidae: *Bombus pensylvanicus*	1
3.vii.2014	Asilidae: *Triorla interrupta*	1
2.vii.2014	Ulidiidae: *Physiphora* sp.	1
5.vii.2014	Chrysomelidae: *Anomoea* sp.	2
3.vii.2014	Nymphalidae: *Asterocampa celtis*	1
3.vii.2014	Nymphalidae	1
7.vii.2014	Nymphalidae	1
4.vii.2014	Sarcophagidae: *Sarcophaga bullata* female	1
24.vi.2014	Histeridae	1
23.vii.2014	Culicidae	1
7.vii.2014	Scarabaeidae: *Onthophagus hecate*	1
7.vii.2014	Sarcophagidae: *Sarcophaga bullata* male	1
7.vii.2014	Sarcophagidae: *Ravinia* sp. male	2
8.vii.2014	Sarcophagidae: *Ravinia* sp. male	3
8.vii.2014	Sarcophagidae male	1
10.vii.2014	Sarcophagidae male	1
D7-R3-Sweep	Fanniidae: *Fannia* sp.	1
D7-C3-Sweep	Diptera	2
D7-R2-Sweep	Ephydridae	1
D7-M1-Sweep	Diptera	1
D10-M3-Sweep	Muscidae: *Hydrotaea aenescens*	1
20.vi.2014	Diptera	1
28.vi.2014	Dermestidae: *Dermestes caninus*	2

Label	Identification	Quantity
D14-M2-PT Collected: 21.vi.2014 Emerged: 11.vii.2014 Pupation: 21 Days	Dermestidae: *Dermestes caninus* Sarcophagidae: *Sarcophaga bullata*	1 8
Collected: 28.vi.2014 Emerged: 15.vii.2014 Pupation: 18 Days	Sarcophagidae: *Sarcophaga bullata*	3
Collected: 4.vii.2014 Emerged: 20.vii.2014 Pupation: 16 Days	Sarcophagidae: *Sarcophaga bullata*	15
Collected: 4.vii.2014 Emerged: 20.vii.2014 Pupation: 16 Days	Sarcophagidae: *Sarcophaga bullata*	10
Collected: 4.vii.2014 Emerged: 20.vii.2014 Pupation: 16 Days	Sarcophagidae: *Sarcophaga bullata*	9

APPENDIX M

OBSERVATIONAL DATA ON TERRESTRIAL ARTHROPOD COMMUNITY

STRUCTURE AND FUNCTION ON PIG CARRION IN SUMMERS 2013 AND 2014

Year effect between 2013 and 2014

Table M1. Comparison of observational terrestrial arthropod community structure data using PERMANOVA between 2013 and 2014 trials in Snook, Texas. There was significant difference detected between years ($p < 0.05$).

Factor	df	F Model	R^2	P value
Year	1	34.214	0.0230	0.001*
Residual	1456		0.9770	
Total	1457		1.0000	

Field Trial 2013

Observational terrestrial arthropod community structure (Order)

Table M2. PERMANOVA on observational terrestrial arthropod community structure data by Order in 2013 trial at Snook, Texas. Significant differences ($p < 0.05$) were found on all factors and interactions.

Factor	df	F Model	R^2	P value
Day	41	4.899	0.1545	0.001*
Phase	1	190.684	0.1467	0.001*
Treatment	2	12.935	0.0199	0.001*
Day x Phase	39	3.835	0.1151	0.001*
Day x Treatment	82	1.460	0.0921	0.001*
Phase x Treatment	2	7.987	0.0122	0.001*
Day x Phase x Treatment	78	1.341	0.0805	0.001*

Observational terrestrial arthropod community structure (Family)

Table M3. PERMANOVA on observational terrestrial arthropod community structure data by Family in 2013 trial at Snook, Texas. Significant differences ($p < 0.05$) were found on all factors and interactions.

Factor	df	F Model	R^2	P value
Day	41	4.521	0.1520	0.001*
Phase	1	103.221	0.0846	0.001*
Treatment	2	5.438	0.0089	0.001*
Day x Phase	39	4.317	0.1380	0.001*
Day x Treatment	82	1.659	0.1115	0.001*
Phase x Treatment	2	7.317	0.0120	0.001*
Day x Phase x Treatment	78	1.397	0.0893	0.001*

Observational terrestrial arthropod community structure (Genus and species)

Table M4. PERMANOVA on observational terrestrial arthropod community structure data by Genus and species in 2013 trial at Snook, Texas. Significant differences ($p < 0.05$) were found on all factors and interactions.

Factor	df	F Model	R^2	P value
Day	41	4.391	0.1473	0.001*
Phase	1	106.085	0.0868	0.001*
Treatment	2	6.012	0.0098	0.001*
Day x Phase	39	4.221	0.1347	0.001*
Day x Treatment	82	1.718	0.1152	0.001*
Phase x Treatment	2	7.558	0.0123	0.001*
Day x Phase x Treatment	78	1.426	0.0910	0.001*

Observational terrestrial arthropod community functions

Table M5. PERMANOVA on observational terrestrial arthropod community functions in 2013 trial at Snook, Texas. Significant differences ($p < 0.05$) were found on all factors and interactions.

Factor	df	F Model	R^2	P value
Day	41	4.589	0.1461	0.001*
Phase	1	129.776	0.1007	0.001*
Treatment	2	4.201	0.0065	0.001*
Day x Phase	39	4.946	0.1498	0.001*
Day x Treatment	82	1.710	0.1088	0.001*
Phase x Treatment	2	8.556	0.0132	0.001*
Day x Phase x Treatment	78	1.527	0.0924	0.001*

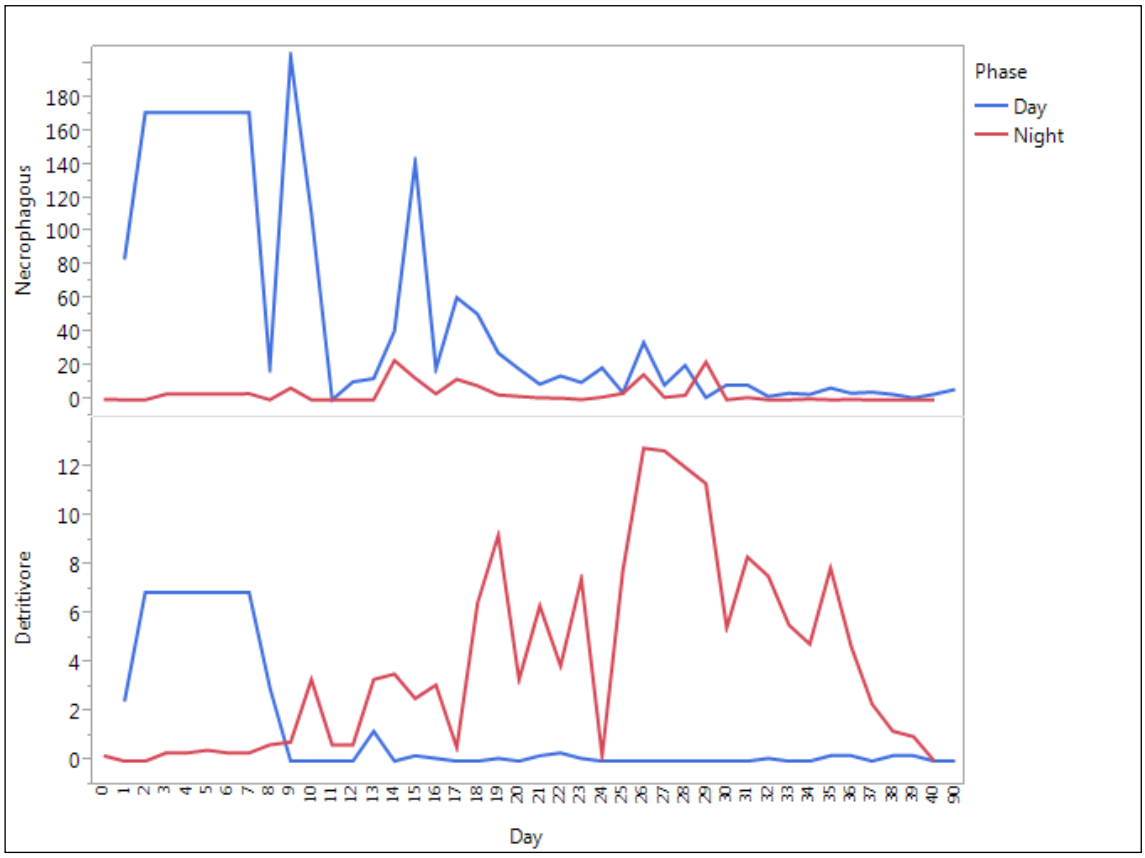


Figure M1. Mean abundance of necrophagous and detritivore communities according to phases (day and night) over day of pig carrion decomposition in summer 2013 at Snook, Texas. Increased population of generalist detritivores was noted during night phase at the study site.

Larval abundance observed on pig carrion in summer 2013

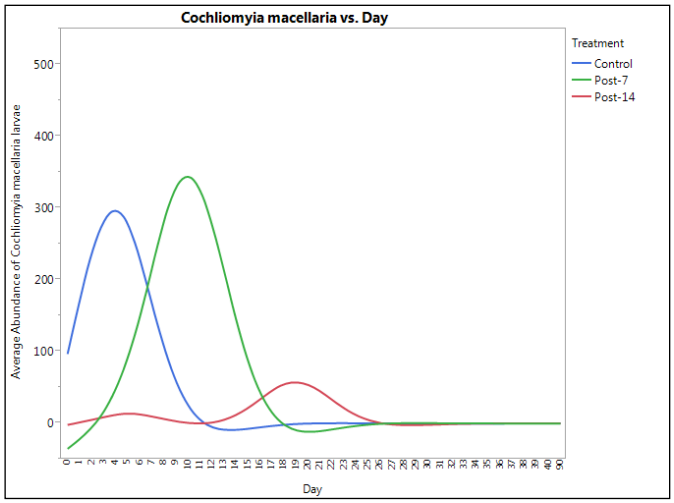


Figure M2. Average abundance of *Co. macellaria* (Diptera: Calliphoridae) larvae observed on pig carrion according to treatments over decomposition day in summer 2013 at Snook. Texas.

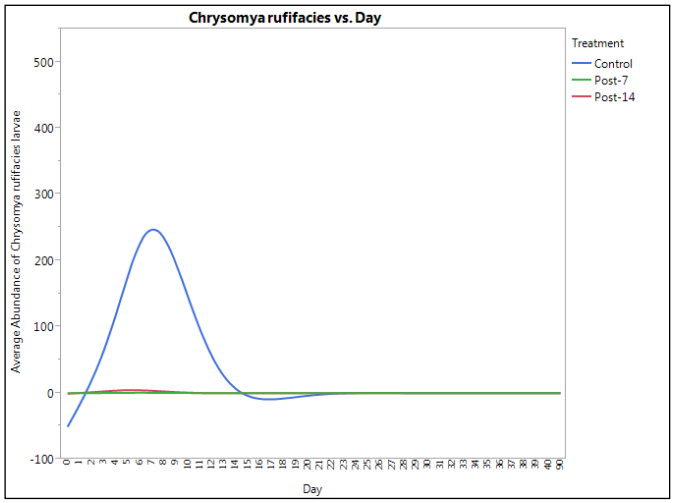


Figure M3. Average abundance of *Ch. rufifacies* (Diptera: Calliphoridae) larvae observed on pig carrion according to treatments over decomposition day in summer 2013 at Snook, Texas.

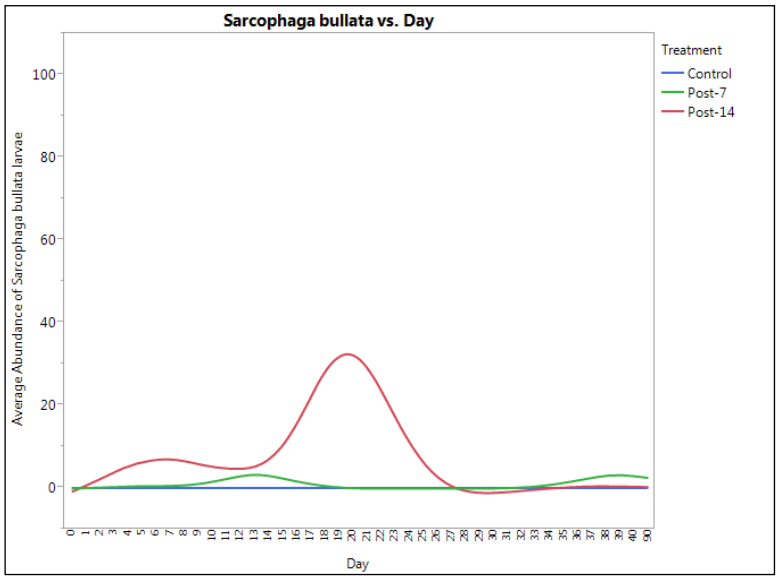


Figure M4. Average abundance of *S. bullata* (Diptera: Sarcophagidae) larvae observed on pig carrion according to treatments over decomposition day in summer 2013 at Snook, Texas.

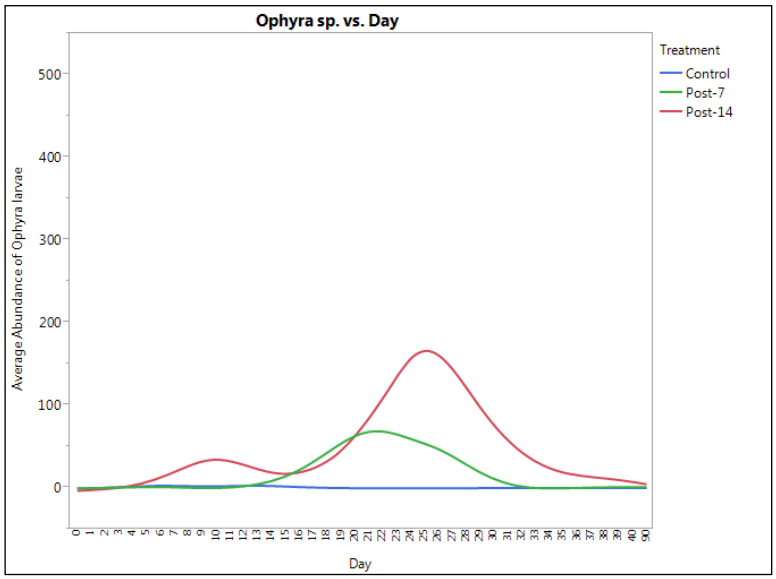


Figure M5. Average abundance of *Hydrotaea* sp. (Diptera: Muscidae) larvae observed on pig carrion according to treatments over decomposition day in summer 2013 at Snook, Texas.

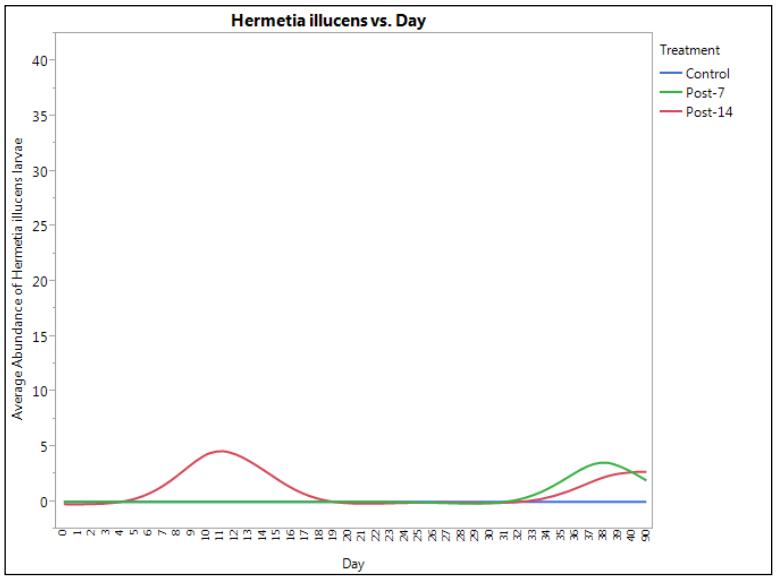


Figure M6. Average abundance of *Hermetia illucens* (Diptera: Stratiomyidae) larvae observed on pig carrion according to treatments over decomposition day in summer 2013 at Snook, Texas

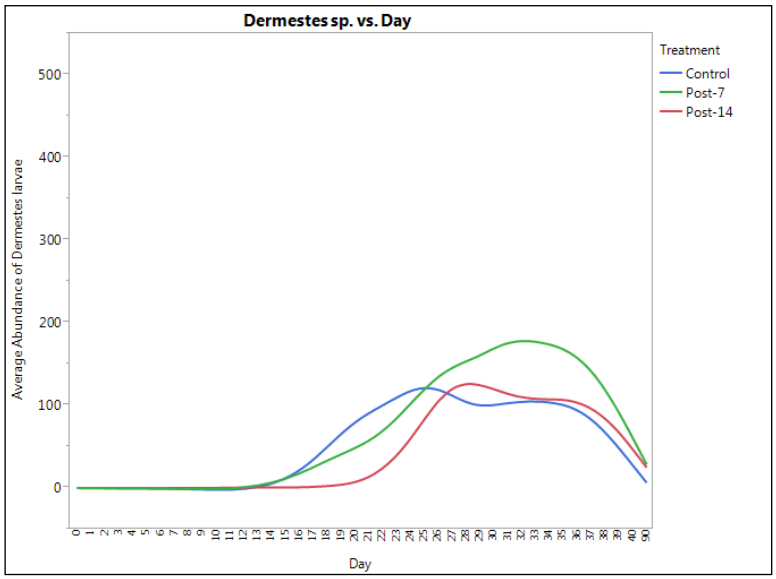


Figure M7. Average abundance of *Dermestes* sp. (Coleoptera: Dermestidae) larvae observed on pig carrion according to treatments over decomposition day in summer 2013 at Snook, Texas.

Circadian rhythms of adult insects observed on pig carrion in summer 2013

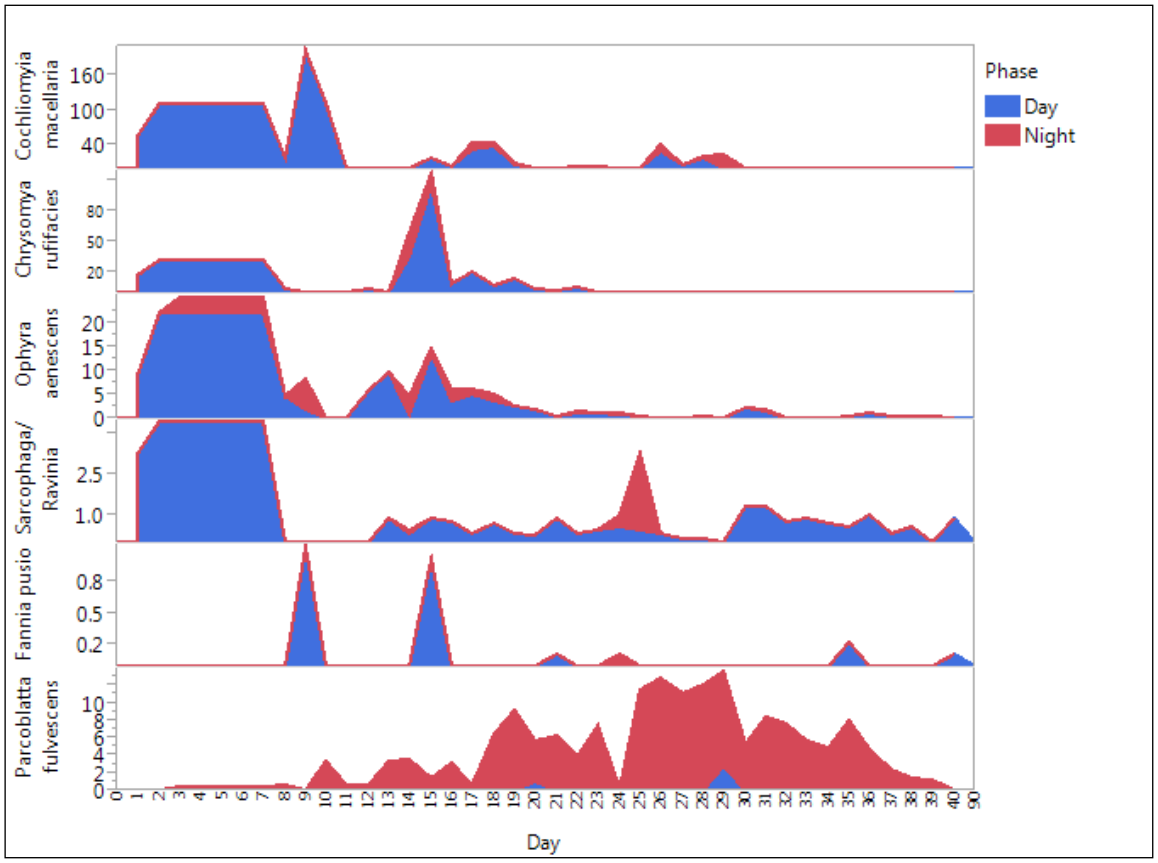


Figure M8. Average abundance of adult insects observed on pig carrion according to phases (day or night) over decomposition day in summer 2013 at Snook, Texas. Note that most of the necrophagous-specialist insects were active diurnally, while the cockroaches, *P. fulvescens*, was active nocturnally on the pig carrion in the field.

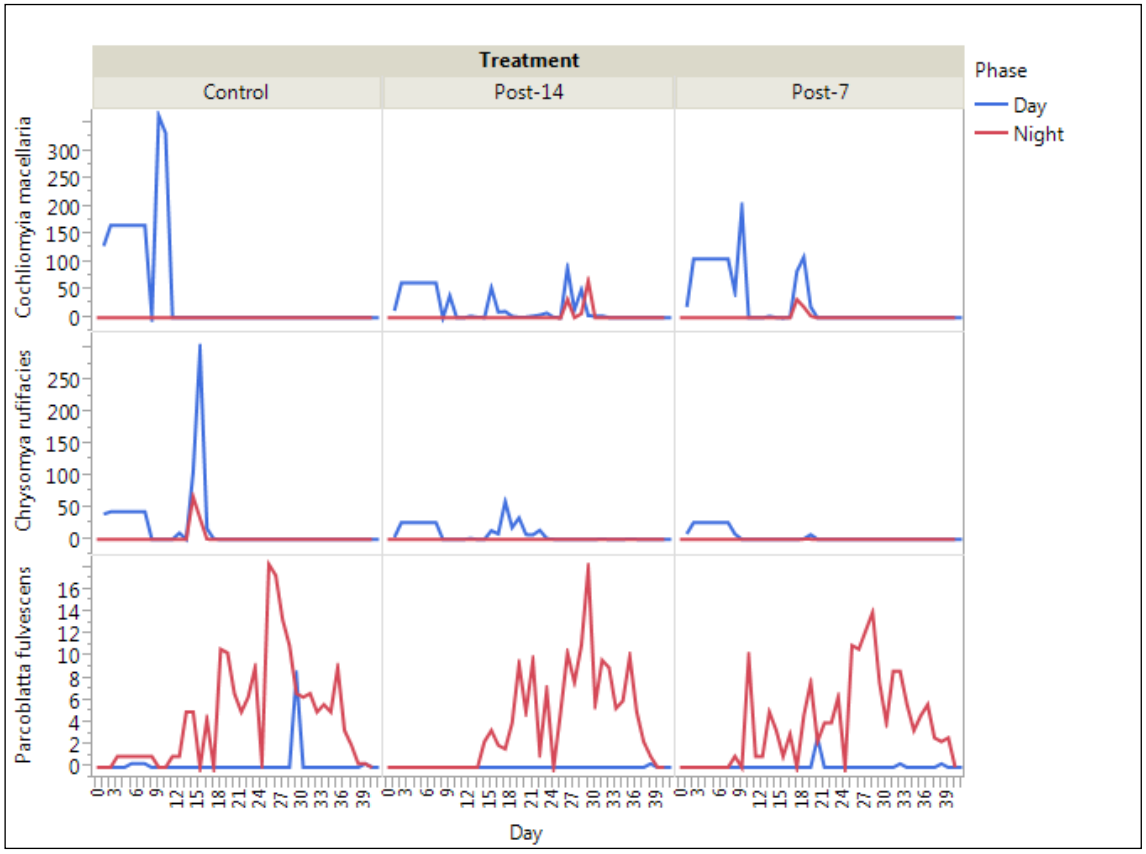


Figure M9. Average abundance of adult insects observed on pig carrion according to treatments (Control, Post-7, and Post-14) and phases (day, and night) over decomposition day in summer 2013 at Snook, Texas. Note that *Co. macellaria* and *Ch. rufifacies* were mostly active diurnally, whileas the cockroaches, *P. fulvescens*, was active nocturnally on the pig carrion in the field.

Dipteran larval community structure on pig carrion during summer 2013

Table M6. PERMANOVA on observational dipteran larval community structure by species in 2013 trial at Snook, Texas.

Factor	df	F Model	R^2	P value
Day	41	8.4590	0.4137	0.001*
Treatment	2	17.9388	0.0428	0.001*
Day x Treatment	82	2.3719	0.2320	0.001*

Table M7. PERMANOVA pairwise comparisons between treatments on the dipteran larval community structure by species in summer 2013 at Snook, Texas.

Treatments	df	F Model	R^2	P value
Control x Post-7	1	3.1313	0.0120	0.009*
Control x Post-14	1	15.738	0.0579	0.001*
Post-7 x Post-14	1	7.0182	0.0266	0.001*

Field Trial 2014

Observational terrestrial arthropod community structure (Order)

Table M8. PERMANOVA on observational terrestrial arthropod community structure data by Order in 2014 trial at Snook, Texas. Significant differences ($p < 0.05$) were detected on all factors and interactions.

Factor	df	F Model	R^2	P value
Day	40	6.216	0.1816	0.001*
Phase	1	99.752	0.0728	0.001*
Treatment	2	20.489	0.0299	0.001*
Day x Phase	38	4.250	0.1179	0.001*
Day x Treatment	80	2.032	0.1187	0.001*
Phase x Treatment	2	16.955	0.0247	0.001*
Day x Phase x Treatment	76	1.865	0.1035	0.001*

Observational terrestrial arthropod community structure (Family)

Table M9. PERMANOVA on observational terrestrial arthropod community structure data by Family in 2014 trial at Snook, Texas. Significant differences ($p < 0.05$) were detected on all factors and interactions.

Factor	df	F Model	R^2	P value
Day	40	6.153	0.1847	0.001*
Phase	1	102.776	0.0771	0.001*
Treatment	2	18.727	0.0281	0.001*
Day x Phase	38	3.835	0.1093	0.001*
Day x Treatment	80	1.983	0.1190	0.001*
Phase x Treatment	2	14.795	0.0222	0.001*
Day x Phase x Treatment	76	1.739	0.0991	0.001*

Observational terrestrial arthropod community structure (Genus and species)

Table M10. PERMANOVA on observational terrestrial arthropod community structure data by Genus and species in 2014 trial at Snook, Texas. Significant differences ($p < 0.05$) were found on all factors and interactions.

Factor	df	F Model	R^2	P value
Day	40	5.879	0.1791	0.001*
Phase	1	113.787	0.0866	0.001*
Treatment	2	19.437	0.0296	0.001*
Day x Phase	38	3.530	0.1021	0.001*
Day x Treatment	80	1.926	0.1173	0.001*
Phase x Treatment	2	14.728	0.0224	0.001*
Day x Phase x Treatment	76	1.674	0.0969	0.001*

Observational terrestrial arthropod community functions

Table M11. PERMANOVA on observational terrestrial arthropod community functions in 2014 trial at Snook, Texas. Significant differences ($p < 0.05$) were found on all factors and interactions.

Factor	df	F Model	R^2	P value
Day	40	6.383	0.1762	0.001*
Phase	1	134.253	0.0926	0.001*
Treatment	2	21.995	0.0303	0.001*
Day x Phase	38	4.668	0.1224	0.001*
Day x Treatment	80	2.145	0.1184	0.001*
Phase x Treatment	2	19.966	0.0275	0.001*
Day x Phase x Treatment	76	1.924	0.1009	0.001*

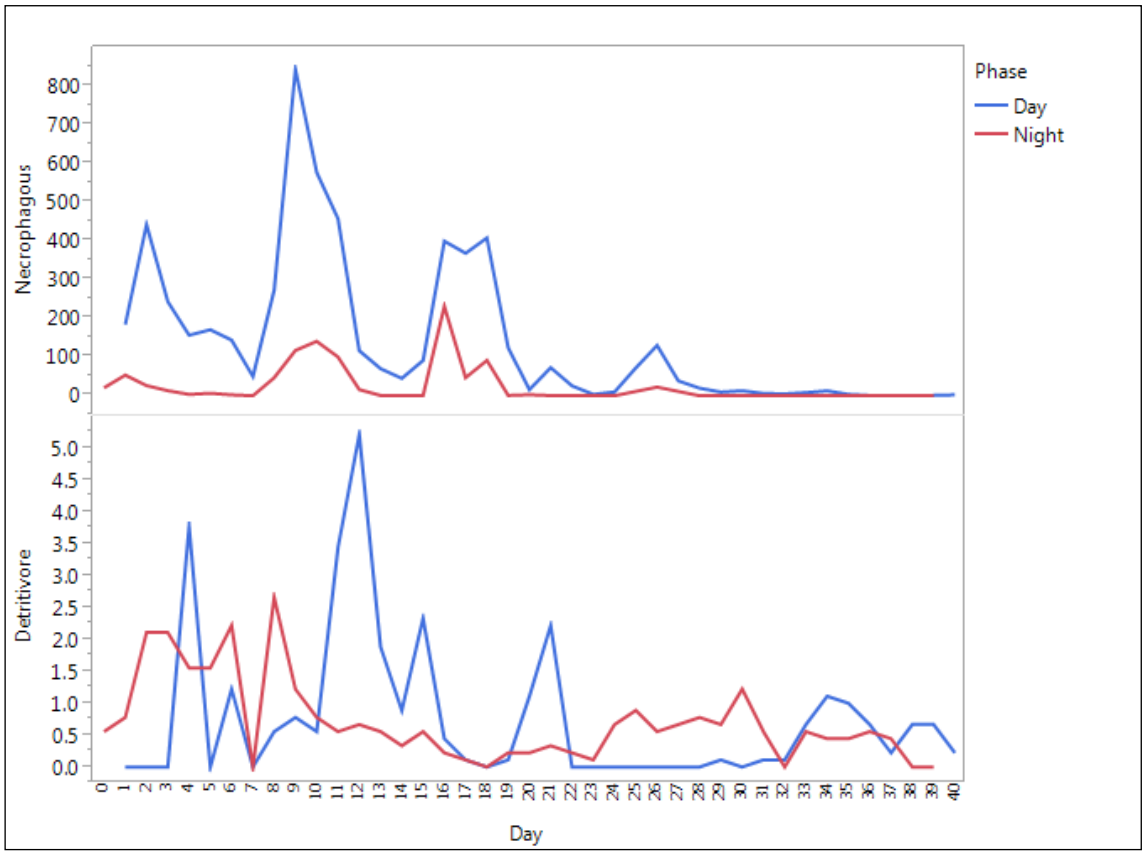


Figure M10. Mean abundance of necrophagous and detritivore communities according to phases (day and night) over day of pig carrion decomposition in summer 2014 at Snook, Texas.

Larval abundance observed on pig carrion in summer 2014

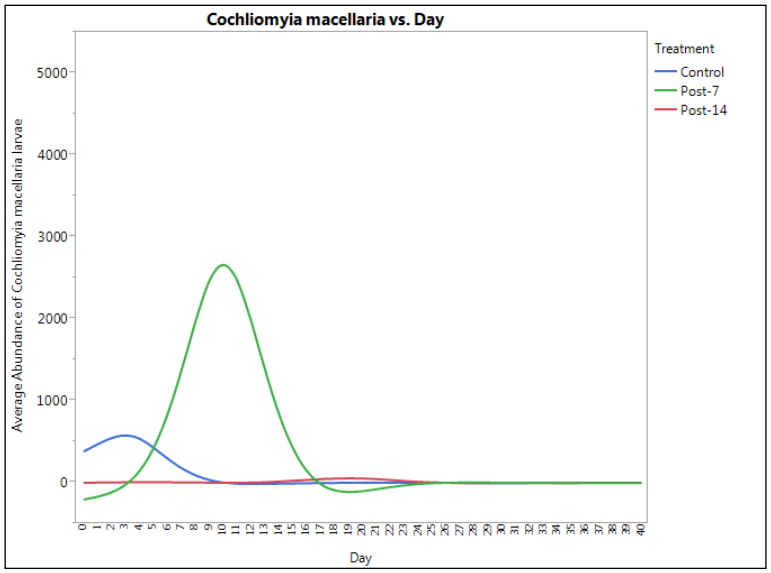


Figure M11. Average abundance of *Co. macellaria* (Diptera: Calliphoridae) larvae observed on pig carrion according to treatments over decomposition day in summer 2014 at Snook, Texas.

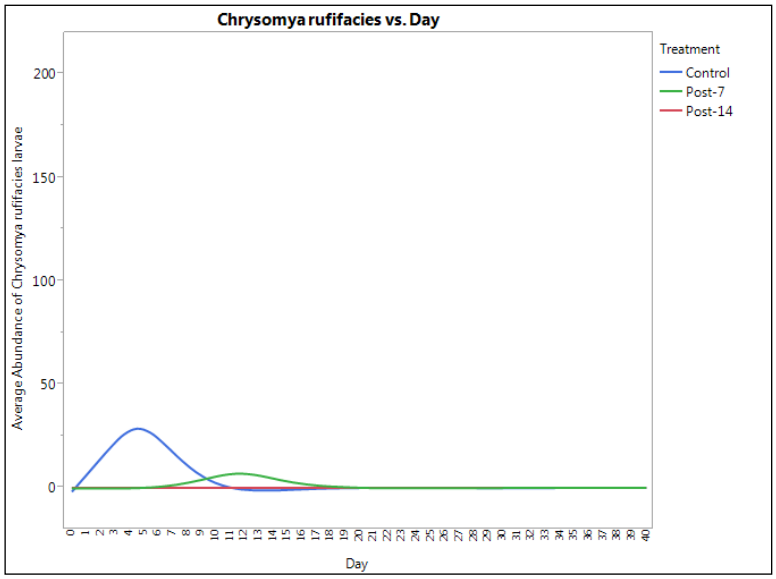


Figure M12. Average abundance of *Ch. rufifacies* (Diptera: Calliphoridae) larvae observed on pig carrion according to treatments over decomposition day in summer 2014 at Snook, Texas.

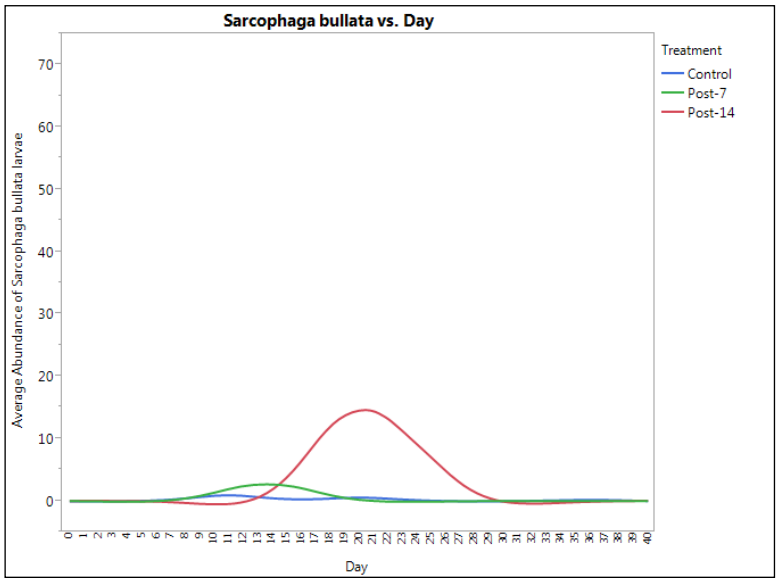


Figure M13. Average abundance of *S. bullata* (Diptera: Sarcophagidae) larvae observed on pig carrion according to treatments over decomposition day in summer 2014 at Snook, Texas.

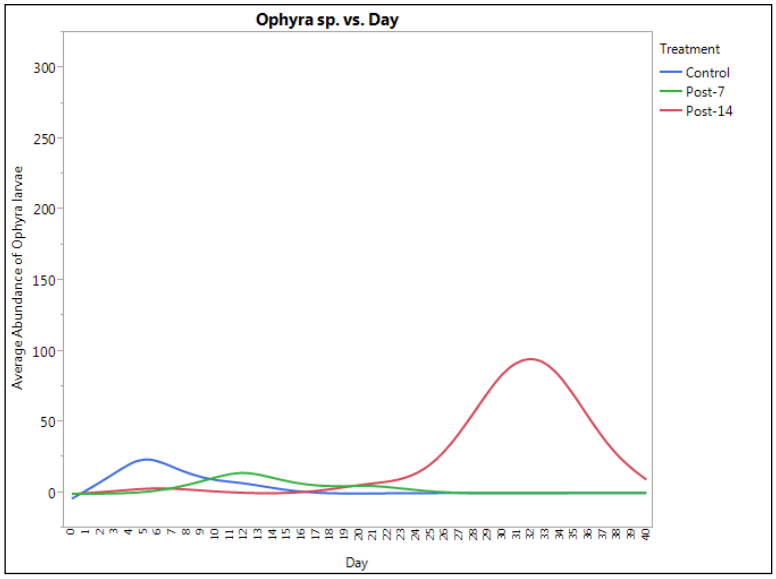


Figure M14. Average abundance of *Hydrotaea* sp. (Diptera: Muscidae) larvae observed on pig carrion according to treatments over decomposition day in summer 2014 at Snook, Texas.

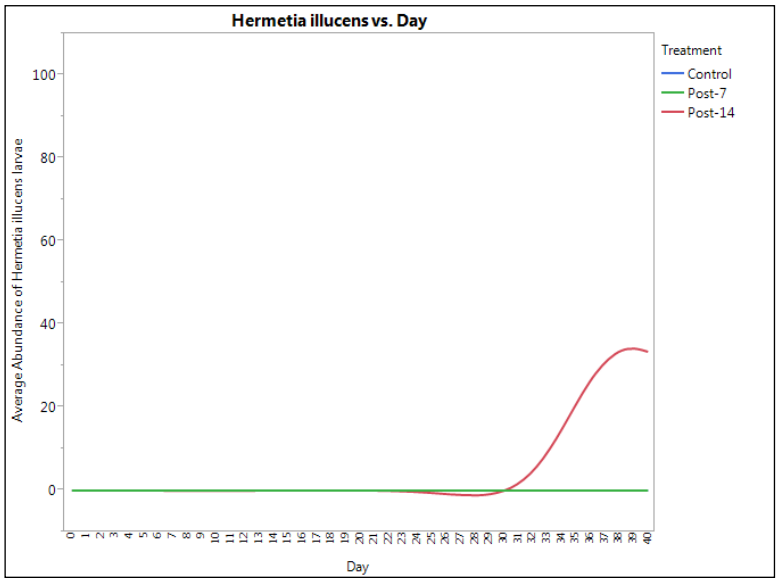


Figure M15. Average abundance of *H. illucens* (Diptera: Stratiomyidae) larvae observed on pig carrion according to treatments over decomposition day in summer 2014 at Snook, Texas.

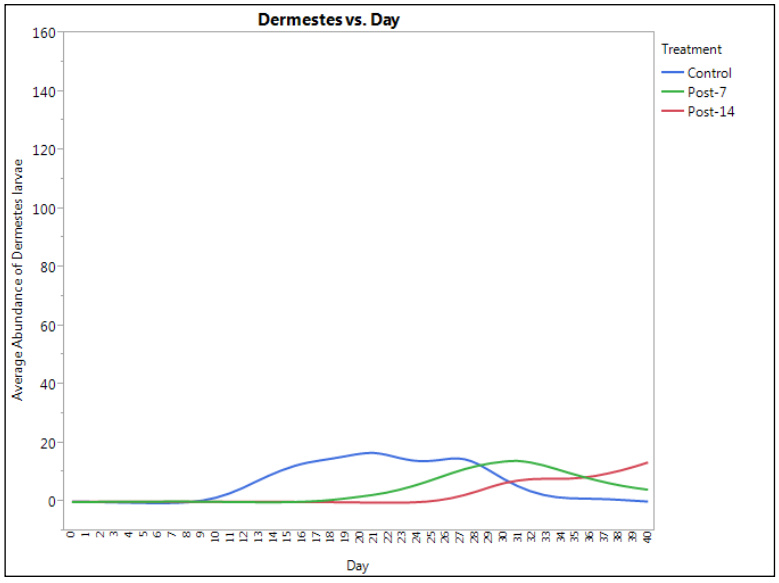


Figure M16. Average abundance of *Dermestes* sp. (Coleoptera: Dermestidae) larvae observed on pig carrion according to treatments over decomposition day in summer 2014 at Snook, Texas.

Circadian rhythms of adult insects observed on pig carrion in summer 2014

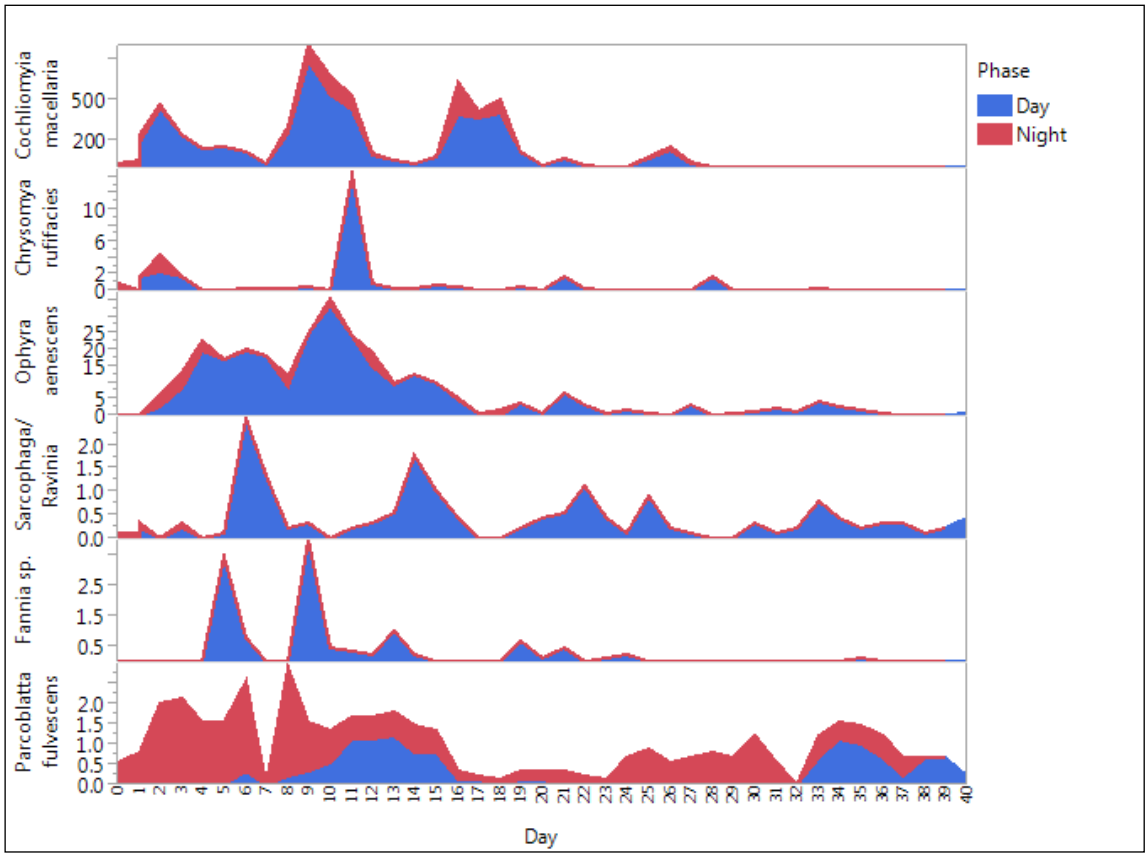


Figure M17. Average abundance of adult insects observed on pig carrion according to phases (day and night) over decomposition day in summer 2014 at Snook, Texas. Note that most of the necrophagous insects were active diurnally, while the cockroaches, *P. fulvescens*, was mainly active nocturnally on the pig carrion.

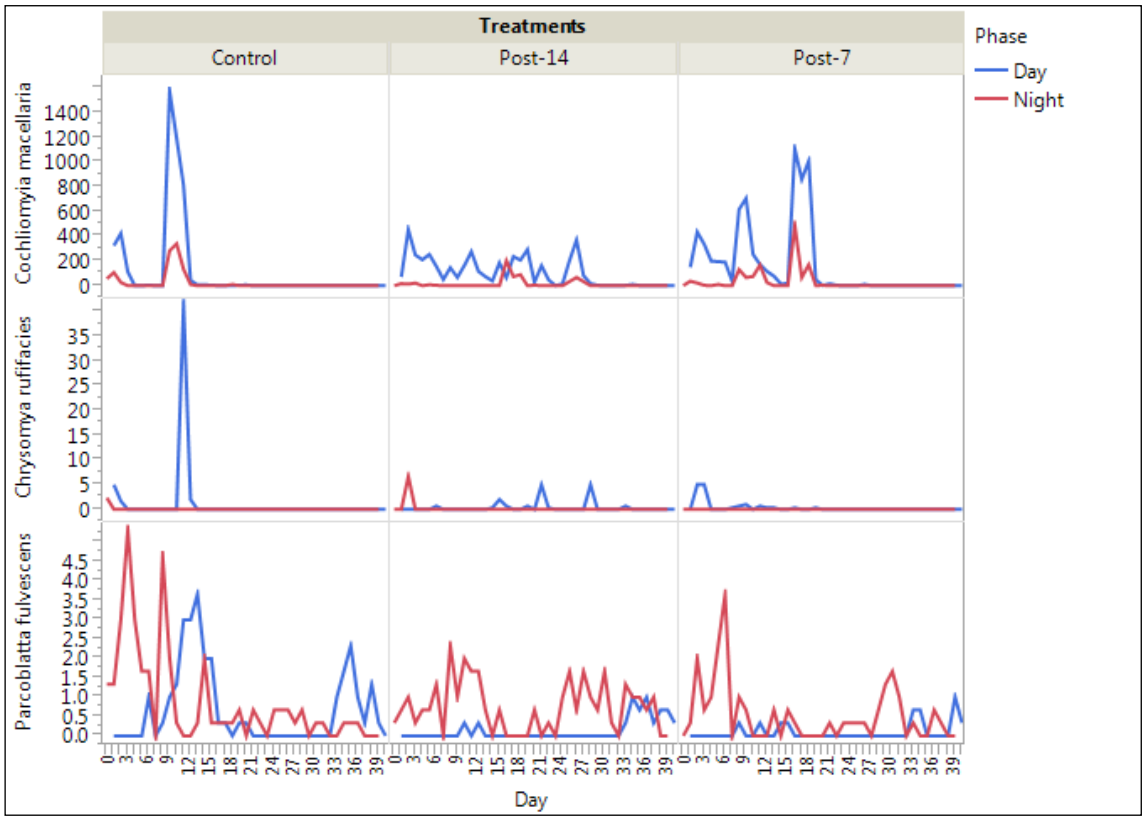


Figure M18. Average abundance of adult insects observed on pig carrion according to treatments (Control, Post-7, and Post-14) and phases (day, and night) over decomposition day in summer 2014 at Snook, Texas. Note that *Co. macellaria* and *Ch. rufifacies* were mostly active diurnally, whileas the cockroaches, *P. fulvescens*, was mostly active nocturnally on the pig carrion.

Dipteran larval community structure on pig carrion during summer 2014

Table M12. PERMANOVA on observational dipteran larval community structure by species in 2014 trial at Snook, Texas.

Factor	df	F Model	R^2	P value
Day	40	2.7128	0.1661	0.001*
Treatment	2	14.6756	0.0449	0.001*
Day x Treatment	80	3.2517	0.3983	0.001*

Table M13. PERMANOVA pairwise comparisons between treatments on the dipteran larval community structure by species in summer 2014 at Snook, Texas.

Treatments	df	F Model	R^2	P value
Control x Post-7	1	0.8078	0.0032	0.502
Control x Post-14	1	13.785	0.0522	0.001*
Post-7 x Post-14	1	11.155	0.0427	0.001*

Colonization pattern of *Chrysomya rufifacies* on pig carrion according to treatments over time

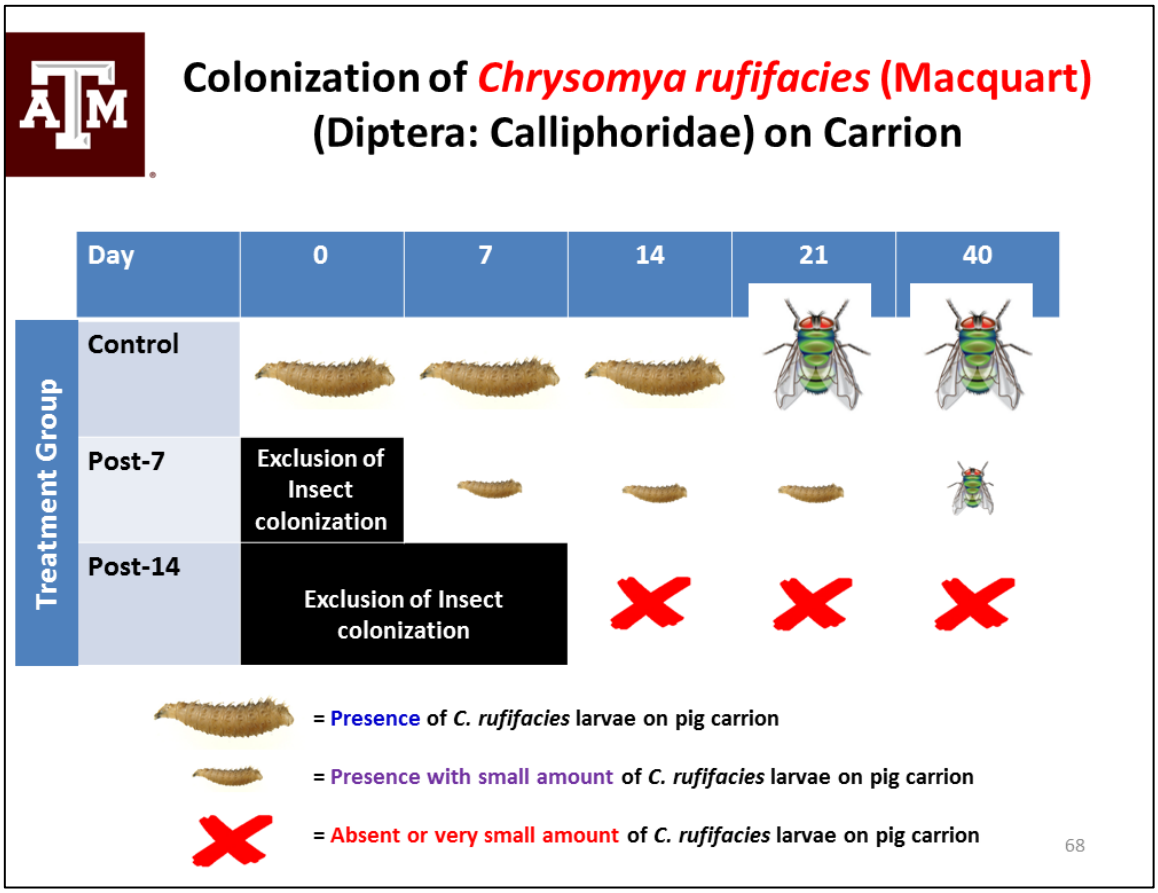


Figure M19. Colonization of *Chrysomya rufifacies* (Diptera: Calliphoridae) larvae on pig carrion by observation according to treatments over time in summers 2013 and 2014 at Snook, Texas. A large population (> 1000 individuals) of *C. rufifacies* larvae could be observed on the Control carrion while smaller amount of individuals were observed on Post-7 (~100 individuals) and Post-14 carrion (< 50 individuals). These apparent changes in the intensity of *C. rufifacies* colonization on carrion according to treatment groups may indicate less plasticity in resource utilization by *C. rufifacies*, and perhaps niche partitioning among the necrophagous guild.

Dipteran larva successional sequence on pig carrion

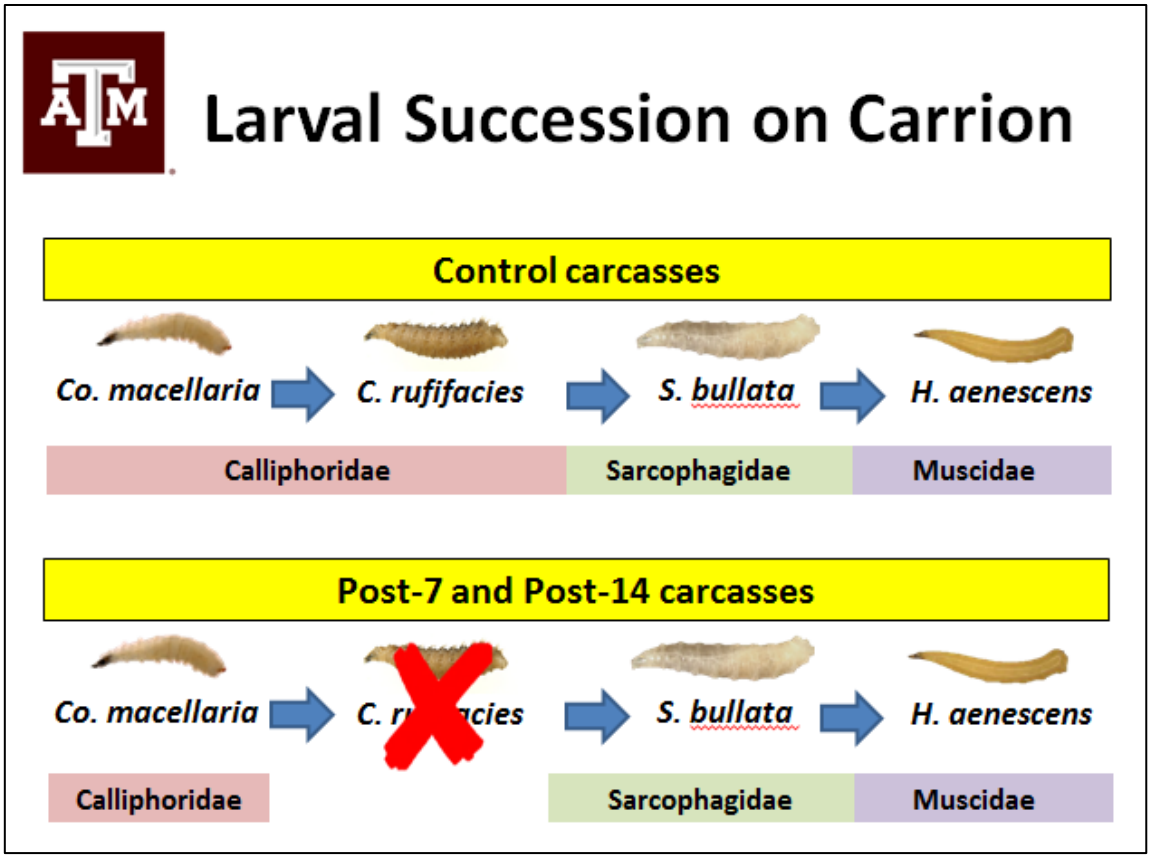


Figure M20. Comparison of larval succession between Control and Delayed (Post-7 and Post-14) carcasses in both summers 2013 and 2014 at Snook, Texas. For Control carcasses, larval succession began with *Co. macellaria*, succeeded by *C. rufifacies*, and then *S. bullata* and lastly by *H. aenescens*. By family level, larval succession is predicatble, as the primary colonizers are always Calliphoridae, followed by Sarcophagidae and then Muscidae. When disturbance of carrion occurred (i.e., delayed of dipteran colonization), the colonization of *C. rufifacies* was affected which led to low population or absent on carrion. As such, under normal or disturbed condition, larval succession on carrion by Family level are predictable (as in Clementsian model), however, there was a change in species response to disturbed carrion when larval succession is recognized at the Genus and species level (as in Gleasonian model). Hence, both models can explain insect succession on carrion, but is dependent on taxonomic scale of the organisms involved.

APPENDIX N

SUMMARY OF INDICATOR SPECIES ANALYSES (ISA)

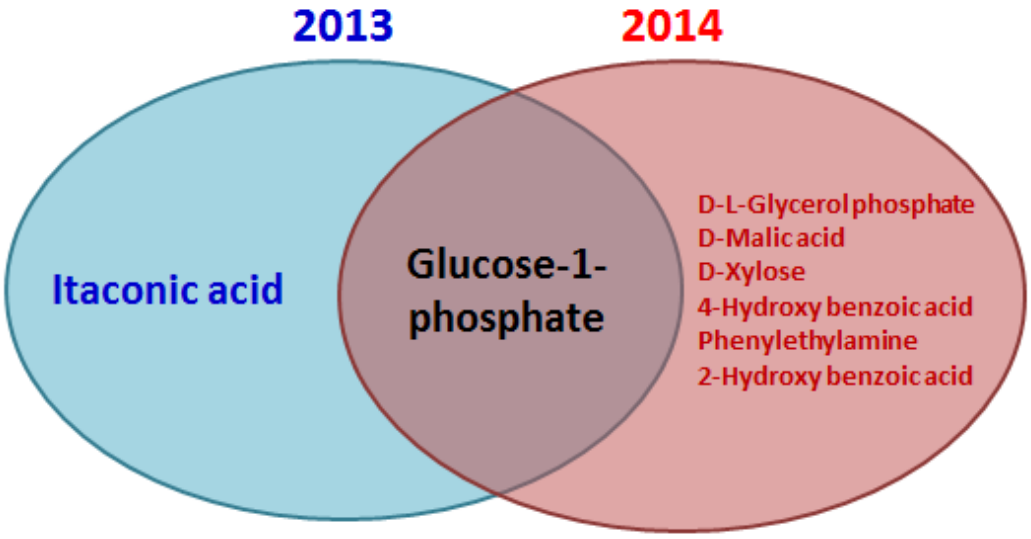


Figure N1. Comparison of indicator carbon sources in all soil samples (collected from beneath, lateral and 5 m from pig carrion) in summers 2013 and 2014 at Snook, Texas.

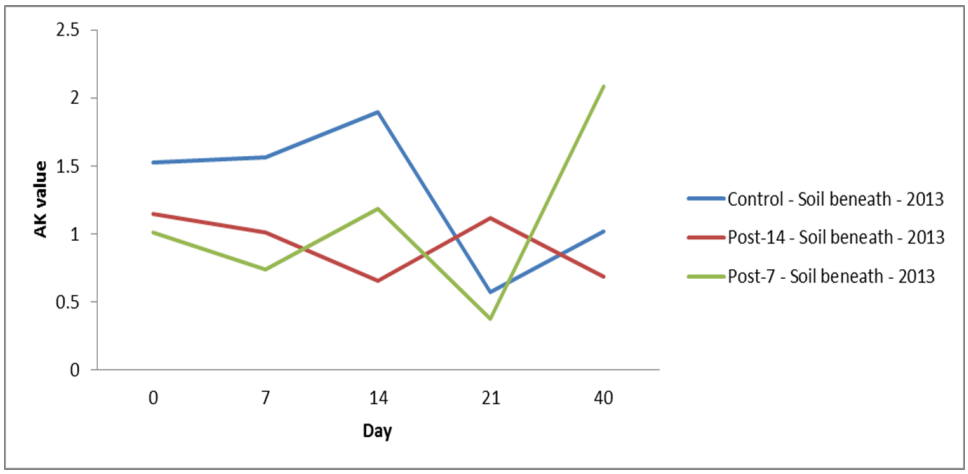


Figure N2. Soil microbe metabolic activity (average Ak value) on glucose-6-phosphate at soil beneath across treatments over day (summer 2013) at Snook, Texas.

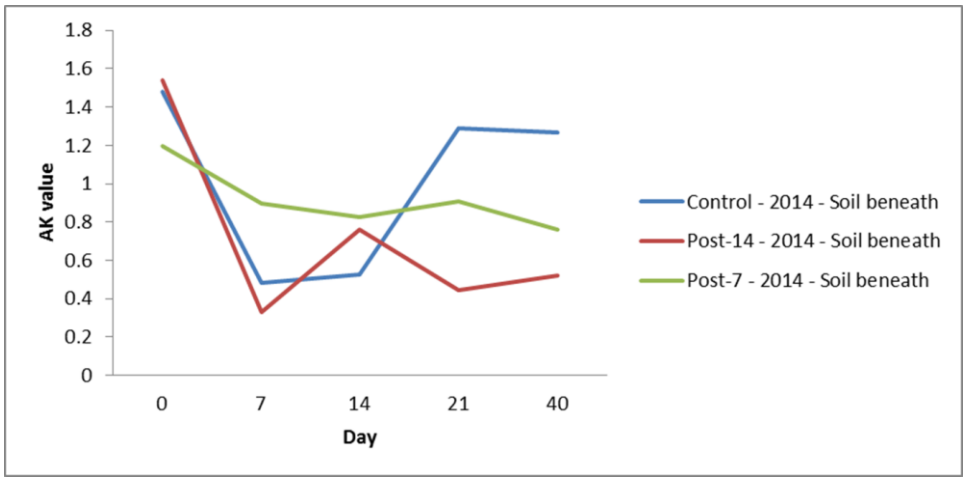


Figure N3. Soil microbe metabolic activity (average Ak value) on glucose-6-phosphate at soil beneath across treatments over day (summer 2014) at Snook, Texas.

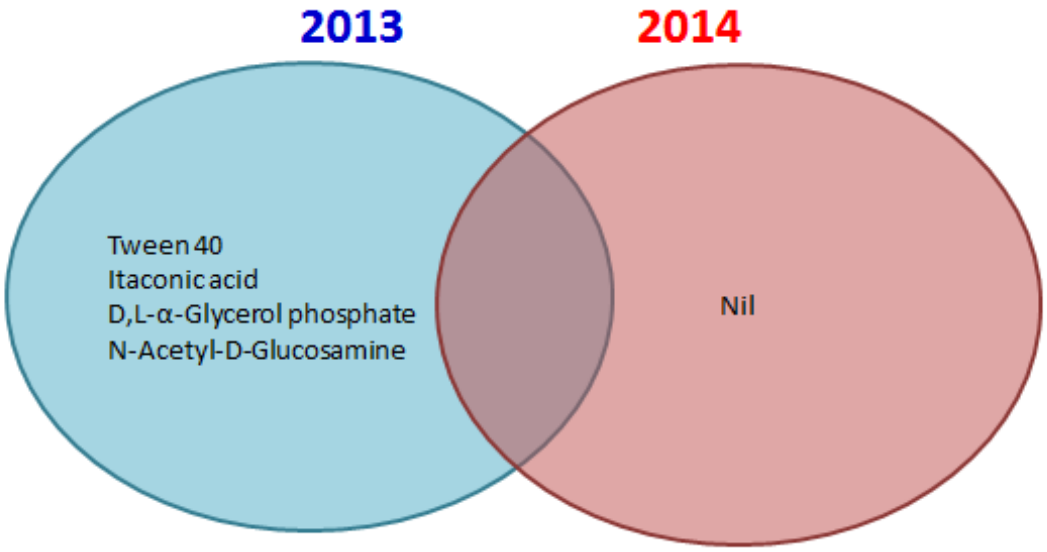


Figure N4. Comparison of indicator carbon sources in all pig samples (collected from oral, skin and anal region) in summers 2013 and 2014 at Snook, Texas.

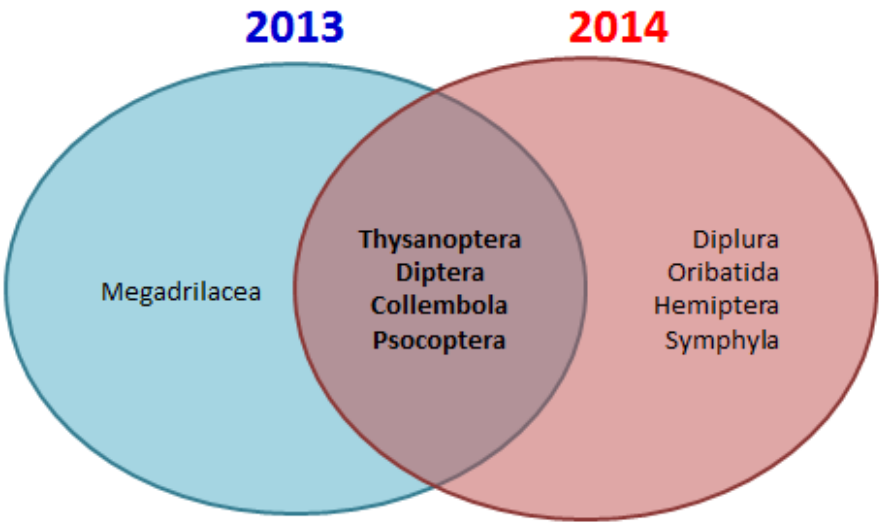


Figure N5. Comparison of indicator species analysis (by Order) of soil arthropods collected from all soil samples (beneath, lateral and 5 m) in summers 2013 and 2014 at Snook, Texas.

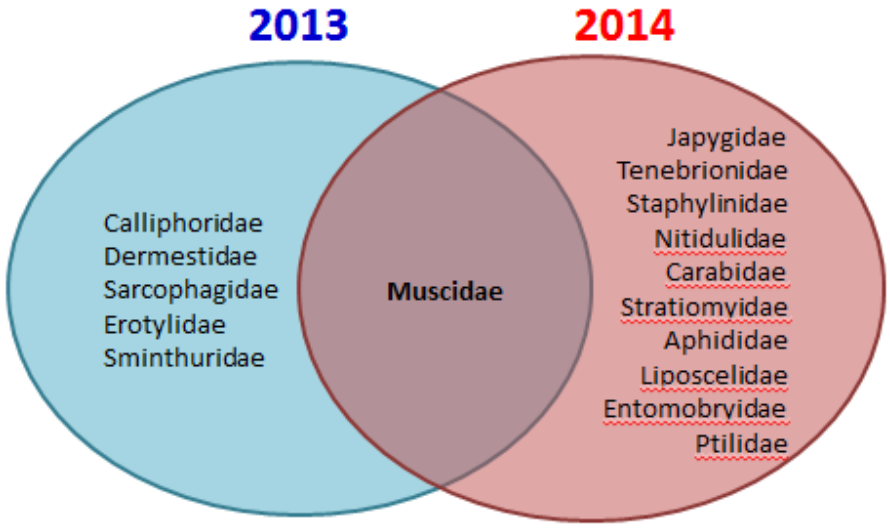


Figure N6. Comparison of indicator species analysis (by Family) of soil arthropods collected from all soil samples (beneath, lateral and 5 m) in summers 2013 and 2014 at Snook, Texas.

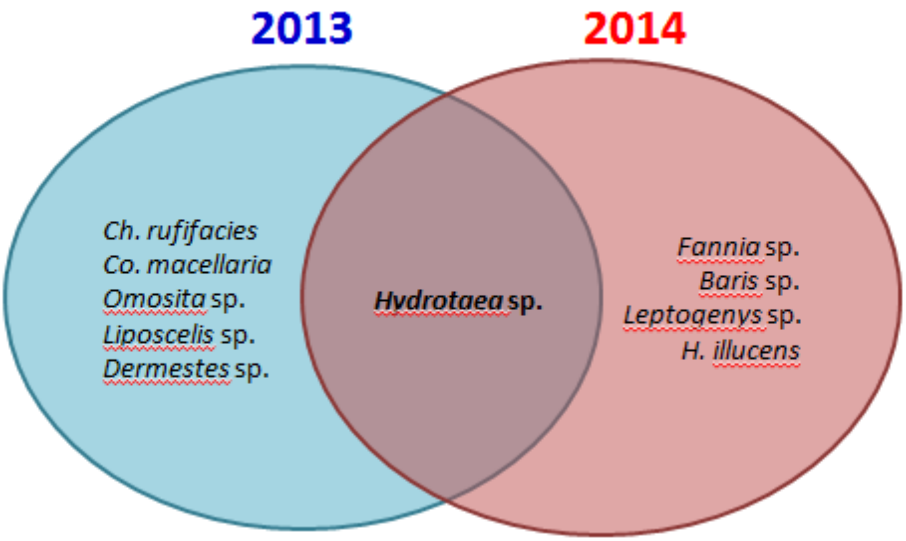


Figure N7. Comparison of indicator species analysis (by Genus and species) of soil arthropods collected from all soil samples (beneath, lateral and 5 m) in summers 2013 and 2014 at Snook, Texas.

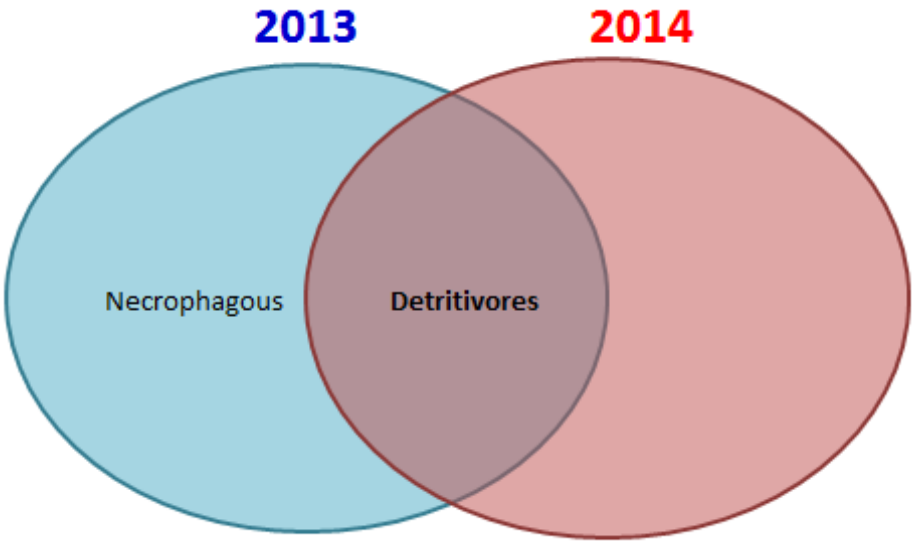


Figure N8. Comparison of indicator species analysis (by Function) of soil arthropods collected from all soil samples (beneath, lateral and 5 m) in summers 2013 and 2014 at Snook, Texas.

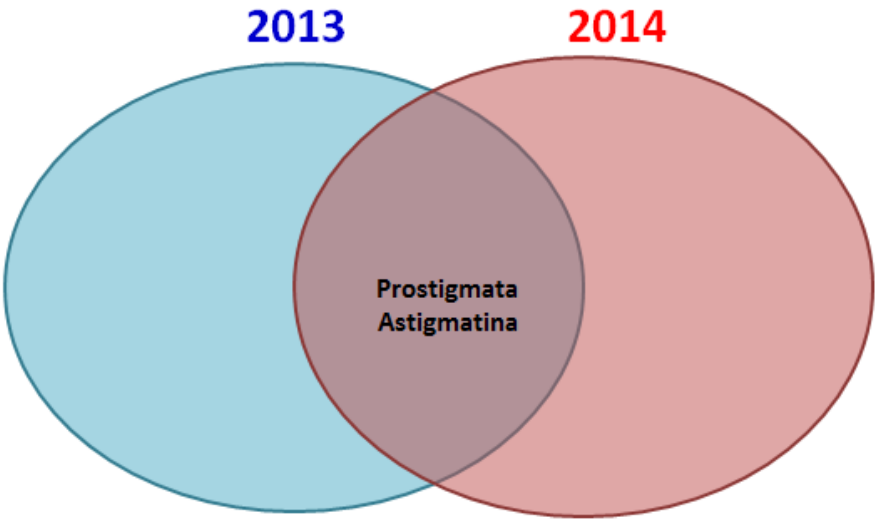


Figure N9. Comparison of indicator species analysis (by Suborder) of soil mites collected from all soil samples (beneath, lateral and 5 m) in summers 2013 and 2014 at Snook, Texas.

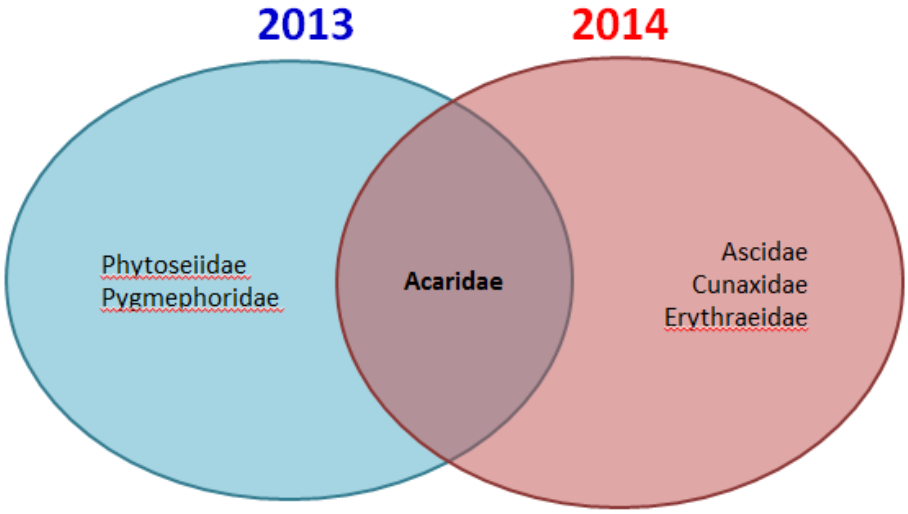


Figure N10. Comparison of indicator species analysis (by Family) of soil mites collected from all soil samples (beneath, lateral and 5 m) in summers 2013 and 2014 at Snook, Texas.

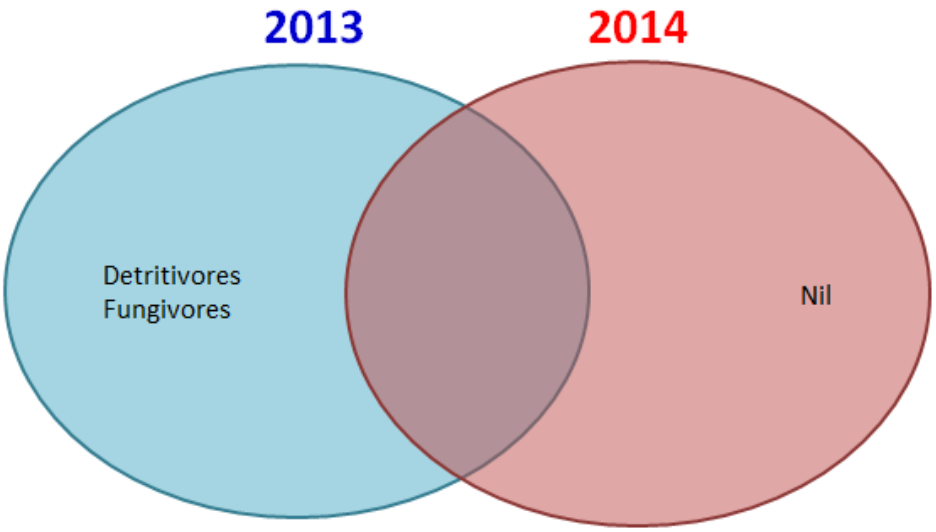


Figure N11. Comparison of indicator function analysis of soil mites collected from all soil samples (beneath, lateral and 5 m) in summers 2013 and 2014 at Snook, Texas.

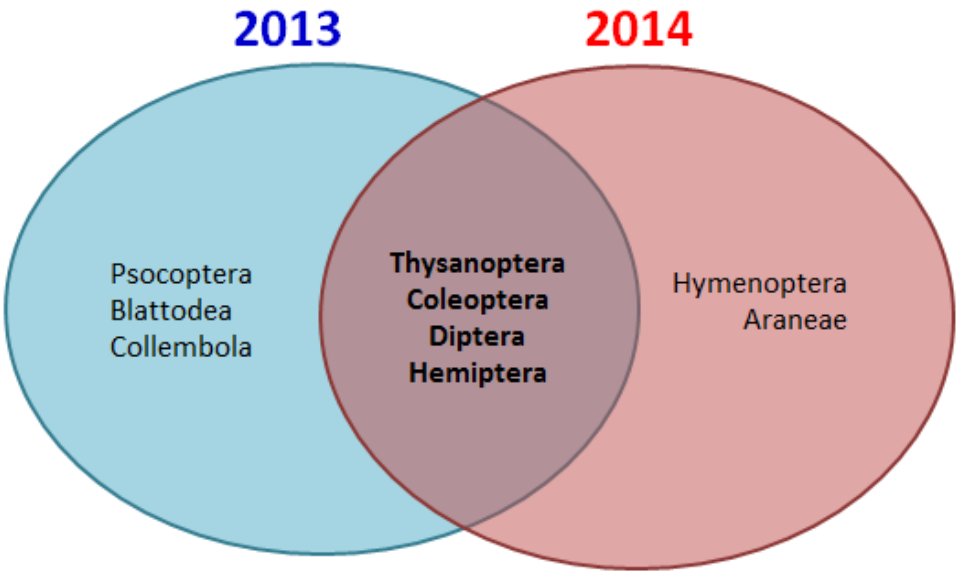


Figure N12. Comparison of indicator species analysis (by Order) of arthropods collected from all sticky traps placed in the field located at Snook, Texas during summers 2013 and 2014.

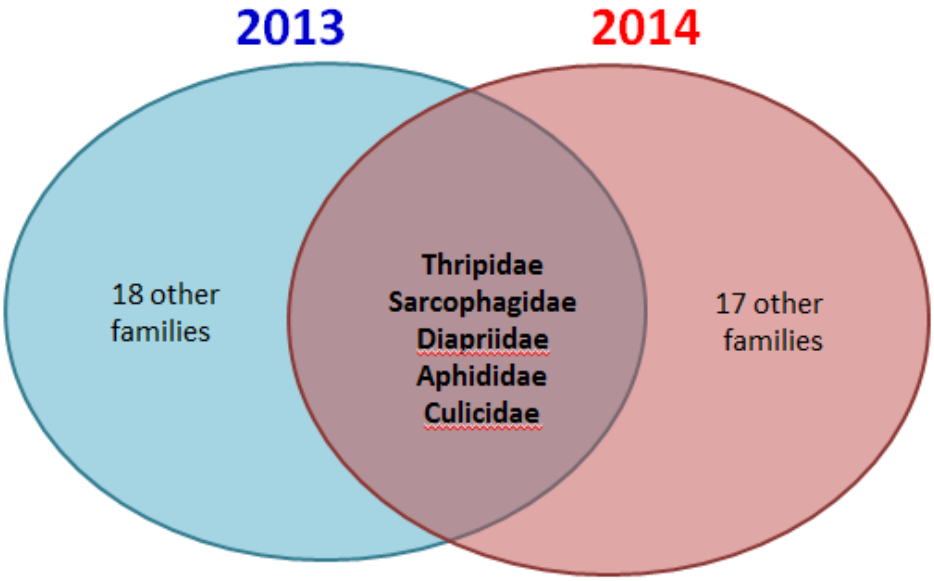


Figure N13. Comparison of indicator species analysis (by Family) of arthropods collected from all sticky traps placed in the field located at Snook, Texas during summers 2013 and 2014.

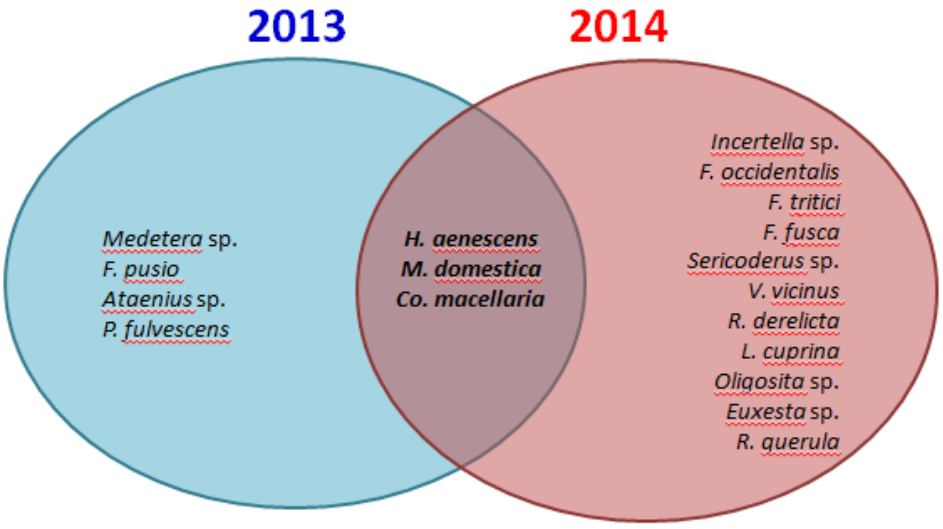


Figure N14. Comparison of indicator species analysis (by Genus and species) of arthropods collected from all sticky traps placed in the field located at Snook, Texas during summers 2013 and 2014.

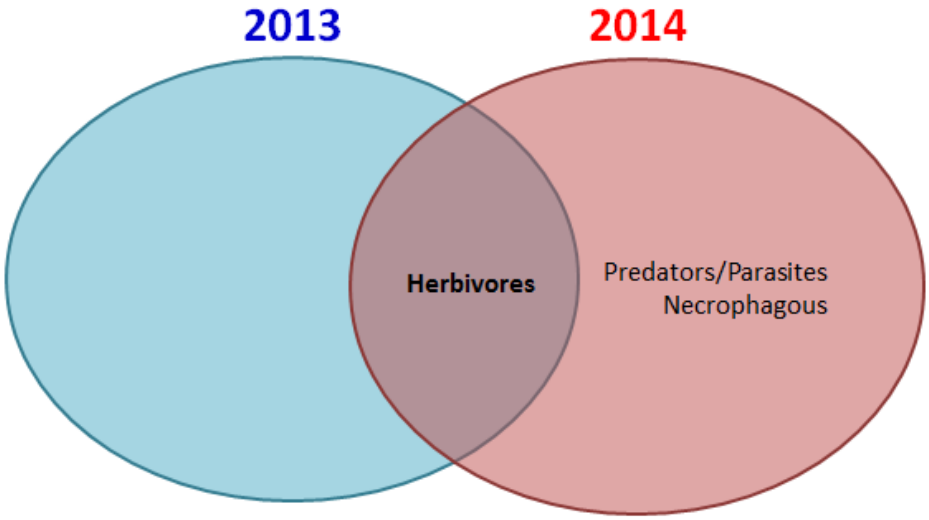


Figure N15. Comparison of indicator function analysis of arthropods collected from all sticky traps placed in the field located at Snook, Texas during summers 2013 and 2014.

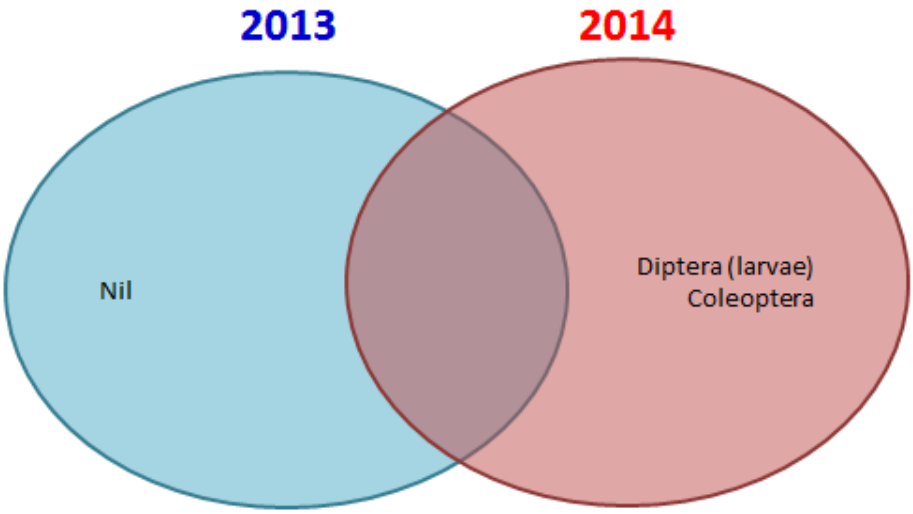


Figure N16. Comparison of indicator species analysis (by Order) of arthropods collected from all pitfall traps placed in the field located at Snook, Texas during summers 2013 and 2014.

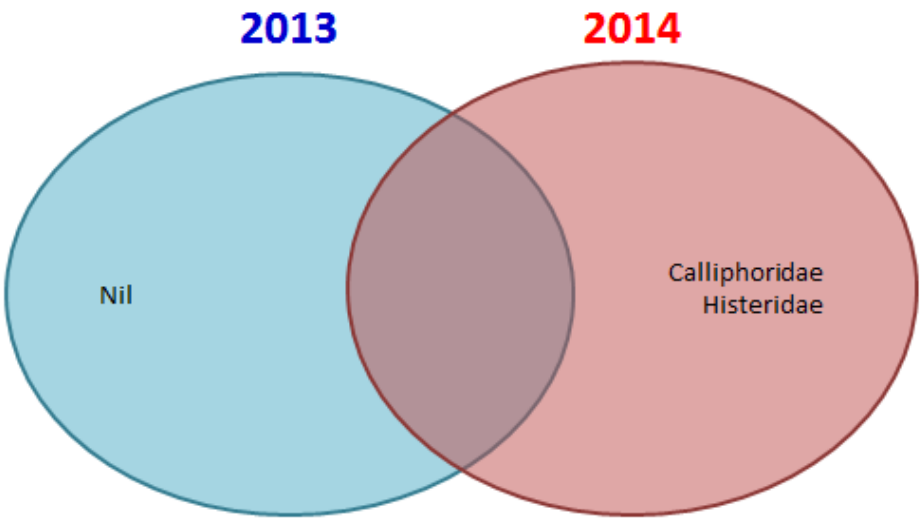


Figure N17. Comparison of indicator species analysis (by Family) of arthropods collected from all pitfall traps placed in the field located at Snook, Texas during summers 2013 and 2014.

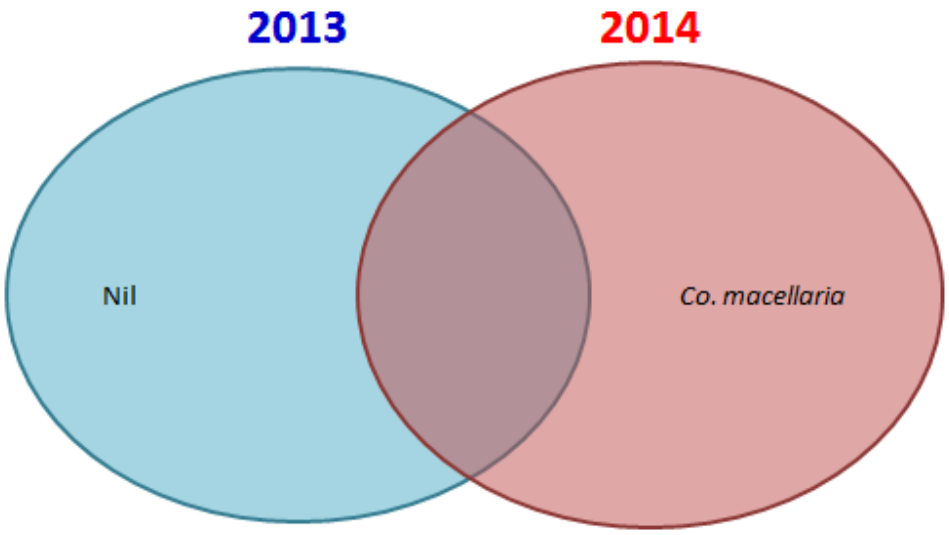


Figure N18. Comparison of indicator species analysis (by Genus and species) of arthropods collected from all pitfall traps placed in the field located at Snook, Texas during summers 2013 and 2014.

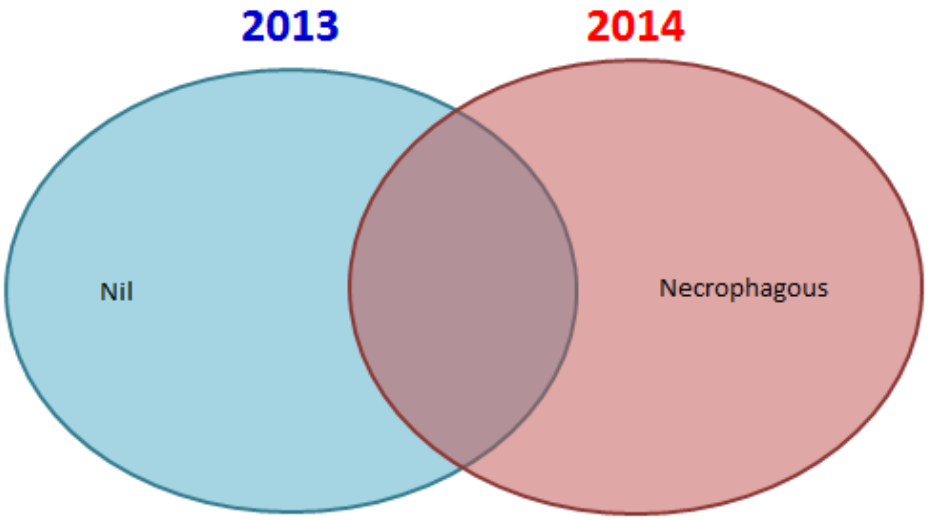


Figure N19. Comparison of indicator function analysis of arthropods collected from all pitfall traps placed in the field located at Snook, Texas during summers 2013 and 2014.

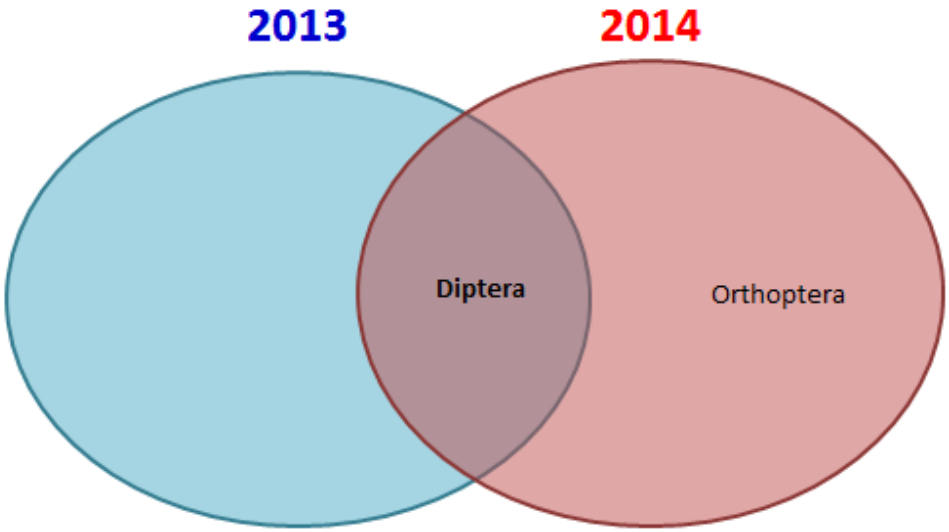


Figure N20. Comparison of indicator species analysis (by Order) of flying arthropods collected by sweep nets in the field located at Snook, Texas during summers 2013 and 2014.

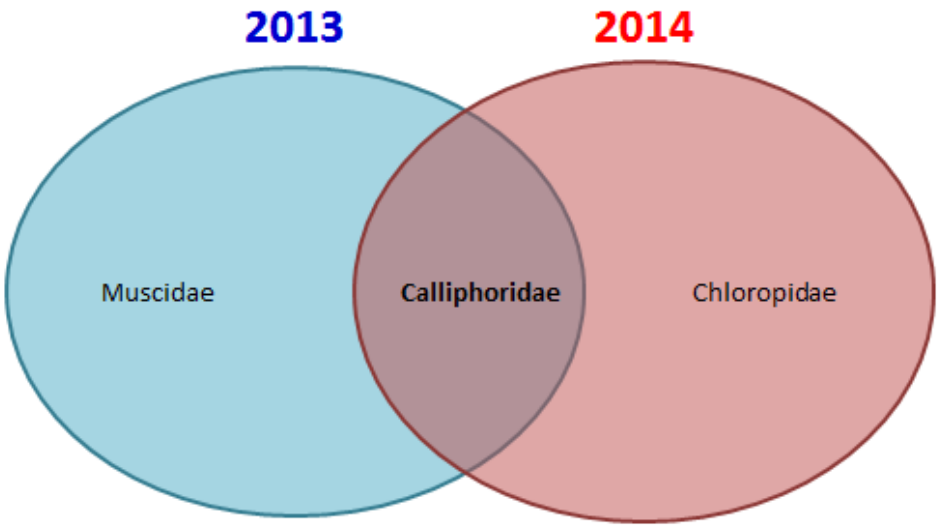


Figure N21. Comparison of indicator species analysis (by Family) of flying arthropods collected by sweep nets in the field located at Snook, Texas during summers 2013 and 2014.

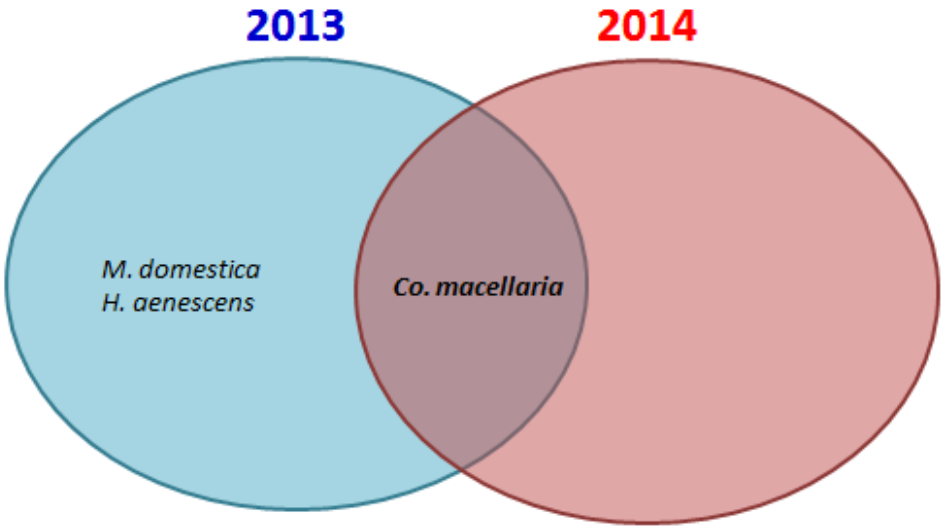


Figure N22. Comparison of indicator species analysis (by Genus and species) of flying arthropods collected by sweep nets in the field located at Snook, Texas during summers 2013 and 2014.

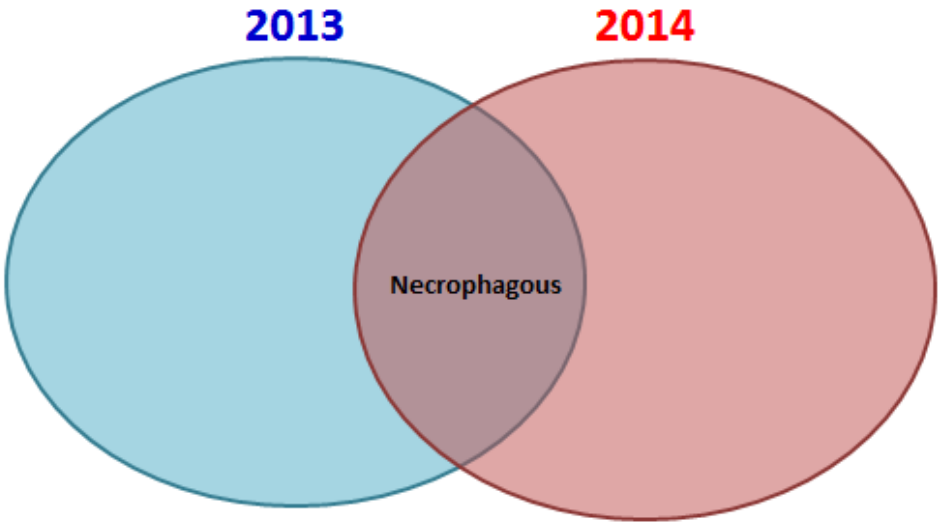


Figure N23. Comparison of indicator function analysis of flying arthropods collected by sweep nets in the field located at Snook, Texas during summers 2013 and 2014.

APPENDIX O

NEWLY-PROPOSED TERMS IN CARRION ECOLOGY

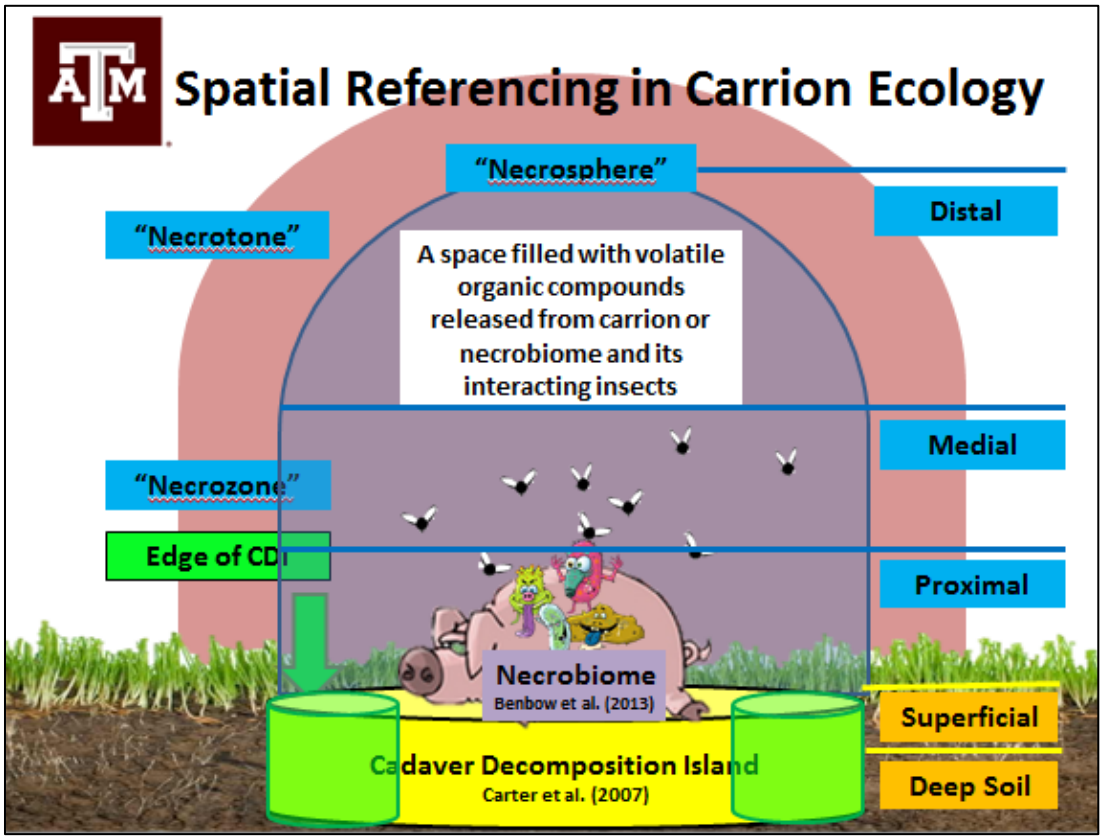


Figure O1. New terms proposed for carrion ecology: "Necrosphere", "Necrotone" and "Necrozone". Necrospehere refers to the active space filled with volatile organic compounds that elicits necrophagous arthropod responses toward carrion. Necrosphere can be further divided according to different altitudes namely proximal, medial and distal. Note that necrosphere may not have fixed shape (the dome-shaped necrosphere above is hypothetical). The contrasting ambient environments between necrophere and the external environment (where no insect responses toward carrion) is termed necrotone (word derived from the combination of necrophere and ecotone). Necrozone refers to cadaver decomposition island, but it is more specific as necrozone can be divided horizontally according to soil depth (e.g., superficial or deep necrozone), and vertically according to soil region (e.g., external necrozone refers to the soil lateral of carrion and internal necrozone refers to the soil beneath of carrion).

APPENDIX P

ECOLOGICAL NETWORKS OF ARTHROPODS ASSOCIATED WITH CARRION

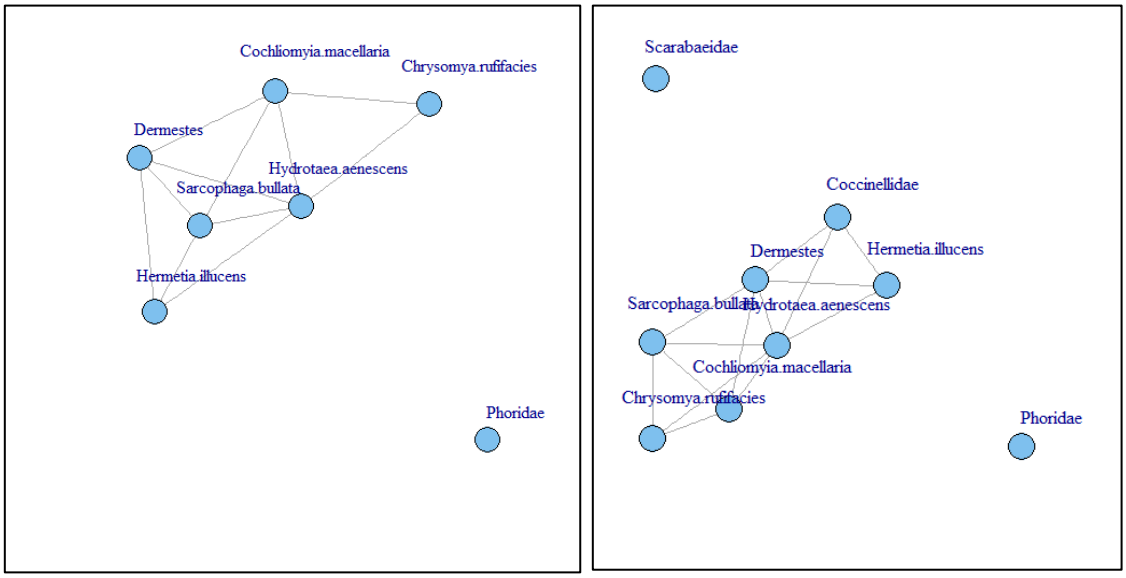


Figure P1. Ecological network of insect larvae (by Genus and species) associated with pig carrion during summer 2013 (left) & 2014 (right) at Snook, Texas.

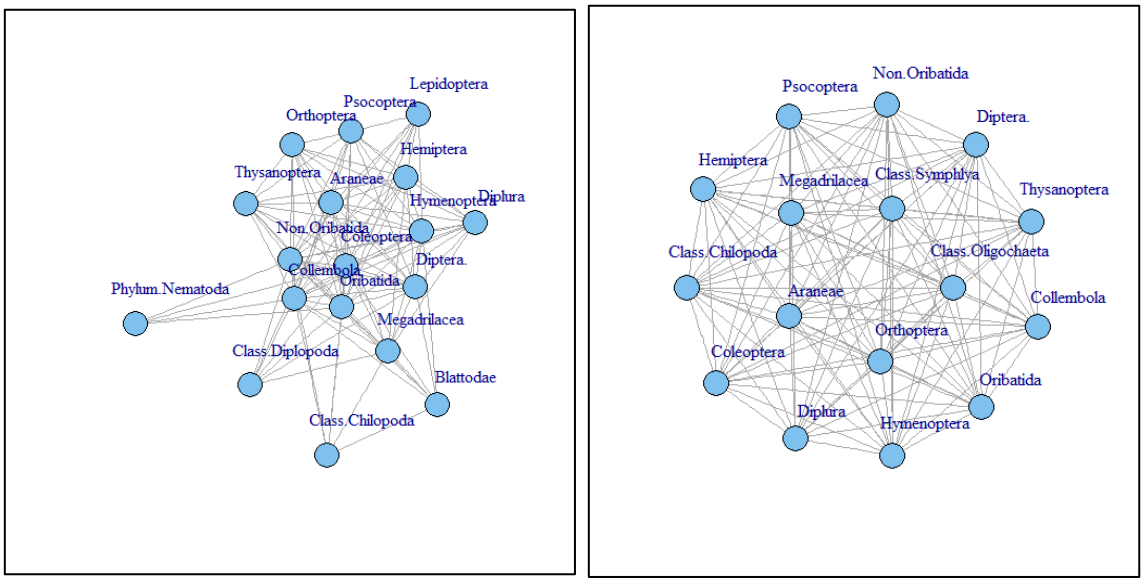


Figure P2. Ecological network of soil arthropods (by Order) associated with pig carrion during summer 2013 (left) & 2014 (right) at Snook, Texas.

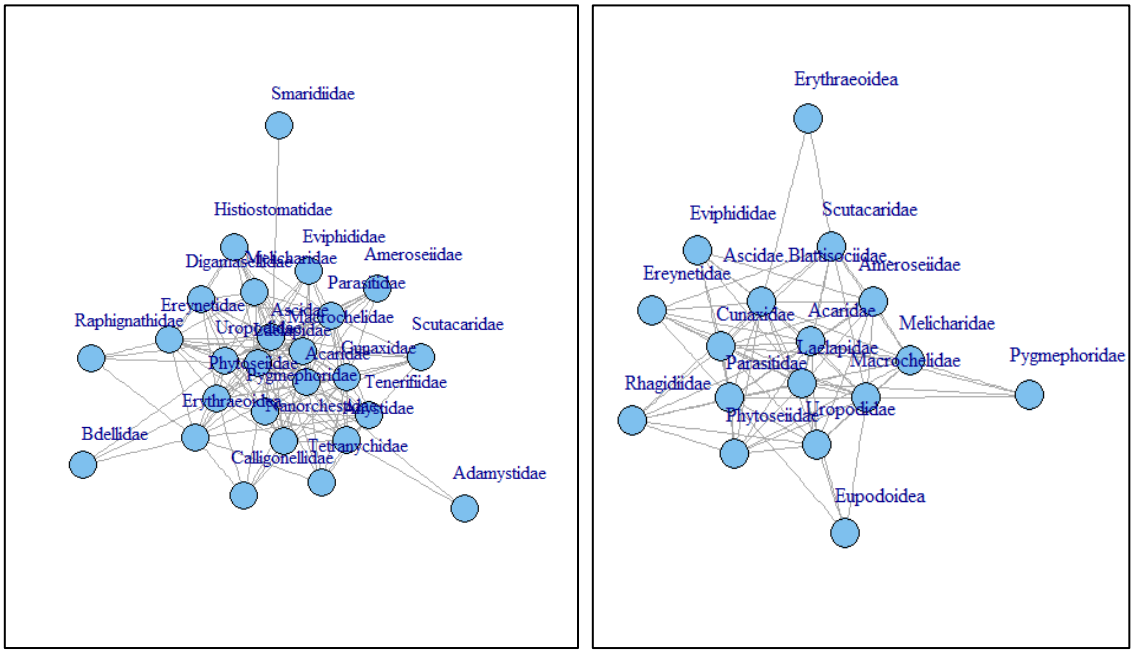


Figure P3. Ecological network of soil mites (by Family) associated with pig carrion during summer 2013 (left) & 2014 (right) at Snook, Texas.

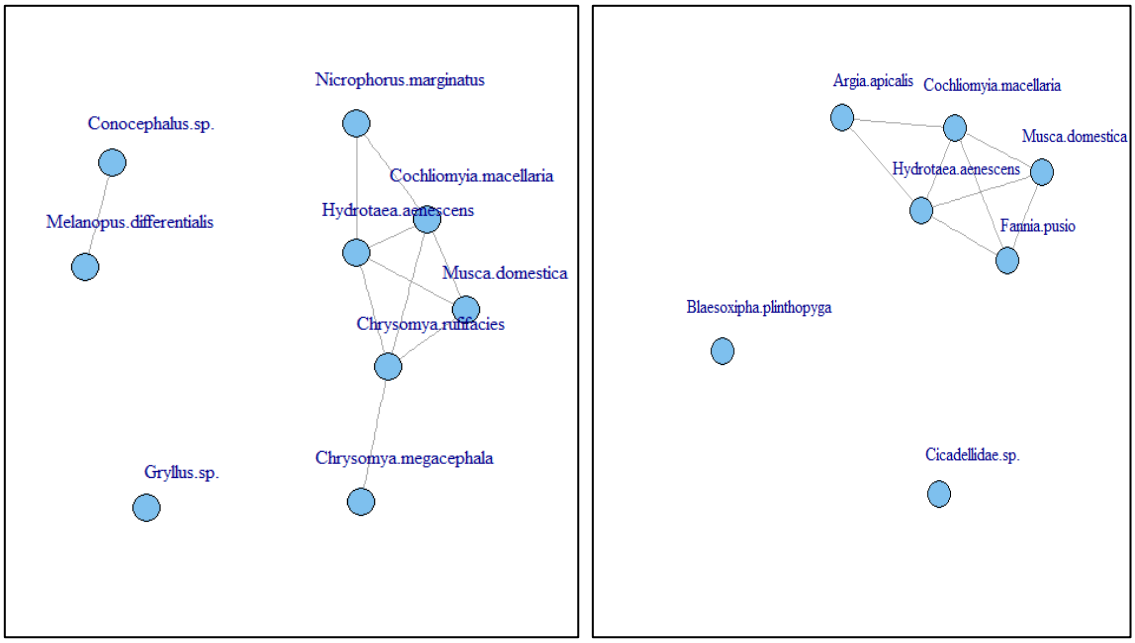


Figure P4. Ecological network of flying arthropods associated with pig carrion (by Genus and species) collected through sweep nets during summer 2013 (left) & 2014 (right) at Snook, Texas.

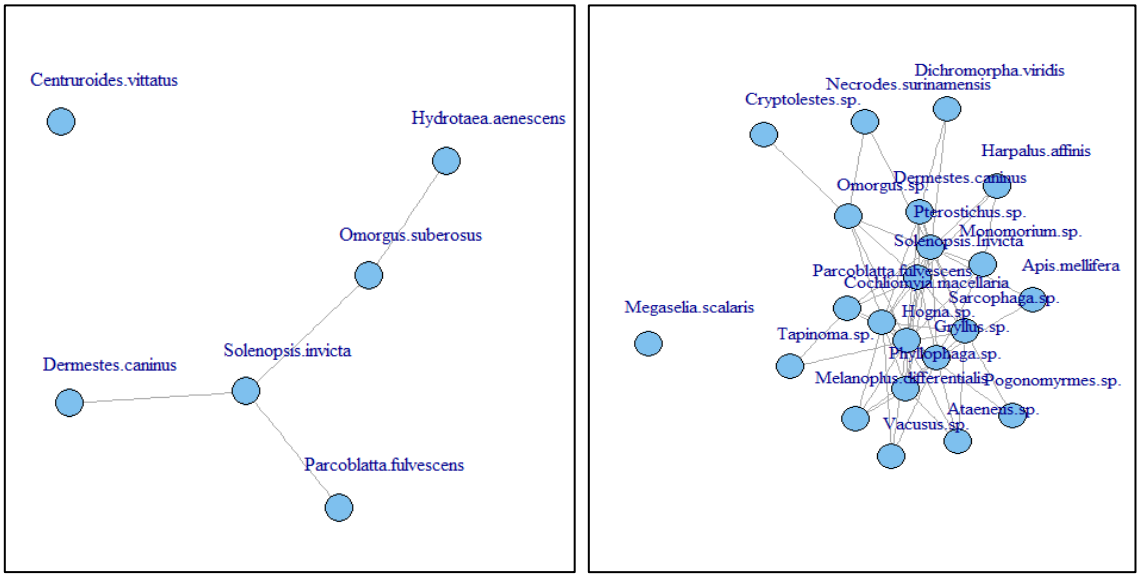


Figure P5. Ecological network of crawling arthropods (by Genus and species) associated with pig carrion collected through pitfall traps during summer 2013 (left) & 2014 (right) at Snook, Texas.

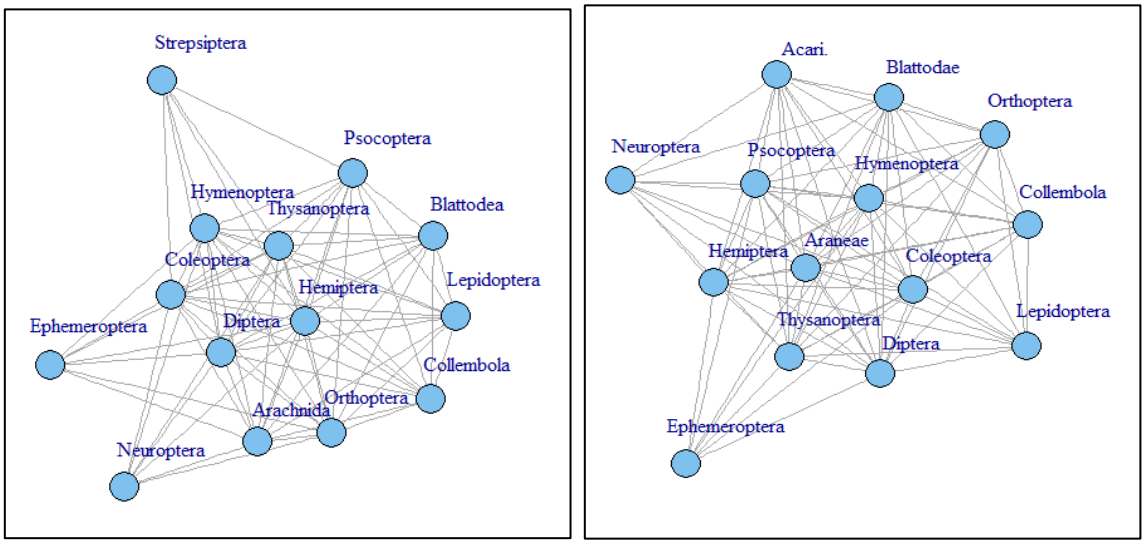


Figure P6. Ecological network of arthropods (by Order) associated with pig carrion collected by sticky traps during summer 2013 (left) & 2014 (right) at Snook, Texas.

APPENDIX Q

SIGNIFICANT PAIRWISE CORRELATIONS BETWEEN VARIABLES

Pairwise Correlations

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
Necrophagous (soil)	Calliphoridae	0.9535	540	0.9452	0.9606	<.0001*
Reduviidae	Trogidae	0.9486	540	0.9394	0.9564	<.0001*
Detritivore (mite)	Acaridae	0.9159	540	0.9011	0.9285	<.0001*
Herbivore (soil)	Aphididae	0.9152	540	0.9004	0.9280	<.0001*
Phenylethylamine	L-Threonine	0.8952	540	0.8771	0.9108	<.0001*
Predator/Parasite (mite)	Macrochelidae	0.8689	540	0.8466	0.8881	<.0001*
Phenylethylamine	2-Hydroxy Benzoic Acid	0.8559	540	0.8316	0.8769	<.0001*
Smaridiidae	Calliphoridae	0.8536	540	0.8289	0.8750	<.0001*
Fungivore (soil)	Latridiidae	0.8443	540	0.8182	0.8669	<.0001*
Necrophagous (soil)	Smaridiidae	0.8152	540	0.7848	0.8417	<.0001*
L-Threonine	2-Hydroxy Benzoic Acid	0.8107	540	0.7796	0.8377	<.0001*
Detritivore (soil)	Muscidae	0.8097	540	0.7785	0.8369	<.0001*
Phenylethylamine	L-Phenylalanine	0.8043	540	0.7724	0.8322	<.0001*
Nectarivore/Pollenivore (mite)	Histiostomatidae	0.7993	540	0.7667	0.8279	<.0001*
Histiostomatidae	Cleridae	0.7993	540	0.7667	0.8279	<.0001*
Nectarivore/Pollenivore (mite)	Cleridae	0.7993	540	0.7667	0.8279	<.0001*
H2O (%)	pH	0.7920	540	0.7582	0.8214	<.0001*
PO4-P	Conductivity	0.7909	540	0.7570	0.8205	<.0001*
Fungivore (mite)	Pygmephoridae	0.7782	540	0.7426	0.8095	<.0001*
L-Threonine	?-Ketobutyric Acid	0.7725	540	0.7361	0.8044	<.0001*
Pyruvic Acid Methyl Ester	Glycogen	0.7717	540	0.7352	0.8038	<.0001*
i-Erythritol	?-Ketobutyric Acid	0.7573	540	0.7189	0.7911	<.0001*
L-Serine	Glycyl-L-Gglutamic Acid	0.7550	540	0.7162	0.7891	<.0001*
Predator and Parasite (soil)	Staphylinidae	0.7452	540	0.7051	0.7805	<.0001*
Rhyparochromidae	Histeridae	0.7450	540	0.7049	0.7803	<.0001*
L-Threonine	i-Erythritol	0.7383	540	0.6974	0.7745	<.0001*
Glucose-1-Phosphate	2-Hydroxy Benzoic Acid	0.7327	540	0.6911	0.7695	<.0001*
L-Threonine	Glucose-1-Phosphate	0.7314	540	0.6896	0.7683	<.0001*
L-Phenylalanine	2-Hydroxy Benzoic Acid	0.7284	540	0.6862	0.7657	<.0001*
Phenylethylamine	Glucose-1-Phosphate	0.7260	540	0.6835	0.7636	<.0001*
Glucose-1-Phosphate	?-Ketobutyric Acid	0.7213	540	0.6783	0.7595	<.0001*
2-Hydroxy Benzoic Acid	?-Ketobutyric Acid	0.7182	540	0.6748	0.7568	<.0001*
Raphignathidae	Erythraeoidea	0.7065	540	0.6615	0.7463	<.0001*

Figure Q1. Pearson's pairwise correlation between soil variables (microbial function, soil chemistry, soil arthropod structure and function) measured during both summers 2013 and 2014 at Snook, Texas.

L-Threonine	L-Phenylalanine	0.7024	540	0.6570	0.7428	<.0001*
Predator and Parasite (soil)	Formicidae	0.6976	540	0.6516	0.7385	<.0001*
Herbivore (mite)	Phytoseiidae	0.6970	540	0.6509	0.7380	<.0001*
H2O (%)	Conductivity	0.6916	540	0.6449	0.7332	<.0001*
L-Serine	L-Phenylalanine	0.6828	540	0.6350	0.7254	<.0001*
i-Erythritol	2-Hydroxy Benzoic Acid	0.6709	540	0.6217	0.7148	<.0001*
L-Serine	D-Mannitol	0.6654	540	0.6156	0.7099	<.0001*
L-Phenylalanine	4-Hydroxy Benzoic Acid	0.6630	540	0.6129	0.7078	<.0001*
Phenylethylamine	?-Ketobutyric Acid	0.6593	540	0.6088	0.7045	<.0001*
Phenylethylamine	i-Erythritol	0.6591	540	0.6086	0.7043	<.0001*
L-Threonine	Glycyl-L-Gglutamic Acid	0.6430	540	0.5907	0.6900	<.0001*
Glycyl-L-Gglutamic Acid	D-Mannitol	0.6217	540	0.5671	0.6709	<.0001*
Glucose-1-Phosphate	D,L-?-Glycerol Phosphate	0.6200	540	0.5652	0.6693	<.0001*
Phenylethylamine	L-Serine	0.6134	540	0.5579	0.6634	<.0001*
Phenylethylamine	Glycyl-L-Gglutamic Acid	0.6109	540	0.5551	0.6612	<.0001*
L-Serine	Glucose-1-Phosphate	0.6076	540	0.5516	0.6583	<.0001*
L-Phenylalanine	Glucose-1-Phosphate	0.6034	540	0.5469	0.6545	<.0001*
L-Threonine	D-Galactonic Acid ?-Lactone	0.5997	540	0.5428	0.6511	<.0001*
L-Threonine	D-Mannitol	0.5962	540	0.5390	0.6480	<.0001*
L-Threonine	L-Serine	0.5850	540	0.5266	0.6379	<.0001*
L-Serine	4-Hydroxy Benzoic Acid	0.5801	540	0.5213	0.6335	<.0001*
Phenylethylamine	D-Mannitol	0.5776	540	0.5185	0.6312	<.0001*
Armadillidiidae	Erotylidae	0.5763	540	0.5170	0.6300	<.0001*
Rophalidae	Erotylidae	0.5763	540	0.5170	0.6300	<.0001*
Phenylethylamine	4-Hydroxy Benzoic Acid	0.5751	540	0.5158	0.6290	<.0001*
Pyruvic Acid Methyl Ester	D,L-?-Glycerol Phosphate	0.5707	540	0.5109	0.6250	<.0001*
Glycyl-L-Gglutamic Acid	4-Hydroxy Benzoic Acid	0.5694	540	0.5095	0.6238	<.0001*
Phenylethylamine	D-Galactonic Acid ?-Lactone	0.5692	540	0.5093	0.6236	<.0001*
L-Phenylalanine	Glycyl-L-Gglutamic Acid	0.5620	540	0.5014	0.6171	<.0001*
Glycyl-L-Gglutamic Acid	Glucose-1-Phosphate	0.5613	540	0.5006	0.6165	<.0001*
Anystidae	Anthocoridae	0.5586	540	0.4977	0.6140	<.0001*
Conductivity	pH	0.5585	540	0.4975	0.6139	<.0001*

Figure Q1. Continued.

Detritivore (soil)	Sarcophagidae	0.5573	540	0.4963	0.6129	<.0001*
Sarcophagidae	Muscidae	0.5567	540	0.4956	0.6123	<.0001*
L-Phenylalanine	D-Mannitol	0.5555	540	0.4943	0.6112	<.0001*
Ameroseiidae	Lumbricidae	0.5544	540	0.4930	0.6102	<.0001*
i-Erythritol	Glucose-1-Phosphate	0.5478	540	0.4859	0.6043	<.0001*
Pyruvic Acid Methyl Ester	?-Hydroxybutyric Acid	0.5438	540	0.4815	0.6006	<.0001*
H2O (%)	PO4-P	0.5424	540	0.4800	0.5993	<.0001*
L-Threonine	4-Hydroxy Benzoic Acid	0.5392	540	0.4765	0.5964	<.0001*
Predator/Parasite (mite)	Dermestidae	0.5344	540	0.4713	0.5921	<.0001*
D-Malic Acid	?-Ketobutyric Acid	0.5331	540	0.4699	0.5909	<.0001*
Predator and Parasite (soil)	H2O (%)	0.5300	540	0.4664	0.5880	<.0001*
D,L-?-Glycerol Phosphate	2-Hydroxy Benzoic Acid	0.5297	540	0.4662	0.5878	<.0001*
Glucose-1-Phosphate	D-Mannitol	0.5296	540	0.4661	0.5877	<.0001*
Glycyl-L-Gglutamic Acid	D-Galactonic Acid ?-Lactone	0.5292	540	0.4656	0.5873	<.0001*
Glucose-1-Phosphate	?-Hydroxybutyric Acid	0.5266	540	0.4628	0.5850	<.0001*
L-Serine	2-Hydroxy Benzoic Acid	0.5266	540	0.4628	0.5850	<.0001*
L-Phenylalanine	?-Hydroxybutyric Acid	0.5251	540	0.4611	0.5836	<.0001*
Predator/Parasite (mite)	H2O (%)	0.5251	540	0.4611	0.5836	<.0001*
Glucose-1-Phosphate	D-Galactonic Acid ?-Lactone	0.5197	540	0.4553	0.5787	<.0001*
Glycyl-L-Gglutamic Acid	?-Hydroxybutyric Acid	0.5142	540	0.4493	0.5737	<.0001*
Acaridae	PO4-P	0.5134	540	0.4484	0.5730	<.0001*
Fungivore (mite)	Nanorchestidae	0.5072	540	0.4417	0.5673	<.0001*
Glycogen	D,L-?-Glycerol Phosphate	0.5065	540	0.4410	0.5667	<.0001*
Scarabaeidae	Staphylinidae	0.5060	540	0.4405	0.5662	<.0001*
Glucose-1-Phosphate	D-Malic Acid	0.4998	540	0.4337	0.5606	<.0001*
Macrochelidae	Dermestidae	0.4933	540	0.4267	0.5546	<.0001*
Glucose-1-Phosphate	4-Hydroxy Benzoic Acid	0.4899	540	0.4230	0.5515	<.0001*
Tween 40	Glycogen	0.4880	540	0.4210	0.5498	<.0001*
Fungivore (mite)	Scutacaridae	0.4867	540	0.4196	0.5486	<.0001*
D-Mannitol	4-Hydroxy Benzoic Acid	0.4865	540	0.4193	0.5484	<.0001*
D,L-?-Glycerol Phosphate	?-Ketobutyric Acid	0.4856	540	0.4184	0.5475	<.0001*
Nitidulidae	Staphylinidae	0.4853	540	0.4180	0.5473	<.0001*

Figure Q1. Continued.

Coccinellidae	Aphididae	0.4778	540	0.4100	0.5404	<.0001*
L-Phenylalanine	?-Ketobutyric Acid	0.4772	540	0.4093	0.5399	<.0001*
L-Serine	?-Hydroxybutyric Acid	0.4744	540	0.4062	0.5372	<.0001*
L-Phenylalanine	D-Galactonic Acid ?-Lactone	0.4687	540	0.4001	0.5320	<.0001*
Predator/Parasite (mite)	Tenebrionidae	0.4672	540	0.3985	0.5306	<.0001*
Lumbricidae	Japygidae	0.4639	540	0.3950	0.5276	<.0001*
D-Mannitol	D-Galactonic Acid ?-Lactone	0.4609	540	0.3918	0.5249	<.0001*
D-Galactonic Acid ?-Lactone	2-Hydroxy Benzoic Acid	0.4599	540	0.3907	0.5239	<.0001*
D-Mannitol	2-Hydroxy Benzoic Acid	0.4594	540	0.3901	0.5234	<.0001*
Predator and Parasite (soil)	pH	0.4573	540	0.3879	0.5216	<.0001*
Detritivore (soil)	Conductivity	0.4570	540	0.3876	0.5213	<.0001*
Itaconic Acid	?-Ketobutyric Acid	0.4553	540	0.3857	0.5197	<.0001*
L-Serine	D-Galactonic Acid ?-Lactone	0.4522	540	0.3824	0.5168	<.0001*
Ereynetidae	Scarabaeidae	0.4520	540	0.3822	0.5167	<.0001*
Herbivore (soil)	Coccinellidae	0.4499	540	0.3800	0.5148	<.0001*
D-Galactonic Acid ?-Lactone	4-Hydroxy Benzoic Acid	0.4488	540	0.3788	0.5137	<.0001*
Digamasellidae	Histiostomatidae	0.4446	540	0.3743	0.5099	<.0001*
Nectarivore/Pollenivore (mite)	Digamasellidae	0.4446	540	0.3743	0.5099	<.0001*
Digamasellidae	Cleridae	0.4446	540	0.3743	0.5099	<.0001*
Erythraeoidea	Anthicidae	0.4444	540	0.3741	0.5097	<.0001*
Predator/Parasite (mite)	Staphylinidae	0.4439	540	0.3735	0.5092	<.0001*
Predator/Parasite (mite)	Ascidae	0.4439	540	0.3735	0.5092	<.0001*
Detritivore (mite)	PO4-P	0.4418	540	0.3712	0.5072	<.0001*
Muscidae	Conductivity	0.4370	540	0.3661	0.5028	<.0001*
?-Ketobutyric Acid	?-Cyclodextrin	0.4370	540	0.3661	0.5028	<.0001*
Pyruvic Acid Methyl Ester	Glucose-1-Phosphate	0.4367	540	0.3658	0.5026	<.0001*
Detritivore (mite)	H2O (%)	0.4363	540	0.3654	0.5022	<.0001*
Predator and Parasite (soil)	Scarabaeidae	0.4360	540	0.3651	0.5019	<.0001*
Putrescine	L-Threonine	0.4351	540	0.3641	0.5011	<.0001*
L-Serine	?-Ketobutyric Acid	0.4350	540	0.3640	0.5010	<.0001*
Phenylethylamine	?-Hydroxybutyric Acid	0.4347	540	0.3637	0.5007	<.0001*
Predator/Parasite (mite)	pH	0.4338	540	0.3627	0.4999	<.0001*

Figure Q1. Continued.

Putrescine	L-Threonine	0.4351	540	0.3641	0.5011	<.0001*
L-Serine	?-Ketobutyric Acid	0.4350	540	0.3640	0.5010	<.0001*
Phenylethylamine	?-Hydroxybutyric Acid	0.4347	540	0.3637	0.5007	<.0001*
Predator/Parasite (mite)	pH	0.4338	540	0.3627	0.4999	<.0001*
Corylophidea	Erotylidae	0.4330	540	0.3619	0.4991	<.0001*
D-Cellobiose	?-Ketobutyric Acid	0.4295	540	0.3582	0.4960	<.0001*
Macrochelidae	Staphylinidae	0.4295	540	0.3581	0.4959	<.0001*
Tween 40	Pyruvic Acid Methyl Ester	0.4284	540	0.3569	0.4949	<.0001*
NH4-N	Conductivity	0.4267	540	0.3551	0.4933	<.0001*
D-Mannitol	?-Ketobutyric Acid	0.4266	540	0.3550	0.4932	<.0001*
N-Acetyl-D-Glucosamine	L-Threonine	0.4265	540	0.3549	0.4932	<.0001*
N-Acetyl-D-Glucosamine	D-Galactonic Acid ?-Lactone	0.4265	540	0.3549	0.4931	<.0001*
Fungivore (soil)	Erotylidae	0.4259	540	0.3543	0.4926	<.0001*
i-Erythritol	D-Xylose	0.4250	540	0.3533	0.4917	<.0001*
Predator and Parasite (soil)	Nitidulidae	0.4247	540	0.3529	0.4914	<.0001*
4-Hydroxy Benzoic Acid	2-Hydroxy Benzoic Acid	0.4246	540	0.3528	0.4913	<.0001*
L-Threonine	Itaconic Acid	0.4237	540	0.3519	0.4906	<.0001*
Predator and Parasite (soil)	Tenebrionidae	0.4226	540	0.3507	0.4895	<.0001*
Predator/Parasite (mite)	Predator and Parasite (soil)	0.4201	540	0.3481	0.4872	<.0001*
Staphylinidae	Conductivity	0.4199	540	0.3478	0.4870	<.0001*
L-Arginine	?-Ketobutyric Acid	0.4196	540	0.3475	0.4867	<.0001*
L-Phenylalanine	i-Erythritol	0.4191	540	0.3470	0.4863	<.0001*
Macrochelidae	Tenebrionidae	0.4186	540	0.3465	0.4859	<.0001*
Fanniidae	PO4-P	0.4184	540	0.3463	0.4857	<.0001*
N-Acetyl-D-Glucosamine	Glycyl-L-Gglutamic Acid	0.4159	540	0.3435	0.4833	<.0001*
?-Ketobutyric Acid	?-Hydroxybutyric Acid	0.4141	540	0.3416	0.4816	<.0001*
Staphylinidae	H2O (%)	0.4116	540	0.3390	0.4794	<.0001*
Detritivore (mite)	pH	0.4110	540	0.3383	0.4788	<.0001*
Staphylinidae	Tenebrionidae	0.4090	540	0.3362	0.4769	<.0001*
L-Threonine	?-Hydroxybutyric Acid	0.4088	540	0.3360	0.4767	<.0001*
Ascidae	pH	0.4084	540	0.3355	0.4763	<.0001*
D,L-?-Glycerol Phosphate	D-Malic Acid	0.4072	540	0.3343	0.4753	<.0001*
Putrescine	?-Ketobutyric Acid	0.4070	540	0.3340	0.4750	<.0001*
L-Arginine	Glucose-1-Phosphate	0.4068	540	0.3339	0.4749	<.0001*
Silvanidae	Isotomidae	0.4062	540	0.3333	0.4744	<.0001*
Erotylidae	Isotomidae	0.4062	540	0.3333	0.4744	<.0001*

Figure Q1. Continued.

Putrescine	?-Ketobutyric Acid	0.4070	540	0.3340	0.4750	<.0001*
L-Arginine	Glucose-1-Phosphate	0.4068	540	0.3339	0.4749	<.0001*
Silvanidae	Isotomidae	0.4062	540	0.3333	0.4744	<.0001*
Erotylidae	Isotomidae	0.4062	540	0.3333	0.4744	<.0001*
Tween 40	D,L-?-Glycerol Phosphate	0.4056	540	0.3327	0.4738	<.0001*
Glycyl-L-Gglutamic Acid	2-Hydroxy Benzoic Acid	0.4026	540	0.3295	0.4710	<.0001*
Macrochelidae	Fanniidae	0.4023	540	0.3291	0.4707	<.0001*
Predator and Parasite (soil)	Conductivity	0.4021	540	0.3289	0.4705	<.0001*
D-Glucosaminic Acid	?-Cyclodextrin	0.4020	540	0.3288	0.4705	<.0001*
L-Threonine	L-Arginine	0.3994	540	0.3260	0.4680	<.0001*
Herbivore (soil)	Araneidae	0.3987	540	0.3252	0.4673	<.0001*
Tween 80	Tween 40	0.3982	540	0.3247	0.4669	<.0001*
Eupodoidea	Aphididae	0.3972	540	0.3237	0.4660	<.0001*
Pygmephoridae	Scutacaridae	0.3941	540	0.3204	0.4631	<.0001*
Predator/Parasite (mite)	Parasitidae	0.3932	540	0.3194	0.4623	<.0001*
H2O (%)	NO3-N	0.3932	540	0.3194	0.4622	<.0001*
i-Erythritol	D-Malic Acid	0.3927	540	0.3189	0.4618	<.0001*
Ascidae	H2O (%)	0.3899	540	0.3159	0.4592	<.0001*
Itaconic Acid	Glycyl-L-Gglutamic Acid	0.3887	540	0.3146	0.4581	<.0001*
L-Arginine	i-Erythritol	0.3872	540	0.3131	0.4567	<.0001*
L-Arginine	2-Hydroxy Benzoic Acid	0.3845	540	0.3102	0.4541	<.0001*
Formicidae	H2O (%)	0.3844	540	0.3101	0.4540	<.0001*
Phenylethylamine	L-Arginine	0.3833	540	0.3089	0.4530	<.0001*
PO4-P	NH4-N	0.3812	540	0.3067	0.4511	<.0001*
Predator and Parasite (soil)	NO3-N	0.3807	540	0.3062	0.4506	<.0001*
Fungivore (soil)	Corylophidea	0.3804	540	0.3058	0.4503	<.0001*
Putrescine	Phenylethylamine	0.3796	540	0.3050	0.4496	<.0001*
4-Hydroxy Benzoic Acid	?-Hydroxybutyric Acid	0.3777	540	0.3030	0.4478	<.0001*
Acaridae	Conductivity	0.3776	540	0.3028	0.4477	<.0001*
Itaconic Acid	?-Hydroxybutyric Acid	0.3769	540	0.3022	0.4471	<.0001*
Acaridae	Histeridae	0.3766	540	0.3018	0.4467	<.0001*
Armadillidiidae	Corylophidea	0.3761	540	0.3012	0.4463	<.0001*
Rophalidae	Corylophidea	0.3761	540	0.3012	0.4463	<.0001*
Lumbricidae	Corylophidea	0.3761	540	0.3012	0.4463	<.0001*
Phenylethylamine	Itaconic Acid	0.3749	540	0.3000	0.4452	<.0001*
D-Mannitol	?-Hydroxybutyric Acid	0.3744	540	0.2995	0.4448	<.0001*

Figure Q1. Continued.

PO4-P	pH	0.3737	540	0.2987	0.4441	<.0001*
Ameroseiidae	Latridiidae	0.3736	540	0.2987	0.4440	<.0001*
Fungivore (soil)	Ameroseiidae	0.3713	540	0.2962	0.4418	<.0001*
Cunaxidae	Rophalidae	0.3706	540	0.2955	0.4412	<.0001*
Ereynetidae	Histiostomatidae	0.3702	540	0.2950	0.4408	<.0001*
Nectarivore/Pollenivore (mite)	Ereynetidae	0.3702	540	0.2950	0.4408	<.0001*
Ereynetidae	Cleridae	0.3702	540	0.2950	0.4408	<.0001*
Herbivore (soil)	Monotomidae	0.3695	540	0.2943	0.4401	<.0001*
Entomobryidae	Sminthuridae	0.3665	540	0.2911	0.4373	<.0001*
Histeridae	Anthicidae	0.3657	540	0.2903	0.4366	<.0001*
L-Threonine	D-Malic Acid	0.3633	540	0.2877	0.4343	<.0001*
Putrescine	i-Erythritol	0.3626	540	0.2870	0.4337	<.0001*
Macrochelidae	H2O (%)	0.3624	540	0.2867	0.4335	<.0001*
Dermestidae	Tenebrionidae	0.3622	540	0.2866	0.4333	<.0001*
Itaconic Acid	4-Hydroxy Benzoic Acid	0.3617	540	0.2861	0.4329	<.0001*
Predator/Parasite (mite)	PO4-P	0.3613	540	0.2857	0.4325	<.0001*
4-Hydroxy Benzoic Acid	?-Ketobutyric Acid	0.3603	540	0.2846	0.4316	<.0001*
Monotomidae	Entomobryidae	0.3602	540	0.2844	0.4314	<.0001*
Detritivore (mite)	Histeridae	0.3584	540	0.2825	0.4298	<.0001*
L-Serine	i-Erythritol	0.3569	540	0.2810	0.4284	<.0001*
Putrescine	2-Hydroxy Benzoic Acid	0.3566	540	0.2807	0.4281	<.0001*
D-Galactonic Acid ?-Lactone	?-Ketobutyric Acid	0.3559	540	0.2799	0.4275	<.0001*
Nitidulidae	Scarabaeidae	0.3543	540	0.2782	0.4259	<.0001*
Tenebrionidae	NO3-N	0.3528	540	0.2766	0.4245	<.0001*
D-Malic Acid	?-Cyclodextrin	0.3514	540	0.2752	0.4233	<.0001*
Tenebrionidae	H2O (%)	0.3508	540	0.2745	0.4226	<.0001*
Predator/Parasite (mite)	Conductivity	0.3505	540	0.2742	0.4224	<.0001*
Macrochelidae	PO4-P	0.3499	540	0.2736	0.4218	<.0001*
Detritivore (mite)	Conductivity	0.3494	540	0.2731	0.4214	<.0001*
D,L-?-Glycerol Phosphate	?-Hydroxybutyric Acid	0.3488	540	0.2724	0.4208	<.0001*
Acaridae	Scarabaeidae	0.3466	540	0.2701	0.4187	<.0001*
Silvanidae	Anthocoridae	0.3463	540	0.2698	0.4184	<.0001*
Detritivore (soil)	PO4-P	0.3463	540	0.2698	0.4184	<.0001*
Aphididae	Araneidae	0.3440	540	0.2674	0.4163	<.0001*
Herbivore (soil)	Eupodoidea	0.3438	540	0.2672	0.4161	<.0001*

Figure Q1. Continued.

Ascidae	Japygidae	0.3435	540	0.2668	0.4158	<.0001*
Histeridae	PO4-P	0.3415	540	0.2648	0.4140	<.0001*
N-Acetyl-D-Glucosamine	L-Serine	0.3404	540	0.2636	0.4129	<.0001*
i-Erythritol	D-Galactonic Acid ?-Lactone	0.3399	540	0.2630	0.4124	<.0001*
Glycogen	?-Hydroxybutyric Acid	0.3398	540	0.2630	0.4124	<.0001*
Scutacaridae	Aphididae	0.3371	540	0.2601	0.4098	<.0001*
Sarcophagidae	Conductivity	0.3361	540	0.2590	0.4088	<.0001*
Formicidae	pH	0.3356	540	0.2586	0.4084	<.0001*
L-Serine	?-Cyclodextrin	0.3346	540	0.2575	0.4075	<.0001*
Parasitidae	H2O (%)	0.3341	540	0.2570	0.4070	<.0001*
Laelapidae	Entomobryidae	0.3339	540	0.2567	0.4068	<.0001*
Predator and Parasite (soil)	PO4-P	0.3335	540	0.2563	0.4064	<.0001*
Carabidae	Corylophidea	0.3329	540	0.2557	0.4059	<.0001*
Nitidulidae	NO3-N	0.3325	540	0.2553	0.4055	<.0001*
i-Erythritol	D,L-?-Glycerol Phosphate	0.3314	540	0.2541	0.4045	<.0001*
Scutacaridae	Coccinellidae	0.3310	540	0.2537	0.4041	<.0001*
Cunaxidae	Thripidae	0.3310	540	0.2537	0.4041	<.0001*
Itaconic Acid	i-Erythritol	0.3306	540	0.2533	0.4038	<.0001*
Entomobryidae	H2O (%)	0.3301	540	0.2528	0.4033	<.0001*
Phenylethylamine	N-Acetyl-D-Glucosamine	0.3296	540	0.2523	0.4028	<.0001*
Silvanidae	Erotylidae	0.3296	540	0.2522	0.4028	<.0001*
Tenebrionidae	pH	0.3270	540	0.2495	0.4003	<.0001*
Itaconic Acid	D-Xylose	0.3269	540	0.2494	0.4002	<.0001*
Detritivore (mite)	Scarabaeidae	0.3266	540	0.2490	0.3999	<.0001*
Predator and Parasite (soil)	Macrochelidae	0.3261	540	0.2486	0.3995	<.0001*
Entomobryidae	Formicidae	0.3258	540	0.2483	0.3992	<.0001*
Silphidae	Conductivity	0.3249	540	0.2473	0.3984	<.0001*
D-Cellobiose	?-Cyclodextrin	0.3248	540	0.2472	0.3983	<.0001*
2-Hydroxy Benzoic Acid	?-Hydroxybutyric Acid	0.3243	540	0.2467	0.3978	<.0001*
i-Erythritol	Glycyl-L-Gglutamic Acid	0.3226	540	0.2449	0.3962	<.0001*
Japygidae	Entomobryidae	0.3221	540	0.2444	0.3957	<.0001*
Detritivore (mite)	Uropodidae	0.3213	540	0.2435	0.3950	<.0001*
Herbivore (soil)	Scutacaridae	0.3202	540	0.2423	0.3939	<.0001*
Nectarivore/Pollenivore (mite)	Laelapidae	0.3201	540	0.2422	0.3938	<.0001*
Laelapidae	Cleridae	0.3201	540	0.2422	0.3938	<.0001*

Figure Q1. Continued.

Histiostomatidae	Laelapidae	0.3201	540	0.2422	0.3938	<.0001*
Staphylinidae	PO4-P	0.3198	540	0.2419	0.3935	<.0001*
Predator/Parasite (mite)	Fanniidae	0.3192	540	0.2413	0.3930	<.0001*
D-Malic Acid	2-Hydroxy Benzoic Acid	0.3191	540	0.2412	0.3929	<.0001*
Acaridae	H2O (%)	0.3191	540	0.2412	0.3929	<.0001*
Herbivore (soil)	Entomobryidae	0.3189	540	0.2410	0.3927	<.0001*
L-Serine	L-Arginine	0.3183	540	0.2404	0.3922	<.0001*
Glycyl-L-Gglutamic Acid	?-Ketobutyric Acid	0.3161	540	0.2381	0.3901	<.0001*
Laelapidae	H2O (%)	0.3159	540	0.2379	0.3899	<.0001*
Fungivore (soil)	Silvanidae	0.3152	540	0.2371	0.3892	<.0001*
Glycogen	Glucose-1-Phosphate	0.3141	540	0.2359	0.3882	<.0001*
Cunaxidae	Erotylidae	0.3139	540	0.2358	0.3880	<.0001*
NO3-N	pH	0.3136	540	0.2355	0.3877	<.0001*
Detritivore (mite)	Predator and Parasite (soil)	0.3129	540	0.2347	0.3870	<.0001*
Glycyl-L-Gglutamic Acid	D-Cellobiose	0.3128	540	0.2346	0.3870	<.0001*
?-Hydroxybutyric Acid	?-Cyclodextrin	0.3128	540	0.2346	0.3869	<.0001*
D,L-?-Glycerol Phosphate	?-Cyclodextrin	0.3128	540	0.2346	0.3869	<.0001*
N-Acetyl-D-Glucosamine	Glucose-1-Phosphate	0.3108	540	0.2325	0.3850	<.0001*
Dermestidae	Staphylinidae	0.3105	540	0.2322	0.3848	<.0001*
Muscidae	PO4-P	0.3102	540	0.2319	0.3845	<.0001*
i-Erythritol	D-Mannitol	0.3095	540	0.2311	0.3838	<.0001*
L-Threonine	D-Xylose	0.3094	540	0.2311	0.3838	<.0001*
Digamasellidae	Ereynetidae	0.3080	540	0.2296	0.3824	<.0001*
Phytoseiidae	Sminthuridae	0.3064	540	0.2279	0.3809	<.0001*
L-Arginine	4-Hydroxy Benzoic Acid	0.3049	540	0.2264	0.3796	<.0001*
Phenylethylamine	D,L-?-Glycerol Phosphate	0.3031	540	0.2244	0.3778	<.0001*
Aphididae	Entomobryidae	0.3022	540	0.2236	0.3770	<.0001*
D-Malic Acid	D-Cellobiose	0.3014	540	0.2227	0.3762	<.0001*
Necrophagous (soil)	Conductivity	0.3007	540	0.2220	0.3756	<.0001*
Scarabaeidae	NO3-N	0.2993	540	0.2205	0.3743	<.0001*
Herbivore (mite)	Sminthuridae	0.2956	540	0.2166	0.3707	<.0001*
D-Malic Acid	D-Glucosaminic Acid	0.2951	540	0.2161	0.3703	<.0001*

Figure Q1. Continued.

APPENDIX R

PREDICTING ADH USING SOIL VARIABLES (SOIL MICROBIAL FUNCTION, SOIL CHEMISTRY, SOIL ARTHROPOD COMMUNITY STRUCTURE AND FUNCTION) ASSOCIATED WITH CARRION DECOMPOSITION

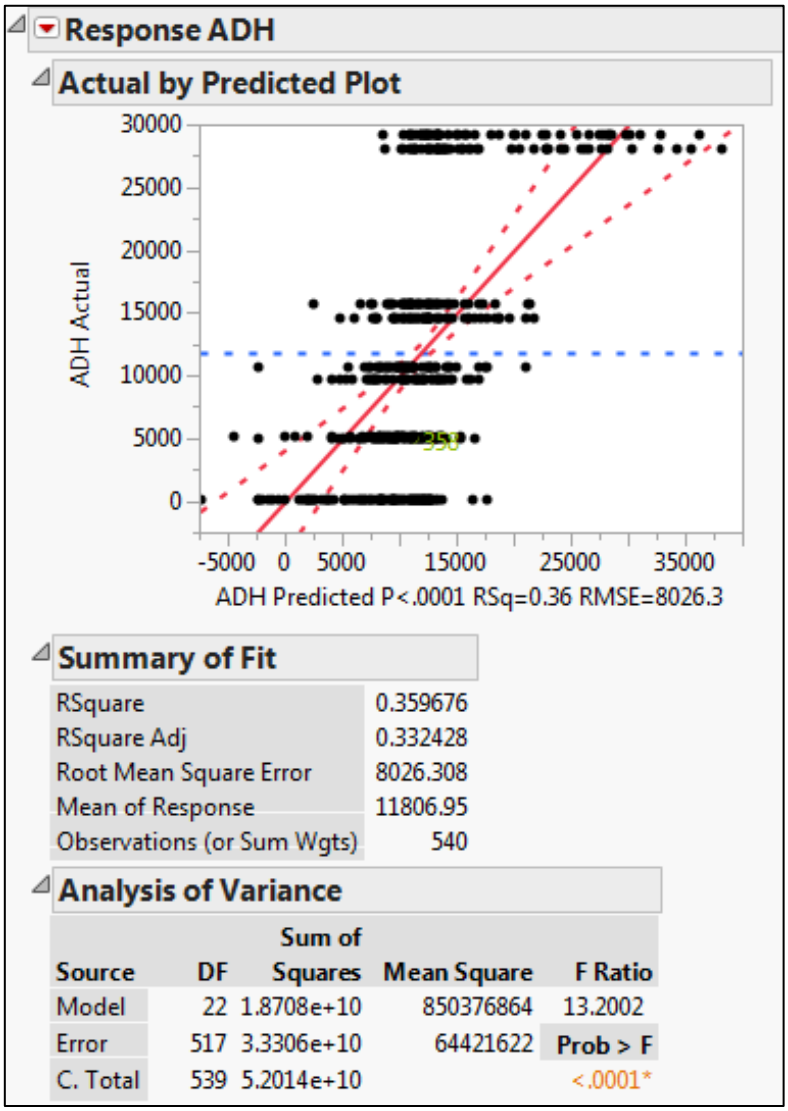
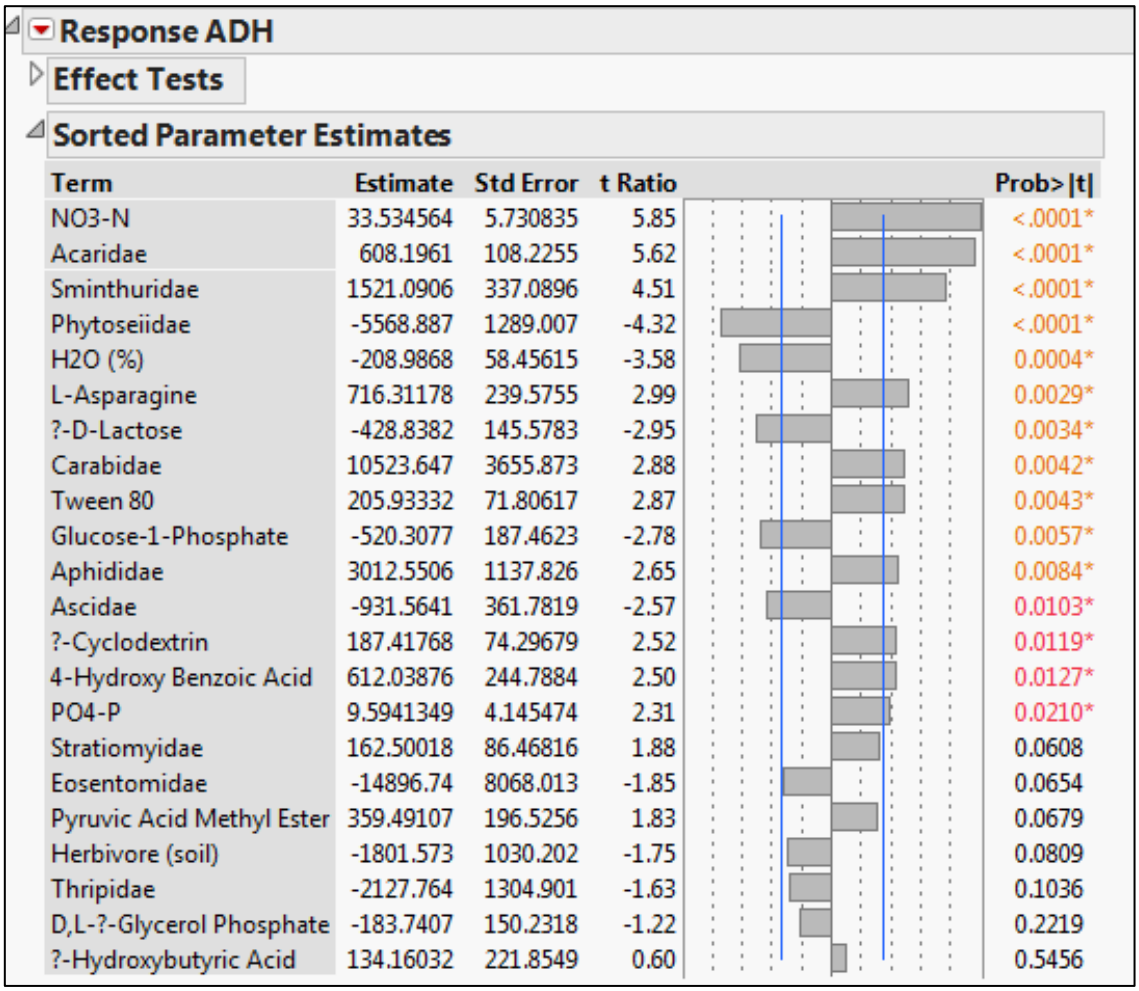


Figure R1. Stepwise model selection in predicting ADH using Max K-Fold Rsquare (kfold = 5) stopping rule with forward direction. $P < 0.0001$; $R^2 = 0.36$; R^2 Adj = 0.33; R^2 K-Fold = 0.30. Analysis performed by JMP Pro version 11.



Term	Estimate	Std Error	t Ratio	Prob>|t|
NO3-N	33.534564	5.730835	5.85	<.0001*
Acaridae	608.1961	108.2255	5.62	<.0001*
Sminthuridae	1521.0906	337.0896	4.51	<.0001*
Phytoseiidae	-5568.887	1289.007	-4.32	<.0001*
H2O (%)	-208.9868	58.45615	-3.58	0.0004*
L-Asparagine	716.31178	239.5755	2.99	0.0029*
?-D-Lactose	-428.8382	145.5783	-2.95	0.0034*
Carabidae	10523.647	3655.873	2.88	0.0042*
Tween 80	205.93332	71.80617	2.87	0.0043*
Glucose-1-Phosphate	-520.3077	187.4623	-2.78	0.0057*
Aphididae	3012.5506	1137.826	2.65	0.0084*
Ascidae	-931.5641	361.7819	-2.57	0.0103*
?-Cyclodextrin	187.41768	74.29679	2.52	0.0119*
4-Hydroxy Benzoic Acid	612.03876	244.7884	2.50	0.0127*
PO4-P	9.5941349	4.145474	2.31	0.0210*
Stratiomyidae	162.50018	86.46816	1.88	0.0608
Eosentomidae	-14896.74	8068.013	-1.85	0.0654
Pyruvic Acid Methyl Ester	359.49107	196.5256	1.83	0.0679
Herbivore (soil)	-1801.573	1030.202	-1.75	0.0809
Thripidae	-2127.764	1304.901	-1.63	0.1036
D,L-?-Glycerol Phosphate	-183.7407	150.2318	-1.22	0.2219
?-Hydroxybutyric Acid	134.16032	221.8549	0.60	0.5456

Figure R1. Continued.

Prediction Expression

ADH = 10309.80 + 187.42*α-Cyclodextrin – 428.84*α-D-Lactose + 134.16*γ-Hydroxybutyric acid + 612.04*4-Hydroxybenzoic acid – 183.74*D,L-α-Glycerol phosphate – 520.31*Glucose-1-phosphate + 716.31*L-Asparagine + 359.50*Pyruvic acid methyl ester + 205.93*Tween 80 + 33.53*NO_3-N + 9.59*PO_4-P – 208.99*H_2O – 2127.76*Thripidae + 1521.09*Sminthuridae + 162.50*Stratiomyidae + 3012.55*Aphididae + 10523.65*Carabidae – 14896.74*Eosentomidae – 931.56*Ascidae – 5568.89*Phytoseiidae + 608.20*Acaridae – 1801.57*Soil herbivores

APPENDIX S

METACOMMUNITY STRUCTURE OF ARTHROPODS ASSOCIATED WITH PIG CARRION AT SNOOK, TEXAS

Sticky trap 2013: Species by Day (Succession)
Method: 'tswap'

Treatment	Coherence			Turnover			Boundary clumping		Idealize pattern
	Abs	p	Mean ± SD	Rep	p	Mean ± SD	I	p	
Control	216	0.347	198 ± 18	3036	0.2583	3844 ± 715	0	0.2058	Random
Post-7	175	0.316	190 ± 15	5281	0.0103	3914 ± 532	0	0.2087	Random
Post-14	193	0.469	184 ± 12	2797	0.7253	2979 ± 519	1.2575	0.3933	Random

Abs = absences; Rep = replacement; I = Morisita's Index

Sticky trap 2014: Species by Day (Succession)
Method: 'tswap'

Treatment	Coherence			Turnover			Boundary clumping		Idealize pattern
	Abs	p	Mean ± SD	Rep	p	Mean ± SD	I	p	
Control	150	0.123	129 ± 12	2275	0.8504	2353 ± 413	0	0.1916	Random
Post-7	138	0.914	138 ± 8	2970	0.0633	2201 ± 413	0	0.1933	Random
Post-14	140	0.887	141 ± 13	2284	0.3525	1986 ± 319	0	0.1899	Random

Abs = absences; Rep = replacement; I = Morisita's Index

Larva 2013: Species by Day (Succession)
Method: 'tswap'

Treatment	Coherence			Turnover			Boundary clumping		Idealize pattern
	Abs	p	Mean ± SD	Rep	p	Mean ± SD	I	p	
Control	4	0.505	4 ± 1	568	0.014	361 ± 84	1.2307	0.052	Random
Post-7	37	0.183	59 ± 16	680	0.694	795 ± 293	1.3076	0.003	Random
Post-14	17	0.079	40 ± 13	974	0.0005	497 ± 138	1.0691	0.063	Random

Abs = absences; Rep = replacement; I = Morisita's Index

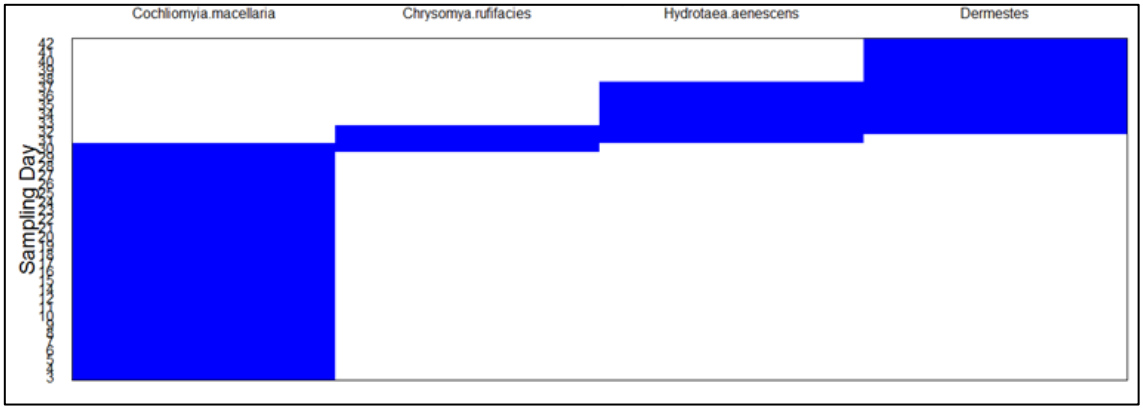


Figure S1. Visualization of insect larva colonization on pig carrion (Control) in Snook, Texas during summer 2013.

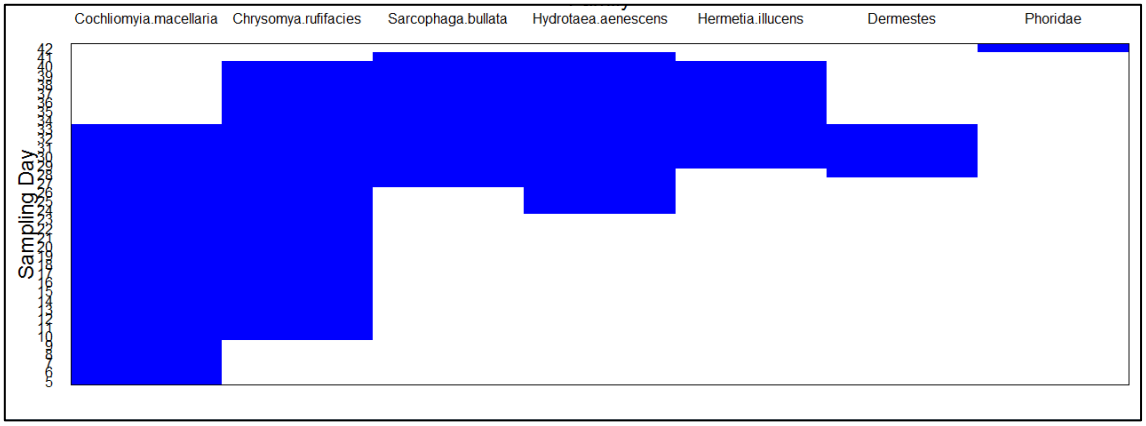


Figure S2. Visualization of insect larva colonization on pig carrion (Post-7) in Snook, Texas during summer 2013.

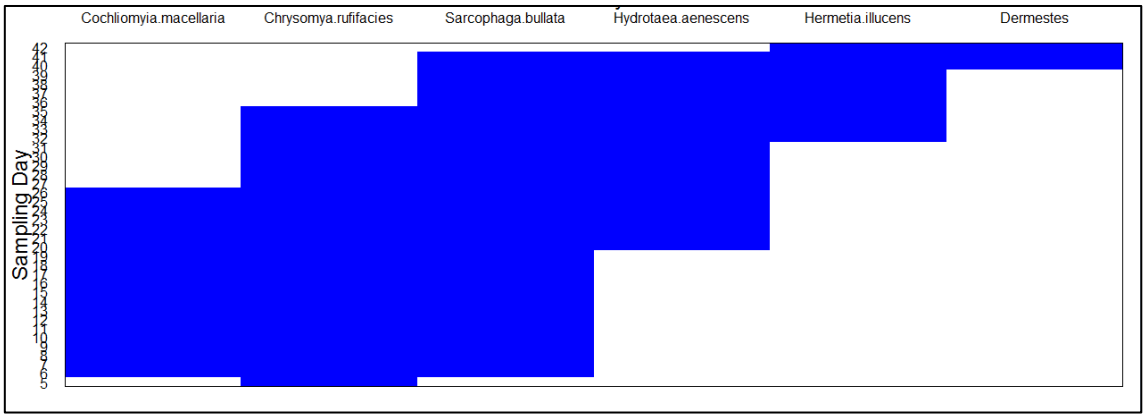


Figure S3. Visualization of insect larva colonization on pig carrion (Post-14) in Snook, Texas during summer 2013.

Larva 2014: Species by Day (Succession)
Method: 'tswap'

Treatment	Coherence			Turnover			Boundary clumping		Idealize pattern
	Abs	p	Mean ± SD	Rep	p	Mean ± SD	I	p	
Control	67	0.001	28 ± 12	468	0.740	510 ± 127	1.188	0.061	**Checker board**
Post-7	16	0.352	25 ± 9	902	0.348	715 ± 199	1.638	0.000	Random
Post-14	2	0.000	46 ± 9	892	0.625	800 ± 186	1.670	0.000	**Checker board**

Abs = absences; Rep = replacement; I = Morisita's Index

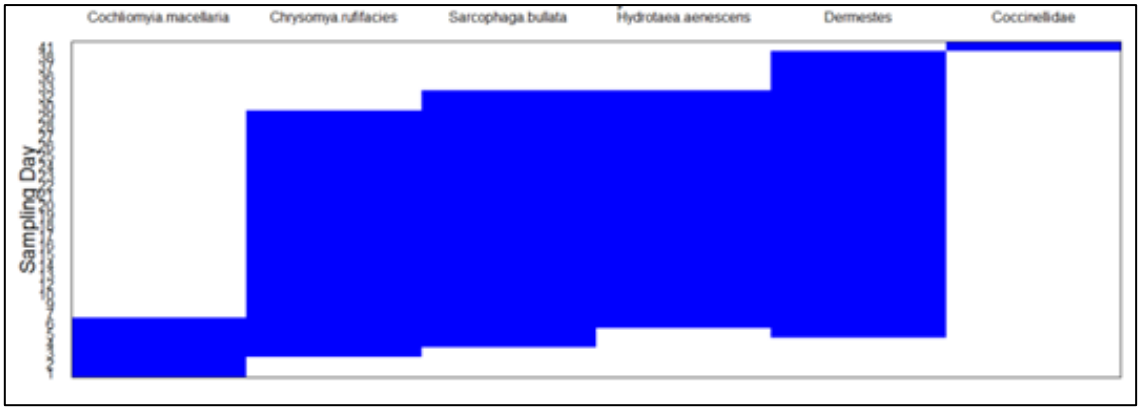


Figure S4. Visualization of insect larva colonization on pig carrion (Control) in Snook, Texas during summer 2014.

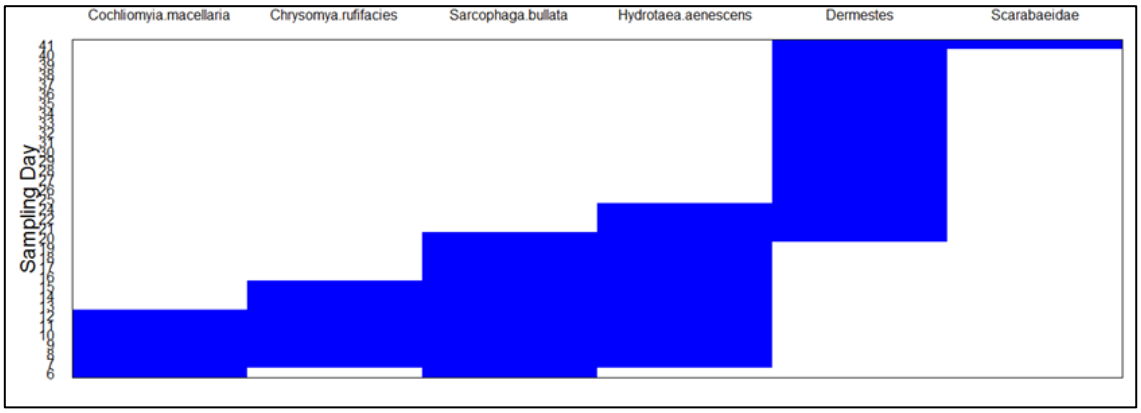


Figure S5. Visualization of insect larva colonization on pig carrion (Post-7) in Snook, Texas during summer 2014.

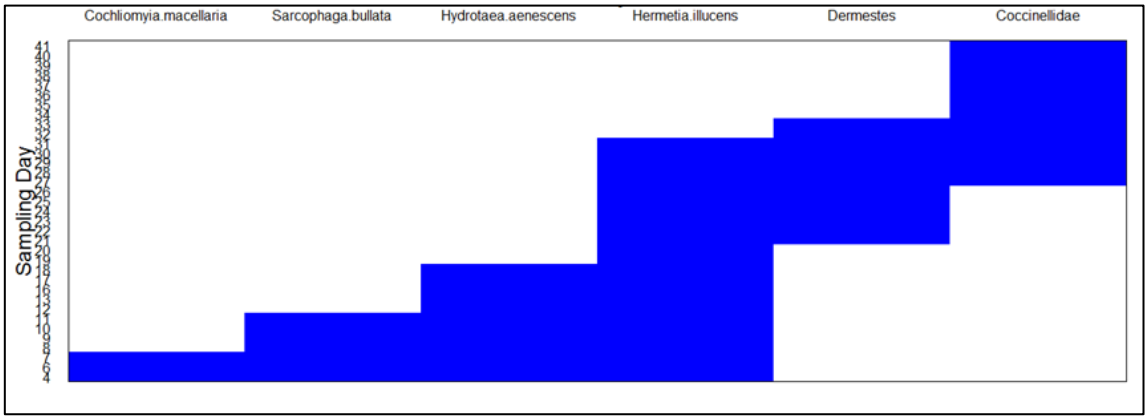


Figure S6. Visualization of insect larva colonization on pig carrion (Post-14) in Snook, Texas during summer 2014.