THE IMPACT OF LARVAL DIGESTION OF DIFFERENT MANURE TYPES BY THE BLACK SOLDIER FLY, *Hermetia illucens*, (L.) (DIPTERA: STRATIOMYIDAE) ON VOLATILE EMISSIONS AND CORRESPONDING ADULT ATTRACTION

A Thesis

by

KELLY VIRGINIA BESKIN

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

MASTER OF SCIENCE

Chair of Committee,	Jeffery K. Tomberlin
Committee Members,	Pete D. Teel
	Tawni L. Crippen
	Michael T. Longnecker
	Jonathan A. Cammack
Head of Department,	David Ragsdale

December 2016

Major Subject: Entomology

ABSTRACT

The black soldier fly, *Hermetia illucens* (L.), is a large, non-pest species whose larvae (BSFL) are known to consume a variety of decaying organic materials. This ability is being pursued for industrialization as a means to recycle wastes and produce protein for use as food and feed. BSFL were reared under laboratory conditions on poultry, swine, and dairy manure at rates of 18.0 and 27.0 g every other day until 40% reached the postfeeding stage. Volatile emissions were collected and analyzed from freshly thawed manure (control) as well as the digested waste when 90% of the BSFL reached the prepupal stage. Volatiles were also collected from manure not inoculated with BSFL and held under similar conditions until 90% of the BSFL had reached the prepupal stage in the treated manure (non-digested). Manure samples were analyzed for relative amounts of nine odorous compounds: phenol, 4-methylphenol, indole, 3-methylindole, propanoic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid and pentanoic acid. BSFL reduced emissions of all compounds by 87% or greater. Complete reductions (i.e. 100%) in relative amounts of compounds were observed for propanoic acid, 2-methly in BSFL digested poultry manure, phenol, 4-methylphenol, indole and all five acids in BSFL digested swine manure and 4-methylphenol, indole, 3-methylindole and all five acids in BSFL digested dairy manure.

This study was the first to identify volatile emissions from manure colonized by BSFL and compare to those found in uncolonized manure. These data demonstrate

additional benefits to using BSFL as a cost effective and environmentally-safe means of livestock manure management in comparison to current methods.

DEDICATION

I would like to dedicate this thesis to my parents, Rick and Lisa, and my brother, Brad, for their constant love and support of all my endeavors.

ACKNOWLEDGEMENTS

First and foremost, I'd like to thank my committee chair, Dr. Jeffery Tomberlin, for his guidance and support throughout this process and for allowing me to come work in the F.L.I.E.S. Facility. I could not have asked for a better mentor and am thankful for each and every opportunity you presented me. Your constant support, guidance and motivation were invaluable. I cannot thank you enough for these experiences and the personal growth you have facilitated over the years.

I'd also like to thank my other committee members, Dr. Pete Teel, Dr. Tawni Crippen, Dr. Michael Longnecker, and Dr. Cammack for their expertise, support, and suggestions for my research.

I owe special thanks to Dr. Jonathan Cammack, in particular, for teaching me the volatile collection and elution techniques in addition to building the manifold in the F.L.I.E.S. Facility. Your willingness to always look over my writing, presentations and offer constructive comments, suggestions, and perspectives was crucial to this process.

I'd like to thank Dr. Tony Knapp at the Geochemical and Environmental Research Group for allowing me to use their GC-MS for sample processing and work with the researchers there. Specifically, my time with Mr. Steve Sweet was integral to this project. I am indebted to him for all the hours he dedicated to helping me with the GC-MS and teaching me about the technique. There would be no data without Mr. Sweet.

There would also be no data here without the help I received from Le Zheng. Thank you, Le, for taking the time to teach me how to use R, especially when you were so busy

with your own work. I owe you so much for all the hours that you spent with me and it is tremendously appreciated.

I'd like to thank all of our manure sources in addition to those that were involved in the collection process. Dr. Craig Coufal and Tucker Allcorn at the Texas A&M Poultry Science Research, Teaching and Extension Center, Mr. Ricky Schroeder at RW Genetics for the swine manure and Dr. David Kattes at the Southwest Regional Dairy Center.

Special thanks to Chelsea Holcomb for offering constant support and help with my research. You're a great friend, lab mate and researcher and I know you'll go on to do incredible work. I'm so glad to have had your ear, experience and advice throughout this endeavor. I cannot imagine having gone through this without you and not having you to share in the laughter, sweat and tears that were put into these projects.

Thanks to my lab mates at the F.L.I.E.S. Facility for their support and being a wonderful group of scientists and researchers. I am so fortunate to have gone through this with such a knowledgeable group of individuals.

Finally, I'd like to thank the Department of Entomology at Texas A&M University and all my friends and peers within it for Friday Happy Hours, trivia, tail gates, and hours of discussion and commiseration. I couldn't have done it without you.

NOMENCLATURE

AFO	Animal Feeding Operation
AA	Amino Acids
ANOVA	Analysis of Variance
AU	Animal Unit
BSF(L)	Black Soldier Fly (Larvae)
CAFO	Concentrated Animal Feeding Operation
CP	Crude Protein
EAG	Electroantennogram
EPA	Environmental Protection Agency
F.L.I.E.S.	Forensic Laboratory for Investigative Entomological Sciences
GC-MS	Gas Chromatography- Mass Spectrometry
HSD	Honest Significant Difference
ISA	Indicator Species Analysis
MRPP	Multi-response Permutation Procedure
MVOC	Microbial Volatile Organic Compound
NIST	National Institute of Standards and Technology
NMDS	Non-metric Multidimensional Scaling
PERMANOVA	Permutational Multivariate Analysis of Variance
Spp.	Species
TAMU	Texas A&M University

USDA	United States Department of Agriculture
VFA	Volatile Fatty Acid
VOC	Volatile Organic Compound
WDGS	Wet Distillers Grains with Solubles

TABLE OF CONTENTS

Page

ABSTRACT ii

DEDICATION iv

ACKNOWLEDGEMENTS v

NOMENCLATURE vii

TABLE OF CONTENTS ix

LIST OF FIGURES xii

LIST OF TABLES xiii

CHAPTER I INTRODUCTION AND LITERATURE REVIEW 1

Introduction and Literature Review 1
Increasing Global Protein Demands 1
Environmental and Human Health Effects of Animal Farming 2
Livestock Manure Characteristics 10
Poultry Manure 11
Swine Manure 11
Dairy Manure 13
CAFO Classifications 13
Poultry 14
Swine 14
Dairy 14
Manure Management Practices 14
Poultry Manure 16
Swine Manure 22
Dairy Manure 27
Cost of Manure Management Practices 32
Volatiles Associated with Manure and Their Origins 33
Poultry Manure 43
Swine Manure 44
Dairy Manure 45
Microbial VOCs as Insect Semiochemicals 46
Manure Associated Volatiles as Dipteran Attractants 49

Background on the Black Soldier Fly 52
Objectives and Hypotheses 60

CHAPTER II VOC ANALYSIS: RESEARCH, RESULTS, AND DISCUSSION 61

Introduction 61
Materials and Methods 66
Acquisition of Flies 66
Acquisition of Manure 67
Experiment Design 68
Volatile Sampling 69
GC-MS Analysis 72
Chemical Standards 74
Moisture Content 74
Statistical Analyses 74
Research Results 76
Moisture Contents 76
Multivariate Work 82
Number of Compounds 82
Select Odorous Compounds in Poultry Manure 86
Select Odorous Compounds in Swine Manure 95
Select Odorous Compounds in Dairy Manure 101
Discussion 107

CHAPTER III OVIPOSITION AND ATTRACTION ASSAYS: RESEARCH, RESULTS, AND DISCUSSION 116

Introduction 116
Materials and Methods 119
Volatile Sampling 119
Acquisition of Flies 120
Oviposition and Attraction Assays 121
Statistical Analyses 124
Research Results 125
Oviposition and Attraction Assays 125
Discussion 128

CHAPTER IV CONCLUSION AND FUTURE STUDIES 134

REFERENCES 142

APPENDIX A 159

APPENDIX B 177

APPENDIX C 198

LIST OF FIGURES

Page

Figure 2.1 Headspace collection set up used to collect volatiles from manure samples. A 236.5 ml Ball® mason jar was outfitted with two holes in the lid: one for a carbon filter to purify ambient air (left) and another for the volatile trap (right). Volatile traps contain 30.0 mg of porous polymer mix Hayesep® Q to which volatiles adhere to. Samples of 20g were placed at the bottom while air was pulled at 1.0 L per min for 1 hr 71

Figure 2.2 VOCs were eluted from the Hayesep® Q into 1.5 ml SureStop™ GC vials containing a 9.0 mm 300 µl insert with 150 µl of dichloromethane (Sigma -Aldrich, St. Louis, MO, USA) injected into the trap which was pushed gently through with N_2. An additional 5.0 µl of n-Octane at a concentration of 80 ng/µl was added to each sample as an internal standard 72

Figure 3.1 Cage used for oviposition and attraction assays June-July, 2016 at the F.L.I.E.S. Facility in College Station, TX. Cups with cardboard were placed inside the cages. Eluate from BSF digested, non-digested manure, and two controls were applied to the cardboard. Adults were allowed to oviposit for eight hours on treatments after which egg masses were collected and weighed 121

Figure 3.2 Block design of cups within cage examining adult oviposition and attraction June 19th, 2016. Three pieces of cardboard, to which the eluate was applied, were taped approximately 4 cm below the lid inside each cup. Different treatments included three replicates of digested manure at 90%, its corresponding non-digested replicate and two controls (dichloromethane and negative control with no eluate applied). Experiments were run from 0800 – 1700 123

Figure 3.3 Cage used for oviposition and attraction assays June-July, 2016 at the F.L.I.E.S. Facility in College Station, TX broken down and examined for positional effects by quadrant and position within each quadrant. The cage was divided into four quadrants east to west and each cup within a quadrant given a position A-F. 125

LIST OF TABLES

Page

Table 1.1 Manure characteristics of poultry layer, lactating swine and dairy cow manure in units of per day per AU. Modified from the Agricultural Waste Management Field Handbook, Chapter 4 – Agricultural Waste Characteristics (NRCS 2008). 12

Table 2.1 Mean moisture percentages ± SEM of poultry, swine, and dairy manure at 18 and 27 g with and without *Hermetia illucens* (L.) larvae compared to control manure maintained at 29 ± 0.3°C with 60 ± 5.1% and 14:10 L:D cycle *(P* < 0.05)..... 76

Table 2.2 ANOVA and Tukey-Kramer HSD on mean moisture percentages ± SEM of poultry, swine, and dairy manure (n^1 = 3) at 18 g with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle *(P* < 0.05)**....** 77

Table 2.3 ANOVA and Tukey-Kramer HSD on mean moisture percentages ± SEM of poultry, swine, and dairy manure (n^1 = 3) at 27 g with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle *(P* < 0.05)... 78

Table 2.4 Mean number of compounds ± SEM of poultry, swine, and dairy manure with and without *Hermetia illucens* (L.) larvae compared to control manure maintained at 29 ± 0.3°C with 60 ± 5.1% and 14:10 L:D cycle (*P* < 0.05). 81

Table 2.5 ANOVA and Tukey-Kramer HSD on mean number of compounds ± SEM of poultry, swine, and dairy manure (n^1 = 3) at 27 g with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle *(P* < 0.05)... 83

Table 2.6 Mean relative peak areas ± SEM of noxious odors emitted from poultry, swine, and dairy manure with and without *Hermetia illucens* (L.) larvae compared to control manure at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle (*P* < 0.05) 87

Table 2.7 Mean relative peak areas ± SEM of noxious odors (cont.) emitted from poultry, swine, and dairy manure with and without *Hermetia illucens* (L.) larvae compared to control manure at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$)..... 88

Table 2.8 ANOVA and Tukey-Kramer HSD on relative amount of amounts of odorous volatile compounds in poultry manure with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% and 14:10 L:D cycle ($P < 0.05$)..... 89

Table 2.9 ANOVA and Tukey-Kramer HSD on relative amount of amounts of odorous volatile compounds in swine manure with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% and 14:10 L:D cycle ($P < 0.05$)..... 96

Table 2.10 ANOVA and Tukey-Kramer HSD on relative amount of amounts of odorous volatile compounds in dairy manure with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% and 14:10 L:D cycle ($P < 0.05$)..... 102

Table 3.1 Mean number ± SEM of adult *Hermetia illucens* (L.) per oviposition quadrant across two trials under field conditions. Each quadrant contained cups outfitted with three layers of corrugated cardboard which was treated with VOCS isolated from manure provided at two different feed rates to larval black soldier flies 126

Table 3.2 Mean number ± SEM of adult *Hermetia illucens* (L.) per oviposition position across two trials under field conditions. Each position represents a cup outfitted with three layers of corrugated cardboard which was treated with VOCS isolated from manure provided at two different feed rates to larval black soldier flies 127

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction and Literature Review

Increasing Global Protein Demands

There has been increasing pressure on the livestock sector to meet the growing demand for animal protein over the past several decades. The world's livestock sector is growing at an unprecedented rate driven by an increasing global population, rising incomes, and urbanization (FAO 2009). Annual meat production is expected to increase from 218 million tons in 1997-1999 to 376 million tons in 2030 (FAO 2009). By the year 2050, the demand for meat and milk is expected to be 58 and 70% higher, respectively, than their levels in 2010, and a large portion of this will come from developing countries (Msangi et al. 2011). In Asia, for example, consumption of animal protein per capita increased by 225% between 1961 and 2007. This consumption in 2007 accounted for nearly 40% of Asian total protein consumption compared to only 15% in 1961 (FAOSTAT 2009). This demand is contrasted to their supply of crop-derived protein for human consumption, which increased only 22% during this time period. Globally, animal derived protein accounts for almost 40% of total protein consumption by humans and is expected to continue to increase significantly by 2050 (FAO 2009).

The livestock industry is important to nations, not only as a source of much-needed animal-derived protein, but also in terms of the economic stability that it provides. Globally, the livestock sector comprises 40% of agricultural gross domestic product and

employs 1.3 billion people (Steinfeld et al. 2006). While important in terms of providing essential nutrients to diets, livestock production is a significant contributor to local and global environmental problems (FAO 2009, Steinfeld et al. 2006). This increase in demand will require more animal production as well as a need to conserve resources that can be utilized by humans rather than livestock. Furthermore, the negative impacts of animal farming are exacerbated by the trend in the livestock industry to raise large numbers of animals in animal feeding operations (AFOs) and concentrated animal feeding operations (CAFOS).

Environmental and Human Health Effects of Animal Farming

The livestock sector affects humans and their environment by occupying and using a large portion of natural resources. Production of this commodity represents the largest anthropogenic user of land occupying 75% of all agricultural land and 30% of all land-surface on the planet (Steinfeld et al. 2006). The global population is experiencing problems of water shortages with an estimated 64% of the population expected to live in water-stressed basins by only 2025 (Steinfeld et al. 2006). Livestock production will only increase this problem as it consumes 8% of global water use, mainly through irrigation of feed crops (FAO 2009, Foley et al. 2011). Livestock production is a huge use of natural resources and these animals compete directly with humans, who could otherwise use them.

Animal production is a detriment to the environment and causes severe environmental issues such as water pollution, land degradation, decline in atmospheric quality and increased health problems (FAO 2009). For example, animal farming is

estimated to account for 55% of soil and sediment erosion, 37% of nationwide pesticide use, 80% of antibiotic usage, and over 30% of the total nitrogen and phosphorus loading to national drinking water (Steinfeld et al. 2006). These negative impacts are strongly tied to the manure production from these intensive farming facilities, which will continue to increase with animal production as it strives to meet consumer demands. The root causes of these negative environmental impacts are the large volumes of animal waste produced, its improper management and disposal, and the unsuitable water usage and soil degradation associated with feed production (EPA 2013). The need to deal with manure is increasing much like it's prevalence. A tremendous amount of animal waste is produced annually. For 2001, the United States Department of Agriculture (USDA) reported over 299,370,964 metric tons of manure were produced, by broiler and egg-laying chickens, beef and dairy cows, and hogs and of this, 86% was said to come from animals in confinement (NASS 2012).

This increased need to deal with manure is also because of a geographic and structural shift. Livestock production is being encroached upon by expanding urban and peri-urban areas (Steinfeld et al. 2006), which puts consumers closer to these animals and their wastes. Animal production has also become more concentrated over the past two decades and is expected to continue along this trend (Ribaudo and Gollehon 2006). This increase in concentration is happening both at the scale of an individual farm and geographically across the United States (EPA 2013). For example, the 2007 USDA Census of Agriculture noted that 40% of swine production occurred in Iowa and North Carolina and 30% of broiler chicken production occurred in Alabama, Georgia, and Arkansas. Head

counts have increased on individual farms, which are becoming smaller, often leading to these animals being raised in high concentrations (NASS 2007).

The increase in prevalence of animal feeding operations (AFOs) and concentrated animal feeding operations (CAFOs) exacerbate this problem of concentration. AFOs are defined as agricultural operations where animals are kept and raised in confined situations such that animals have been, are, or will be stables or confined and fed or maintained for a total of 45 d or more in any 12-month period. The standards to be qualified as a CAFO are presented in subsequent sections.

Another term often used in the literature is “factory farm”. Food and Water Watch analyzed data from the USDA Censuses of 1997, 2002, 2007, and 2012 and created their own analyses of the livestock industry. They defined factory farms as having over 500 dairy cows, over 1,000 hogs or over 100,000 layer chickens. Their analysis found that the total number of livestock on the largest factory farms rose by 20% from 2002 to 2012. In 2008, it was reported that 54% of all United States’ food animals were concentrated on only 5% of the farms (Food and Water Watch 2015).

Whereas animals in pasture can distribute this resource more evenly and over a greater area of land, CAFOs produce more manure per square foot than can be applied to the land (Ribaudo and Gollehon 2006). Manure, traditionally viewed as a welcome source of nutrients for soil amendment, has now become a liability and problematic resource. This excess of manure must be dealt with via other methods such as land application, storage, or removal all of which increase the cost to the farmer (Nowak et al. 1998).

Additionally, these methods may lead to pollution as contaminants associated with manure can move through the soil and wind to waterways. In the USA, it is estimated that 278,416.51 km of national waters are impacted by runoff from agricultural sources (Pew Commission on Industrial Farm Animal Production 2008). Improper or malfunctioning storage and natural disasters can also lead to environmental exposure to manure. In 1999, Hurricane Floyd hit North Carolina, flooding much of the land, including 50 lagoons used for manure storage, causing three to burst. The result was the release of over 3.7 million L of manure mixed with the floodwaters in addition to the deaths of millions of animals, which drowned during the flooding (Henderson 1999). In 2009, a clogged pipe caused the leak of over 94,000 L of dairy manure in Pipestone County, MN, which spilled into a local tributary, killing fish and resulting in a state park closing to swimmers after elevated levels of coliform bacteria were found in the park's waters (Kuphal 2009). In 2010, the Environmental Protection Agency (EPA) mandated a feedlot in Grand View, ID to cease the discharge of fecal bacteria-contaminated water from its stock watering system into a tributary of the Snake River (EPA 2010).

Animal farming can also impact water environments by depleting limited freshwater sources by contaminating surface and groundwaters, often seen in air regions and floodplains, respectively (Burkholder et al. 2007, Mallin et al. 2003). Aquatic environments can also be negatively impacted by the overabundance of nutrients such as nitrogen and phosphorus, another issue associated with improper manure management (Kellogg 2003). In 2010 a broiler chicken operation with over 100,000 animals was ordered to cease the discharge of pollutants from large piles of uncovered manure, which

were leaching nitrogen and phosphorus into a nearby tributary of the Shenandoah River, VA. (EPA 2010).

Nitrogen-containing pollutants, such as ammonium, nitrate and nitrite, pose both ecological and human health threats, and can reach the environment through leaching and surface runoff (Burkholder et al. 2007). Nitrogen in animal waste, is largely present as ammonium and is quickly converted to nitrate by microorganisms in aerobic conditions. Nitrate, which is highly soluble, moves with waters into rivers and groundwater. Nitrogen is a limiting nutrient in marine and estuarine environments, and therefore increased loading of this element can significantly contribute to downstream effects such as eutrophication and oxygen depletion (hypoxia), which are responsible for massive fish kills (Rabalais et al. 1999). Manure discharged from a dairy in O'Brien County, IA polluted a 45 km stretch of stream and killed over 860,000 fish. The Iowa Department of Natural Resources estimated the value of these fish to be over $160,000 (Iowa Department of Natural Resources 2014). Nitrate is also an important water contaminant that is regulated by the EPA's Safe Drinking Water Act. High levels of exposure to nitrates can cause methomoglobinemia, or blue baby syndrome (Ward et al. 2005) in infants and is linked to different cancers, insulin-dependent diabetes and neurodevelopmental defects in adults (Burkholder et al. 2007). Phosphorus is another important contaminant linked to manure and intense livestock farming. Like nitrogen, phosphorus is a limiting nutrient in many aquatic environments. Through manure disposal, leaching, and runoff, phosphorus can reach these environments and result in eutrophication (PEW 2008).

There are many other pollutants and pathogens associated with manure such as those relative to human health (e.g., bacteria, viruses and protozoans) (Cole et al. 1998). These pathogens include bacteria such *Escherichia coli* 0157:H7, *Salmonella* spp., and *Campylobacter* spp., viruses such as *Rotavirus,* avian influenza virus and Hepatitis E and protozoa such as *Cryptosporidium parvum* and *Giardia lamblia.*

The decomposition of manure is also responsible for environmental pollutant emissions such as greenhouse gases, ammonia and other volatile organic compounds. Livestock production is responsible for more greenhouse emissions globally than transportation (i.e. vehicles) and produces more carbon dioxide emissions than manufacturing of chemical fertilizers for animal feed (FAO 2009). Greenhouse gases, specifically, methane, carbon dioxide, and nitrous oxide, are given off by the animals during the digestion process in the gut (FAO 2009). The livestock sector produces 68% of anthropogenic nitrous oxide, which has 296 times the global warming potential of carbon dioxide and can remain in the atmosphere for up to 150 years. Nitrous oxide contributes to ozone depletion and therefore global warming. Livestock is also responsible for 64% of anthropogenic ammonia emissions (FAO 2009), which contribute to the acidification of ecosystems and acid rain. Livestock production is also responsible for 35-40% of anthropogenic methane, which has 23 times the global warming potential of carbon dioxide (FAO 2009). According to the EPA, over the last 15 years, greenhouse gas emissions have risen significantly; methane emissions from swine and dairy cows have increased by 37% and 50%, respectively (EPA 2013). Greenhouse gas and other harmful

environmental emissions will only continue to increase as intensive livestock practices become more commonplace as demand continues to rise.

The decomposition of manure is also responsible for emissions of volatile organic compounds (VOCs), which are pollutants and pose potential health risks (FAO 2009). Studies have indicated that between 100 and 330 VOCs and volatile fatty acids are generated by CAFOs, depending on management practices and the species of animal involved (Cai et al. 2006, Powers and Bastyr 2004, Schiffman et al. 2001). The Pew Commissions on Industrial Farm Animal Production demonstrated that both working in and living near intense livestock farming increased respiratory problems, including asthma (Trusts and Hopkins 2008). Studies have shown that as much as 25% of workers in confined swine production suffered from ailments such as chronic bronchitis and non-allergenic asthma (Donham 2000). Rushton et al. (2007) demonstrated mucous membrane irritation, bronchitis, asthma and chronic obstructive pulmonary disease in pig farmers as well. Additionally, workers exposed to hydrogen sulfide at levels only slightly above the odor threshold were found to have accelerated deterioration of neurobehavioral function (Kilburn 1999). In 2010, a manure lagoon liner from a 1,650 cow dairy operation in Randolph County, IN, became detached and inflated with gases associated with decomposing manure. The operator of the farm could not afford the costs associated with fixing the liner, causing the county to shut down local roads to the area due to the risk posed by the potential lease of noxious odors or even explosion (Etter 2010). In Portage County, Wisconsin, 2016, a farmer and 16 cattle were overcome and killed by deadly

amounts of sulfur oxide fumes from a manure holding tank when agitation caused the surface line to crack and release deadly fumes into the environment (Cerullo 2016).

Finally, in addition to experiencing some of the respiratory problems mentioned above, those living in the area of heavy animal production have been found to experience higher levels of tension, depression and anger (Barrett 2006). Reports have associated odor from animal production facilities in rural areas with other issues such as harassment from outspoken community members to farmers and a general negative perception by local residents (Thu 1997). Odors, which are so noxious they overtake an entire area, have been linked with a decline in local retail purchases, degradation of community fabric, and decline in land and property values (Goldschmidt 1979).

Although the EPA is currently examining emission control solutions with programs such as carbon credits and credit trading (Jensen 2006), these emissions are largely unregulated. Furthermore, much of the studies and potential regulation focuses on greenhouse gas emissions and those, as previously mentioned, that are byproducts of the animals themselves versus non greenhouse gas VOCs. Regulating these compounds will be more difficult as it is unlikely that the number of animals being farmed in the USA will decrease in the near future. North Carolina has recently passed legislation to set high performance standards for swine AFOs with substantial reductions in emissions of ammonia, odors and pathogens required (General Assembly of North Carolina 2007). Beyond this, legislators to significantly deal with these harmful and noxious emissions have done little. Reducing emissions of greenhouse gases and odorous VOCs is of important interest to those in farming, environmental and public sectors.

Livestock Manure Characteristics

The 2012 Census of Agriculture reported a total of 89.9 million cattle and calves, 66 million hogs and pigs, and 350.7 million layers in the United States. The analyses done by the Food and Water Watch found that factory-farmed livestock produced 369 million tons of manure in 2012, about 13 times as much sewage produced by the entire U.S. population (Food and Water Watch 2015). Advances in production and conversion efficiencies are being made every year, therefore previous and dated estimates of waste production and composition may not always be the most accurate estimators and will often overestimate both the total volume of production and composition of the waste produced (Sweeten et al. 1998).

When describing manure characteristics, several terms are often used. Some terms, which will be used below, are herein defined (NRCS 2008). Moisture content of manure is the part of the waste removed by evaporation and oven drying at 103°C for 24 h. Total solids are the residue remaining after water is removed from waste material by evaporation, also known as dry matter content. Moisture content (%) plus total solids (%) equals 100%. Dry matter is assed in the same fashion as moisture content. Volatile solids are defined as the part of total solids driven off volatile (combustible) gases when heated to 600°C for one hour. Fixed solids are the part of the total solids remaining after volatile gases are driven off. Data such as moisture content is given on an “as excreted” basis and different handling system can affect such aspects of manure (i.e., a liquid handling system or dry storage). The sections below give aspects of these animals for their average size. However, this can be hard to compare across animal types as a layer hen is much smaller

than dairy cow. Therefore, these values have been calculated on a per animal unit (AU) (453.59 kg animal) basis to allow comparison across animals. These values are presented in Table 1.1 (NRCS 2008)

Poultry Manure

Poultry litter, which is a mixture of manure, bedding material, wasted feed, feathers, and soil that is picked up during recovery, is generally considered to be the most valuable animal manure owing to its relatively low moisture and high macronutrient content (Wilkinson 1979). An l.36 kg layer produces 0.09 kg of waste a day at 75% moisture. This equates to only 8.77×10^5 m^3. This manure breaks down into 0.02 kg of total solids and 0.02 kg of volatile solids. Daily amounts of nitrogen, phosphorus and potassium equal 0.001, 0.0005, and 0.0005 kg respectively (NRCS 2000).

Swine Manure

The amount of manure produced by swine varies between gestating and lactating sow and boars. A 199.58 kg gestating sow produces 4.99 kg of manure a day, which equals approximately 0.005 m^3. This manure is 90% moisture and contains 0.50 kg of total solids and 0.45 kg of volatile solids. Per day, a sow produces 0.03 kg of nitrogen, 0.009 kg of phosphorus and 0.02 kg of potassium. A 191.87 kg lactating sow will generate more manure than a gestating sow. On average these pigs produce 11.33 kg of manure a day at 90% moisture, equaling approximately 0.011 m^3. Of this waste, 1.13 kg

Table 1.1 Manure characteristics of poultry layer, lactating swine and dairy cow manure in units of per day per AU. Modified from the Agricultural Waste Management Field Handbook, Chapter 4 – Agricultural Waste Characteristics (NRCS 2008).

Component	Layer	Lactating sow	Lactating cow producing 27.21 kg per day of milk
Manure weight (kg)	25.85	26.76	58.97
Volume (m^3)	0.03	0.03	0.06
Moisture (%)	75	90	87
Total Solids	15	2.67	7.71
Volatile Solids	6.80	2.45	5.90
Nitrogen (kg)	0.50	0.20	0.36
Phosphorus (kg)	0.15	0.06	0.06
Potassium	0.18	0.13	0.21

are total solids, and 1.04 are volatile solids. Per day, this animal produces 0.09 kg of nitrogen, 0.02 kg of phosphorus and 0.05 kg of potassium. A 199.58 kg boar produces less manure than either of the sows with only 3.81 kg a day, about 0.003 m^3. Like the others types this manure is 90% moisture and however contains less total and volatile solids than the other two types 0.38 and 0.34 kg, respectively. The boar produces 0.03 kg of nitrogen, 0.009 kg of phosphorus and 0.02 kg of potassium a day (NRCS 2000).

Dairy Manure

A 623.96 kg lactating milk cow that produces 22.6 kg of milk a day excretes 60.33 kg of manure a day equating to 0.06 m^3. On average, the moisture content of this manure is 87% with 7.71 kg of total solids and 6.35 kg of volatile solids. Per day, this equates to 0.41 kg of nitrogen, 0.07 kg of phosphorus and 0.19 kg of potassium. While moisture content remains the same, the more milk a cow produces daily, the more manure it produces and therefore higher levels of these nutrients. For example, a cow of the same weight which produces 46.36 kg of milk a day will produce 74.39 kg of manure breaking down into 9.52 kg of total solids and 8.16 kg of volatile solids. A cow that produces this increased amount of milk will also produce more nitrogen, phosphorous and potassium but only by 15.55, 26.66 and 19.51%, respectively (NRCS 2000).

A 544.31 kg beef cow produces 56.70 kg of manure a day. This manure is 88% moisture with 6.80 kg of total solids and 5.89 kg of volatile solids. Per day this equates to 0.19 kg of nitrogen, 0.04 kg of phosphorus and 0.14 kg of potassium (NRCS 2000)

CAFO Classifications

The EPA has guidelines to determine whether an operation should be classified as a CAFO. Large CAFOs confine at least the number of animals listed below, while medium CAFOs fall within a range of sizes listed below and either 1) has a manmade ditch or pipe which carried manure or wastewater to surface waters, or 2) the animals come into contact with surface waters that pass through the area where they are confined. Even regardless of size, if an operation is found to be significant enough contributor of pollutants, the EPA

may designate a medium-sized facility as a CAFO. Small CAFOs confine fewer animals than listed below and have been designated as a CAFO by a permitting authority as a significant contributor of pollutants.

Poultry

A small laying hen or broiler CAFO is defined as having less than 9,000 birds. A medium sized CAFO has 9,000-29,000 birds and large CAFOs have 30,000 or more birds.

Swine

Swine must weigh over 24.95 kg to be included in the consideration for CAFO status. Small CAFOs are those with less than 750 animals while medium sized CAFOS contain 750-2,499 swine. A large swine CAFO is that which confines over 2,500 animals.

Dairy

A small dairy CAFO confines less than 200 cows. A medium-sized CAFO confines 200-699 while a large CAFO is any operation which confines more than 700 animals.

Manure Management Practices

Proper manure management can be a cost-saving tool for farmers as manure can be a resource that contains nutrients needed by crops. By taking advantage of this aspect, farmers can reduce their fertilizer costs significantly. For example, the Purdue Extension

Service estimated that those taking advantage of poultry manure as a fertilizer could save up to $50 in commercial fertilizer costs per acre per application (England et al. 1978). Although a potential valuable resource because of its nitrogen and phosphorus content, livestock farming, especially that which occurs at a concentrated scale where there is more manure available than can be taken up by the land, must deal with the overabundance of manure in other ways by implementing different practices of management.

Different management practices are often needed due to needs of the land receiving the manure. For example, poultry manure may need to be stored because schedule of flock may not coincide with crop production and the application of the manure as a fertilizer (Ritz et al. 2009). As such, poultry producers may be forced to store the manure temporarily until application times are appropriate. Regardless of size, CAFOs rely on two types of biological processes: aerobic and anaerobic (USDA 2000). In aerobic treatment, oxygen is available or provided to speed up the decomposition of organic compound found in the manure. The bacteria that thrive in this oxygenated environment tend to produce fewer odors than anaerobic bacteria. Anaerobic treatments occur when manure is decomposed in the absence of oxygen, which results in the production of biogas (USDA 2000). Biogas is a mixture of methane, carbon dioxide and other gases, which can be captured for energy recovery (Lusk 1998). The anaerobic process involves approximately three steps of microbial action. First, insoluble materials such as carbohydrates, fats and proteins are transformed into soluble materials (liquefaction). Second, volatile fatty acids (VFAs) are formed from the soluble material. The VFA's are a major source of the odors associated with manure. Finally VFAs are converted into biogas – partly methane- by

methane forming bacteria. Manure that is treated anaerobically emits significantly less odor than untreated or raw manure (Van Horn 1994).

Poultry Manure

Systems for dealing with the mass amounts of poultry manure revolve about the removal of the manure from the poultry (either layer or broiler) houses, pretreating it and transporting it to a field for application. The different means by which it is handled is often due to and controlled by its moisture content (Moore et al. 1995), size of farm and region where it is occurring (USDA 2000). A total cleanout of poultry litter from production houses is typically accomplished with tractor-mounted box scrapers or blades and machinery capable of scooping the material, such as front-end loaders. Upon removal from poultry houses, this material may be directly applied to land or temporarily stored (Moore et al. 1995).

Liquid poultry manures (those containing less than 40 g dry matter kg^{-1}) are generated when manure is scraped or flushed into storage reservoirs, such as tanks, detention basins, aerobic or anaerobic lagoons, and oxidation ditches. Most of the liquid poultry manure is generated in laying-hen operations (Miner 1977). Although these materials are generally amenable to hydraulic pumping, those containing between 40 and 150 g dry matter kg^{-1}, referred to as slurries, can present problems to pumping equipment because of their viscosity and potential to plug orifices (Miner 1977). Solid-liquid separation via sedimentation or filtration may be necessary when liquid poultry manures with higher amounts of solids are to be pumped.

Poultry manure that is being stored is subject to nitrogen loss based on the method of storage. The maximum value of poultry litter as a fertilizer occurs at the time of its removal from the poultry house when nitrogen content is greatest (Ritz et al. 2009). The longer the manure is held the more nitrogen that is lost into the atmosphere as ammonia; this loss is compounded when the manure is stored uncovered.

Poultry manure can be stored in several different ways such as covered stockpiles, stockpiles with ground liners, permanent storage structures or stack houses (Ritz et al. 2009). Nitrogen losses are minimized when the liquids or slurries are added to the bottom of storage reservoirs instead of to the surface (Carpenter 1992). Like other types of manure, poultry manure can also be applied to the land with the use of Nutrient Management Plans to prevent over application. Except for small amounts used in animal feed, the major portion (greater than 90%) of poultry litter is applied to agricultural land (Carpenter 1992).

The transport of solid poultry manure to the field, depending on the distance, is typically done with spreader trucks. Slurries may be pumped from storage reservoirs into tank-bearing vehicles for transport to the field, which requires agitation (Miner 1977). Liquid poultry manures having less than 40 g dry matter kg^{-1} may be handled in the same manner as slurries or may be pumped directly from storage reservoirs though pipeline systems to irrigation equipment at the site of application. The cost of moving poultry litter is a major obstacle facing the more efficient use of this resource (Moore et al. 1995).

The type of spreading equipment used depends on the method of storing and handling poultry manure. Traditionally, poultry litter is broadcast directly from the house,

using a variety of spreaders. Manure stored in deep pits is removed by scraping and is applied with a spreader. In a few cases, manure stored in shallow pits is removed by flushing and, after large solids have been removed by sedimentation and/or filtration, is applied with an irrigation system. Spreading equipment can vary among contractors (Moore et al. 1995).

Two USDA agencies, the Animal and Plant Health Inspection Service and the National Agricultural Statistics Service conducted a thorough evaluation of the nation's layer (versus broiler) industry in 2013. This questionnaire was administered to table-egg farms with 3,000 or more hens, that had been registered with the Food and Drug Administration (FDA). The study characterizes the manure handling practices by region and by size. The regions considered were the Northeast (Indiana, Michigan, Ohio, Pennsylvania, Connecticut, Maine, Massachusetts, New Hampshire and Vermont), the Southeast (Alabama, Florida, Georgia, and North Carolina), Central (Arkansas, Illinois, Iowa, Minnesota, Nebraska and Wisconsin) and West (California, Texas and Washington). Small farms were considered to have between 3,000-29,000 hens, while medium farms were those with 30,000-99,999 hens and finally, large farms were those with over 100,000 hens. The method used to house these animals significantly impacts the management of their waste. Just under half of the farms surveyed had a single layer house on site (46.5%) and about one fifth had either two or more than six houses (20.8 and 20.0%, respectively). Finally, 12.7% of farms had 3-5 layer houses on site (USDA 2014).

In terms of the number of animals housed within each layer house, only 0.5% of houses kept less than 1,000 birds in a single house. Around half of farms (51.9%) had a

capacity anywhere from 1,000-29,000 birds accounting for 28.1% of houses. Close to a third (37.2%) of farms had houses with a capacity of 30,000-99,999 birds, which accounted for 38.1% of houses surveyed. Approximately one-fifth of farms had layer houses with a capacity of 100,000-199,000 birds, accounting for 26.3% of all houses. Overall, 6.2% of farms had at least one house that could hold 200,000 or more birds. Layer houses with a capacity of 200,000 birds or more accounted for 7.1% of houses. The majority of farms, regardless of region use some sort of cage for housing whether these be conventional or enriched cages. The greatest percentage of cage use occurs in the West (68.0%) and the least in the Central region (45.1%). Enriched cages are those that provided perch, scratch and nesting areas. The use of enriched cages alone accounted for less than 3% of farms in any given region. Around one third to one half of farms across region employ some sort of cage free housing type (whether certified organic or not) with the highest percentage being 55.6% in the Central region and the lowest being 37.1% in the Southeast (USDA 2014).

The type of housing used at farms was also correlated with the size of the farm. Smaller farms typically employed a cage free system (95.4%) while this become less common with medium and larger farms (40.0 and 7.6%, respectively). In medium and large sized farms, conventional cages were the most common housing type (64.7 and 93.8%, respectively) (USDA 2014).

Typically, across regions, the layer hens do not have outdoor access. The percentage of farms with outdoor access for birds decreased as farm size increased. For example, the percent of farms with houses that do not have any outdoor access is 35.1%

in small farms, 84.3% in medium farms and 98.2% in large farms. The vast majority of farms that provide their layers with outside access were certified organic operations (94.9%) (USDA 2014).

A large portion of producers in several regions of the USA used high-rise housing (pit at ground level with house above) to deal with their manure. A majority of farms in the Northeast (60.9%) employ this method in addition to 42.2% in the Southeast, 27.0% in the Central region and only 12.4% in the West. The use of deep pits (i.e., below ground) is an uncommon practice across regions with the West using this method the most (6.8%). Likewise, the use of shallow pits at ground level is also not common with the great proportion of farms using this method occurring in the Southeast and West (11.0 and 11.9%, respectively). The use of raised slats over the floor without the use of a manure belt was used in roughly one fifth to one fourth of farms in the Northeast, Southeast, and West (21.7, 28.6, and 23.0%, respectively) with the Central region making more use of this method (44.0%). The use of flush systems to a lagoon are more widely used in the Southeast and the West (12.1 and 15.4%) compared to the other regions where such methods were used in less than 1.0% of farms. Manure belts are another management practice used, although not in a large portion of farms. The Central and West regions use this method the most with 18.3% and 15.3% of farms using this practice, respectively, compared to only 4.9% in the Northeast and 6.0% in the Southeast. Finally, a scraper system without a flush or pit are used but not commonly with farms in the West using this method the most (15%) compared to the other three regions where less than 5% used this method (USDA 2014).

Not only are high-rise buildings more popular across regions, they are also more commonly found in larger scale operations. The use of these set ups is associated with larger operations as only 14.5% of small farms used this method compared to medium and large farms (61.1 and 62.0%, respectively). Other practices associated with a larger proportion of large farms are manure belts, and flush systems. The use of manure belts is more common in large farms (21.9%) compared to medium (4.4%) and small farms (0.8%). Flush systems were found the highest proportion in large farms (5.7%) compared to medium and small farms (3.8 and 0.0%, respectively) (USDA 2014).

Conversely, the use of raised slats over floors was associated with smaller farms and their use decreases as farm size increases. A large proportion of small farms (62.9%) use this method compared to medium (21.6%) and large (0.8%) farms. Other practices associated with smaller farms are the use of shallow pits and scraper systems. These two systems were used more in small farms (14.0 and 7.3%, respectively) compared to medium farms (4.1 and 3.1%, respectively) and large farms (5.1 and 3.2%, respectively).

Poultry manure is a valuable resource for its nitrogen and phosphorus content making it a good alternative to commercially available fertilizers. However, due to crop timings, often the manure must be store before it is applied. Manure can be stored in different types of places, however, the location of these structures fall into three categories: in a building, in an open structure such as a lean to, or outside (USDA 2014).

The manure can be stored on site at the farm or elsewhere. In large farms, 59.9% of operations used some sort of on site storage. This trend decreases slightly as operation size decreases: 49.2% of small farms and 52.1% of medium sized farm used an on site

storage system. Specifically, larger farms stored manure in a building (51.4%) compared to an open-structure (3.1%) or outside (8.2%). This is similar to medium sized farms where 44.8% of operations stored their manure on site inside a building compared to 3.4% in an open structure and 5.8% outside. Smaller operations made more use of outside storage with 30.9% of farms storing manure outside or in an open structure (4.5%) and only 16.6% of operations storing manure in a building. A large percent (48.0%) of farms that stored manure on site had the storage facility attached to the layer house or the distance between the manure storage building and the layer house was less than 30.5 m (36.7%) (USDA 2014).

Swine Manure

While several states participate in dairy farming, for example, the majority of pork production occurs in the Midwest (Ohio to Nebraska and Minnesota to Missouri) and North Carolina. According to the Bureau of the Census from 1989, 96.6% of pigs marketed in 1987 were produced in the north-central region of the US. In 2012, the top three producers, North Carolina, Iowa and, Minnesota, accounted for 55% of the value of U.S. hog and pig sales and 56% of the 66 million hog and end-of-year inventory (NASS 2012). Contrary to commercial nature of dairy of poultry farming, 83% of operations, accounting for 41% of hog sales, were from family or individual owned farms. This sector was followed by corporations, which account for 8% of all farms but 34% of sales. The remaining operations are owned in partnership or categorized as “other” (NASS 2012).

Despite the high nutrient quality of swine manure, Hatfield et al. (1998) noted that most manure management practices for swine were not geared towards retaining the nutrients in the manure. The authors noted a reason for this being that land application for manure is limited and many facilities use aerobic lagoons to digest the manure solids allowing it be handled as liquid. These lagoons may volatilize up to 70 to 90% of the nitrogen, which is then converted into ammonia and lost into the atmosphere (Hatfield et al. 1998). This volatilization of nitrogen allows the land requirements to be decreased by 10% of the land required for application of slurry manure. The use of these lagoons helps with the issue over nitrifying surround landscapes (Hatfield et al. 1998).

Unlike manure from ruminants, swine manure is a relatively homogenous substance from farming unit to unit (Hatfield et al. 1998). Similar to the diets of poultry, swine in the U.S. are fed diets formulated with corn, or grain sorghum and soybean meal and vitamins and minerals necessary to prevent deficiency. Because diets across production units are relatively stable, the major differences in the composition of swine manure are dependent upon methods of collection, dilution and storage rather than diet (Hatfield et al. 1998). About 85% of the nitrogen in a typical corn and soybean diet is digested (McConnell et al. 1972) and the majority of nitrogen excreted from the pig is in the form of uric acid in the urine while organic nitrogen forms in the feces. Anywhere from 40-60% of phosphorus in a corn and soybean diet is digested as well (National Research Council 1988).

Systems for handling solid manure are the least common in that less than 15% of swine in the U.S. are raised on farms using systems designed for solid manure (Hatfield

et al. 1998). These systems are most commonly found in the western Corn Belt and in facilities were smaller production systems are likely to make use of extensive housing systems where small roofed buildings are used to handle solid manure. Small production facilities may also make use of pastures or open feedlots for distributing and handling solid manure.

Like with dairy manure, pasture production allows the manure to be spread "naturally" by the swine as they graze. Pasture production is most common in states were small swine farms are more common and in the mid and southern Corn Belt (Hatfield et al. 1998). Pasture production, however, accounts for a small proportion of swine production as it is estimated that no more than 5% of swine are raised on pasture (Hatfield et al. 1998).

Small- and moderate-sized operations also make use of open feedlot systems. These systems are not covered by a roof and their surfaces often have an accumulated manure layer, from which the solid manure is scraped periodically in intervals that vary from once or twice a week to monthly (Hatfield et al. 1998). Some manure is lost through runoff from rain or snowmelt and therefore unless some runoff containment system is in place, surface contamination is possible if the runoff from a feedlot enters a body of water before the manure solids settle or infiltrate into soils during transport in the runoff.

Studies have shown that anywhere from 5-20% of manure deposited in a feedlot can be transported via water runoff (Hatfield et al. 1998). To prevent this, solid storage systems are required to store manure between land disposal events and prevent the contamination risk associated with runoff. These storage units generally consist of an on-

grade concrete pad with low walls surrounding it to allow manure to be pushed into storage and removed with either a blade or a front-end loader (Hatfield et al. 1998). Nutrient values of manure from solid systems are quite variable as nitrogen losses during storage have been reported to range from 20-40% (National Research Council 1988).

Other systems dealing with solid manure may also use different types of bedding such as straw, wood chips or newspaper, which are used to absorb urine as well as provide insulation for animals in unheated buildings (Hatfield et al. 1998). As noted earlier, the presence of bedding can have impacts on the nutrient quality of manure. Finally, solid manure can be applied to the field using a box spreaders or side discharge flail-type spreaders (Hatfield et al. 1998).

According to Hatfield et al. (1998), most large-scale swine production facilities have totally roofed confinement systems in which bedding is purposefully not used so the manure can be handled as either slurry or a liquid. Manure that is converted into a slurry is not diluted much, however, liquid manure is diluted significantly in that water is added to assist with its transport, treatment and land application.

Slurry systems are most common in the north-central region where manure can be taken back to cropland land and where cooler temperatures make the use of lagoons less conducive. Approximately 50-60% of producers use slurry systems (Hatfield et al. 1998). Several different types of storage structures are commonly used in slurry systems but the moat common system is the blow-floor pit covered with a slatted floor (Hatfield et al. 1998). Until recently, a high proportion of operations used this deep-pit system, however, recent years have seen elevated concern with air quality and odor issues that arise from

the long-term storage of manure in these types of settings. Alternatives to this type of indoor system are in-ground storage tanks located remotely from the confinement building, aboveground tanks and earthen structures. In-ground tanks may be uncovered or covered and aboveground tanks are often constructed from concrete and glass-fused steel (Hatfield et al. 1998). While earthen structures are cheaper than other storage structures, certain levels of soil investigation and construction controls must be implemented to prevent and minimize potential groundwater pollution. Slurry can be applied to the land by use of spreaders or directly injected into the land. Direct injection allows for the immediate covering of manure to prevent nutrient loss by volatilization, reduces the potential for surface runoff and reduced odor potential (Hatfield et al. 1998).

Hydraulic flushing systems have been used over the past two decades as a quick and efficient means of removing manure from swine confinement buildings. These flushing systems require larger manure storage systems as significant amounts of water are added to the manure during flushing. Lagoons are used extensively for these and several types exist. Lagoon water is often recycled and treated which cuts down on storage requirements. This water is often used for irrigation throughout the year, however, in places where adequate freshwater is available year round, the use of recycled lagoon water is not common (Hatfield et al. 1998).

Anaerobic lagoons are popular in areas with a limited land since high loss of nitrogen is expected from these systems. These lagoons convert manure that is low in solids to a liquid, facilitating transport and application. Traditional irrigation equipment can be employed to apply the liquid manure to the land. While higher volumes of waster

are generated with these systems, the cost and labor requirements are relatively lower than for slurries and solids (Hatfield et al. 1998). However, with these systems come high concerns for odor, potential leakage, overflow and over application of lagoon effluent. It is estimated that 80-90% of input Nitrogen is lost to the atmosphere through ammonia volatilization in anaerobic lagoon systems (Hatfield et al. 1998).

Lagoons can also be maintained under aerobic conditions. While odors are minimized, the cost of mechanically aerating these lagoons is relatively high. Capital requirements, energy and maintenance are all cost prohibitive for the use of these systems typically.

The majority of anaerobic and aerobic lagoon use occurs in warmer climates. The majority of larger operations (over 1,000 animals) use anaerobic systems to minimize land applications areas. These areas are concentrated in the Southeast, the southern Corn Belt and the southwest Plains. It is estimated that 20-30% of manure from swine production is processed in liquid manure systems (Hatfield et al. 1998).

Dairy Manure

The majority of dairy operations employ conventional operations (63.9%), compared to organic, grazing or a combination of the two (USDA 2007). Organic operations are similar to grazing, however, the pasture used in grazing must be USDA certified organic. In conventional operations forage is harvested and “delivered” to cows compared to grazing operations were forage is “harvested” by cows. These conventional operations accounted for a majority of dairy cows in the country (82.2%). Of larger dairies

(over 500 animals), 91.5% are conventional operations. This is compared to the percentage of conventional operations for smaller dairies at 57.1% and 79.9% for small (less than 100 animals) and medium-sized (100-499 animals) dairies, respectively (USDA 2007).

Across operations of all sizes, lactating cows were housed primarily in tie stalls/stanchions (49.2%) or in freestalls (32.6%) with only a smaller portion housed in pastures (9.9%) (USDA 2007). Smaller operations make larger use of tie stalls/stanchions (63.0%) while medium and larger-sized operations mainly use freestalls (67.5 and 72.6%, respectively). There is also a regional influence to the use of different housing methods; across all operations in the west (Texas, New Mexico, California, Washington and Idaho), freestalls, drylot/multiple-animal outside area and pasture are the most popular (49.7, 29.8 and 15.0 %, respectively). These housing preferences are compared to the east (Virginia, Kentucky, Missouri, Iowa, Minnesota, Michigan, Wisconsin, Indiana, Ohio, Pennsylvania, New York and Vermont) where tiestalls/stanchions and freestalls are most widely used (53.1 and 31.2%, respectively) (USDA 2007).

Cow manure handling practices often varied depending on the number of cows present at the dairy. In smaller operations, a gutter cleaner handles 58.5% of manure, 17.2% with an alley scraper (either mechanical or via tractor), and 8.7% is scraped from the drylots. In medium-sized operations, alley scrapers are used in 64.1% of operations in addition to gutter cleaners (11.1%) and drylot scraping (8.7%). In larger operations, drylot scraping was used in 30.1%, alley scrapers in 33.5%, and alley flushing using recycled water in 27.4%. Other methods used across operations include leaving the manure on the

pasture, using a slotted floor or packing the manure to be used later as bedding (USDA 2007).

Manure management practices also varied between regions. Across operations, the most common practice for the west were drylot scraping, use of alley scrapers or flushing the alley with recycled water (38.2, 23.4 and 21.0%, respectively). In the east, the majority of operations used either gutter cleaners or alley scrapers (47.0 and 30.7%, respectively) (USDA 2007).

There are also certain practices that are strongly associated with a type of housing. For example, 82.5% of operations that housed their cows in tiestalls/stanchions used gutter cleaners and 72.1% of operations that housed their cows in freestalls used alley scrapers. In operations where animals were housed on a drylot or an outside area, the majority of lots were scraped or the manure is packed for bedding (50.3 and 32.6%, respectively). When cows are allowed in pasture, 27.3% of operations leave the manure on the pasture and 40.7% use gutter cleaners (USDA 2007).

Manure is also stored and treated in different ways depending on herd size and region. In operations with smaller herds, a large proportion employ a spreader (50.4%) or store slurry or liquid manure in an untreated basin (24.4%). Smaller operations frequently also pack manure for bedding (55.8%) and/or store outside either within the drylot (24.0%) or outside of the drylot (44.0%) (USDA 2007).

In medium-sized operations, 18.3% use a below floor slurry or deep pit, 44.0% store manure in a spreader, 21.6% store slurry in a tank, 45.7% store liquid manure or slurry in an untreated earthen basin, 63.4% pack manure for bedding. Additionally,

manure is often store outside either within the drylot (20.9%) or outside of it (32.4%) (USDA 2007).

In larger operations, 18.8% use a below floor slurry or deep pit, 43.1% store liquid manure or slurry in untreated earthen, 31.0% pack manure, and outside storage is frequently used either within the drylot (29.1%) or outside of it (65.2%). A notable proportion of larger operations will also compost manure (26.4%) or use a solid separator (36.2%). Treatment lagoons either with (18.7%) or without (49.7%) mechanical aeration are used most commonly in larger operations (USDA 2007).

Storage and treatment methods varied regionally across operations of all sizes, most noticeably, in practices such storage in manure spreader (49.9%, East; 7.5%, West), storing slurry or liquid manure in untreated earthen basins (29.7%, East; 44.1%, West), packing manure for bedding (60.4%, East; 12.4%, West), outside storage within a drylot (43.1%, East; 21.6%, West), and using solid separators (28.8%, East; 0.9%, West) (USDA 2007).

The storage and treatment of solid versus both solid and liquid manure depends on herd size and region as well. In small operations, 47.6% store and treat both solid and liquid manure. The proportion of operations that deal with both types of waste increases with herd size; 75.5% of medium sized operations will store and treat both types and 99.8% of larger operations surveyed did as well. Overall, 58.0% of all dairy operations surveyed dealt with both types compared to 42.0% of operations that only dealt with solid manure. Almost all operations in the west (96.0%) store and/or treated both solid and liquid manure compared with 54.3% of operations in the eastern region (USDA 2007).

Different sized facilities also have different manure storage capacities, that is, the time that can pass before manure needs to be removed from the storage facility. In operations will small herd sizes, 32.6% responded with fewer than 7-d, 10.8% indicated 90-179-d, 26.4% indicated 180-364-d and 16.7% indicated 365-d or more. The majority of responses for medium sized operations fell in the same time periods: 21.7% indicated fewer than 7 d, 16.7% indicated 90-179 d, 37.4% indicated 180-364 and 13.5% indicated 365-d or more. The majority of operations with larger herd sizes were unsurprising able to store manure for longer periods of time as larger facilities tend to have larger storage structures (USDA 2007): 15.7% responded with 90-179-d, 32.3% responded with 180-364-d and 39.6% responded with 365-d or more.

Almost all operations applied the manure- either liquid, solid or both- to the land (99.1%). A higher percentage of larger operations sold manure or received other compensation, gave manure away or used composted manure as bedding compared to smaller operations. Manure applied to the land can be done so in several ways such as using a broadcast/solid spreader, surface application, subsurface injection, and irrigation/sprinkler. The majority of operations, regardless of size, used broadcast/solid spreaders (91.5%) followed by surface application (40.7%). A higher percentage of operation in the west (60.0%) applied manure via irrigation/sprinkler compared to the east (2.5%). Other methods were conducted in similar proportions across the two regions (USDA 2007).

The survey found that the reason or dictator for manure application and how much fell into four major categories: to suit crop nitrogen requirements, to suit crop phosphorous

requirements, manure volume and/or acreage availability and improvement of soil quality. Regardless of size, no single reason was significantly more common than another. Manure is often applied to pasture or hay, forage to be ensiled, and grain or oilseed (USDA 2007).

Cost of Manure Management Practices

Several major factors impact the cost of managing manure; however, loading, transportation and application to the land are of particular importance (Wright et al. 1998). Each of these activities can require specialized equipment, which can come at considerable cost to the farmer. For example, a manure scraper can cost $22,000 (USDA 2000). The storage of manure can require ancillary systems suck as tanks, lagoons or buildings. The cost for a 148 m^2 timber shed for solid waste storage for a typical broiler house is estimated to cost $12,403 with a cost of $7.00 per ton per year (USDA 2000). Installation costs per 3.78 L for ponds/lagoons are 2.2 cents per 3.78 L for those with capacities less than 3,785,411.78 L, 1.8 cents per 3.78 L for capacities 3,785,411.78 – 11,356,235.35 L and 1.5 cents per 3.78 L for capacities larger than 11,356,235.35 L (USDA 2000). The estimated cost for a liquid collection flush system on a 100-head dairy farm is $19,451 in capital costs compared to $32,973 for a 200-head farm and $46, 495 for a 300-head farm making the annual operating cost per head $11.84 (USDA 2000). Rental rates for pumping systems can run $17.50 per hour translating to about $0.20 per ton (USDA 2000).

Different manure handling systems cost different amounts and can range depending on the area of the country. Depending on the region of the country, liquid storage can have an annual cost of $14,188-$15,777 per house for layer operations and

$28.45-34.85 per AU for swine operations and $32.36-$42.40 per AU for dairy operations (USDA 2000). Total installation costs range from $105,817-$380,814 for layers, $17,481-$485,227 for swine, and $23,793-$78,251 for dairy (USDA 2000).

Slurry storage can have annual costs of run $5.43-$11.35 per head of swine and $11.35 - $15.05 per head of dairy with total installation costs ranging from $6,322-$78,689 and $12,342-$30,294, respectively (USDA 2000). The cost to manage manure is animal dependent, with layers having an average annual cost of $16.00 per head compared to $18.00 per head of swine and $22.00 per head of dairy cattle (USDA 2000). By region, the average annual cost of manure management varies as well with the pacific region having the highest total cost of $7,731 compared to that of the Lake States, which have the lowest average annual cost of $1,669. Across all regions, the average total cost for manure management and wastewater handling and storage per farm is $2,509, which does not include capital costs such as equipment and installation (USDA 2000).

Other costs associated with manure management can include gas, wages for those operating systems and transportation. Regardless if the cost is considered high or low, manure management is an ancillary cost to the farmer that must be addressed and can present considerable limitations for a farmer's budget.

Volatiles Associated with Manure and Their Origins

The volatile emissions from livestock manure have been studied to determine their sources as well as how to practically manage them. Manure is a complex mixture of residues from undigested diet, endogenous secretions, and bacterial cells. Microbes

present in this mixture lead to the fermentation of said products and the anaerobic decomposition of these materials results in the production of volatile organic compound (VOC) emissions. When decomposing manure has a surface that is exposed to the environment, volatiles and their intermediates are released into the atmosphere (Sun et al. 2008). VOCs can be broken down into two categories: those associated with odor and human health and those impacting the environment, such as greenhouse gases and ammonia. Both types are regarded as important as higher levels of greenhouse gases have been attributed to a global warming effect, and odors produced from livestock production have been related to health issues such as accelerated decline in lung function, bronchitis, sinusitis, inflamed nasal mucosa, throat irritation and headaches (Schenker et al. 1996, Donham 2000, Mitloehner and Calvo 2008).

The odors generated from these facilities come from several different sources including feed, animal bodies, but in particular, urine, feces and the mixture of the two (Le et al. 2005). Fresh manure, its decomposition during collection, storage, handling, and spreading are all significant sources of odor. This odor production is influenced by many factors but a large part of manure-associated odors is attributed to the diet of the animal, which dictates the microbial conversions of non-utilized nutrients and endogenous products secreted in the gastrointestinal tract under anaerobic conditions; i.e. the fermentation and hydrolysis of undigested nutrients produce odors directly or provide the precursors for odor formation in the manure (Le et al. 2005). These odors can be broken down into four main groups: VFAs, sulfurous compounds, phenols and indoles, and ammonia and volatile amines. Many different odors are associated with animal production

facilities. O'Neill and Phillips (1992) summarized 168 odorous compounds studies in various animal facilities and production operations. Schiffman et al. (2001) identified a total of 331 different compounds from pig production facilities in North Carolina.

The most commonly found VFAs are acetic, propanoic, butanoic, 2-methylpropanoic (isobutanoic), 3-methylbutanoic (isovaleric), pentanoic (n-valeric) and capric acids (McGill and Jackson 1977, Cooper and Cornforth 1978, Spoelstra 1980). VFAs with higher carbon numbers have relatively lower detection thresholds than shorter chain acids (Mackie 1998). The majority of VFAs associated with manure come from the microbial breakdown of dietary plant fibers and protein residues in the large intestine (Le et al. 2005). These dietary plant fiber residues may include cellulose, hemicellulose and lignin. Cellulose and hemicellulose are first hydrolyzed by enzymes into oligomers and/or monomers. Microbes then convert monomers into VFAs such as acetic, propanoic and butanoic acids. The proportion of acids produced is dependent upon the type of substrate, composition of intestinal flora and pH (Le et al. 2005). Conversely, lignin is very difficult to degrade under the anaerobic conditions of the large intestines. The pathways of carbohydrate metabolism in the rumen of cattle has been described by Russell et al. (1983); however, the same pathways are assumed to occur in the large intestines of single stomached animals as well although end-product amounts and ratios may differ. Mortensen et al. (1987) and Rasmussen et al. (1988) state that carbohydrates are easily converted into acetic, propanoic and butanoic acids in fecal incubation systems, however, the production of branch-chained VFAs like propanoic, 2-methyl and butanoic, 3-methyl acids did not occur in these systems. 2-Methylpropanoic acid results from the breakdown

of peptides, which are hydrolyzed into amino acids (AA) by peptolytic bacteria. These AA are then deaminated and decarboylated into these branch-chained VFAs (Le et al. 2005). In addition to carbohydrates, acetic, propanoic and butanoic acids are also produced by the deamination of AA such as L-glutamate, L-lysine, and L-alanine (Le et al. 2005). End-products of this deamination-decarboxylation include ammonia, CO_2 and [H]. In short, carbohydrates are transformed to straight-chain VFAs only while proteins are transformed to both straight- and branched-chain VFAs. According to Müller and Kirchgessner (1985) anywhere from 66-99% of short-chained VFAs produced in the large intestine are capable of being absorbed and used as an energy source in the host animal. This along with their high odor detection make the short chained VFA's less of concern for nuisance odor, however, during manure storage, are capable of volatilizing and causing malodor.

Sulfurous compounds are generally regarded to be the most offensive compounds and often have lower detection thresholds (O'Neill and Phillips 1992). Hydrogen sulfide is an important compound because it is capable of causing animal and human deaths at low thresholds (Donham et al. 1982). The production and emission of this gas appears to be heavily influenced by the type of housing and manure management systems in animal facilities. For example, regular flushing of manure versus long-term pit storage may cause significant differences in its production (Le et al. 2005). In pig manure, the most commonly reported sulfurous compounds responsible for odor are hydrogen sulfide and methanethiol (methylmercaptan) (Spoelstra 1980) and these two compounds have been reported to account for as much as 70-97% of total sulfur volatilized in manure (Banwart and Bremmer 1975). These authors also reported that in pig and poultry manure,

methanethiol production exceeded that of hydrogen sulfide (Banwart and Bremmer, 1975). In addition to these compounds, carbon disulfide, 2-propanethiol, dimethyl disulfide, diemthyl trisulfide, 2-methylthiopropane, methanethiocyclopentane, 1-methylthiopentane, dimethyl tetrasulfide and dimethyl hexasulfide have been found to cause odor in livestock manure (Odam et al. 1986). Compared to VFAs, the detection thresholds of sulfurous compounds are lower and the concentrations found in the air of these facilities are higher (Le et al. 2005). For example, the low to high detection thresholds of dimethyl disulfide is 0.0001-0.3465 mg/m^3 compared to 0.0840-60.000 mg/m^3 for propanoic acid (Ruth 1986). Sulfur-containing compounds are produced by anaerobic bacteria through sulfate reduction and metabolism of sulfur containing AA such as methionine and cysteine (Mackie et al. 1998). Various bacteria are capable of performing this task where these AA are carbon and energy sources utilized by the microbes (Le et al. 2005). During this process, intermediate products may also volatilize and create odor. For example, the hydrolysis of methionine creates methanethiol as an intermediate product, which is further degrades to sulfide (American Society of Agricultural Engineers 1989). Methanethiol can be chemically converted to dimethyl disulfide or dimethyl trisulfide in the presence of copper oxide (Parliament et al. 1982) or ascorbate and ferric oxide (Chin and Lindsay 1994). Another source of sulfide production is sulfate. For examine, in urine, the primary form of sulfur excreted is sulfate. Spoelstra (1980) stated the primary origin of sulfide in manure is the reduction of sulfate into sulfide, which proceeds through an assimilation or dissimilation pathway. In the assimilation pathway, bacteria produce sulfur in a reduced form for the synthesis of cysteine and

methionine. In dissimilation pathways, sulfate is used to accept an electron for anaerobic respiration much like the role of oxygen in aerobic respiration (Clanton and Schmidt, 2001). The bacteria that are sulfate reducers belong to the genera *Desulfovibrio, Desulfotomaculum, Desulfobacter, Desulfococcus* and *Desulfonema* (Spoelstra 1980). As byproducts, these sulfate-reducing bacteria may also produce trace amounts of CO_2, CS_2 and methyl, ethyl and propyl mercaptans (Spoelstra 1980). In short, sulfurous compounds are produced under anaerobic conditions either from sulfate in the urine or from proteins or AA containing sulfur in manure with various bacteria aiding in these processes.

In terms of phenols and indoles, phenol, 4-methylphenol (p-cresol), 3-methylphenol (m-cresol), and 4-ethylphenol are important representatives of phenolic compounds while indole and 3-methylindole (skatole) are important indolic compounds (Le et al. 2005). Spoelstra (1980) indicated that the concentration of phenol increased over a measuring period of 150 days while indole, 4-methylphenol and 3-methylindole concentrations increased initially but then decreased after day 40, 65, and 70, respectively. 4-Methylphenol has been found to have a higher in air concentration than other phenols and indoles and furthermore has a lower odor detection threshold than these compounds making it an important compound in terms of odor nuisance compared to other compounds in this group (Le et al. 2005). Following 4-methylphenol, indole and 3-methylindole are the next most important odorous compounds in this group. While phenol has been found to have a high concentration in headspace air in previous studies, its high detection threshold (0.1788-22.43 mg/m^3 (Ruth 1986)) and aromatic smell make it less of a nuisance odor compared to the other indolic and phenolic compounds. Like VFAs, the origin of

these compounds is the metabolism of AA. Specifically, indole and 3-methylindole are end-products of tryptophan metabolism (MacFarlane 1995) while phenol and 4-methylphenol are products of tyrosine fermentation (Mackie et al. 1998). Phenol, 4-methylphenol and 4-ethylphenol originate from the microbial degradation of L-tyrosine occurring either in manure storage or in the intestinal tract of animals. L-Tyrosine can also be split directly to release ammonia, phenol and pyruvic acid by *Clostridium tetanomorphum* (Brot et al. 1965) and *Escherichia coli* (Ichihara et al. 1956). Pig manure incubated with L-Tyrosine and L-tryptophan forms 4-methylphenol (Hammond et al. 1968). Yokoyama et al. (1982) were able to isolate an anaerobic Gram-positive bacterium from the caecal contents of weaning pigs, which produced 4-methylphenol via the decarboxylation of 4-hydroxyphenylacetic acid. Other phenolic compounds are produced as metabolites of other compounds. For example, 3-methylphenol was found to be a metabolite of dihydroxyphenylalanine (DOPA), which is the precursor of such neurotransmitters like dopamine, noradrenaline and adrenaline (Drasar and Hill 1974). DOPA is produced via the oxidation of L-tyrosine by the oxygen dependent enzyme monophenol mono-oxygenase (Drasar and Hill 1974). Phenolics are absorbed by the large intestine of the host animal and detoxified by the liver resulting in glucuronides, or sulfuric acid that results in sulfates (Le et al. 2005). In manure, urinary glucuronides are hydrolyzed to release phenolic compounds. Indole and 3-methylindole are produced in the large intestines as a result of L-tryptophan fermentation. Indoles may be partly absorbed and detoxified by the liver to glucuronides and indolic detoxification products, such are excreted via urine. Unabsorbed indole and 3-methylindoles are excreted via the feces and

therefore can be found in fresh feces (Le et al. 2005). Because feces contain high levels of beta-glucuronidase of bacterial origin, which hydrolyzes glucuronides, the mixes of feces of urine causes the level of free indolic compounds to rise. The ability for indole to form by tryptophan is a taxonomic feature, which distinguishes enterobacteria. For example, *E. coli* and *Proteus* (except *Proteus mirabilis),* some *Shigella* spp., *Aeromonas liquefaciens,* some *Fusobacterium*, *Bacteroides melaniogenicus*, *Bacillus alvei*, some *Clostridium, Propionibacterium acnes, Photobacterium harveyi,* and *Micrococcus aerogenes* are all capable of forming indole from tryptophan (Le et al. 2005). Jensen et al. (1995) demonstrated in *in vitro* experiments that the production of indole and 3-methylindole were pH dependent with the highest rate of production occurring between a pH of 6.0 and 7.0 with less than one half of the maximum production occurring at a pH of either 5.0 or 8.0. It was found that high pH favored the production of indole while low pH values favored the production of 3-methylindole (Jensen et al. 1995). In short, phenol and 4-methylphenol come from L-tyrosine while indole and 3-methylindole come from L-tryptophan. The three sources of indole and phenol in manure are (1) the degradation of L-tyrosine and L-tryptophan in manure, (2) direct excretion from the large intestines of animals via the feces after being formed from these two AA and (3) finally the release from glucuronides in urine when put in contact with feces

Ammonia and volatile amines have pungent smells. Ammonia, commonly present in the form of NH_4^+, can be produced by the deamination of AA as well as from urea and nitrate. Ammonia is an important source of nitrogen from many anaerobic bacterial species and when present is preferred over AA and peptides by many bacteria (Mackie et

al. 1998). Volatile amines make up a small proportion of volatile nitrogenous compounds found in these facilities but include methylamine, ethylamine, trimethylamine, cadaverine, and putrescine (Le et al. 2005).

The source of ammonia is mainly urea (Spoelstra 1980), which is formed in the liver as a product of the animal's metabolism, which breaks down proteins and is then excreted by the kidneys. After excretion by the kidneys, it is hydrolyzed by urease found in feces and then converted into ammonium ions some of which will disassociate and form free ammonia. This activity by urease is commonplace with intestinal bacteria and has been observed in many species such as *Bacteroides multiacidus, Bacteriodes ruminicola, Bifidobacterium bifidum* and others (Suzuki et al. 1979). Ammonia can also be released from the deamination of proteins and AA when used as a source of energy. A gateway used by bacteria converts the amino acid L-glutamate into ammonia and respective fatty acid and other residual structures through oxidative deamination processes (Zhu 2000). The emission of ammonia into the air is a slow process which is controlled by several environmental factors such as the concentration of it in the air, temperature and pH with this last factor being especially important as the volatilization of ammonia increases with rising pH (Aarnink 1997). At pH below 7, ammonia is almost only present as NH_4^+, which thereby reduces its volatilization as ammonia gas (Aarnink 1997).

Volatile amines are frequently produced from products containing proteins under anaerobic conditions (Le et al. 2005). The microbial formation of volatile amines is capable through three different mechanisms. First, AA are capable of undergoing decarboxylation within the gastrointestinal tract and during the storage of fresh manure

(Bast et al. 1971). Certain genera of bacteria with decarboxylating activity include the enterobacteria *Bacteroides, Bifidobacterium, Selenomonas,* and *Streptococcus* (Spoelstra 1979). Secondly, Bast et al. (1971) indicated the formation of different amines through the amination of corresponding aldehydes. Examples he cited included the formation of hexylamine and ethylamine by *Sarcina lutea,* hexylamine by *E. coli* and isobutylamine by *Aerobacter aerogenes* (Bast et al. 1971). Finally, the urine in manure may provide a source of amines. For example, in an average man, the daily excretion of dimethylamine is estimated to be 20mg half of which originates from choline and the activity of gut flora (Drasar and Hill 1974). Choline is subsequently degraded to either ethylamine plus ethanolamine or to triethylamine, which is subsequently de-methylated (Drasar and Hill 1974). In short, ammonia is produced when AA are deaminated through their use as energy by bacteria and additionally through the hydrolysis of urea, the main source of ammonia in animal facilities, in urine by urease. Volatile amines are produces through the decarboxylation of amino acid, the amination of aldehydes, and the demethylation of choline (Le et al. 2005).

In summary, many microbial activities are responsible for odorous compounds generated either in the large intestines of livestock animals and poultry or during the storage of manure. These compounds can be intermediate or end-products from microbial conversions under anaerobic conditions. Diet heavily influences the generation of these compounds with proteins and fermentable carbohydrates being the most important macronutrients at play (Le et al. 2005).

Poultry Manure

Deibel (1967) found that the chief volatiles of decomposing poultry manure were butanoic acid, ethanol, and acetoin. Deibel noted that butanoic acid was the most malodorous and attributed to the offensive odor associated with accumulated poultry manure. Burnett and Dondero (1969) trapped gases from poultry manure in a column submerged in an acetone-dry ice bath and identified eight compounds: acetic, propanoic, iso-butanoic, n-butanoic, iso-valeric, and n-valeric acid, and indole and 3-methylindole. Another study identified 72 compounds associated with poultry manure, using gas chromatography-mass spectrometry (Yasuhara 1987). This study found that the volatile profile of manure changed through time, and the key odorous compounds were butanoic acid, 3-methylbutanoic acid, dimethyl trisulphide, indole and 3-methylindole with phenol being the most abundant compound in every sample. It was noted that certain compounds' concentrations increase with the decomposition and rotting of the manure. These compounds included carboxylic acids, sulfur containing compounds, phenols and indoles. The concentrations of compounds such as alcohols and aldehydes were found to increase in the early stages of decomposition but then decrease in the long term. Burnett and Dondero (1969) implicated sulfur compounds, organic acids, and 3-methylindole as being important malodorous compounds involved in air pollution.

Like with swine manure, odor emissions have been tied to diet quality and an excess of protein (Jacob et al. 1994). Similar to that of swine a general guide indicates that for each 1% unit reduction in dietary crude protein (CP), along with AA supplementation, the estimated ammonia loses in poultry manure is around (Jacob et al. 1994).

Swine Manure

Shiffman et al. (2001) reported a diverse group of compounds found in swine barn air and lagoon wastewater. These 324 VOCs and seven fixed gases identified were classified into acids, alcohols, aldehydes, amides, amines, aromatics, esters, ethers, fixed gases, halogenated hydrocarbons, hydrocarbons, ketones, nitriles, other nitrogen-containing compounds, phenols, sulfur-containing compounds and steroids. An additional 16 compounds were placed in an "unclassified" group. Relatively abundance VOCs that have been reported in at least two or more independent studies include acetic acid, butanoic acid, dimethyl sulfide, dimethyl disulfide, iso-valeric, 4-methylphenol, propanoic acid, 3-methylindole, trimethyl amine and valeric acid (Ni et al. 2012). Cooper and Cornforth (1978) tested 20 pig slurry samples and found that pig slurry contained more volatile fatty acids than cow slurry. The number of VOCs found in manure itself is less than in swine facilities. The largest number of VOCs analyzed in manure samples came from a study conducted by Yasuhara and Fuwa (1983) were volatiles were analyzed from manure, urine and a mixture of the two in which 36 VOCs were identified. The number of VFAs in swine manure varies by study but the first study to report on their abundance was by Roustan et al. (1997). This study reported the abundance of acetic, propanoic, butanoic, iso-butanoic, iso-valeric and valeric acids in decreasing order. Yasuhara and Fuwa (1977) conducted an earlier study were reported the major components of odor in swine manure were butanoic, iso-valeric, benzoic, phenyl acetic acids and 4-methylphenol.

In swine, excessive ammonia and odorous compound emissions have been tied to excess protein or AA in the diet (Le et al. 2005). The excessive protein in the diet will either be excreted in the urine as urea, glucuronides and sulfate, non-digested proteins in the feces, bacterial proteins in the feces. Diets with reduced CP contents and supplemented with essential AA have been shown to have reduced amounts of fecal nitrogen excretion by 25-30% (Cromwell and Coffey 1994). Not only does this lowered CP reduce nitrogen excreted in manure, Sutton et al. (1990) demonstrated that the pH of manure was also lowered thus reducing ammonia volatilization. Studies have indicated that, in general, for each 1% unit reduction in dietary CP, along with AA supplementation, estimated ammonia loses are reduced by 10% in swine (Sutton et al. 1990, Kay and Lee 1997).

Dairy Manure

Hales et al. (2012) examined volatiles from cattle manure and found that phenol; 4-methyl was responsible for 67.3% of odor activity. Bethea and Narayan (1972) examined beef cattle manure and found four classes of compounds: alcohols, amines, aldehydes and esters. Indole, 3-methylindole, ammonia and hydrogen sulfide were also identified (Bethe and Narayan 1972). A study that compared the VOC emissions of dairy cows and that of fresh manure found that fresh manure did not produce notable fluxes in CH_4 but rather it is the cattle who're responsible for the production of this gas (Sun et al. 2008). After land application of cattle manure, Woodbury et al. (2014) found isovaleric acid, butanoic acid and 4-methylphenol to account for 28.9, 18.0 and 17.7% of odor activity, respectively.

The amount of wet distillers grains with solubles (WDGS) fed to feedlot cattle influences the uptake and excretion of phosphorus, nitrogen and sulfur, by these animals. Feeding WDGS to cattle has been linked to the production of odorous VOCs such as VFAs and phenol (Spiehs and Varel 2009). However, increases in specific VFAs may not lead to overall increases in total VFA emissions. Similar studies found that total VFA concentrations in manure can decrease as WDGS increase in the diet and that the concentration of other aromatic compounds (4-methylphenol, indole, 3-methylindole) in cattle feces does not change (Spiehs and Varel 2009, Parker et al. 2013). Another study found that VOC flux or odor activity values did not differ between measured VOC emissions from feces and urine from cattle fed corn diets containing 0, 15, 30, or 45% WDGS (Hales et al. 2012).

Microbial VOCs as Insect Semiochemicals

Volatile emissions have been shown to serve as cues to communicate different information between insects (Price et al. 2011). Often, volatile organic compounds (VOCs) are the mechanism behind the initiation of colonization of an ephemeral resource (Benbow et al. 2015). These emissions can be derived from a variety of sources such as plants and microbes, and from decaying organic material like carrion and manure.

Livestock manure is biologically-active resource that is home to many different microbes, which utilize the energy contained in manure (Zhu 2000). Microbial activity is part of the natural process of manure decomposition and VOCs are often the intermediate and end-products of this process.

Many insect-plant interactions have been studied by examining the volatiles that perhaps stimulate many symbiotic relationships between two organisms. However, many volatiles that are microbial in origin such as those from fungi, yeast and bacteria play significant roles within an ecosystem and especially within ephemeral resource ecology.

Microbes have long been recognized for their responsibility regarding arthropod colonization of resources. Many insects are sensitive to odors that relay information about resources, potential mates and habitat suitability (Price et al. 2011). Microbial VOCS (MVOCs) can play a role in oviposition and its site selection for many insects including Diptera. Many dipteran species have expressed semiochemical-related oviposition behavior as it relates to odors from ephemeral sources like decaying organic material in the form of carrion and animal wastes.

MVOCs can serve as either repellents or attractants for certain dipterans. For example, Lindh et al. (2008) found that bacteria-containing water stimulated oviposition in the mosquito *Anopheles gambiae* (Giles) (Diptera: Culicidae), while Huang et al. (2006) demonstrated that volatiles from bacteria cultured in agarose media were deterrents to gravid mosquitoes of the same species. Trexler et al. (2003) isolated bacteria from the rearing water of *Aedes albopictus* (Skuse) (Diptera: Culicidae) and evaluated the response of gravid females in behavioral assays. The authors found that water-containing bacteria elicited a significantly ($P < 0.01$) greater oviposition response than sterile control water. Volatiles collected from larval rearing water elicited significant ($P < 0.05$) electroantennogram responses in females (Trexler et al. 2003).

Other studies have demonstrated that blow fly (Diptera: Calliphoridae) oviposition is stimulated by the ammonia production that occurred during bacterial putrefaction (Holdaway 1930, Seddon 1931). Gravid house flies are able to evaluate volatile profiles from microbes on conspecific eggs, which in turn help females ensure conducive conditions for larval development (Lam 2007)

Bacteria that produce sulphur-containing compounds are highly attractive to gravid *Lucilia* spp. (Diptera: Calliphoridae) whereas oviposition was stimulated by ammoniacal volatiles resulting from bacterial putrefaction on sheep (Ashworth 1994).

Chaudhury et al. (2002) inoculated bovine blood with eight species of coliform bacteria and found that gravid *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) responded positively to odors generated after incubation in comparison to uninocluated blood. Their results concluded that inoculated blood, when incubated for 48-72 h, gave off volatiles chemicals, which were attractive to gravid females and furthermore contained oviposition stimulants (Chaudhury et al. 2002).

Robacker and Moreno (1995) tested the hypothesis that Mexican fruit flies, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), were attracted to odor produced by tryptic soy broth cultures of *Staphylococcus aurous* (Rosenbach). Their results indicated that bacterial odor was driving the attraction as the flies sought our proteinaceous resources (Robacker and Moreno 1995).

For black soldier flies *Hermetia illucens* (L.) (Diptera: Stratiomyidae), Zheng et al. (2013) found that bacteria from conspecific eggs attracted gravid females to lay eggs.

This attraction was hypothesized to be due to associated volatile emissions (Zheng et al. 2013).

Manure Associated Volatiles as Dipteran Attractants

Intermediate and end-products of the microbial degradation of manure include VOCs, many of which are responsible for the malodor associated with manure (Zhu 2000). Attractiveness to volatiles associated with animal waste and manure has been studied in several species particularly for phenols, indoles and VFAs.

A study conducted by Jeanbourquin and Guerin (2007) found that the stable fly, *Stomoxys calictrans* (L.) (Diptera: Muscidae) uses olfactory cues to locate suitable substrates for oviposition at distances between 50 and 130 cm and furthermore found dimethyl trisulphide, butanoic acid and 4-methylphenol to attract *S. calcitrans*. Another stable fly study identified compounds from cattle manure slurry and with an olfactometer found that phenol, 4-methylphenol, and 3-methylphenol were attractive to adults. Furthermore, adults were most attracted to blends of these compounds rather than a single phenolic (Tangtrakulwanich et al. 2015).

Several studies have focused on using animal waste and/or known associated compounds to study the effects these compounds have on different Tabanidae (Diptera) species. In Turkey, Krčmar et al. (2009) found that canopy traps baited with a combination of octanol, acetone and ammonia trapped 15 times more tabanid flies than the control. Traps baited with donkey urine, lactic acid, and fresh human urine collected 12, 4 and 2.5 times as many tabanids, respectively, than the unbaited control traps (Krčmar et al. 2009).

Altunsoy and Afacan (2014) used malaise traps that were baited with either 1-octen-3-ol or 4-methylphenol. Of the 5153 adult tabanid specimens collected, 53.37% of them were with the octanol trap, 34.59% with the 4-methylphenol trap and only 12.04% with the unbaited control trap. In Canada, Mihok and Lange (2012) observed in five *Hybomitra* (Diptera: Tabanidae) species, that baits containing ammonia and/or octenal in addition to phenol saw a 1.7-4.1 fold increase in adults caught relative to the untreated trap. Neither ammonia alone nor in combination with octenol was attractive for these flies indicating a synergism between ammonia and phenol, the combination of which is found in aged urine. Baldacchino et al. (2014) assessed the electrophysical and behavioral responses of females of two tabanid species, *Tabanus bromius* (L.) and *Atylotus quadrifarius* (Loew) to ammonia, octenol, phenols and aged horse urine. Electroantennogram responses in both species to octenol, 4-methylphenol, phenol, 3-propyl and a phenol mixture of the two aforementioned compounds increased in a dose dependent fashion. The most effective stimulus was 4-methylphenol. The horse urine elicited strong responses in both species as well. Twenty-nine compounds were identified in the horse urine using GC-MS, with 4-methylphenol being the most abundant compound (~80%) (Baldacchino et al. 2014).

Mulla et al. (1977) examined synthetic fly attractants of *Musca domestica* (L.) (Diptera: Muscidae) on poultry ranches and found the chemical constituents showing maximum attractancy were trimethylamine hydrochloride, ammonium salt, linoleic acid and indole, with the first and the last compounds being the main attractants. Butanoic acid was found to increase attractiveness, however, not significantly (Mulla et al. 1977). Cossé and Baker (1996) used wind tunnels and electroantennographic (EAG) assays to examine

house fly behavior to swine manure volatiles. Compounds such as dimethyl disulfide, butanoic acid, 3-methylbutanoic acid, phenol, indole and 3-methylindole were found to be EAG active. Additionally, antennal response in females increased with dose in the case of butanoic acid, phenol and indole. Butanoic acid, 3-methylbutanoic acid, indole and 3-methylindole elicited the greatest response in females compared to other compounds examined (Cossé and Baker 1996). A study with *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) found that their attraction to putrefying substances was largely due to the presence of indole, skatole and ammonium carbonate and when dilution of this substances were placed on sheep fleece, female *L. sericata* were attracted to oviposit (Hobson 1936).

Bursell et al. (1988) found that the attractiveness of cattle urine to *Glossina morsitans* (Westwood) and *G. pallidipes* (Austen) (Diptera: Glossinidae) was attributed to the phenolic compounds in urine. Eight phenolics were identified and four of those (4-methylphenol, 3-methylphenol, 3-ethylphenol, and 3-propylphenol) were electroantennographically active (Bursell et al. 1988). Level of attraction of *Hippelates* gnats (Diptera: Chloropidae) to aqueous solutions of chicken whole-egg power was significantly enhanced when solutions of propanoic acid were added and attraction was also increased when indole or skatole was added to the mixture (Hwang 1976).

Fatty acids, which contribute to the noxious smell of livestock manure (Burnett and Dondero 1969, Zhu 2000), are also attractive to many dipterans. Perry and Fay (1967) reported that gravid *Aedes aegypti* (L.) (Diptera: Culicidae) exhibited an olfactory response to fatty acids in a laboratory setting with propanoic and butanoic acids eliciting the greatest response in females (Perry and Fay 1967). Another study found that baits

with attractant mixtures with butanoic and pentanoic acid trapped 5-20 times more *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) than the liver standard (Urech et al. 2004). Trap increases were also seen for *Chrysomya* spp. (0.85-2.7) and *Calliphora* spp. (Diptera: Calliphoridae) (0.020-0.2) (Urech et al. 2004).

Background on the Black Soldier Fly

Black solider fly (BSF), *Hermetia illucens* (L.), (Diptera: Stratiomyidae) larvae (BSFL) are an attractive alternative means of manure management because of their abilities to reduce organic matter (Nguyen 2013) including human feces (Banks 2014), livestock manure (Sheppard et al. 1994, Newton et al. 2005, Myers et al. 2008), pathogens (Erickson et al. 2004, Liu et al. 2008), pollutants (Myers et al. 2008) and house fly populations (Furman et al. 1959, Bradley and Sheppard 1984, Sheppard et al. 1994, Tingle et al. 1975, Kilpatrick and Schoof 1959). Larval stock can be sold as feedstuff for livestock and aquacultures (Bondari and Sheppard 1981, Bondari and Sheppard 1987, St-Hilaire et al. 2007, Sealey et al. 2011, Kroeckel et al. 2012), and the processed manure as fertilizer (Čičková et al. 2015). The utilization of wild black soldier fly populations is also cheaper than traditional manure management practices and requires no additional equipment (Newton et al. 2005, Čičková et al. 2015). Furthermore, it has been estimated that the use of BSFL larvae as a manure management tool could increase net revenue by $25,000 per poultry layer house per year (Newton et al. 2005). The black soldier fly is distributed throughout the sub-tropic and tropic regions of the world (James 1935, Callan 1974). This species is relatively large with larvae achieving 18-20 mm in length (Nartshuk 1988). They

have a life cycle of 40 days at 27°C with larval stages lasting ~22-24 days (Tomberlin et al. 2002). The adults will colonize a variety of decaying organic and animal matter (James 1935), ovipositing in cracks and crevices associated with the resource (Copello 1926, Gonzales et al. 1963). Adults do not need to feed as they rely on nutrients accumulated during their larval development (Tomberlin and Sheppard 2002). Because adults do not feed, they do not compete with humans for food resources and therefore have not been shown to vector human pathogens. These reasons and they non-synanthropic nature have earned them the label of a non-pest species.

BSF will colonize a variety of livestock manures (Zhou et al. 2013) including poultry, swine and cattle (Sheppard et al. 1994, Newton et al. 2005, Myers et al. 2008). The amount of manure that BSFL are able to consume is dependent upon resource availability; for example, those fed 27 g of dairy manure daily, reduced dry matter by 58%, while those fed 70 g daily reduced dry matter by 33% (Myers et al. 2008). Banks et al. (2014) also observed that the ability to digest manure could be larval density dependent with 10 larvae being fed a feed ratio of 100 mg of human feces per larvae per day reducing the wet weight of waste by 49.7 ± 1.03% compared to that of 100 larvae fed the same ratio reducing the wet weight by 54.2 ± 0.86% (Banks et al. 2014). Across larval densities and feeding regimes, however, Banks et al. (2014) observed waste reductions from 25-55%. Another study which examined the ability of BSFL to digest human feces saw a 73% reduction on total solids and a 75% reduction in volatile solids compared to only a 30 and 34% decrease, respectively, in control feces without larvae (Lalander et al. 2013). A third

study indicated a 54.7% reduction in the dry weight of fecal sludge by BSFL (Diener et al. 2011).

Sheppard et al. (1994) estimated a dry matter reduction in poultry manure 50% or more, while previous studies reported 56 and 42% reductions in manure accumulation (Sheppard 1983). Oonincx et al. (2015) observed a ~37% reduction in dry matter across three manure types (poultry, swine and dairy cow). Zhou et al. (2013) recorded 31-61% reduction in poultry manure, 28-53% reductions in swine manure and 34-57% reductions in dairy manure. Finally, Li et al. (2011) observed a 53% reduction in dry matter of dairy manure.

Additionally, it has been shown that waste reduction plasticity occurs across strains. Zhou et al. (2013) examined the ability of three different strains of BSFL to reduce livestock manure. Two strains from provinces in China, Wuhan and Guangzhou, were examined in addition to one from Texas. It was found that larvae from the Wuhan strain reduced manure dry matter greater than the Guangzhou strain by 48.4, 46.0 and 40.1% for poultry, swine, and dairy manure, respectively, and 7.9, 6.9 and 7.2% greater than the Texas strain (Zhou et al. 2013)

During the decomposition of dairy manure, black soldier flies also reduce concentrations of nitrogen and phosphorus. In a study where BSFL were allowed to feed on poultry, swine and dairy manure, nitrogen content was reduced by 80, 37 and 30% respectively (Oonincx et al. 2015). Another study that examined these three manure types saw nitrogen reduced by 24-51% in poultry manure, 22-49% in swine manure and 25-53% in dairy manure (Zhou et al. 2013).

Another study indicated a 62% reduction in nitrogen content of poultry manure fed on by BSFL (Sheppard 1983). Li et al. (2011) observed a 43.6% reduction in nitrogen from dairy manure fed upon by BSFL while Myers et al. (2008) observed reductions of nitrogen and phosphorus in dairy manure by 30-50% and 61-70%, respectively. Newton et al. (2005) observed a 55.1% decrease in nitrogen and a 44.1% decrease in phosphorus in swine manure processed by BSFL.

Manure decomposed by BSFL is also suitable for use as fertilizer (Čičková et al. 2015). During the degradation of manure, its temperature rises, pH shifts to alkaline, moisture decreases and ammonia release increases. Human feces which began in the experiment with a pH of 6.0 ± 0.0, finished with a pH of 7.5 ± 0.0 after being processed with BSFL.

Dairy manure digested by BSFL had a dry matter percent of 54.4 ± 0.3 compared to that of 46.5 ± 0.2 in fresh manure and the authors noted that no odor was smelled from the digest manure (Li et al. 2011). Odor emissions and humidity of manure that was treated with larvae were also decreased (Čičková et al. 2015).

BSFL reduce pathogens such as *Escherichia coli* in poultry and dairy manure (Erickson et al. 2004, Liu et al. 2008), When poultry manure colonized by larvae was maintained at temperatures of 27 or 32°C, populations of *E. coli* O157:H7 were reduced by 1.5-log and 5-log, respectively (Erickson et al. 2004) . However, the ability of BSFL to reduced *E. coli* O157:H7 was dependent on the manure type. For example, *E. coli* O157:H7 populations were amplified in swine manure that had larvae in comparison to manure without larvae (Erickson et al. 2004). A 2.5 log reduction in populations of

Salmonella enterica in poultry manure treated with BSFL was also found. However, the larvae became infected with the pathogen after feeding on contaminated manure.

The ability of BSFL to decrease *E. coli* in dairy manure was dependent upon how much manure was provided to the larvae. Regardless, pathogen populations were greatly reduced in all treatments (Liu et al., 2008). The greatest reduction in pathogens loads occurred when the larvae were fed a single dose of 125 g of manure. *E. coli* was inoculated into dairy manure at 7.0 log cfu/g manure, and after 72 hours, only 0.23±3.39 log cfu/g remained when larvae were given 125g of manure. This study determined BSFL were most successful at reducing *E. coli* counts in dairy manure maintained at 27 and 31°C. A study that examined the ability of BSFL to reduce human waste found that their presence reduced *Salmonella* spp. by 6 log in eight days, compared to a 2 log reduction in the control over the same time period (Lalander et al. 2013).

Manure colonized by BSFL inhibits colonization by house flies, Furman and Catts 1959, Kilpatrick and School 1959, Axtell and Edwards 1970, Tingle 1975, Sheppard 1983, Bradley and Sheppard 1984, Sheppard et al. 1994). Furman and Catts (1959) demonstrated that as the number of actively feeding BSFL in manure increased, the number of successfully developing house flies decreased. Furthermore, field tests conducted in the San Joaquin Valley of California, USA noted that house flies were outcompeted by dense populations of BSFL in manure; house flies were absent from both dry and moistened manure that had been inoculated with BSFL after the second week of treatment (Furman and Catts, 1959). Artificial infestation of BSFL prevented house fly breeding while adjacent manure not infested with BSFL experienced heavy infestation by house flies

(Furman and Catts 1959). Kilpatrick and Schoof (1959) found that pit privies treated with insecticides such that BSF populations were eliminated experiences dense populations of house flies compared to untreated privies, which maintained BSFL populations, and therefore minimum adult house fly presence and zero larval colonization.

Axtell and Edwards (1970) found that when larviciding poultry houses to control for BSF adults, their absence produced a dramatic invasion of house flies compared to their complete absence when BSFL were present in the poultry manure. Observations by Tingle et al. (1975) supported the findings of Furman and Catts (1959) that BSFL can be a significant factor in controlling house fly populations at poultry operations.

This relationship was demonstrated in south Georgia, USA where there was a significant ($P \leq 0.05$) negative correlation between black soldier fly populations on house fly and lesser house fly, *Fannia canicularis* (L.), (Diptera: Muscidae) numbers. In fact, no other dipteran larvae were observed in treatments where BSFL were dense. Treatments saw over 94-100% reductions in both house fly and lesser house fly populations (Sheppard, 1983).

Bradley and Sheppard (1984) observed that this relationship between the two species was density dependent where house flies would readily oviposit in poultry manure when BSFL populations were less dense i.e. one larvae and would not oviposit in manure where BSFL were densely populated i.e. 10 or 100 larvae. These authors were the first to attempt to characterize the mechanism behind this interspecific competition and suggested that an allomone could be driving this relationship.

The impact of BSFL on manure volatiles has not been researched. Green and Popa (2012) found that BSFL feeding on leachate from decaying food scraps increased ammonium (NH_4^+) concentrations five to six fold relative to leachate that was not fed on by larvae. BSFL increased nitrogen mineralization by elevating the ammonia concentration while feeding on the leachate. Furthermore, the larvae assimilated nitrogen and carbon that would have otherwise been released into the environment as gases such as ammonia, nitrogen oxide, and nitrous oxide (Green and Popa 2012).

Black soldier fly mating and oviposition has been examined in a few studies. Tingle et al. (1975) observed that males could "call" females to an area with mating occurring on the ground with the genders facing opposite directions. Other authors have noted in-flight mating (Copello, 1926) and as well as lekking behaviors (Tomberlin and Sheppard 2001). In another study by Tomberlin and Sheppard (2002), the authors noted that 69% of mating occurred two days after emergence and 70% of oviposition occurred four days after emergence. Factors such as sunlight, time of day, temperature, and humidity significantly ($P < 0.0001$) correlated with oviposition (Tomberlin and Sheppard 2002).

Zheng et al. (2013) demonstrated that bacteria mediate oviposition for BSF (Zheng et al. 2013). Significantly ($P < 0.05$) more eggs were laid in sites with bacteria isolated from conspecifics on decomposing material. It was hypothesized that this attraction by gravid females was due to volatile emissions (Zheng et al. 2013) Furthermore, it was ascertained that a mixture of bacteria versus a single species, differentially influenced the

behavior of adults. Bacteria species and concentration also had different effects on adult fly behavior

The relationship between microbes and black soldier flies has been investigated by Yu et al. (2011) who found that larvae fed manure that had been inoculated with bacteria were 9-22% heavier than larvae fed sterile manure and overall had enhanced development such as shorter larval durations. Zheng et al. (2013) used 16S rDNA pyrosequencing to survey the bacterial diversity of successive life stages of BSF. It was found that bacteria from the phyla Bacteroidetes and Proteobacteria were most dominant and accounted for two thirds of the identified bacteria.

Objectives and Hypotheses

The research objectives are as follows:

1. Examine how feeding by BSFL at different feed rates affects volatile emissions from three manure types.

    $H_{01:}$ The overall VOC community will not be different between freshly-thawed, BSF digested and non-digested manures.

    $H_{a1:}$ The overall VOC community will be different between freshly-thawed, BSF digested and non-digested manures.

    $H_{02:}$ The diversity of VOC compounds will not be different between freshly-thawed, BSF digested and non-digested manures.

    $H_{a2:}$ The diversity of VOC community will be different between freshly-thawed, BSF digested and non-digested manures.

    $H_{03:}$ The relative amounts of select odorous VOCs will not be different between freshly-thawed, BSF digested and non-digested manures.

    $H_{03:}$ The relative amounts of select odorous VOCs will be different between freshly-thawed, BSF digested and non-digested manures.

2. Examine the effect of volatiles found on site selection and oviposition by the black soldier fly.

    $H_{01:}$ Attraction and oviposition preferences will not be different between VOCs of different manure types and their treatments.

    $H_{a1:}$ Attraction and oviposition preferences will be different between VOCs of different manure types and their treatments.

CHAPTER II

VOC ANALYSIS: RESEARCH, RESULTS, AND DISCUSSION

Introduction

With a rise in the global human population and increase in demand for meat over the past several decades, especially within developing countries, livestock producers have been forced to increase their output to match the growing demand (FAO 2009, Msangi et al. 2011). Furthermore, animal farming has become increasingly concentrated as more animals are housed over smaller areas of land (EPA 2013). Between 1997 and 2012 the number of dairy cows on factory farms doubled, the number of hogs increased by one-third, and the number of egg-laying hens increased nearly one quarter, reaching numbers of 5.6 million, 46.1 million and 269 million animals, respectively by 2012 (USDA 2014). These animals produced over 369 million tons of manure in 2012 equating to over 13.8 billion cubic feet of waste to be dealt with (USDA 2014).

Manure can be a valuable resource, such as on-farm fertilizer, but its use is less feasible with the increase in number of confined animal feeding operations (CAFOs) and factory farms. Whereas animals in pasture can distribute this resource more evenly and over a greater area of land, CAFOs produce more manure per square foot than can be applied as fertilizer (Ribaudo et al. 2003). In 1997, animal feeding operations controlled over 73 million acres of cropland and permanent pasture, which was estimated to assimilate only 40% and 30% of recoverable nitrogen and phosphorus, respectively, from manure (Gollehon et al. 2001). Large farming facilities (more than 1000 animals), which

constituted only 2% of farms, accounted for nearly half of the excess on-farm nutrients (Gollehon et al. 2001). The excess of manure must be dealt with via alternative methods such as storage in tanks and lagoons or transportation elsewhere which require the purchase of ancillary equipment such as tanks or the construction of pits and lagoons all at increased cost to the farmer (Nowak et al. 1998).

Furthermore, these methods may lead to further pollution as contaminants associated with livestock and their manure can move through the soil and wind to waterways through a variety of circumstances (Mawdsley et al. 1995). In 1999, Hurricane Floyd hit North Carolina, flooding much of the land, including 50 lagoons used for manure storage, causing three to burst. The result was the release of over 3.7 million L of manure mixed with the floodwaters in addition to the deaths of millions of animals, which drowned during the flooding (Henderson and Suchetka 1999).

There are many microbial contaminants associated with manure including pathogenic organisms, such as bacteria, viruses and protozoans (Cole et al. 1998). In 2009, a clogged pipe caused the leak of over 94,000 L of dairy manure in Pipestone County, MN, which spilled into a local tributary, killing fish and resulting in a state park closing to swimmers after elevated levels of coliform bacteria were found in the park's waters (Kuphal 2009). In 2010, the Environmental Protection Agency (EPA) mandated a feedlot in Grand View, ID to cease the discharge of fecal bacteria-contaminated water from its stock watering system into a tributary of the Snake River (EPA 2010).

The overabundance of nitrogen and phosphorus in the environment is another issue associated with improper manure management (Kellogg 2003). In 2010 a broiler chicken

operation with over 100,000 animals was ordered to cease the discharge of pollutants from large piles of uncovered manure, which were leaching nitrogen and phosphorus into a nearby tributary of the Shenandoah River in Virginia (EPA 2010). Nitrogen-containing pollutants pose both ecological and human health threats, and can reach the environment through leaching and surface runoff (Burkholder et al. 2007). Nitrogen is a limiting nutrient in marine and estuarine environments, and therefore increased loading of this element through forms such as ammonium and nitrate, can significantly contribute to downstream effects such as eutrophication and oxygen depletion, which are responsible for massive fish kills (Rabalais et al. 2002). Manure discharged from a dairy in O'Brien County, Iowa polluted a 45 km stretch of stream and killed over 860,000 fish. The Iowa Department of Natural Resources estimated the value of these fish to be over $160,000 (Iowa Department of Natural Resources 2014). In 2009, over 150,000 L of swine manure was released over a farm field in Mitchell County, IA, which was responsible for killing over 150,000 fish over a six km stretch of local stream. In 2009, over 150,000 L of swine manure was released over a farm field in Mitchell County, IA, which was responsible for killing over 150,000 fish over a six km stretch of local stream (Wilkton 2004). Nitrate is also an important water contaminant that is regulated by the EPA's Safe Drinking Water Act. High levels of exposure to nitrates can cause methomoglobinemia, or blue baby syndrome (Ward et al. 2005) in infants and is linked to different cancers, insulin-dependent diabetes and neurodevelopmental defects in adults (Burkholder et al. 2007).

Phosphorus is another important contaminant linked to manure and intense livestock farming. Like nitrogen, phosphorus is a limiting nutrient in many aquatic

environments. Through manure disposal, leaching, and runoff, phosphorus can reach these environments and result in eutrophication (Sharpley1999).

The decomposition of manure is also responsible for environmental emissions such as greenhouse gases, ammonia and other volatile organic compounds (VOCs), which are pollutants and pose potential health risks (FAO 2009). Studies have indicated that between 100 and 330 VOCs and volatile fatty acids are generated by CAFOs, depending on management practices and the species of animal involved (Cai et al. 2015, Powers and Bastyr 2004, Schiffman et al. 2001). The compounds most associated with or responsible for the odor of manure are phenols, indoles, skatoles, alcohols, organic sulphides, and volatile fatty acids (Hales et al. 2012, Kuroda et al. 1996, El-Mashad et al. 2011). For example, Hales et al. (2012) found that 4-methylphenol was responsible for 67.3% of odor activity in dairy manure. Another study found that the key odorous compounds in poultry manure were butanoic acid, 3-methylbutanoic acid, dimethyl trisulphide, indole and skatole (Yasuhara 1987). These VOCs, which are noxious odors, can also negatively affect humans by posing potential health risks to those living in areas surrounding communities (PEW 2008). VOCs responsible for noxious odors contribute to higher levels of tension, depression and anger experiences by those working or living in close proximity to areas with heavy animal farming (Barrett 2006). Buildup of such gases can also pose certain safety risks. In 2010, a manure lagoon liner from a 1,650 cow dairy operation in Randolph County, Indiana, became detached and inflated with gases associated with decomposing manure. The operator of the farm could not afford the costs associated with fixing the liner, causing the county to shut down local roads to the area due to the risk posed by the

potential lease of noxious odors or even explosion (Etter 2010). In Portage County, Wisconsin, 2016, a farmer and 16 cattle were overcome and killed by deadly amounts of sulfur oxide fumes from a manure holding tank when agitation caused the surface line to crack and release deadly fumes into the environment (Cerullo 2016).

With the increasing amount of manure and the need for a sustainable method of management, fly (Diptera) larvae have become an alternative means to deal with this resource. Black solider fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae (BSFL) have been studied as a means of manure management because of several beneficial abilities. First, BSFL reduce organic matter such as livestock manure. Significant reductions in poultry manure were observed by Sheppard (1994) who observed an estimated 50% reduction in poultry manure in addition to a previous study, which reported 56 and 42% reductions in dry weight (Sheppard 1983). Myers et al. (2008) saw a 58% and 33% reduction in dry matter of manure from BSFL fed 27g and 70 g of dairy manure daily, respectively. Newton et al. (2005) observed a 39% reduction in the dry weight of swine manure processed by BSFL. In a study comparing poultry, swine, and dairy manure, the dry matter of all three manure types was reduced by ~37% (Oonincx et al. 2015).

In addition to the reduction of dry matter of manure, nutrients present in manure, which in excess can be detrimental to the environment, were decreased. During the decomposition of dairy manure, BSFL reduced concentrations of nitrogen and phosphorus by 30-50% and 61-70%, respectively (Myers et al. 2008). Another study indicated a 62% reduction in nitrogen content of poultry manure consumed by BSFL (Sheppard 1983). In a study where BSFL were allowed to feed on dairy, swine, and poultry manure, nitrogen

content was reduced by 30, 37, and 80%, respectively (Oonincx et al. 2015). However, to date, no one has examined the impact of black soldier fly digestion of manure on noxious odor production. The purpose of this study was to assess how the presence and digestion of these manure types by BSFL impacts select noxious VOCs.

Materials and Methods

Acquisition of Flies

Hermetia illucens larvae used in this experiment came from a colony maintained at the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University in College Station, TX. This colony was established in 2014 from eggs received from a laboratory colony maintained at the Coastal Plains Experiment Station, University of Georgia, Tifton, GA. Adult flies were maintained in a 2.6 × 1.2 × 1.3 m wooden cage fitted with metal screening in a greenhouse maintained at approximately 27°C. Adults were allowed to oviposit on three 7.0 × 5.0 × 0.3 cm pieces of corrugated cardboard (Booth and Sheppard, 1984) held together with masking tape and placed on the lid of a 30.0 × 15.0 × 11.0 cm plastic shoe box containing one kilogram of Gainesville diet (Hogsette 1992) saturated with water. A 13.0 × 5.0 cm portion of the lid was removed and replaced with metal screening on which the cardboard pieces were placed; this allowed volatiles to escape from the wet Gainesville diet and attract the flies, but prevented the flies from contacting and/or ovipositing directly into the media instead of the cardboard. The cardboard was removed from the cage after eight hours, and eggs were removed from cardboard using a sterile plastic spatula and weighed. One gram of

eggs were then placed in a 0.5 L plastic container, covered with a paper towel secured with a rubber band, stored in a walk-in environmental chamber (29 ± 0.3°C with 60 ± 5.1% RH and 16:8 L:D) and checked every 12 h until hatch. Two hundred grams of Gainesville diet at 70% moisture was added to the container once larvae emerged. Newly-emerged larvae were allowed to feed for four days in the environmental chamber prior to use in the experiment.

Acquisition of Manure

Three different livestock manure types were used in this study. Poultry manure was collected from layer hens housed at the Poultry Science Research, Teaching, and Extension Center at Texas A&M University in College Station, TX. The hens were fed a mixture of corn and soybean meal that is considered typical layer diet, consisting of 18.5% crude protein, 2.5% crude fat and 2.4% crude fiber. Dairy manure was collected from cows maintained at the Southwest Regional Dairy Center in Stephenville, TX. The diet for these animals consisted of 16.1% crude protein, 5.0% crude fat and 28.1% crude fiber. The majority of this diet is composed of a mixture of corn silage (32.0%), ground corn (22.5%), and concentrate pre-mix (19.4%) composed of canola and soybean meal. Swine manure was collected from sows raised by Schroeder Genetics in Anderson, TX. The sows were maintained on cubes containing 14.0% crude protein, 2.8% crude fat, and 6.5% crude fiber formulated for gilts, sows and adult boars.

Each manure type was collected on site within 12 h of excretion, using a shovel and two 19 L buckets with lids (Home Depot®) that had been sterilized prior to use for

manure collection. The manure was transported to the F.L.I.E.S. Facility where it was homogenized in the buckets and transferred to individual 3.78 L self-sealing plastic freezer bags and frozen at -20°C until used. Manure was removed from the freezer and allowed to thaw for 24 h at room temperature before use. Thawed manure was stored in a refrigerator at 4°C.

Experiment Design

One hundred 4-d-old larvae were placed in 88.7 ml plastic bathroom cups and assigned to one of the three manure types and one of two feed rates (18.0 or 27.0 g every other day). Feed rates used were based off the methods of Myers et al. (2008) who used 300 larvae and feed rates of 27, 40, 54 and 70 g of manure per day. The feed rates used in this study are therefore modified from this due to only 100 larvae being used and feeding occurring every other day. Preliminary experiments were conducted to confirm these feed rates.

Containers without larvae were used as controls and subsequently referred to as non-digested manure. These containers received manure assigned at a given feed rate in similar fashion to those with larvae. Three replicates for each feed rate and manure type with and without larvae were used. Containers were placed in a randomized block design among three levels of a shelving unit in the environmental chamber. The experiment was replicated twice.

Initially, larvae in each replicate were provided manure at the assigned amount and allowed to feed for four days. Larvae and contents of the bathroom cup were then

transferred to a 1.89 L Reditainer™ EXTREME FREEZE™ deli container (Clear Lake Enterprises, Port Richey, FL) and fed every other day. Manure was weighed directly into the containers using a Scout® Pro Balance (Ohaus, Parsippany, NJ). Containers were then covered with a 25.4 × 25.4 cm piece of tulle for ease of visualization and returned to the environmental chamber.

Containers were checked daily for post-feeding larvae (i.e., prepupae Sheppard et al. 1994), which were then subsequently removed. Prepupae could be visually identified by the cuticle turning from opaque to black (May 1961). Additionally, to monitor the progress of larval feeding, manure in each container was shifted using forceps that had been sterilized with 70% ethanol. Separate forceps were used for each manure type to prevent cross contamination. Feeding of larvae terminated when 40% of the larvae reached the prepupal stage (Sheppard et al. 2002). VOCs were sampled when approximately 90% of the larvae reached the prepupal stage. This level of pupation was selected, as it would also represent industrialized production of BSFL. When a replicate from a treatment reached 90% prepupation, a replicate from the non-digested group of the same manure type and feed rate was randomly selected and volatiles were collected from both samples.

Volatile Sampling

Volatile organic compounds were collected from the following manure samples: 1) freshly-thawed also referred to as the control, 2) replicates of each treatment when approximately 90% of the larvae had reached the prepupal stage, referred to as BSF digested, and 3) a replicate without larvae corresponding to the second sample, referred to

as non-digested. Larvae remaining in a replicate that had reached 90% prepupation were removed and the remaining waste homogenized. A subsequent 20.0 g sample was transferred to a 236.5 ml Ball® mason jar (Ball Corporation, Broomfield, CO, USA) and covered with a metal lid. The lid was equipped with two holes equidistant from each other in the center: one held a 14.6 cm glass Labcraft Pasteur pipet (Curtin Matheson Scientific, Inc., Morris Plains, NJ, USA) filled with approximately 0.75 g Black Diamond® activated carbon (Marineland, Cincinnati, OH, USA) as a means to purify incoming air. The second hole was fitted with a volatile trap packed with approximately 30.0 mg of Hayesep® Q porous polymer (Volatile Assay Systems, Rensselaer, NY, USA) as a means to collect VOCs (Figure 2.1). The volatile traps were attached to a length of 6.4 mm diameter Tygon® tubing (Saint-Gobain S.A., Malvern, PA, USA). The opposing end of the Tygon® tubing was attached to an intake port on a flow meter (Dwyer Instruments, Inc., Michigan City, IN, USA). The exhaust port on the flow meter was attached to Tygon® tubing which led to an AC110V, 60Hz Rocker 300 oil free vacuum pump (Rocker, Scientific Co., Ltd., New Taipei City, Taiwan). The pump and flow meters allowed purified air to be pulled over the samples at a rate of 1 L min^{-1} for

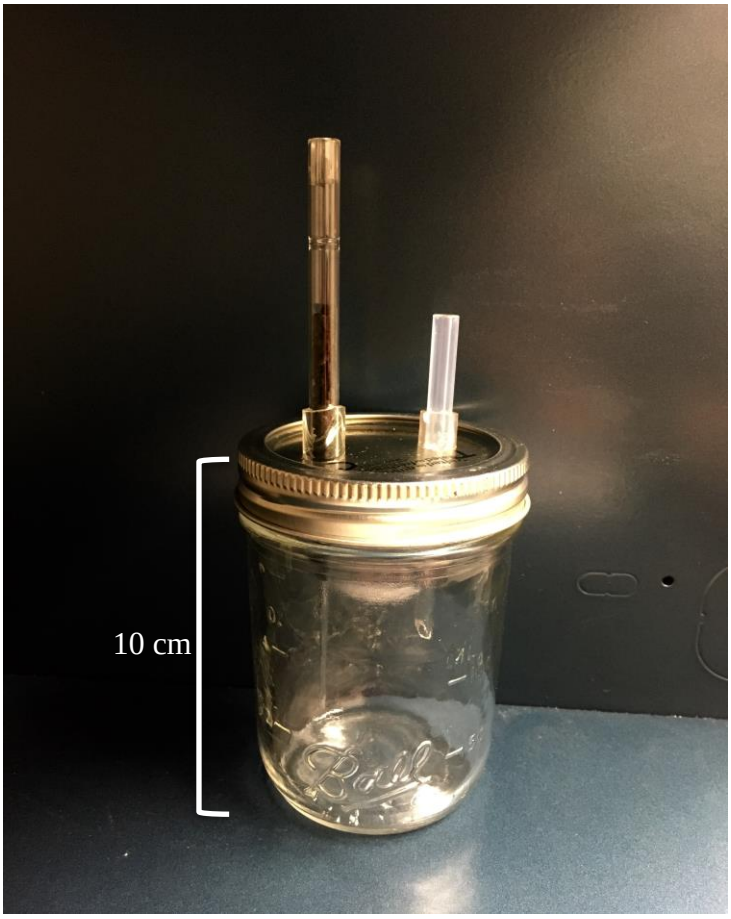

Figure 2.1. Headspace collection set up used to collect volatiles from manure samples. A 236.5 ml Ball® mason jar was outfitted with two holes in the lid: one for a carbon filter to purify ambient air (left) and another for the volatile trap (right). Volatile traps contain 30.0 mg of porous polymer mix Hayesep® Q to which volatiles adhere to. Samples of 20g were placed at the bottom while air was pulled at 1.0 L per min for 1 hr.

two hours under lab conditions. A total of three volatile samples were taken from each 20.0 g sample of manure; one to be processed in a GC-MS and two to be used in subsequent oviposition and attraction assays.

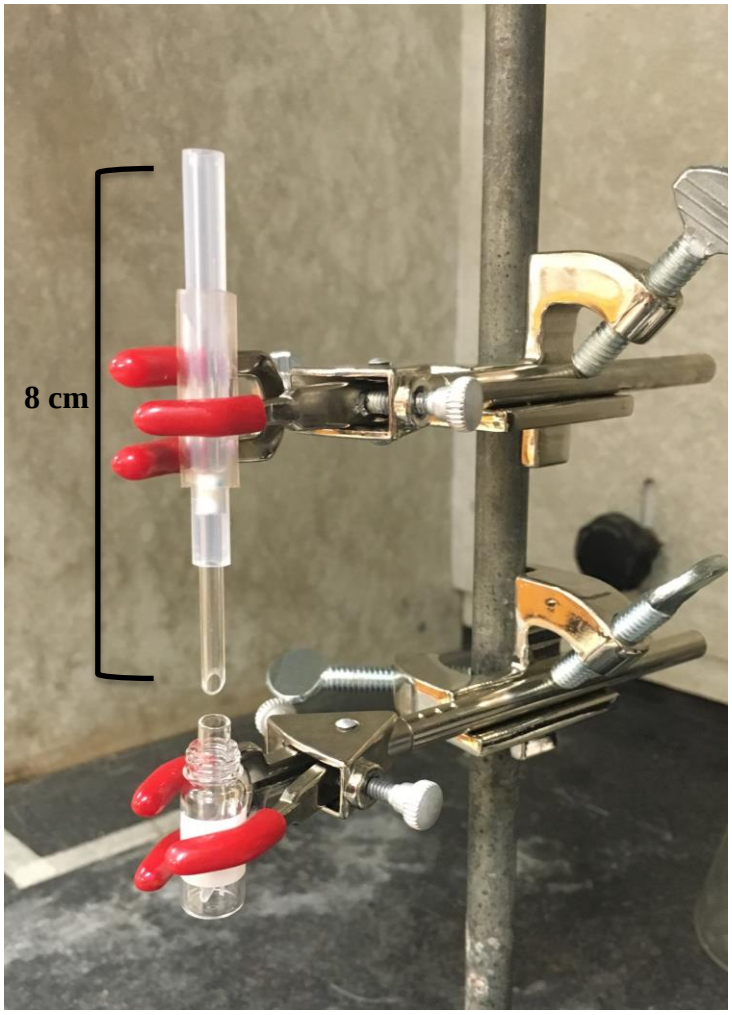

Figure 2.2. VOCs were eluted from the Hayesep® Q into 1.5 ml SureStop™ GC vials containing a 9.0 mm 300 µl insert with 150 µl of dichloromethane (Sigma-Aldrich, St. Louis, MO, USA) injected into the trap which was pushed gently through with N_2. An additional 5.0 µl of n-Octane at a concentration of 80 ng/µl was added to each sample as an internal standard.

GC-MS Analysis

VOCs were eluted from the Hayesep® Q into 1.5 ml SureStop™ GC vials (Thermo Fisher Scientific, Waltham, MA, USA) containing a 9.0 mm 300 µl insert (Thermo Fisher

Scientific, Waltham, MA, USA) with 150 µl of dichloromethane (Thermo Fisher Scientific, Waltham, MA, USA) injected into the trap which was pushed gently through with N_2 (Figure 2.2). An additional 5.0 µl of n-Octane (Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 80 ng/µl was added to each sample as an internal standard. Samples were then capped and stored at -20°C until taken to the Geochemical Environmental Research Group at Texas A&M University in College Station, Texas to be analyzed using a using an Agilent 6890 gas chromatograph with an Agilent 5973 mass selective detector (Agilent Technologies, Santa Clara, CA, USA). The column employed for the separation of VOCs was a fused silica DB-5MS capillary column (30 m x 0.25 mm ID, 0.50 µm film thickness) (Agilent Technologies, Santa Clara, CA, USA).

Injections of 1µL were performed in splitless mode with an injection temperature of 250°C. The column temperature program was as follows: an initial temperature of 35°C was held for 8 min then increased at a rate of 4°C min^{-1} until 60°C was attained. This temperature was maintained for one minute followed by an increase in temperature at a rate of 6°C min^{-1} until 160°C was reached and maintained for 1 min. Finally the temperature was increased to 300°C at a rate of 20°C min^{-1} and held for 10 minutes. Zero-grade helium was used as the carrier gas at a flow rate was 1.29 mL min^{-1}. Electron impact ionization was at 70 eV and mass range was from m/z 45-450. Compounds were identified using standards where available in addition to comparing their mass spectra fragmentation patterns with those stored in the NIST05 mass spectra library.

Chemical Standards

Acetic acid (99% purity), propanoic acid (99.5%), 2-methylpropanoic acid (99%), butyric acid (99%), 3-methylbutanoic acid (99%), pentanoic acid (99%), pentanoic acid, 4-methyl (99%), hexanoic acid (99.5%), heptanoic acid (97%), 3-methylindole (98%), 4-methylphenol (99%), benzaldehyde (99.5%), indole (99%), phenol (99%) were purchased from Absolute Standards, Inc. (Hambed, CT, USA). Octane (99%), dichloromethane (99.8%) and dimethyl disulfide (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Beyond octane, the internal standard, and dichloromethane, the elution solvent, additionally chemicals herein were purchased and used to confirm the identifications of select compounds of interest, which were further examined.

Moisture Content

Moisture content of the freshly-thawed manure was assessed at the beginning of the experiment and compared to the treatment at 90% pupation and its corresponding control. Moisture content was determined gravimetrically using a Scout® Pro Balance (Ohaus, Parsippany, NJ). Ten g of manure in three replicates was weighed out into an aluminum pie dish and dried for 24 h at 55°C in a Precision Scientific Thelco Oven (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analyses

Numbers of compounds present in treatments (control manure, BSF digested manure and non-digested manure were compared using a two-way analysis of variance

(ANOVA) using JMP Pro 12 statistical software (SAS Institute, Cary, NC, USA). Significant differences in means were further separated using Tukey-Kramer Honest Significant Difference (HSD) ($P \leq 0.05$). Identified compounds were quantified using peak areas obtained from each chromatogram. The peak area of the identified compound was divided by the peak area of the internal standard, n-Octane, to obtain relative areas for use in analyses. Relative peak areas were also compared using a two-way ANOVA using JMP Pro 12 statistical software with significant differences further separated using Tukey-Kramer Honest Significant Difference (HSD) ($P \leq 0.05$).

Additional analyses were performed using the vegan 2.0-9 library in the R statistical package (Anderson, 2001; R Core Team, 2013). Differences among volatile profiles of treatments were assessed using a permutational multivariate analysis of variance (PERMANOVA) with the adonis function; a nonparametric technique based on the Bray-Curtis dissimilarity matrix. Bray-Curtis distance with non-metric multidimensional scaling (NMDS) was performed to visualize similarities among treatments (initial freshly-thawed manure, digested treatment manure and non-digested manure) and does not assume linearity between variables (McCune and Grace, 2002). A multiple response permutation procedure (MRPP) was employed to test for statistical differences between covariates. Finally, an Indicator Species Analysis (ISA) was used to determine statistically unique compounds present in volatile sources.

Research Results

Moisture Content

The moisture contents of BSF digested and non-digested manure compared to freshly-thawed manure are provided in Table 2.1 separated by manure type and feed rate. Corresponding ANOVAs and Tukey HSD tests are provided in Table 2.2 for the 18 g feed rate and 2.3 for the 27 g feed rate. The moisture contents of freshly-thawed among manure types were significantly different ($F_{2,12}$ = 159.66, $P \leq 0.0001$). Each manure type was significantly different ($P \leq 0.05$) from one another.

Table 2.1. Mean moisture percentages ± SEM of poultry, swine, and dairy manure (n[1] = 3) at 18 and 27 g with and without *Hermetia illucens* (L.) larvae compared to control manure maintained at 29 ± 0.3°C with 60 ± 5.1% and 14:10 L:D cycle ($P < 0.05$).

Manure	Treatment	18 g	27 g
Poultry	Control	77.25 ± 0.28a[2]	77.25 ± 0.28a
	BSF Digested	13.43 ± 0.98b	42.00 ± 6.73b
	Non-Digested	25.90 ± 7.03b	52.99 ± 1.82b
Swine	Control	73.77 ± 0.338a	73.77 ± 0.338a
	BSF Digested	13.50 ± 0.84b	36.74 ± 8.51b
	Non-Digested	11.90 ± 0.92b	43.52 ± 7.75b
Dairy	Control	84.16 ± 0.50a	84.16 ± 0.50a
	BSF Digested	10.83 ± 0.47b	38.50 ± 13.59b
	Non-Digested	10.40 ± 1.15b	37.35 ± 12.37b

[1]n = replicates; [2]Different letters within a feed rate and manure type indicate significant difference ($P < 0.05$).

Table 2.2. ANOVA and Tukey-Kramer HSD on mean moisture percentages ± SEM of poultry, swine, and dairy manure ($n^1 = 3$) at 18 g with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle *(P* < 0.05).

Poultry					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	13731.3	6865.6	68.0	<**0.0001**
Error	15	1515.1	101.0		
C. Total	17	15246.4			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	63.8	5.8	48.8	78.9	<**0.0001**
Control vs Non-Digested	51.3	5.8	36.3	66.4	<**0.0001**
BSF Digested vs Non-Digested	12.5	5.8	-2.6	27.6	0.1131
Swine					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	14924.6	7462.3	2253.4	<**0.0001**
Error	15	49.7	3.3		
C. Total	17	14974.3			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	60.3	1.0	57.5	63.0	<**0.0001**
Control vs Non-Digested	61.9	1.0	59.1	64.6	<**0.0001**
BSF Digested vs Non-Digested	1.6	1.0	-1.1	4.3	0.3091
Dairy					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	21638.1	3016.2	3016.2	<**0.0001**
Error	15	53.8	3.6		
C. Total	17	21691.9			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	73.3	1.1	70.5	76.2	<**0.0001**
Control vs Non-Digested	73.8	1.1	70.9	76.6	<**0.0001**
BSF Digested vs Non-Digested	0.4	1.1	-2.4	3.3	0.9204

Table 2.3. ANOVA and Tukey-Kramer HSD on mean moisture percentages ± SEM of poultry, swine, and dairy manure ($n^1 = 3$) at 27 g with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Poultry

ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	3903.9	1951.9	20.0	**<0.0001**
Error	15	1461.4	97.4		
C. Total	17	5365.2			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	35.3	5.7	20.4	50.1	**<0.0001**
Control vs Non-Digested	24.3	5.7	9.5	39.1	**0.0019**
BSF Digested vs Non-Digested	11.0	5.7	-3.8	25.8	0.1650

Swine

ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	4664.4	2332.2	8.8	**0.0030**
Error	15	3976.7	265.1		
C. Total	17	8641.1			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	37.0	9.4	12.6	61.4	**0.0035**
Control vs Non-Digested	30.2	9.4	5.8	54.7	**0.0150**
BSF Digested vs Non-Digested	6.8	9.4	-17.6	31.2	0.7547

Dairy

ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	8555.7	4277.8	6.3	**0.0101**
Error	15	10131.5	675.4		
C. Total	17	18687.2			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	45.7	15.0	6.7	84.6	**0.0211**
Control vs Non-Digested	46.8	15.0	7.8	85.8	**0.0181**
BSF Digested vs Non-Digested	1.2	15.0	-37.8	40.1	0.9967

The mean moisture content of BSF digested manure was marginally significantly different among manure types ($F_{2,15} = 3.70$, $P = 0.0496$) for 18 g. However, Tukey-Kramer HSD did not find their separation to be significant from one another. The mean final moisture content of BSF digested manure was not significantly different among manure types ($F_{2,12} = 0.07$, $P = 0.9316$) for the 27 g feed rate.

The mean moisture content of non-digested manure was significantly different among manure types ($F_{2,15} = 4.25$, $P = 0.0345$) for the 18 g feed rate. The moisture content of poultry manure was significantly different ($P = 0.0459$) from dairy; however, the differences between poultry and swine ($P = 0.0740$) and swine from dairy ($P = 0.9645$) were not. The mean moisture content of non-digested manure was not significantly different among manure types ($F_{2,12} = 0.8613$, $P = 0.4425$) for 27 g.

Feed rate was significant for poultry manure ($F_{1,22} = 26.90$, $P \leq 0.0001$) and therefore analyses were done separately. Trial was significant in neither the freshly-thawed poultry manure ($F_{1,4} = 0.70$, $P = 0.4477$) nor the 18 g ($F_{1,10} = 0.32$, $P = 0.5838$) nor the 27 g ($F_{1,10} = 2.59$, $P = 0.1385$) feed rates of BSF digested or non-digested manure. Treatment was significant in both the 18 g feed rate ($F_{2,15} = 67.97$, $P \leq 0.001$) and the 27 gram feed rate ($F_{2,15} = 20.03$, $P \leq 0.0001$). The freshly-thawed poultry manure had an average moisture content of 77.25 ± 0.28% compared to that of 13.43 ± 0.98 % of the BSF digested manure and 25.90 ± 7.03% in the non-digested manure in the lower feed rate. In the higher feed rate of 27 g, the average moisture content of the BSF digested manure was 42.00 ± 6.73% and 52.99 ± 1.82% in the non-digested manure. On average, black soldier flies reduced the moisture content of poultry manure by 82.61% in the low feed rate and

45.63% in the high feed rate. This is compared to the non-digested manure, which saw an average reduction of 66.47% in the low feed rate and 31.40% in the high feed rate. The BSF digested manure, therefore, experienced greater reductions in moisture content at both feed rates compared to the non-digested manure.

Feed rate was significant for swine manure ($F_{1,22} = 23.84$, $P \leq 0.001$) and therefore analyses were done separately. Trial was significant in neither the freshly-thawed swine manure ($F_{1,4} = 0.23$, $P = 0.6584$) nor the 18 g ($F_{1,10} = 1.60$, $P = 0.2351$) nor the 27 g ($F_{1,10} = 0.05$, $P = 0.8239$) feed rates of BSF digested or non-digested manure. Treatment was significant in both the 18 g feed rate ($F_{2,15} = 2253.42$, $P \leq 0.0001$) and the 27 gram feed rate ($F_{2,15} = 8.80$, $P = 0.0030$). The freshly-thawed swine manure had an average moisture content of 73.77 ± 0.34% compared to that of 13.50 ± 0.84 % of the BSF digested manure and 11.90 ± 0.92% in the non-digested manure in the lower feed rate. In the higher feed rate of 27 g, the average moisture content of the BSF digested manure was 36.74 ± 8.51% and 43.52 ± 7.75% in the non-digested manure. On average, black soldier flies reduced the moisture content of swine manure by 81.69% in the low feed rate and 50.20% in the high feed rate. This is compared to the non-digested manure, which saw an average reduction of 83.87% in the low feed rate and 41.00% in the high feed rate. The BSF digested manure, therefore, experienced a greater reduction in moisture than the non-digested manure at the higher feed rate whereas the treatments had comparable reductions in moisture at the lower feed rate.

Black soldier flies, therefore, reduced moisture content to a higher feed rate than the non-digested manure in the higher feed rate however, non-digested manure was drier in the lower feed rate.

Feed rate was significant for dairy manure ($F_{1,22} = 9.68$, $P = 0.0051$) and therefore analyses were done separately. Trial was significant in neither freshly-thawed dairy manure ($F_{1,4} = 0.19$, $P = 0.6856$) nor the 18 g ($F_{1,10} = 0.31$, $P = 0.5874$) nor 27 g ($F_{1,10} = 1.49$, $P = 0.2498$) feed rates of BSF digested or non-digested manure. Treatment was significant in both the 18 g feed rate ($F_{2,15} = 3016.21$, $P \leq 0.0001$) and the 27 gram feed rate ($F_{2,15} = 6.33$, $P = 0.0101$ The freshly-thawed dairy manure had an average

Table 2.4. Mean number of compounds ± SEM of poultry, swine, and dairy manure with and without *Hermetia illucens* (L.) larvae compared to control manure (n[1] = 3) maintained at 29 ± 0.3°C with 60 ± 5.1% and 14:10 L:D cycle (*P* < 0.05).

Manure	Treatment	Number of Compounds
Poultry	Control	35.00 ± 2.51a[2]
	BSF Digested	17.83 ± 2.04b
	Non-Digested	30.42 ± 1.35a
Swine	Control	26.00 ± 1.53a
	BSF Digested	15.25 ± 1.71b
	Non-Digested	16.00 ± 1.66b
Dairy	Control	21.67 ± 1.45a
	BSF Digested	17.33 ± 1.78a
	Non-Digested	15.17 ± 1.46a

[1]n = replicates; [2]Different letters within a subset of a column indicate significant difference (*P* < 0.05).

moisture content of 84.16 ± 0.50% compared to that of 10.83 ± 0.47 % of the BSF digested manure and 10.40 ± 1.15% in the non-digested manure in the lower feed rate. In the higher feed rate of 27 g, the average moisture content of the BSF digested manure was 38.50 ± 13.59% and 37.35 ± 12.37% in the non-digested manure. On average, black soldier flies reduced the moisture content of dairy manure by 87.13% in the low feed rate and 54.25% in the high feed rate. This is compared to the non-digested manure, which saw an average reduction of 87.64% in the low feed rate and 55.62% in the high feed rate. Black soldier flies, therefore, reduced moisture content to comparable levels of that of non-digested manure.

Multivariate Work

Data generated from multivariate analyses were inconclusive. All data generated are presented in Appendices A-C.

Number of Compounds

A summary of the number of compounds per manure type and treatment is provided in Table 2.4 and the corresponding ANOVAs and Tukey HSD tests in Table 2.5. The number of compounds in a given treatment differed significantly ($F_{2,78} = 7.84$, $P = 0.008$) among the three manure types. In the control manure, the number of compounds was significantly different by manure type ($F_{2,6} = 12.87$, $P = 0.0067$) with the number of compounds in control poultry manure significantly different from dairy ($P = 0.0060$) and swine ($P = 0.0060$). However, the number of compounds in control swine

Table 2.5. ANOVA and Tukey-Kramer HSD on mean number of compounds ± SEM of poultry, swine, and dairy manure ($n^1 = 3$) at 27 g with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Poultry					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	1220.5	610.3	18.0	**<0.0001**
Error	24	812.7	33.9		
C. Total	26	2033.2			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	16.2	3.8	6.8	25.5	**0.0007**
Control vs Non-Digested	3.5	3.8	-5.9	12.9	0.6259
BSF Digested vs Non-Digested	12.6	2.37	6.7	18.6	**<0.0001**
Swine					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	290.4	145.2	4.6	**0.0201**
Error	24	764.3	31.8		
C. Total	26	1054.7			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	10.75	3.6	1.7	19.8	**0.0184**
Control vs Non-Digested	10.0	3.6	0.9	19.1	**0.0293**
BSF Digested vs Non-Digested	0.8	2.3	-5.0	6.5	0.9434
Dairy					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	106.4	53.2	1.8	0.1876
Error	24	711.0	29.6		
C. Total	26	817.4			

manure was not significantly different from that of control dairy manure ($P = 0.3097$). In BSF digested manure, the number of compounds was significantly different by manure type ($F_{2,33} = 32.91$, $P \leq 0.0001$) with the number of compounds in control poultry manure significantly different from that of dairy ($P \leq 0.0001$) and of swine ($P \leq 0.0001$). However, the number of compounds in control swine manure was not significantly different from that of control dairy manure ($P = 0.9181$). In non-digested manure, the number of compounds was not significantly different by manure type ($F_{2,33} = 0.5504$, $P = 0.5819$). Further analyses on the number of compounds were conducted within a given manure type.

Poultry Manure

Treatment and feed rate did not significantly ($F_{1,17} = 0.84$, $P = 0.3725$) interact as it related to the number of compounds present. Furthermore, neither trial ($F_{1,22} = 1.23$, $P = 0.2791$) nor feed rate were significant ($F_{1,22} = 1.72$, $P = 0.2030$) and were therefore excluded from analyses. The number of compounds between control, BSF digested and non-digested manure was significantly different ($F_{2,24} = 18.42$, $P \leq 0.0001$). The control manure had a mean of 35.00 ± 2.51 compounds compared to 17.83 ± 2.04 in the BSF digested manure and 30.42 ± 1.35 in the non-digested manure. Black soldier flies reduced the number of compounds in manure by 49.06% compared to non-digested manure, which experienced a 13.05% reduction in the number of compounds.

Swine Manure

Treatment and feed rate did not significantly ($F_{1,17} = 0.94$, $P = 0.3400$) interact as it related to the number of compounds present. Furthermore, neither trial ($F_{1,22} = 1.23$, $P = 0.2194$ nor feed rate were significant ($F_{1,22} = 0.01$, $P = 0.9176$) and were therefore excluded from analyses. The number of compounds between control, BSF digested and non-digested manure was significantly different ($F_{2,24} = 4.56$, $P = 0.0210$). The control manure had a mean of 26.00 ± 1.53 compounds compared to 15.25 ± 1.71 in the BSF digested manure and 16.00 ± 1.66 in the non-digested manure. Black soldier flies and non-digested manure, therefore, saw similar decrease in the number of compounds compared to the control with 41.45 and 38.46% reductions, respectively.

Dairy Manure

Treatment and feed rate did not significantly ($F_{1,17} = 0.37$, $P = 0.5486$) interact as it related to the number of compounds present. Furthermore, neither trial ($F_{1,22} = 3.68$, $P = 0.0681$) nor feed rate were significant ($F_{1,22} = 0.02$, $P = 0.8863$) and were therefore excluded from analyses. The number of compounds between control, BSF digested and non-digested manure did not differ significantly ($F_{2,24} = 1.80$, $P = 0.1876$). While not significant, the control manure had a greater number of compounds (21.67 ± 1.45) than BSF digested (17.33 ± 1.78) and non-digested (15.17 ± 1.46) manure. Black soldier flies, therefore, did not reduce the number of compounds more than non-digested manure with only a 20.02% reduction compared to a 30.00% reduction, respectively.

Select Odorous Compounds in Poultry Manure

A summary of the average relative amounts of select odorous compounds in poultry manure is presented in Table 2.6 and 2.7. ANOVA and Tukey-Kramer HSD tables for each compound are provided in Table 2.8.

Treatment and feed rate did not significantly ($F_{1,17} = 1.6$, $P = 0.2229$) interact as related to phenol. Furthermore, neither trial ($F_{1,22} = 1.8$, $P = 0.1949$) nor feed rate ($F_{1,22} = 0.0$, $P = 0.9960$) were significant and were therefore excluded from the analyses. Level of phenol present across manure types was significantly different ($F_{2,24} = 247.8$, $P \leq 0.0001$). The level of phenol production between BSF digested and non-digested was not significantly different ($P = 0.3485$), while the differences between both the control and BSF digested and the control and the non-digested were significant ($P \leq 0.0001$).

Table 2.6. Mean relative peak areas[2] ± SEM of noxious odors emitted from poultry, swine, and dairy manure ([1]n = 3) with and without *Hermetia illucens* (L.) larvae compared to control manure at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Manure	Treatment	Volatile			
		Phenol	4-Methylphenol	Indole	3-Methylindole
Poultry	Control	462.05 ± 57.48a[3]	512.06 ± 70.39a	82.90 ± 12.34a	24.50 ± 3.80a
	BSF Digested	3.00 ± 2.61b	2.06 ± 1.51b	0.03 ± 0.3b	0.04 ± 0.04b
	Non-Digested	22.11 ± 6.44b	16.03 ± 2.80b	15.32 ± 8.28b	18.09 ± 7.18b
Swine	Control	243.67 ± 64.18a	860.95 ± 215.77a	177.61 ± 37.79a	283.3 ± 224.2a
	BSF Digested	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.0 ± 14.3b
	Non-Digested	9.04 ± 9.04b	33.49 ± 33.49b	19.54 ± 19.54b	13.2 ± 14.3b
Dairy	Control	93.70 ± 17.99a	414.00 ± 22.0a	8.99 ± 4.50a	66.03 ± 17.30a
	BSF Digested	0.27 ± 0.94b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
	Non-Digested	0.77 ± 2.68b	0.85 ± 0.85b	0.38 ± 0.38b	0.07 ± 0.07b

[1]n = replicates; [2]Relative areas were obtained by dividing the peak area of the compound by the peak area of the internal standard, n-Octane, obtained from the chromatogram; [3]different letters within a subset of a column indicate significant difference ($P < 0.05$).

Table 2.7. Mean relative peak areas[2] ± SEM of noxious odors emitted from poultry, swine, and dairy manure ([1]n = 3) with and without *Hermetia illucens* (L.) larvae compared to control manure at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Manure	Treatment	Volatile				
		Propanoic Acid	2-Methylpropanoic Acid	Butanoic Acid	3-Methylbutanoic Acid	Pentanoic Acid
Poultry	Control	63.61 ± 27.30a[3]	17.49 ± 9.68a	1871.98 ± 1583.60a	347.34 ± 46.75a	832.40 ± 365.20a
	BSF Digested	7.64 ± 7.64b	0.00 ± 0.00a	32.55 ± 32.60b	17.18 ± 16.86b	12.94 ± 12.94b
	Non-Digested	4.80 ± 3.45b	13.58 ± 9.35a	39.34 ± 28.30b	49.96 ± 36.04b	49.52 ± 31.39b
Swine	Control	42.01 ± 2.46a	16.27 ± 5.22a	170.05 ± 43.71a	177.95 ± 30.03a	N/A
	BSF Digested	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	N/A
	Non-Digested	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	N/A
Dairy	Control	162.01 ± 13.89a	86.27 ± 4.21a	146.71 ± 6.58a	90.28 ± 5.87a	74.46 ± 4.86a
	BSF Digested	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
	Non-Digested	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b

[1]n = replicates; [2]Relative areas were obtained by dividing the peak area of the compound by the peak area of the internal standard, n-Octane, obtained from the chromatogram; [3]different letters within a subset of a column indicate significant difference ($P < 0.05$).

Table 2.8. ANOVA and Tukey-Kramer HSD on relative amount of amounts of odorous volatile compounds in poultry manure (n = 3) with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Phenol					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	540960.0	270480.0	247.8	<**0.0001**
Error	24	26193.0	1091.0		
C. Total	26	567153.0			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	459.0	21.3	405.8	512.3	<**0.0001**
Control vs Non-Digested	439.9	21.3	386.7	493.2	<**0.0001**
BSF Digested vs Non-Digested	19.1	13.5	-14.5	52.8	0.3485
4-Methylphenol					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	675884.3	337943.0	261.1	<**0.0001**
Error	24	31067.3	1294.0		
C. Total	26	706951.6			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	510.0	23.2	452.0	568.0	<**0.0001**
Control vs Non-Digested	496.0	23.2	438.0	554.0	<**0.0001**
BSF Digested vs Non-Digested	14.0	14.7	-22.7	50.6	0.6144
Indole					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	16488.2	8244.1	19.9	<**0.0001**
Error	24	9958.7	415.0		
C. Total	26	26446.9			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	82.9	13.1	50.0	115.7	<**0.0001**
Control vs Non-Digested	67.6	13.1	34.7	100.4	<**0.0001**

Table 2.8 (Continued)

Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
BSF Digested vs Non-Digested	15.3	8.3	-5.5	36.1	0.1788
3-Methylindole					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	2590.9	1295.5	4.5	**0.0218**
Error	24	6897.4	287.4		
C. Total	26	9488.3			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	24.5	10.9	-2.7	51.8	0.0854
Control vs Non-Digested	6.4	10.9	-20.9	33.7	0.8289
BSF Digested vs Non-Digested	18.1	6.9	0.8	35.3	**0.0395**
Propanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	8830.6	4415.3	9.6	**0.0009**
Error	24	11066.1	461.1		
C. Total	26	19896.7			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	56.0	13.9	21.4	93.4	**0.0013**
Control vs Non-Digested	58.8	13.9	24.2	94.4	**0.0008**
BSF Digested vs Non-Digested	2.8	8.8	-19.1	24.7	0.9442
2-Methylpropanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	1411.6	705.8	1.4	0.2658
Error	24	12092.3	503.8		
C. Total	26	13503.9			
Butanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	8989566.0	4494783.0	7.1	**0.0039**
Error	24	15291817.0	637150.0		

Table 2.8 (Continued)

Source	Df	SS	MS	F Ratio	*P* Value
C. Total	26	24281383.0			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	1839.4	515.3	552.7	3126.2	**0.0043**
Control vs Non-Digested	1832.6	515.3	545.9	3119.4	**0.0044**
BSF Digested vs Non-Digested	6.8	325.9	-807.0	820.6	0.9999
3-Methylbutanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	268981.7	134491.0	14.5	**<0.0001**
Error	24	222297.0	9262.0		
C. Total	26	491278.8			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	330.2	62.1	175.0	489.3	**<0.0001**
Control vs Non-Digested	297.4	62.1	142.2	452.5	**0.0002**
BSF Digested vs Non-Digested	32.8	39.3	-65.3	130.9	0.6858
Pentanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	1719690.0	859845.0	21.7	**<0.0001**
Error	24	952763.4	39698.0		
C. Total	26	2672453.4			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	819.5	128.6	498.3	1140.6	**<0.0001**
Control vs Non-Digested	782.9	128.6	461.7	1104.1	**<0.0001**
BSF Digested vs Non-Digested	36.6	81.3	-5166.6	239.7	0.8950

The mean amount of phenol was reduced by 99.35% in the BSF digested manure and 95.21% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,17} = 1.0$, $P = 0.3385$) interact as it related to 4-methylphenol. Furthermore, neither trial ($F_{1,22} = 0.9$, $P = 0.3587$) nor feed rate ($F_{1,22} = 0.92$, $P = 0.3490$) were significant and were therefore excluded from the analyses. The amount of 4-methylphenol production across manure types was significantly different ($F_{2,24} = 261.1$, $P \leq 0.0001$). The level of 4-methylphenol production between BSF digested and non-digested was not significantly different ($P = 0.6144$) while the differences between both the control and BSF digested and the control and the non-digested were significant to a level of $P \leq 0.0001$. The mean amount of 4-methylphenol was reduced by 99.60% in the BSF digested manure and 96.87% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,17} = 1.0$, $P = 0.3385$) interact as it related to indole. Furthermore, neither trial ($F_{1,22} = 1.0$, $P = 0.3324$) nor feed rate ($F_{1,22} = 1.37$, $P = 0.2550$) were significant either and were therefore excluded from the analyses. Amount indole production across manure types was significantly different ($F_{2,24} = 19.9$, $P \leq 0.0001$). The level of indole production between BSF digested and non-digested was not significantly different ($P = 0.1788$) while the differences between both the control and BSF digested and the control and the non-digested were significant to a level of $P \leq 0.0001$. The mean amount of indole was reduced by 99.96% in the BSF digested manure and 81.52% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,17} = 1.0$, $P = 0.3385$) interact as it related to 3-methylindole. Furthermore, neither trial ($F_{1,22} = 0.5$, $P = 0.4729$) nor feed rate

($F_{1,22}$ = 1.99, P = 0.1721) were significant either and were therefore excluded from the analyses. The amount of 3-methylindole production across manure types was significantly different ($F_{2,24}$ = 17.6, $P \leq 0.0001$). The level of 3-methylindole production between BSF digested and non-digested was not significantly different (P = 0.9237) while the differences between both the control and BSF digested and the control and the non-digested were significant to a level of $P \leq 0.0001$. The mean amount of 3-methylindole was reduced by 99.35% in the BSF digested manure and 95.21% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,17}$ = 2.6, P = 0.1210) interact as it related to propanoic acid. Furthermore, neither trial ($F_{1,22}$ = 1.3, P = 0.2691) nor feed rate ($F_{1,22}$ = 0.11, P = 0.7385) were significant either and were therefore excluded from the analyses. The amount of propanoic acid production across manure types was significantly different ($F_{2,24}$ = 261.1, P = 0.0009). The level of propanoic acid production between BSF digested and non-digested was not significantly different (P = 0.9442) while significant differences between both the control and BSF digested (P = 0.0013) and the control and the non-digested (P = 0.0018) were observed. The mean amount of propanoic acid was reduced by 87.99% in the BSF digested manure and 92.45% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,17}$ = 1.3, P = 0.2668) interact as it related to 2-methylpropanoic acid. Furthermore, neither trial ($F_{1,22}$ = 2.1, P = 0.1603) nor feed rate ($F_{1,22}$ = 1.19, P = 0.2873) were significant either and were therefore excluded from the analyses. The amount of 2-methylpropanoic acid across manure types was not significantly different ($F_{2,24}$ = 1.4, P = 0.2658). The mean amount of propanoic acid, 2-

methy was reduced by 100% in the BSF digested manure and 22.36% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,17} = 2.8$, $P = 0.1133$) interact as it related to butanoic acid. Furthermore, neither trial ($F_{1,22} = 1.6$, $P = 0.2179$) nor feed rate ($F_{1,22} = 0.01$, $P = 0.9053$) were significant either and were therefore excluded from the analyses. The amount of butanoic acid production across manure types was significantly different ($F_{2,24} = 17.6$, $P = 0.0039$). The level of butanoic acid production between BSF digested and non-digested was not significantly different ($P = 0.9999$) while significant differences between both the control and BSF digested ($P = 0.0043$) and the control and the non-digested ($P = 0.0044$) were observed. The mean amount of butanoic acid was reduced by 98.26% in the BSF digested manure and 97.90% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,17} = 2.4$, $P = 0.1431$) interact as it related to 3-methylbutanoic acid. Furthermore, neither trial ($F_{1,22} = 2.2$, $P = 0.1522$) nor feed rate ($F_{1,22} = 0.42$, $P = 0.5223$) were significant either and were therefore excluded from the analyses. The amount of 3-methylbutanoic acid production across manure types was significantly different ($F_{2,24} = 17.6$, $P \leq 0.0001$). The level of 3-methylbutanoic acid production between BSF digested and non-digested was not significantly different ($P = 0.6858$) while significant differences between both the control and BSF digested ($P \leq 0.0001$) and the control and the non-digested ($P = 0.0002$) were observed. The mean amount of 3-methylbutanoic acid was reduced by 95.05% in the BSF digested manure and 85.62% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,17} = 2.46$, $P = 0.13354$) interact as it related to pentanoic acid. Furthermore, neither trial ($F_{1,22} = 1.8$, $P = 0.1973$) nor feed rate ($F_{1,22} = 0.54$, $P = 0.4692$) were significant either and were therefore excluded from the analyses. The amount of pentanoic acid production across manure types was significantly different ($F_{2,24} = 21.66$, $P \leq 0.0001$). The level of pentanoic acid production between BSF digested and non-digested was not significantly different ($P = 0.8950$) while the differences between both the control and BSF digested and the control and the non-digested were significant to a level of $P \leq 0.0001$. The mean amount of pentanoic acid was reduced by 98.45% in the BSF digested manure and 94.05% in the non-digested manure.

Select Odorous Compounds in Swine Manure

A summary of the average relative amounts of select odorous compounds in swine manure is presented in Tables 2.6 and 2.7. ANOVA and Tukey-Kramer HSD tables for each compound are provided in Table 2.9. The amounts of all select volatiles in swine manure were significantly reduced ($P < 0.05$) in both the BSF digested and non-digested treatments compared to the control manure.

Treatment and feed rate did not significantly ($F_{1,18.1} = 1.0$, $P = 0.3195$) interact as it related to phenol. Furthermore, neither trial ($F_{1,22} = 1.0$ $P = 0.3282$) nor feed rate ($F_{1,22}$

Table 2.9. ANOVA and Tukey-Kramer HSD on relative amount of amounts of odorous volatile compounds in swine manure (n = 3) with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Phenol					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	153005.3	76502.6	51.7	**<0.0001**
Error	24	35499.6	1479.1		
C. Total	26	188504.9			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	243.7	24.8	181.7	305.7	**<0.0001**
Control vs Non-Digested	234.6	24.8	172.6	296.6	**<0.0001**
BSF Digested vs Non-Digested	9.0	15.7	-30.2	48.3	0.8342
4-Methylphenol					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	1907193.4	953597.0	53.5	**<0.0001**
Error	24	427414.1	17809.0		
C. Total	26	2334607.5			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	860.9	86.1	645.8	1076.1	**<0.0001**
Control vs Non-Digested	827.5	86.1	612.3	1042.6	**<0.0001**
BSF Digested vs Non-Digested	33.5	54.4	-102.6	169.5	0.8135
Indole					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	77412.9	38706.4	15.8	**<0.0001**
Error	24	58970.1	2457.1		
C. Total	26	136383.0			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	177.6	32.0	97.7	257.5	**<0.0001**

Table 2.9 (Continued)

Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs Non-Digested	158.1	32.0	78.2	238.0	**<0.0001**
BSF Digested vs Non-Digested	19.5	20.2	-31.0	70.1	0.6051
3-Methylindole					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	205151.3	102576.0	41.7	**<0.0001**
Error	24	58979.6	2457.0		
C. Total	26	264130.9			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	283.3	32.0	203.4	363.2	**<0.0001**
Control vs Non-Digested	270.0	32.0	190.1	350.0	**<0.0001**
BSF Digested vs Non-Digested	13.2	20.2	-37.3	63.8	0.7921
Propanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	4706.4	2353.2	1558.9	**<0.0001**
Error	24	36.2	1.5		
C. Total	26	4742.6			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	42.0	0.8	40.0	44.0	**<0.0001**
Control vs Non-Digested	42.0	0.8	40.0	44.0	**<0.0001**
BSF Digested vs Non-Digested	0.0	0.5	-1.3	1.3	1.0000
2-Methylpropanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	706.3	353.1	51.8	**<0.0001**
Error	24	163.7	6.8		
C. Total	26	870.0			

Table 2.9 (Continued)

TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	16.3	1.7	12.1	20.5	<**0.0001**
Control vs Non-Digested	16.3	1.7	12.1	20.5	<**0.0001**
BSF Digested vs Non-Digested	0.0	1.1	-2.7	2.7	1.0000
Butanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	77107.4	38553.7	80.7	<**0.0001**
Error	24	11462.5	477.6		
C. Total	26	88569.9			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	170.0	14.1	134.8	205.3	<**0.0001**
Control vs Non-Digested	170.0	14.1	134.8	205.3	<**0.0001**
BSF Digested vs Non-Digested	0.0	8.9	-22.3	22.3	1.000
3-Methylbutanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	84439.9	42219.9	145.9	<**0.0001**
Error	24	6946.2	289.4		
C. Total	26	91386.0			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	177.9	11.1	150.5	205.4	<**0.0001**
Control vs Non-Digested	177.9	11.1	150.5	205.4	<**0.0001**
BSF Digested vs Non-Digested	0.0	6.9	-17.3	17.3	1.000
Pentanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	1719690.0	859845.0	21.7	<**0.0001**

Table 2.9 (Continued)

Source	Df	SS	MS	F Ratio	*P* Value
Error	24	952763.4	39698.0		
C. Total	26	2672453.4			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	819.5	128.6	498.3	1140.6	**<0.0001**
Control vs Non-Digested	782.9	128.6	461.7	1104.1	**<0.0001**
BSF Digested vs Non-Digested	36.6	81.3	-5166.6	239.7	0.8950

= 1.00, $P = 0.3282$) were significant either and were therefore excluded from the analyses. Level of phenol present across manure types was significantly different ($F_{2,24} = 51.7$, $P \leq 0.0001$). The level of phenol production between BSF digested and non-digested was not significantly different ($P = 0.8342$), while the differences between both the control and BSF digested and the control and the non-digested were significant ($P \leq 0.0001$). The mean amount of phenol was reduced by 99.35% in the BSF digested manure and 95.21% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,18.1} = 1.0$, $P = 0.3195$) interact as it related to 4-methylphenol. Furthermore, neither trial ($F_{1,22} = 1.0$, $P = 0.3282$) nor feed rate ($F_{1,22} = 1.00$, $P = 0.3282$) were significant either and were therefore excluded from the analyses. Amount of 4-methylphenol production across manure types was significantly different ($F_{2,24} = 53.5$, $P \leq 0.0001$). The level of 4-methylphenol production between BSF digested and non-digested was not significantly different ($P = 0.8135$) while the differences between both the control and BSF digested and the control and the

non-digested were significant to a level of $P \leq 0.0001$. The mean amount of 4-methylphenol was reduced by 100% in the BSF digested manure and 96.11% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,18.1} = 1.0$, $P = 0.3195$) interact as it related to indole. Furthermore, neither trial ($F_{1,22} = 1.0$, $P = 0.3282$) nor feed rate ($F_{1,22} = 1.00$, $P = 0.3282$) were significant either and were therefore excluded from the analyses. Indole production across manure types was significantly different ($F_{2,24} = 15.8$, $P \leq 0.0001$). The level of indole production between BSF digested and non-digested was not significantly different ($P = 0.6051$) while the differences between both the control and BSF digested and the control and the non-digested were significant to a level of $P \leq 0.0001$. The mean amount of indole was reduced by 100% in the BSF digested manure and 89.0% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,18.1} = 1.0$, $P = 0.3182$) interact as it related to 3-methylindole. Furthermore, neither trial ($F_{1,22} = 1.0$, $P = 0.3268$) nor feed rate ($F_{1,22} = 1.00$, $P = 0.3268$) were significant either and were therefore excluded from the analyses. 3-Methylindole production across manure types was significantly different ($F_{2,24} = 41.7$, $P \leq 0.0001$). The level of 3-methylindole production between BSF digested and non-digested was not significantly different ($P = 0.7921$) while the differences between both the control and BSF digested and the control and the non-digested were significant to a level of $P \leq 0.0001$. The mean amount of 3-methylindole was reduced by 100% in the BSF digested manure and 95.34% in the non-digested manure.

The select volatile fatty acids examined (propanoic acid, 2-methylpropanoic acid, butanoic acid, and 3-methylbutanoic acid) were only present in the control manure and not detected (i.e., 100% reduction) in either the BSF digested or the non-digested samples and thus statistically significant (Table 2.7). Pentanoic acid was not present in swine manure samples.

Select Odorous Compounds in Dairy Manure

A summary of the average relative amounts of select odorous compounds in dairy manure is presented in Tables 2.6 and 2.7. ANOVA and Tukey-Kramer HSD tables for each compound are provided in Table 2.10. The amounts of all select volatiles in dairy manure were significantly reduced ($P < 0.05$) in both the BSF digested and non-digested treatments compared to the control manure.

Treatment and feed rate did not significantly ($F_{1,18} = 1.6$, $P = 0.229$) interact as it related to phenol. Furthermore, neither trial ($F_{1,22} = 0.4$ $P = 0.5476$) nor feed rate($F_{1,22} = 0.37$, $P = 0.5476$) were significant either and were therefore excluded from the analyses. Level of phenol present across manure types was significantly different ($F_{2,24} = 136.8$, $P \leq 0.0001$). The level of phenol production between BSF digested and non-digested was not significantly different ($P = 0.9902$), while the differences between both the control and BSF digested and the control and the non-digested were significant ($P \leq 0.0001$). The mean amount of phenol was reduced by 99.71% in the BSF digested manure and 99.17% in the non-digested manure.

Table 2.10. ANOVA and Tukey-Kramer HSD on relative amount of amounts of odorous volatile compounds in dairy manure (n = 3) with and without *Hermetia illucens* (L.) larvae compared to control manure. Experiments were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Phenol					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	2313.4	11576.7	136.8	<**0.0001**
Error	24	2031.5	84.6		
C. Total	26	25184.9			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	93.4	5.9	78.6	1083.0	<**0.0001**
Control vs Non-Digested	92.9	5.9	78.1	107.8	<**0.0001**
BSF Digested vs Non-Digested	0.5	3.8	-8.9	9.9	0.9902
4-Methylphenol					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	456129.9	228064.0	156.9	<**0.0001**
Error	24	34893.8	1454.0		
C. Total	26	491022.7			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	414.0	24.6	352.5	475.5	<**0.0001**
Control vs Non-Digested	413.2	24.6	351.7	474.6	<**0.0001**
BSF Digested vs Non-Digested	0.8	15.6	-38.0	39.7	0.9984
Indole					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	206.5	103.3	17.6	<**0.0001**
Error	24	140.4	5.9		
C. Total	26	346.9			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	9.0	1.6	5.1	12.9	<**0.0001**

Table 2.10 (Continued)

Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs Non-Digested	8.6	1.6	4.7	12.5	**<0.0001**
BSF Digested vs Non-Digested	0.4	0.4	-2.1	2.8	0.9237
3-Methylindole					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	2590.9	1295.5	4.5	**0.0218**
Error	24	6897.4	287.4		
C. Total	26	9488.3			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	24.5	10.9	-2.7	51.8	0.0854
Control vs Non-Digested	6.4	10.9	-20.9	33.7	0.8289
BSF Digested vs Non-Digested	18.1	6.9	0.8	35.3	**0.0395**
Propanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	69993.1	34996.6	726.0	**<0.0001**
Error	24	1156.8	48.200.0		
C. Total	26	71149982.0			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	162.0	4.5	150.8	173.2	**<0.0001**
Control vs Non-Digested	162.0	4.5	150.8	173.2	**<0.0001**
BSF Digested vs Non-Digested	0.0	2.8	-7.1	7.1	1.0000
2-Methylpropanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	19848.7	9924.4	22243.1	**<0.0001**
Error	24	106.2	4.4		
C. Total	26	19954.9			

Table 2.10 (Continued)

TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	86.3	1.4	82.9	89.7	<**0.0001**
Control vs Non-Digested	86.3	1.4	82.9	89.7	<**0.0001**
BSF Digested vs Non-Digested	0.0	0.9	-2.1	2.1	1.0000
Butanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	57398.1	28699.0	2650.9	<**0.0001**
Error	24	295.8	10.8		
C. Total	26	57657.9			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	146.7	2.1	141.4	152.0	<**0.0001**
Control vs Non-Digested	146.7	2.1	141.4	152.0	<**0.0001**
BSF Digested vs Non-Digested	0.0	1.3	-3.4	3.4	1.000
3-Methylbutanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	21734.5	10867.3	1260.2	<**0.0001**
Error	24	207.0	8.6		
C. Total	26	21941.5			
TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	90.3	1.9	85.5	95.0	<**0.0001**
Control vs Non-Digested	90.3	1.9	85.5	95.0	<**0.0001**
BSF Digested vs Non-Digested	0.0	1.2	-3.0	3.0	1.000
Pentanoic Acid					
ANOVA					
Source	Df	SS	MS	F Ratio	*P* Value
Treatment	2	14785.3	7392.7	1254.1	<**0.0001**
Error	24	141.5	5.9		
C. Total	26	144926.8			

Table 2.10 (Continued)

TUKEY HSD					
Level	Differences	Std Err Diff	Lower CL	Upper CL	*P* Value
Control vs BSF Digested	74.5	1.6	70.5	78.4	**<0.0001**
Control vs Non-Digested	74.5	1.6	70.5	78.4	**<0.0001**
BSF Digested vs Non-Digested	0.0	1.0	-2.5	2.5	1.000

Treatment and feed rate did not significantly ($F_{1,18} = 0.97$, $P = 0.3385$) interact as it related to 4-methylphenol. Furthermore, neither trial ($F_{1,22} = 1.0$, $P = 0.3282$) nor feed rate ($F_{1,22} = 1.0$, $P = 0.3282$) were significant either and were therefore excluded from the analyses. Amount of 4-methylphenol production across manure types was significantly different ($F_{2,24} = 156.9$, $P \leq 0.0001$). The level of 4-methylphenol production between BSF digested and non-digested was not significantly different ($P = 0.9984$) while the differences between both the control and BSF digested and the control and the non-digested were significant to a level of $P \leq 0.0001$. The mean amount of 4-methylphenol was reduced by 100% in the BSF digested manure and 99.79% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,18} = 0.96$, $P = 0.3385$) interact as it related to indole. Furthermore, neither trial ($F_{1,22} = 1.0$, $P = 0.3282$) nor feed rate ($F_{1,22} = 1.0$, $P = 0.3282$) were significant and were therefore excluded from the analyses. Amount of indole production across manure types was significantly different ($F_{2,24} = 17.6$, $P = \leq 0.0001$). The level of indole production between BSF digested and non-digested

was not significantly different (P = 0.9237) while the differences between both the control and BSF digested and the control and the non-digested were significant to a level of $P \leq$ 0.0001. The mean amount of indole was reduced by 100% in the BSF digested manure and 95.77% in the non-digested manure.

Treatment and feed rate did not significantly ($F_{1,18}$ = 0.97, P = 0.3385) interact as it related to 3-methylindole. Furthermore, neither trial ($F_{1,22}$ = 1.0, P = 0.3282) nor feed rate ($F_{1,22}$ = 1.0, P = 0.3282) were significant either and were therefore excluded from the analyses. 3-methylindole production across manure types was significantly different ($F_{2,24}$ = 77.5, P = $\leq$ 0.0001). The level of 3-methylindole production between BSF digested and non-digested was not significantly different (P = 0.9998) while the differences between both the control and BSF digested and the control and the non-digested were significant to a level of $P \leq$ 0.0001. The mean amount of 3-methylindole was reduced by 100% in the BSF digested manure and 99.89% in the non-digested manure.

The select volatile fatty acids examined (propanoic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid and pentanoic acid) were only present in the control manure and not detected (i.e., 100% reduction) in either the BSF digested or the non-digested samples and thus statistically significant (Table 2.7).

Discussion

I was able to determine that colonization and digestion of manure by BSFL reduces the emissions of select odorous VOCs across all manure types and feed rates compared to amounts present in the control manure. The black soldier fly was adept at reducing select volatile emissions at rates of 87% or greater. In the case of 4-methylphenol, indole, skatole and the five fatty acids examined, no levels of compounds were detected in either digested swine or dairy manure (i.e., 100% reduction).

This ability of BSFL to reduce VOC emissions, particularly those associated with the offensive smell of manure, demonstrates another added benefit to using these larvae as a manure management tool in addition to the benefits presented in previous studies such as reductions in dry matter (Sheppard 1994), nitrogen and phosphorus (Myers et al. 2008) and pathogenic Gram-negative bacteria (Erickson et al. 2004; Liu et al. 2008). With black soldier flies manure is rendered into a substance that is more environmentally and human health friendly.

The reduction of select odorous VOCs is believed to be in part due to a decrease of moisture. VOC emissions are heavily dependent on environmental factors such as moisture content, pH, temperature, O_2 availability, and biodegradability of a substrate (Brinton 1998).

Moisture content data (Table 2.1) indicates that both treatments were lower in moisture than the freshly-thawed manure. In the low feed rate, BSFL reduced moisture the greatest in dairy manure (87.13%), followed by poultry (82.61%) and swine (81.69%). Lower levels of reduction were seen in the higher feed rate. Dairy manure was reduced

the greatest by 54.25% followed by swine (50.20%) and poultry (45.63%). The non-digested manures saw similar trends in manure types with dairy manure moisture reduced the greatest followed by swine and poultry in both 18 g (87.64, 83.87 and 66.47%, respectively) and 27 g feed rates (55.62, 41.00 and 31.40%, respectively) with reduction being greater in the lower feed rate.

This change in moisture by manure type was expected due to the longer larval development times seen in swine and dairy manure compared to poultry. For example, time to 90% pupation significantly different among manure types at both the 18 g feed rate ($F_{2,33} = 30.61$, $P \leq 0.0001$) and the 27 g feed rate ($F_{2,33} = 21.92$, $P \leq 0.0001$). For both 18 g and 27 g feed rates, poultry larvae reached 90% significantly quicker than swine ($P \leq 0.0001$, $P \leq 0.0001$) and dairy ($P \leq 0.001$, $P = 0.0021$). Dairy and swine did not reach 90% pupation at significantly different rates in the 18 g feed rate ($P = 0.8121$) but did in the 27 g feed rate ($P = 0.0176$). On average, poultry larvae reached 90% pupation 46.50 and 44.23% more quickly than swine and dairy manure, respectively, at the 18 g feed rate. For the 27 g feed rate, poultry developed 27.89 and 40.81% more quickly on swine and dairy manure, respectively. This data is supported by previous studies where larvae fed pig manure developed slower and were smaller compared to larvae feed a control of poultry feed (Zhou et al. 2013). This long larval development time was believed to be to due to the lower nutritional quality of the manure (i.e. calories, protein and fat content) when compared to the other feed treatments such as kitchen waste. Oonincx et al. (2015) observed longer development times with larvae fed poultry, swine and cow manure compared to a control diet of chicken feed (144-215 vs 20-d).

In this study, the increased development times observed in swine and dairy manure gave larvae more time to reduce the moisture content via digestion and aeration. Similarly, because VOCs were taken at 90% pupation, corresponding controls for these manure types sat for longer periods of time than either feed rates of poultry manure and dried out extensively in the environmental chamber. Thus, the dairy and swine manure, especially at the lower feed rates, had even higher reduced moisture contents in both the digested and non-digested treatments than poultry manure. The greater level of moisture reduction in these manure types and feed rates could therefore be the reason that compounds such as a volatile fatty acids (VFAs) are completely absent from both swine and dairy manure samples compared to present in the poultry manure samples. However, in some instances, the BSFL were more adept at reducing VOCs than manure without larvae. For example, the lower moisture content of BSF digested manure compared to non-digested manure in the higher feed rate could be the reason we see a complete reduction (i.e. 100%) of certain compounds such as 2-methylpropanoic acid in poultry manure and phenol, 4-methylphenol, and indole in swine manure compared to higher amounts present in the non-digested manure (Tables 2.4 and 2.5).

Moisture data aligned with previous studies, which demonstrated other saprophagous species' abilities to decrease the moisture content of manure. For example, larval feeding by *Musca domestica* (L.) (Diptera: Muscidae) decreased the moisture of poultry manure by as much as 89% (Barnard et al. 1998) compared to reductions ranging from 81.69-87.13% in the lower feed rate of this study.

The ability of the black soldier fly to reduce the moisture content of the substrate on which it feeds, particularly manure, impacts microbial populations which whose are responsible for generating many odorous VOCs (Zhu 2000). Tate (1978) determined survival of *E. coli* to be greatest in organic soils that were under flooded conditions and Hagedorn et al. (1978) similarly determined *E. coli* populations to be highest in a water table following a major rainfall. Similarly, *Streptococcus faecalis* degrades rapidly under low soil moisture conditions (Kibbey et al. 1978). Moisture content of manure and the effect on bacteria survival has been less thoroughly studied, however, Wang et al. (1996) suggested dehydration of manure could contribute to the die off *E. coli* O157:H7 and observed this with fecal *Streptococci* spp. in a later study (Wang et al. 2004). Another study examining the survival of *E. coli* O157:H7 and *Salmonella typhimrum* in cow manure and slurry observed a similar effect with these microbes persisting in wastes with higher moisture contents (Himathongkham et al. 1999).

A reduction in microbial populations in manure digested by the BSFL could also contribute to reduction in targeted VOCs. Lalander et al. (2013) saw a 6 $\log_{10}$ reduction in *Salmonella* spp. in human feces with BSFL digestion compared to a less than 2 $\log_{10}$ reduction in the control feces, which had no larvae. Poultry manure inoculated with *Salmonella enterica* serovar Enteritidis that was digested by BSFL experienced pathogen populations 2.5 log lower than control samples without larvae after three days (Erickson et al. 2004). Lalander et al. (2015) observed a 10^7 CFU g^{-1} to below the detection limit of 1 CFU g^{-1} reduction in *Salmonella* spp. in compost reactors that were supplied a mix of human and pig manure, and organic wastes and allowed to be digested by BSFL. Liu et

al. (2008) inoculated dairy manure with *E. coli* and observed that larvae were successful at reducing the pathogen loads in the manure across all treatments; however, their ability to reduce the pathogen was affected by the amount of manure they were provided and the temperature at which the experiment was conducted. The greatest reduction of *E. coli* occurring with a manure amount of 50 g at 27°C which after 72 h went from 7.0 log CFU g^{-1} to 0.23 ± 3.39 log CFU g^{-1}.

Reduction in microbial populations will reduce many odors associated with manure, as VOCs are the coproducts of different metabolic pathways, which serve as waste products or potential signals for the microbes (Mayrhofer 2006). Bacteria from several genera are responsible for many of the odorous compounds found in manure (Mackie et al. 1998). *Streptococcus* spp. are capable of producing ammonia, five of which have been found in swine manure (Russell 1979). *Peptostreptococcus* spp. will metabolize peptone and AA into VFAs including acetic, formic, propionic, caproic, isobutryic, isovaleric, and isocaproic acids (Mackie et al. 1998). Other bacteria in genera such as *Eubacteria, Lactobacillus, Escherichia, Clostridium Propionibacterium, Bacteroides and Megasphaera* are all responsible for the production of odorous compounds such as VFAs, indole and sulfur containing compounds all of which can contribute significantly to odor (Mackie et al. 1998; Zhu, 2000; Le et al. 2005).

A study by Mayrhofer et al. (2006) found positive correlations between the production of VOCs and the enhancement of microbial growth and conversely negative correlations between microbial inhibition and VOC emissions during the breakdown of household biowastes over a course of 16-d (Mayrhofer et al. 2006). This relationship

between microbes and specific VOC emissions has been demonstrated by several authors (Senecal et al. 2002, Lechner et al. 2005, Jünger et al. 2012) with the hopes of uniquely identifying human bacterial pathogens from specific volatile fingerprints and therefore circumventing biases, which may arise from DNA extraction procedures (Mayrhofer et al. 2006). These studies all support the idea that the microbial structure of manure would affect VOC emissions.

This study should be conducted at industrial scale to determine if the results are consistent. Diener et al. (2009) indicated the challenges that may be faced when trying to take a bioconversion system with the black soldier fly to a larger scale which included altered environmental conditions (e.g. humidity, solar radiation, different predator/prey interactions), system operability and design issues that could lead to potentially problematic contents such as heavy metals and pathogens (Diener et al. 2009). For example, mass production of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) for biological control has proven unsuccessful in many countries such as Australia and the United States compared to the mass rearing success seen in the silkworm industry of China, despite successful small scale laboratory rearing (Consoli et al. 2010). Efforts to eradicate the New World screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) through sterile release programs have warranted the successful mass rearing of these insects, however, Babilonia and Maki (1991) noted that the requirements for a stable rearing environment in artificial rearing systems can be met only in varying degrees but never entirely. Screwworm rearing plants in Mexico observed only a maximum rearing efficiency of 60 to 70%, a decreased efficiency attributed to quality and

quantity of diet, rearing temperature and relative humidity, and higher levels of physical handling than would occur with natural insect densities (Babilonia and Maki, 1991). Despite this, Sheppard et al. (1995) determined black soldier fly production was scalable from the laboratory to the field. They witnessed an increased in efficacy of manure-to-feedstuff conversion from 1-6 to 7-8% when they increased the size from 11 to 230 hens per basin indicating that the black soldier fly may favor larger scales.

Additional limitations of my study were in part due to the availability of fresh manure, which would have been ideal to work with because fresh manure would more closely resemble field or commercial operation conditions that this research aims to be applied to. Additionally, black soldier flies are posited to develop optimally on fresh manure (Sheppard 1983). Despite this, working with fresh manure daily was not feasible due to the distance of the farms from which it was collected.

The freezing of the manure may have had effects on the microbial structure of the manure. Previous studies have indicated that the microbial structure of manure differs with different freezing temperature. For example, a study by Kudva et al. (1998) found that survival of *Escherichia coli* O157:H7 in bovine manure was dependent on manure storage temperatures. Because many manure odors are microbial in origin (Le et al. 2005), a change in the microbial structure of the manure may have had effects on the volatile emissions of the manure as it subsequently was thawed and allowed to decompose or be digested. However, because all manure types were treated the same way we do not propose differences seen between manure types to be only due to manure freezing. The freezing of manure could, however, explain the trial effect observed as the manure used in second

trial was frozen for two months longer than that of the first trial. Georgsson et al. (2006) found that the amount of *Campylobacter* on broiler carcasses was significantly ($P > 0.05$) different on those frozen at 7°C for 31 d compared to 73 d using the most probably number technique with Preston broth. This difference in bacteria populations was not seen, however, in carcasses frozen at 22°C. However, while the complex profile of volatiles differed between trials, the amounts and changes of select odorous volatile compounds did not differ, and the effects seen in both the BSF digested and non-digested manure were the same between trials.

Feed rates used in this experiment were based off those used in Myers et al. (2008). They employed four feed rates (27, 40, 54 and 70 g/day) and two significant ones were chosen (54 and 70 g/day) and reduced by approximately two thirds to produce the ones used in this study (18 and 27 g/day). Initially, a preliminary study was conducted using lower feed rates (4.5 and 9.0 g/day), however, after 100 days it was established that these feed rates were not sufficient to carry out larval development and newer, higher feed rates were chosen. The limitations are due to Myers et al. (2008) only examining the ability of BSFL to reduce dairy manure versus this study, which also examined swine and poultry manure. Feed rates used in my study possibly were not optimal for larval development, waste reduction, and therefore VOC reduction across all three manure types. Further studies should examine the effect of different feed rates in an attempt to optimize the system based on the goal and application of the research whether it is to maximize waste reduction or VOCs or to optimize larval development.

Future studies should examine different treatments (e.g., storage method and temperature) of manure and how this affects the digestion and subsequent VOC emissions. Yasuhara (1983) saw a difference in volatile profiles from samples of fresh poultry manure, manure that was immediately frozen and manure was allowed to age before being frozen. Husted (1993) found that methane production from dairy manure was increased when stored at higher temperatures. Gibson et al. (1987) noted that different temperature regimes affected the growth of *Clostridium botulinum* spores in pork slurry. Therefore, it is reasonable to hypothesize that different temperatures regimes could affect the microbial community of manure and therefore alter VOC emissions.

CHAPTER III

OVIPOSITION AND ATTRACTION ASSAYS: RESEARCH, RESULTS, AND DISCUSSION

Introduction

Insects are highly adapted to detect a wide range of both volatile and soluble chemicals in an environment (Dethier 1948). Compared to gustatory receptors, which react to higher concentrations of liquids and solutions, olfactory receptors respond to very low concentrations of compounds, which are volatile at regular temperature (Dethier 1948). The most-studied method of insect chemoreception is olfaction as many insects are sensitive to odors that relay different information about resource availability, habitat suitability, potential predators, prey and mates to name a few (Price et al. 2011). Additionally, many insects utilize chemical signaling produced by other organisms for their benefit (Endler 1993). This "eavesdropping" is widespread among animals. For example, many parasitoids will exploit sex pheromones of their hosts as a means to locate them (Vinson 1984).

Volatile emissions have been shown to serve as cues to communicate different information between insects. Often, volatile organic compounds (VOCs) are the mechanism behind the initiation of colonization of an ephemeral resource (Benbow et al. 2015). These emissions can be derived from a variety of sources, such as decomposing plants, animals or associated microbes.

Livestock manure is an ephemeral resource that is home to many different types of microbes (Zhu 2000). Microbial activity is an important part in the process of manure decomposition with VOCs intermediate and end-products (Zhu 2000). Microbes play an important role in terms of insect interactions; however, much previous research has been focused within the context of a certain role (e.g., nutrient recyclers and biological control). Nevertheless, many volatiles that are microbially derived play significant roles within an ecosystem and especially within ephemeral resource ecology.

Microbes have long been recognized for their responsibility regarding arthropod colonization of resources. Many insects are sensitive to odors that relay information about resources, potential mates, and habitat suitability (Price 2011). Microbial volatile organic compounds (MVOCs) can play a role in oviposition and its site selection for many insects including dipterans (Davis et al. 2013).

Many dipteran species have expressed semiochemical related oviposition behavior as it relates to odors from ephemeral sources like decaying organic material in the form of carrion and animal wastes. For example, it has been observed that blow fly (Diptera: Calliphoridae) oviposition is stimulated by the ammonia production that occurs during bacterial putrefaction (Holdaway 1930, Seddon 1931). Lindh et al. (2008) found that water-containing bacteria stimulated oviposition by the mosquito *Anopheles gambiae* (Giles) (Diptera: Culicidae), while Huang et al. (2006) demonstrated that cultured bacterial volatiles in agarose media deterred gravid mosquitoes of the same species. Chaudhury et al. (2002) found that blood inoculated with bacteria released different VOCs

and attracted gravid *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) and stimulated oviposition more so than uninocluated control blood.

Specifically, as it pertains to manure VOCs, attractiveness has also been studied in several dipteran species. A study conducted by Jeanbourquin and Guerin (2007) found that the stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae) used olfactory cues dimethyl trisulphide, butanoic acid and 4-methylphenol to locate suitable substrates for oviposition at a distance. In Turkey, Krčmar et al. (2009) found that traps baited with donkey urine, lactic acid, and fresh human urine collected 12, 4 and 2.5 times as many tabanids, respectively, than the unbaited control traps (Krčmar et al. 2009). Hobson (1936) found that attraction for *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) to putrefying substances was largely due to the presence of indole and skatole - two compounds associated with manure and responsible for its characteristic fecal smell (Burnett and Dondero 1969). These compounds were attractive to *L. sericata* and stimulated oviposition when diluted and applied to live sheep fleece.

The black soldier fly (BSF), *Hermetia illucens* (L.), (Diptera: Stratiomyidae) is a temperate and tropical species (James 1935) reaching 18-20 mm in length that acquires the nutrients necessary for life during larval development (Tomberlin 2002). Adults, which therefore do not need to feed, do not compete with humans for food resources. This and other added benefits have earned them the label of non-pest species. Such benefits include their ability to decompose a wide variety of decaying organic matter including human (Banks 2014) and livestock manure (Bradley and Sheppard 1984, Myers et al. 2008). Larval digestion of manure reduced moisture content and dry matter by 50% or

more (Newton et al. 2005). Additional benefits include the reduction of nitrogen and phosphorus by as much as 30-50% and 61-70%, respectively, as well as reductions in pathogens such as gram-negative bacteria (Erickson et al. 2004, Liu et al. 2008). Furthermore, manure colonized by BSFL in dense proportions see anywhere from significant reduction to a complete absence of house fly populations (Furman 1959, Kilpatrick and Schoof 1959, Bradley and Sheppard 1984).

How volatiles associated with decomposing manure and animal wastes affect the behavior and oviposition preference of the black soldier fly is currently unknown; the objective of this study was to examine how these compounds extracted from black soldier fly larval digested and non-digested manures impact adult BSF attraction and oviposition. A reduction in noxious odors with increased adult attraction and oviposition would provide an added benefit to using these insects as an alternative means of manure management in addition to providing insight into the potential mechanisms that drive these insects to colonize this resource.

Materials and Methods

Volatile Sampling

Volatile samples used in attraction and oviposition assays were collected using methods outlines in Chapter II. Volatiles from this chapter were collected in triplicate with the first set used for GC-MS analysis and the second and third replicates used for behavior work.

Acquisition of Flies

Hermetia illucens larvae used in this experiment originated from a colony maintained at the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University in College Station, TX. This colony was established in 2014 from eggs received from a laboratory colony maintained at the Coastal Plains Experiment Station, University of Georgia, Tifton, GA. Adult flies were maintained in a 2.6 × 1.2 × 1.3 m wooden cage fitted with metal screening in a greenhouse maintained at approximately 27° ± 1.34 C. Adults were allowed to oviposit on three 7.0 × 5.0 × 0.3 cm pieces of corrugated cardboard (Booth and Sheppard 1984) held together with masking tape and placed on the lid of a 30.0 × 15.0 × 11.0 cm plastic shoe box containing one kilogram of Gainesville diet (Hogsette 1992) saturated with water. A 13.0 × 5.0 cm portion of the lid was removed and replaced with metal screening on which the cardboard pieces were placed; this allowed volatiles to escape from the wet Gainesville diet and attract the flies, but prevented the flies from contacting and/or ovipositing directly into the media instead of the cardboard. The cardboard was removed from the cage after eight hours and eggs were removed from cardboard using a sterile plastic spatula and weighed. One gram of eggs were placed in a 0.5 L plastic container, covered with a paper towel secured with a rubber band, stored in a walk-in environmental chamber (30˚C, 60% RH, and 16:8 L:D) and checked every 12 h until hatch. Two hundred grams of Gainesville diet at 70% moisture was added to the container once larvae emerged. Larvae were raised on the Gainesville diet and fed *ad libitum* until 40% reach pupation at which time feeding ceased.

Larvae were maintained in the walk-in environment until adulthood. Adults, ages 24 h to 6-d-old were used in the assays.

Oviposition and Attraction Assays

Figure 3.1. Cage used for oviposition and attraction assays June-July, 2016 at the F.L.I.E.S. Facility in College Station, TX. Cups with cardboard were placed inside the cages. Eluate from BSF digested, non-digested manure, and two controls were applied to the cardboard. Adults were allowed to oviposit for eight hours on treatments after which egg masses were collected and weighed.

The two samples of eluate from each manure sample not used in the GC-MS analysis were used in two trials of oviposition and attraction assays. A 3.05 × 1.82 × 1.82 m cage was set up outside the F.L.I.E.S. Facility in late June and July, 2016 (Figure 3.1. The four corners were placed 19 L buckets (Home Depot®) that had been sterilized prior to use and secured using approximately 18 kg of sand. Approximately 5,000 adult flies, with a 50:50 sex ratio, were released within the cage over the course of five days to allow for mating and oogenesis to occur (Tomberlin and Sheppard 2002). During this time, adults were misted with water approximately three times per day in order to improve adult survivorship (Tomberlin et al. 2002). On the following day, three rows of eight 473 ml drinking cups were laid out in the tent in a randomized block design (Figure 3.2). Each cup, outfitted with approximately 80 g of sand and three pieces of cardboard, as previously described, taped to the inside approximately 4 cm below the lid had 150 µL from the volatile extractions of one of six treatments or two controls applied to it. The six treatments were as follows: A replicate of each treatment when approximately 90% of the larvae had reached the prepupal stage for each of the three manure types and its corresponding non-digested replicate for each of the three manure types. The two controls consisted of: one control of plain solvent, dichloromethane, which was used in the eluting process and an additional negative control which consisted of plain cardboard that had nothing applied to it. Treatments were applied to the cardboard with a pipette approximately 30 minutes

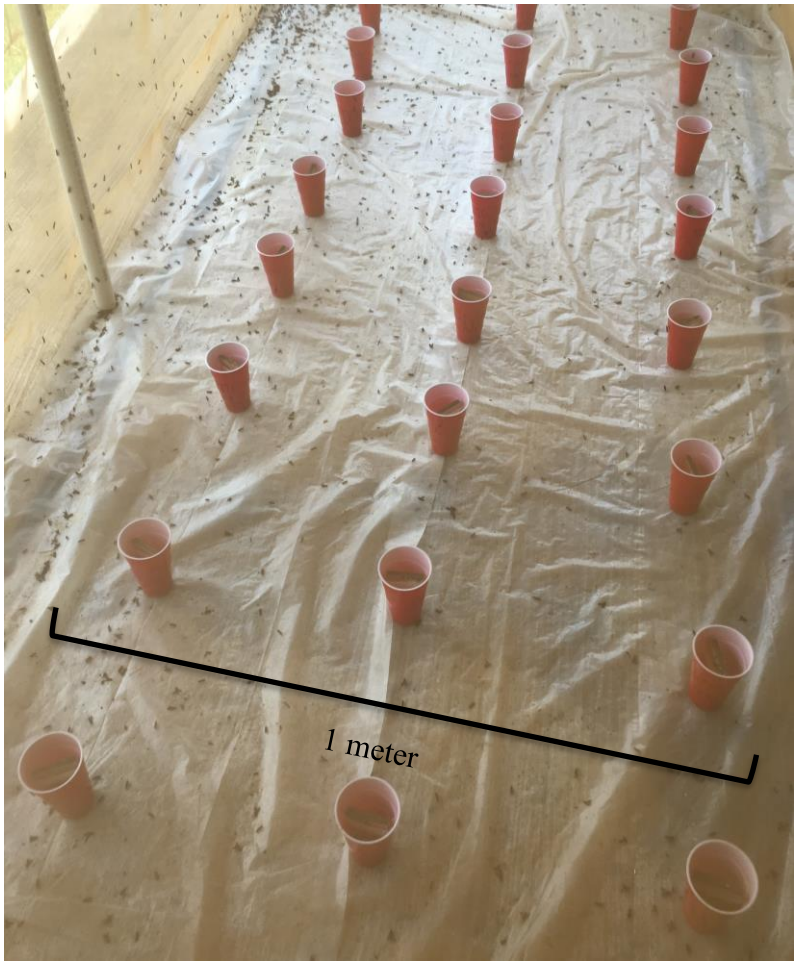

Figure 3.2. Block design of cups within cage examining adult oviposition and attraction June 19th, 2016. Three pieces of cardboard, to which the eluate was applied, were taped approximately 4 cm below the lid inside each cup. Different treatments included three replicates of digested manure at 90%, its corresponding non-digested replicate and two controls (dichloromethane and negative control with no eluate applied). Experiments were run from 0800 – 1700.

prior to the initiation of the experiment. Treatments and controls were assigned a position within the row at random with each row having one of each treatment and control.

Experiments were conducted from 8:00 am to 5:00 pm on the sixth day after adult emergence, during which adult attraction was assessed at every hour by counting the number of adults on the oviposition substrate (cardboard), or the cup (both inside and outside). At the conclusion of each trial, the cups were removed and the cardboard was inspected for egg masses which were subsequently weighed using a Scout® Pro Balance (Ohaus, Parsippany, NJ). Each experiment was replicated twice with trials for each of the two feed rates being carried out separately.

Statistical Analyses

Differences in oviposition between treatments for behavioral assays were determined using the proportion of eggs laid per treatments. Differences in oviposition were examined using a one-way analysis of variance (ANOVA) using JMP Pro 12 statistical software (SAS Institute, Cary, NC, USA). Significant differences in means were further separated using Tukey-Kramer Honest Significant Difference (HSD) ($P \leq 0.05$). Differences in attraction were also determined using a one-way ANOVA to assess differences in the number of adults landing on each treatment. Similarly, Tukey's Honest Significance Difference (HSD) test was used to determine differences between treatments ($P < 0.05$).

Research Results

Oviposition and Attraction Assays

There were no eggs laid on any of the treatments or controls in any of the trials. With regards to attraction, treatment and trial did not significantly ($P > 0.05$) interact in either the 18 g trials ($F_{1,7} = 0.4$, $P = 0.8743$) or the 27 g trials ($F_{1,7} = 0.4$, $P = 0.8743$). Furthermore, treatment was not significant in either the 18 g trials ($F_{7, 40} = 0.6$, $P = 0.7281$) or the 27 g trials ($F_{7, 40} = 0.6$, $P = 0.7608$).

During the trials, adult flies were observed to congregate heavily in both the northeast and southeast corners of the cage, which received significant sunlight during the day. Therefore, data were analyzed for quadrant and positional effects by breaking the

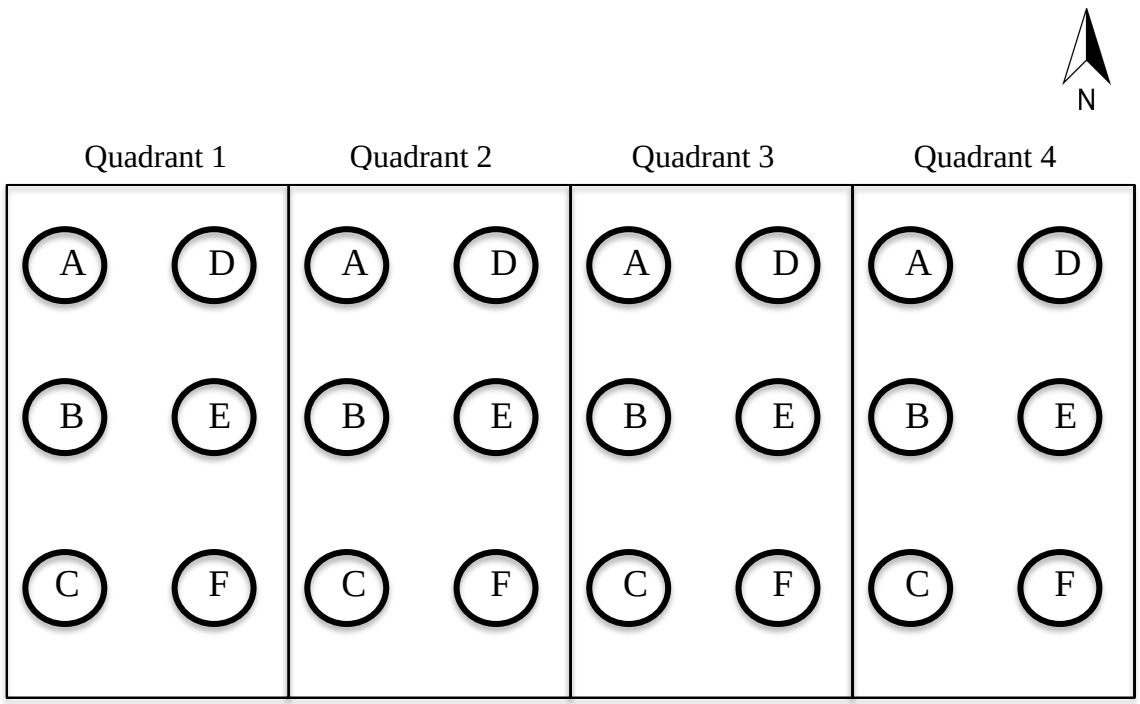

Figure 3.3. Cage used for oviposition and attraction assays June-July, 2016 at the F.L.I.E.S. Facility in College Station, TX broken down and examined for positional effects by quadrant and position within each quadrant. The cage was divided into four quadrants east to west and each cup within a quadrant given a position A-F.

cage into four quadrants from east to west with each quadrant having six positions assigned letters A through F (Figure 3.3). A significant quadrant effect was not observed for the 18 g trials ($F_{3, 44} = 1.3$, $P = 0.2873$) but was observed in the south most quadrant ($F_{3, 44} = 4.6$, $P = 0.0072$) for the 27 g trials (Table 3.1). A significant position effect was observed in the 18 g trials ($F_{5, 42} = 7.4$, $P \leq 0.001$) with positions C and F being significantly different from the other letters (Table 3.2). Position was not significant for the 27 g trials ($F_{5, 42} = 1.5$, $P = 0.2100$). A treatment effect was then re-examined with the significant quadrant removed for the 27 g trials and the significant positions removed from the 18 g trials. With these modifications, treatment was still not a significant effect for either the 18 g trials ($F_{7, 24} = 0.5$, $P = 0.8130$) or the 27 g trials ($F_{7, 26} = 0.6$, $P = 0.9826$)

Table 3.1. Mean number ± SEM of adult *Hermetia illucens* (L.) per oviposition quadrant across two trials under field conditions. Each quadrant contained cups outfitted with three layers of corrugated cardboard which was treated with VOCS isolated from manure provided at two different feed rates to larval black soldier flies.

Quadrant	18 g	27 g
1	11.33 ± 3.76a[1]	71.25 ± 22.23a
2	5.58 ± 1,12a	25.75 ± 5.71b
3	6.33 ± 1.36a	15.83 ± 3.73b
4	7.83 ± 1.69a	21.00 ± 5.40b

[1] Different letters within a subset of a column indicate significant difference ($P < 0.05$).

Table 3.2. Mean number ± SEM of adult *Hermetia illucens* (L.) per oviposition position across two trials under field conditions. Each position represents a cup outfitted with three layers of corrugated cardboard which was treated with VOCS isolated from manure provided at two different feed rates to larval black soldier flies.

Position	18 g	27 g
A	3.50 ± 1.09c[1]	15.63 ± 5.74a
B	4.25 ± 1.19bc	36.13 ± 11.43a
C	17.00 ± 4.08a	57.63 ± 24.25a
D	2.87 ± 0.55c	10.25 ± 3.12a
E	6.13 ± 1.52bc	27.88 ± 9.53a
F	12.88 ± 2.37ab	53.25 ± 25.49a

[1] Different letters within a subset of a column indicate significant difference ($P < 0.05$).

Discussion

No eggs were laid on any treatments during any trials of the assays, therefore, it can be suggested that volatiles from the treatments were not sufficient to stimulate an oviposition response in BSF adults. This lack of response may have occurred due to several factors.

Both digested and non-digested manure types had considerably lower moisture contents than their fresh counterpart (Table 2.1 from Chapter II). The freshly-thawed manures had average moisture contents of 77.25, 73.77, and 84.16% for poultry, swine and dairy, respectively. BSFL digested manure had moisture contents of 25.90, 13.50, and 10.83% and 42.00, 36.74, and 38.50% in the low and high feed rates for poultry, swine and dairy manure, respectively. This is compared to non-digested manure, which had average moisture contents of 13.43, 11.90 and 10.40% and 52.99, 44.52, and 37.35% for the low and high feed rates of poultry, swine and dairy manure, respectively.

As demonstrated here, previous studies have shown that larval fly digestion reduces the moisture content of manure anywhere from 48 to 89% (Miller et al. 1974, Barnard et al. 1998). Black soldier fly digestion is capable of rendering the manure into a drier substance that is a less suitable habitat for microorganism such as bacteria responsible for volatile production. For example, many strains of *Escherichia coli* (90-100%) produce indole, a very odorous compound in manure (Zhu 2000). Furthermore, indole is a known fly attractant for species like the house fly, *Musca domestica* (L.) (Diptera: Muscidae), (Mulla et al. 1977) and *L. sericata* (Hobson 1936). Studies have

demonstrated that digestion of manure by BSFL reduces populations of *Escherichia coli* O157:H7 and *Salmonella enterica* in poultry manure and *E. coli* in dairy manure (Erickson et al. 2004, Liu et al. 2008).

Different bacteria strains require different moisture contents, temperatures and pH levels to survive and replicate in a substrate. BSFL digestion is capable of changing these factors in manure, such as raising temperatures, shifting manure pH from neutral to alkaline, and decreasing moisture (Čičková 2015). All of these factors affect the suitability of manure as a habitat for VOC producing microbes. For example, *E. coli* loads in manure are temperature dependent with the organism surviving longer in manures of lower temperatures (5°C) compared to those of higher temperatures (22°C and 37°C) (Wang et al. 1996). Another study indicated that the pathogen's survivorship increases in nonaerated ovine versus aerated ovine manure from 4 to 21 months, respectively (Kudva et al. 1998).

Additionally, several bacteria species that are responsible for malodor and general VOC production are obligately anaerobic and therefore their fate in aerobic conditions is limited (Zhu 2000). For example, certain *Eubacteria* and *Clostridium* species are capable of producing large amount of volatile fatty acids and indole, which both contribute significantly to odor in manure but are obligate anaerobes.

Another genera of bacteria, *Lactobacillus,* which produces lactic acid, a known attractant for culicids (Acree et al. 1968) and glossinids (Vale 1979), grows best in slightly acidic media (pH 4.5-6.4) with growth rates reduced when an environment becomes neutral or alkaline (Zhu 2000). This and other bacteria have optimal pH ranges that are

often are neutral or slightly acidic, making the alkaline conditions of BSFL rendered manure unfavorable.

In summary, changes in moisture, temperature and pH levels, whether driven by BSFL digestion or not, are capable of alternating the environment of manure and rendering it into a less suitable habitat for microorganisms producing VOCs that are known fly attractants.

Other factors could be at play beyond a complete absence of chemical cues needed for oviposition i.e. a lack of or inadequate chemoreception. For example, volatiles alone are not enough and that other conditions are necessary for certain responses. For example, a study by Larsen et al. (1968) examined the olfactory and oviposition responses of the house flies to different manure types. They found that manure with air passed over the surface was not effective at eliciting positive responses from house flies inside the olfactometer. However, the authors found that the odor source, when taken from air passed through a mixture of manure and water, was effective at stimulating positive responses from adult flies. The authors concluded that while odor from manure appeared to be strong, constant moisture may be required to maintain concentrations high enough for effective detection by flies and to compensate for the drying out effect that the constant passage of air over the manure possibly had (Larsen et al. 1968).

Olfactory stimulation is a major factor involving the attraction of house flies which seek to sate natural drives of hunger and oviposition (Crumb and Lyon 1917); however, Larsen et al. (1968) also noted that olfactory stimuli alone cannot be used as criterion for most-favorable oviposition sites. For example, it is known that moisture of a substrate can

play a role in the oviposition of adult black soldier flies. Fatchurochim et al. (1989) observed BSF oviposition in different poultry manures with moisture contents ranging from 20-90% and found the majority of oviposition to occur in manure with moisture contents of 40-70%. The authors concluded that little fly oviposition and larval development would be expected from manure with moisture contents less than 40% (Fatchurochim et al. 1989).

Detection of volatiles by insects also requires that a threshold of response be met, i.e. that the compound exists in concentrations high enough for the insect to perceive it. For example, *Geotrupes sylvaticus* (Panzer) (Coleoptera: Geotrupidae) has a threshold of response of 0.003-0.009 mg/L (Warnke 1931) to skatole. If volatiles from manure have been reduced through black soldier fly digestion, it is possible that thresholds of response are not being met to such a level to elicit oviposition responses.

Olfactory thresholds of response are significantly influenced by environmental factors such as temperature and humidity (Dethier 1948). Furthermore, in behavioral studies, responses are based off the locomotion of insects whose overall activity is influenced by temperature and humidity (Fraenkel and Gunn 1961). A study by Tomberlin et al. (2009) examined the development of the black soldier fly in relation to temperature and found that adult survival was better at 27 and 30°C with 74-97% adult survival when compared to 36°C, with only 0.1% adult survival. The paper illustrated temperature differences of only 3°C are capable of significantly influencing the life history of BSF. Another study found that factors such as sunlight, time of day, temperature, and humidity significantly ($P < 0.0001$) correlated with oviposition (Tomberlin and Sheppard 2002).

Behavioral assays were conducted from late June to the third week of July in Texas with temperatures on experiment days averaging 30.27 ± 1.87°C with highs averaging 35.55 ± 2.20°C and lows averaging 24.44 ± 1.55°C. The average relative humidity was 67.50 ± 2.10%. Holmes et al. (2012) examined the effects of different relative humidity levels (25, 40 and 70%) on adult BSF longevity at 25°C and noted that adult longevity was highest with the highest at 70% RH. While the humidity of the ambient temperature may not have had deleterious effects on adults, the high ambient temperatures of the outside environment could have affected the adults in a way previously not encountered as behavioral experiments are often conducted inside or within greenhouses with regulated temperature parameters.

Finally, the small amount of eluate, 150µL, applied to the cardboard may not have been adequate to elicit a response and is a limitation of the study. In previous studies that examined behavioral responses of flies to manure volatiles, larger amounts were used. For example, Cossé and Baker (1996) formulated their odor sources in 10 ml of water in a Petri dish for wind-tunnel behavioral assays and experiences positive responses. Krčmar (2007), whose traps were baited with 4-methylphenol captured 16 times more tabanids than unbaited traps, used 4 ml of bait in his study. However, previous studies which employed an electroantennographic detector were capable of eliciting antennal responses from house flies and stables files with as little as 10 and 100µL, respectively (Cossé and Baker 1996, Jeanbourquin and Guein 2007). While small amounts are a compound of interest are capable of eliciting some kind of response in adult flies, oviposition responses may be dependent on larger doses. The small amount used in this study was to allow

comparability with the samples collected in Chapter II to be processed via GC-MS. While changes in the design might have been implemented, the limited quantity of the resource would not permit the experiment to be replicated enough times with the new design to affirm that any results were legitimate and not simply due to a change in the experiment design

Future work should keep in mind the effects that variable weather can have on behavioral studies and consider the benefits of conducting experiments in environmentally-controlled settings. It has been noted that controlling for these factors is a key component in behavioral assays (Dethier 1948). While environmentally-controlled settings may be optimal for controlling factors such as temperature and humidity, they are often not an option in BSF studies as limited oviposition has been observed with artificial light sources (Zhang et al. 2010). Studies could also examine different aspects, such as age of adults, to see if this could be playing a role in the lack of oviposition and if perhaps response is associated with age. In this study, in order to maintain large enough numbers, adults ranging several days were used versus a shorter range. In this study, all manure types were tested together including BSF digested and non-digested manure treatments. Future studies may consider running assays one manure type at a time in a single choice assay to yield perhaps more concise results. The F.L.I.E.S. facility has continued success with their methods of laying cardboard over a contained with moistened Gainesville diet (Hogsette 1992) as a method of procuring BSF eggs. An altered experiment design that places the cardboard with VOC eluate over a similar set up may yield an oviposition response.

CHAPTER IV

CONCLUSION AND FUTURE STUDIES

I determined that digestion by black soldier fly larvae (BSFL) of poultry, swine, and dairy manures significantly reduced noxious volatile organic compound (VOC) emissions of control manure ($P < 0.05$) (Table 2.6 and 2.7). I hypothesize that the significant ($P < 0.05$) reduction in moisture content of both BSF digested and non-digested manure (Table 2.1) contributed to this reduction in odorous compounds by rendering the manure an unsuitable habitat for microorganisms responsible for producing these VOCs.

Furthermore, I determined that volatiles from manure alone are perhaps not enough to elicit an oviposition response from adults. In terms of attraction, there was no difference in attraction to any of the treatments (i.e., the BSF digested versus non-digested manure) or the controls. This was demonstrated in both trials and between both the high and low feed rates of 18 and 27 g.

Odors associated with intensive animal farming are noxious and potentially harmful to human health (FAO 2009). Those who work or live in close proximity to these farming areas suffer a decreased quality of life in the form of elevated levels of tension, anger and depression (Barrett 2006). Previous research has already presented the black soldier fly as an attractive candidate as an alternative means of manure management for poultry (Sheppard et al. 1983), swine (Newton et al. 2005) and dairy manure (Myers et al. 2008). Using this insect would provide an on-site treatment option that would be cheaper to the farmers than conventional and currently used methods. For example, Bentley et al.

(2016) calculated that the cost of storing, hauling and applying dairy manure averaged $306.13 per cow per year. Not only would this option be cheaper, but the benefits of BSFL processing of manure are numerous and their ability to lower noxious odors emitted from manure is another reason to use this fly over other dipteran species to manage the copious amounts of manure that are being produced from livestock. This amount will only continue to increase as consumer demands more meat grow (FAO 2011). The need to deal with this resource is ever growing and pressing.

Another potential of BSFL is their use as a livestock feed additive. Pupae have been found to be an adequate substitute in diets of swine (Newton et al. 1977), poultry (Hale 1973), and several aquaculture species (Bondari et al. 1981, Bondari and Sheppard 1987, Sealey et al. 2011, Kroeckel et al. 2012, St-Hilaire et al. 2007). If the commercial production of BSFL for feed purposes is to succeed, these animals will have to be grown in mass amounts to meet the demands of the animals consuming them i.e. the growing aquaculture and livestock markets (Msangi et al. 2011, Brown 2002). A key to the success of this potential industry will be to generate enough BSF eggs to produce quantities of pupae to match demands. Large yields of pupal stock will require mass amounts of egg-laying by adult black soldier flies. Black soldier flies are attracted to lay eggs on decaying organic matter (James 1935); however, the mechanism behind this attraction has not been explained. In the second objective, oviposition was not observed on any of the treatments and attraction was not significantly different on any particular treatment or on controls suggesting there are other factors, whether abiotic or biotic, at play that are required to elicit an oviposition response from adults. Once the mechanisms which drive oviposition,

perhaps in addition to volatiles, are elucidated, then the volatiles found in manure could be easily produced synthetically (Cossé and Baker 1996) and applied to a substrate in a commercial production operation. The ability to manipulate and increase oviposition would greatly aide the mass production of BSF pupae and would increase the ability for these animals to be farmed in large numbers. Future research should continue to uncover what other factors determine this response from adults such as moisture.

While the results from my study were informative, limitations were determined. First and foremost, the small scale on which my study was conducted is a limitation and the ability to translate these results to an industrial scale should be done with caution. Diener et al. (2009) indicated the challenges that may be faced when trying to take a bioconversion system with the black soldier fly to a larger scale including altered environmental condition such as humidity, solar radiation, different predator/prey interactions, system operability and design and the issue of potentially problematic contents such as heavy metals and pathogens (Diener et al. 2009). For example mass production of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) for biological control have proven unsuccessful in many countries (Brazil, France, Australia and the United States) when compared to the mass rearing success in the silkworm industry of China, despite successful small scale laboratory rearing (Consoli et al. 2010). Efforts in eradicating the New World screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) through sterile release programs have warranted the successful mass rearing of these insects, however, Babilonia and Maki (1991) noted that in artificial rearing systems the requirements for a stable rearing environment can be met only in varying

degrees but never entirely. Screwworm rearing plants in Mexico observed only a maximum rearing efficiency of 60 to 70%. This decrease in efficiency was attributed to their quality and quantity of diet, rearing temperature and relative humidity, and higher levels of physical handling that would occur with natural insect densities (Babilonia and Maki 1991). Despite this, Sheppard et al. (1995) found a large-scale commercial application to his small study on black soldier flies and their ability to digest manure of layer hens to be feasible. They witnessed an increased in efficacy of manure-to-feedstuff conversion from 1-6 to 7-8% when they increased the size from 11 to 230 hens per basin indicating that the black soldier fly may favor larger scales. Additional limitations of my study were in part due to the availability of manure. It would have been optimal to work with fresh manure each day, as this would more closely resemble the field or commercial operation conditions that this research aims to be applied to and black soldier flies are posited to develop optimally on fresh manure (Sheppard 1983), however there is so data to support this hypothesis. Regardless, the use of fresh manure was not feasible as only the poultry manure was collected locally. The swine manure came from a facility approximately 40 km away and the dairy manure came from a center approximately 250 km away. Because of this distance, the manure had to be collected beforehand and frozen prior to use in the study. The freezing of the manure may have also had effects on the microbial structure of the manure. Previous studies have indicated that the microbial structure of manure differs with different freezing temperature. For example, a study by Kudva et al. (1998) found that survival of *Escherichia coli* O157:H7 in bovine manure was dependent on manure storage temperatures. However, because all manure types were

treated the same way we do not propose differences seen between manure types to be because the manure was frozen.

The colony used for this experiment has been in use at the F.L.I.E.S. Facility since 2014, and it is possible that genetic shifts within this strain may have occurred between previous studies and the time in which this experiment was conducted. For example, Francuski et al. (2014) found a reduction in genetic diversity in *Eristalis tenax* (L.) (Diptera: Syrphidae) in the fourth and eight generations of a laboratory colony compared with earlier and natural populations. Berlocher and Friedman (1981) demonstrated a loss of genetic variation in laboratory-reared *Phormia regina* (Diptera: Calliphoridae) after a single generation. Behavior changes can also be a result of laboratory rearing and subsequent inbreeding; Waldbauer (1983) observed that parasitoids raised for many generations began to flee at the sight of their natural host.

While similar to previous findings conducted at the F.L.I.E.S. Facility, these results are still specific to this strain and different results are possible due to variations between different BSF strains. For example, Zhou et al. (2013) found a significant difference in the dry matter reduction of swine manure between a BSF strain from Wuhan, China (53.4 ± 0.3%) and two other strains from Guangzhou, China (28.8± 0.2%) and Texas, USA (49.7 ± 0.4%). However, the differences in dry matter reduction for dairy and chicken manure did not vary by strain and the differences in nitrogen reduction did not vary by strain in any manure type (Zhou et al. 2013). Furthermore, wild black soldier fly adults typically weigh more than those reared in laboratory settings due to different nutritional sources provided to larvae (Tomberlin 2002). It is therefore possible that wild

black soldier flies may then be able to digest manure differently than larvae from a laboratory maintained colony.

Feed rates used in these experiments were based off those used in Myers et al. (2008) and reduced to compensate for our use of 100 larvae compared to their 300 g. Myers employed four feed rates (27, 40, 54, and 70 g/day) and of these two significant ones were chosen (54 and 70 g/day) and reduced by approximately two thirds to produce the ones used in this study (18 and 27g/day). Initially, a preliminary study was conducted using lower feed rates (4.5 g and 9.0 g/day), however, after 100 days it was established that these feed rates were not sufficient to carry out larval development and newer, higher feed rates were chosen. While feed rates were initially based off Myers et al. (2008), their study examined BSFL feeding only on dairy manure and therefore feed rates, which were satisfactory in that study may not have been satisfactory for larvae feeding on poultry or swine manure. Therefore, it is possible that feed rates used in my study were not optimal for larval development, waste reduction, and therefore VOC reduction across all three manure types. Further studies should examine the effect of different feed rates in an attempt to optimize the system based on the goal and application of the research whether it is to maximize reduction or VOCs or optimize larval development.

Additional future studies should examine different treatments of manure and how this affects the digestion and subsequent VOC emissions. Different variables to be examined could be using different ages of manure. This study used freshly-thawed manure but subsequent ones could investigate the effect of aging the manure on VOC emissions. Yasuhara (1983) saw a difference in volatile profiles from samples of fresh poultry

manure, manure that was immediately frozen and manure was allowed to age before being frozen. Another variable to examine would be to conduct this experiment at different temperatures. This experiment was run in an environmental chamber at a constant temperature but future studies could examine the effects that a lower and higher temperature would have on the variables examined here. Husted (1993) found that methane production from dairy manure was increased when stored at higher temperatures. Zhu (1999) hypothesized that odor from swine manure was a product of volatile fatty acids and the presence of two bacterial genera, *Eubacterium* and *Clostridium*. Gibson et al. (1987) noted that different temperature regimes affected the growth of *Clostridium botulinum* spores in pork slurry. Therefore it is reasonable to hypothesize that different temperatures regimes could affect the microbial community of manure and therefore altering VOC emissions.

Furthermore, as the development of black soldier fly is temperature dependent (Tomberlin et al. 2009), different rates of digestion due to temperature shifts could affect VOC emissions observed. Similarly, future studies should examine the effect of freezing manure at different temperature to see if this would effect the volatile emissions of the manure. Finally, subsequently studies could examine the effect of different larval densities on their ability to process the manure and how that subsequently affects VOC emissions. Previous studies on other species have indicated that larval raised at different densities often have altered life history traits such as differences in pupal weight, adult emergence and weight, and mortality as demonstrated for *Musca domestica* (Sullivan and Sokal

1963), different blow fly species (Ulyett 1950) and *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) (Chiang and Hodson 1950).

REFERENCES

Aarnink, A., D. Swierstra, A. Van den Berg, and L. Speelman. 1997. Effect of type of slatted floor and degree of fouling of solid floor on ammonia emission rates from fattening piggeries. J. Agr. Eng. 66: 93-102.

Acree, F., R. Turner, H. Gouck, M. Beroza, and N. Smith. 1968. L-Lactic acid: A mosquito attractant isolated from humans. Science 161: 1346-1347.

Altunsoy, F., and M. Y. Afacan. 2014. Response of Tabanidae (Diptera) species to malaise traps baited with 1-octan-3-ol and 4-methylphenol. J. Entomol. Res. Soc. 16: 111.

American Society of Agricultural Engineers. 1989. Sulfide in wastewater collection and treatment systems. Am. Soc. Civ. Engin. 13: 4-15.

Ashworth, J. R., and R. Wall. 1994. Responses of the sheep blowflies *Lucilia-Sericata* and *L. cuprina* to odor and the development of semiochemical baits. Med. Vet. Entomol. 8: 303-309.

Axtell, R. C., and T. Edwards. 1970. *Hermetia illucens* control in poultry manure by larviciding. J. Econ. Entomol. 63: 1786-1787.

Babilonia, E., and D. Maki. 1991. Mass production of sterile New World screwworm flies in southern Mexico. World Anim. Rev. 3: 36-41.

Baldacchino, F., S. Manon, L. Puech, B. Buatois, L. Dormont, and P. Robert. 2014. Olfactory and behavioural responses of tabanid horseflies to octenol, phenols and aged horse urine. Med. Vet. Entomol. 28: 201-209.

Banks, I. J., W. T. Gibson, and M. M. Cameron. 2014. Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. Trop. Med. Int. Health 19: 14-22.

Banwart, W., and J. Bremner. 1975. Formation of volatile sulfur compounds by microbial decomposition of sulfur-containing amino acids in soils. Soil Biol. Biochem. 7: 359-364.

Barnard, D., R. Harms, and D. Sloan. 1998. Biodegradation of poultry manure by house fly (Diptera: Muscidae). Environ. Entomol. 27: 600-605.

Barrett, J. R. 2006. Hogging the air - CAFO emissions reach into schools. Environ. Health Persp. 114: A241-A241.

Bast. E., T. Hartmann, and M. Steiner. 1971. Uber eine Valincarboxylase aus Bacillus sphaericus. Arch. Mikrobiol. 79: 12-24.

Benbow, M. E., J. K. Tomberlin, and A. M. Tarone. 2015. Carrion Ecology, Evolution, and their Applications, CRC Press. Florida. 127.

Bentley, J., L. L. Timms, L. F. Tranel, G. Brenneman, K. Kohl, and R. Lenth. 2016. Economics of dairy manure management in Iowa. Anim. Indust. Rep. 36: 662.

Berlocher, S. H., and S. Friedman. 1981. Loss of genetic variation in laboratory colonies of *Phormia regina*. Entomol. Exp. Appl. 30: 205-208.

Bethea, R., and R. Narayan. 1972a. Identification of beef cattle feedlot odors. T ASAE 15: 1135-1137.

Bondari, K., and D. C. Sheppard. 1981. Soldier fly larvae as feed in commercial fish production. Aquaculture 24: 103-109.

Bondari, K., and D. C. Sheppard. 1987. Soldier fly, *Hermetia illucens* L., larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). Aquac. Res. 18: 209-220.

Booth, D. C., and C. Sheppard. 1984. Oviposition of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): eggs, masses, timing, and site characteristics. Environ. Entomol. 13: 421-423.

Bradley, S. W., and D. C. Sheppard. 1984. Housefly oviposition inhibition by larvae of *Hermetia-Illucens*, the black soldier fly. J. Chem. Ecol. 10: 853-859.

Brot, N., Z. Smit, and H. Weissbach. 1965. Conversion of L-tyrosine to phenol by *Clostridium tetanomorphum*. Arch. Biochem. Biophys. 112: 1-6.

Burkholder, J., B. Libra, P. Weyer, S. Heathcote, D. Kolpin, P. S. Thome, and M. Wichman. 2007. Impacts of waste from concentrated animal feeding operations on water quality. Environ. Health Persp. 3: 308-312.

Burnett, W. E., and N. C. Dondero. 1969. Microbiological and chemical changes in poultry manure associated with decomposition and odor generation. Anim. Wase. Manage. 14: 105-108.

Bursell, E., A. Gough, P. Beevor, A. Cork, D. Hall, and G. Vale. 1988. Identification of components of cattle urine attractive to tsetse flies, *Glossina* spp.(Diptera: Glossinidae). Bull. Entomol. Res. 78: 281-291.

Cai, L., J. A. Koziel, S. Zhang, A. J. Heber, E. L. Cortus, D. B. Parker, S. J. Hoff, G. Sun, K. Y. Heathcote, and L. D. Jacobson. 2015. Odor and odorous chemical emissions from animal buildings. T. ASABE 58: 1333-1347.

Callan, E. 1974. *Hermetia illucens* (L.)(Diptera, Stratiomyidae), a cosmopolitan American species long established in Australia and New Zealand. Entomol. Mon. Mag. 109: 232-234.

Carpenter, G. 1992. Current litter practices and future needs. Nat. Poul. Waste Manage. Symp. Proceed. 2: 268-273

Cerullo, M. 2016. Fumes from manure take kill Wisconsin farmer, 16 cows, New York Daily News Mortimer Zuckerman, New York.

Chaudhury, M., J. Welch, and L. A. Alvarez. 2002. Responses of fertile and sterile screwworm (Diptera: Calliphoridae) flies to bovine blood inoculated with bacteria originating from screwworm-infested animal wounds. J. Med. Entomol. 39: 130-134.

Chiang, H., and A. Hodson. 1950. An analytical study of population growth in *Drosophila melanogaster*. Ecol. Monogr. 20: 173-206.

Chin, H. W., and R. C. Lindsay. 1994. Ascorbate and transition-metal mediation of methanethiol oxidation to dimethyl disulfide and dimethyl trisulfide. Food Chem. 49: 387-392.

Čičková, H., G. L. Newton, R. C. Lacy, and M. Kozanek. 2015. The use of fly larvae for organic waste treatment. Waste Manage. 35: 68-80.

Clanton, C.J. and D. R. Schmidt. 2001. Geotextile fabric-straw manure storage covers for odor, hydrogen sulfide, and ammonia control. Appl. Eng. Agri. 17: 849.

Cole, D. J., V. R. Hill, F. J. Humenik, and M. D. Sobsey. 1999. Health, safety, and environmental concerns of farm animal waste. Occup. Med. State Art 14: 423-448.

Consoli, F. L., J. R. Parra, and R. A. Zucchi. 2010. Egg parasitoids in agroecosystems with emphasis on *Trichogramma*. Springer Science & Business Media. 123.

Cooper, P., and I. S. Cornforth. 1978. Volatile fatty acids in stored animal slurry. J. Sci. Food Agri. 29: 19-27.

Copello, A. 1926. Biologia de *Hermetia illucens.* Latr. Rev. Soc. Entomol. 1: 23-27.

Cossé, A. A., and T. C. Baker. 1996. House flies and pig manure volatiles: wind tunnel behavioral studies and electrophysiological evaluations. J. Agric. Entomol. 13: 301-317.

Cromwell, G., and R. Coffey. 1993. Future strategies to diminish nitrogen and phosphorus in swine manure. Proc. Environ. Symp. 2: 20-32.

Crumb, S., and S. Lyon. 1921. Further observations on the effect of chemicals upon opposition in the house fly. J. Econ. Entomol. 14: 461-465.

Davis, T. S., T. L. Crippen, R. W. Hofstetter, and J. K. Tomberlin. 2013. Microbial volatile emissions as insect semiochemicals. J. Chem. Ecol. 39: 840-859.

Deibel, R. H. 1967. Biological aspects of the animal waste disposal problem. Agri. Quali. Environ. Publ. 85: 395-399.

Dethier, V., and L. Chadwick. 1948. Chemoreception in insects. Physiol. Rev. 28: 220-254.

Diener, S., C. Zurbrugg, and K. Tockner. 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste. Manage. Res. 27: 603-610.

Diener, S., N. Gutiérrez, F. Zurbrugg and K. Tockner. 2011. Biological treatment of municipal organic waste using black soldier fly larvae. Waste Biomass Valor. 2: 357-363.

Donham, K., K. Thu, and S. Melvin. 1982. Understanding the impacts of large-scale swine production. Proceed. Interdiscip. Sci. Work.

Donham, K. J. 2000. The concentration of swine production - Effects on swine health, productivity, human health, and the environment. Vet. Clin. Am. Food 16: 559-565.

Drasar, B. S., and M. J. Hill. 1974. Human intestinal flora. Academic Press. London. 263.

El-Mashad, H. M., R. Zhang, V. Arteaga, T. Rumsey, and F. M. Mitloehner. 2011. Volatile fatty acids and alcohols production during anaerobic storage of dairy manure. T. ASABE. 54: 599-607.

Endler, J. A. 1993. Some general comments on the evolution and design of animal communication systems. Biol. Sci. 340: 215-225.

England, D. C., D. Jones, and D. Younkin. 1978. Pork Industry Handbook. Cooperative Extension Service Purdue University. Indiana.

EPA. 2010. EPA orders Simplot Cattle Feeding Company to change stock watering practices at Grand View, ID, feedlot to protect the snake river. Washington, DC.

EPA. 2010. EPA orders two Virginia farms to cease unpermitted waste discharges to the Shenandoah River. Washington, DC.

EPA. 2013. Literature review of contaminants in livestock and poultry manure and implications for water quality. Washington, DC.

Erickson, M. C., M. Islam, C. Sheppard, J. Liao, and M. P. Doyle. 2004. Reduction of *Escherichia coli* O157 : H7 and *Salmonella enterica* serovar enteritidis in chicken manure by larvae of the black soldier fly. J. Food Protect. 67: 685-690.

Etter, L. 2010. Manure Raises New Stink. The Wall Street Journal. Dow Jones & Company New York.

FAO 2009. The State of Food and Agriculture 2009. Livestock in the balance. FAO Agriculture Series.

FAOSTAT, F. 2009. Statistical databases. Food and Agriculture Organization of the United Nations.

Foley, J. A. 2011. Can we feed the world & sustain the planet? Sci. Am. 305: 60-65.

Food and WaterWatch. 2015. Factroy Farm Nation, Washington, DC.

Fraenkel, G. S., and D. L. Gunn. 1961. The Orientation of Animals: Kineses, Taxes and Compass Reactions. University Microfilms. 352.

Francuski, L., M. Djurakic, J. Ludoški, P. Hurtado, C. Pérez-Bañón, G. Ståhls, S. Rojo, and V. Milankov. 2014. Shift in phenotypic variation coupled with rapid loss of genetic diversity in captive populations of *Eristalis tenax* (Diptera: Syrphidae): consequences for rearing and potential commercial use. J. Econ. Entomol. 107: 821-832.

Furman, D. P., R. D. Young, and E. P. Catts. 1959. *Hermetia-Illucens* (Linnaeus) as a factor in the natural control of *Musca-Domestica* Linnaeus. J. Econ. Entomol. 52: 917-921.

Georgsson, F., Á. E. Þorkelsson, M. Geirsdóttir, J. Reiersen, and N. J. Stern. 2006. The influence of freezing and duration of storage on *Campylobacter* and indicator bacteria in broiler carcasses. Food Microbiol. 23: 677-683.

Gibson, A. M., N. Bratchell, and T. Roberts. 1987. The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinu*m type A in pasteurized pork slurry. J. Appl.Bacteriol. 62: 479-490.

Goldschmidt, W. 1979. Large-scale farming and the rural social structure. Rural Sociol. 45: 4.

Gonzales, J. V., W. R. Young, and M. R. Genel. 1963. Reducion de la problacion de *mosca domestica* en gallinzaz por la mosca soldado en el Tropica. Agric. Tec. 2: 53-57.

Green, T. R., and R. Popa. 2012. Enhanced ammonia content in compost leachate processed by black soldier fly larvae. Appl. Biochem. Biotech. 166: 1381-1387.

Hagedorn, C., D. Hansen, and G. Simonson. 1978. Survival and movement of fecal indicator bacteria in soil under conditions of saturated flow. J. Environ. Qual. 7: 55-59.

Hale, O. M. 1973. Dried *Hermetia illucens* larvae (Diptera: Stratiomyidae) as a feed additive for poultry. Ga. Entomol. Soc. J. 8: 16-20.

Hales, K. E., D. B. Parker, and N. A. Cole. 2012. Potential odorous volatile organic compound emissions from feces and urine from cattle fed corn-based diets with wet distillers grains and solubles. Atmos. Environ. 60: 292-297.

Hammond, C., D. Day, and E. Hansen. 1968. Can lime and chlorine suppress odors in liquid hog manure. Agr. Eng. 49: 340-343.

Hatfield, J., M. Brumm, and S. Melvin. 1998. Swine manure management. Agri. Municip. Anim. Indust. Byprod. 44: 78-90.

Henderson, B. 1991. Backhoes bury most of the hogs killed in floods, USA Today Cauchon, Dennis, Washington D.C.

Himathongkham, S., S. Bahari, H. Riemann, and D. Cliver. 1999. Survival of *Escherichia coli* O157: H7 and *Salmonella typhimurium* in cow manure and cow manure slurry. FEMS Microbiol. Lett. 178: 251-257.

Hobson, E. 1936. Sheep blow fly investigations iii. Observations on the chemotropism of *lucilia sericata* mg. Ann. Appl.Biol. 23: 845-851.

Hogsette, J. A. 1992. New diets for production of house-flies and stable flies (Diptera, Muscidae) in the Laboratory. J. Econ. Entomol. 85: 2291-2294.

Holdaway, F. G. 1930. Field Populations and natural control of *Lucilia sericata*. Nature 126: 648-649.

Holmes, L., S. Vanlaerhoven, and J. Tomberlin. 2012. Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). Environ. Entomol. 41: 971-978.

Huang, J., J. R. Miller, S. C. Chen, J. M. Vulule, and E. D. Walker. 2006. *Anopheles gambiae* (Diptera: Culicidae) oviposition in response to agarose media and cultured bacterial volatiles. J. Med. Entomol. 43: 498-504.

Husted, S. 1994. Seasonal variation in methane emission from stored slurry and solid manures. J. Environ. Qual. 23: 585-592.

Hwang, Y.-S., M. S. Mulla, and H. Axelrod. 1976. Attractants for synanthropic flies. Identification of attractants and coattractants for *Hippelates* eye gnats (Diptera, Chloropidae). J. Agr. Food Chem. 24: 164-169.

Ichihara, K. 1956. Studies on phenol formation. J. Biochem. 43: 803-810.

Iowa Department of Natural Resources. 2014. DNR determines fish kill counts, source of Clayton County fish kill. Iowa.

Jacob, J., R. Blair, D. Bennett, T. Scott, and R. Newberry.1994. The effect of dietary protein and amino acid levels during the grower phase on nitrogen excretion of broiler chickens. Proceed. Can. Anim. Sci.: 20-32.

James, M. T. 1935. The genus Hermetia in the United States (Diptera: Stratiomyidae). Bull. Brook. Entomol. Soc. 30: 165-170.

Jeanbourquin, P., and P. M. Guerin. 2007. Sensory and behavioural responses of the stable fly S*tomoxys calcitrans* to rumen volatiles. Med. Vet. Entomol. 21: 217-224.

Jensen, M. T., R. P. Cox, and B. B. Jensen. 1995. 3-Methylindole (skatole) and indole production by mixed populations of pig fecal bacteria. Appl.Environ. Microbiol. 61: 3180-3184.

Jünger, M., W. Vautz, M. Kuhns, L. Hofmann, S. Ulbricht, J. I. Baumbach, M. Quintel, and T. Perl. 2012. Ion mobility spectrometry for microbial volatile organic compounds: a new identification tool for human pathogenic bacteria. Appl. Microbiol. Biot. 93: 2603-2614.

Kay, R., and P. Lee. 1997. Ammonia emission from pig buildings and characteristics of slurry produced by pigs offered low crude protein diets. Proceed. Internat. Symp. Ammon. Anim. Prod. Fac.: 253-260

Kellogg, L. E., and S. D. Bridgham. 2003. Phosphorus retention and movement across an ombrotrophic-minerotrophic peatland gradient. Biogeochem. 63: 299-315.

Kibbey, H., C. Hagedorn, and E. McCoy. 1978. Use of fecal *Streptococci* as indicators of pollution in soil. Appl. Environ. Microbiol. 35: 711-717.

Kilburn, K. H. 1999. Evaluating health effects from exposure to hydrogen sulfide: central nervous system dysfunction. Environ. Epidemiol. Toxicol. 1: 207-216.

Kilpatrick, J. W., and H. F. Schoof. 1959. Interrelationship of water and *Hermetia-Illucens* breeding to *Musca-Domestica* production in human excrement. Am. J. Trop. Med. Hyg. 8: 597-602.

Krčmar, S. 2007. Responses of Tabanidae (Diptera) to canopy traps baited with 4-methylphenol, 3-isopropylphenol, and naphthalene. J. Vector Ecol. 32: 188.

Krčmar, S., A. Mikuska, and V. Radolic. 2009. Comparison of sampling tabanids (Diptera: Tabanidae) by four different potential attractants. J. Appl.Entomol. 134: 608.

Kroeckel, S., A. G. E. Harjes, I. Roth, H. Katz, S. Wuertz, A. Susenbeth, and C. Schulz. 2012. When a turbot catches a fly: Evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute - Growth performance and chitin degradation in juvenile turbot (*Psetta maxima)*. Aquaculture 364: 345-352.

Kudva, I. T., K. Blanch, and C. J. Hovde. 1998. Analysis of *Escherichia coli* O157: H7 survival in ovine or bovine manure and manure slurry. Appl. Environ. Microbiol. 64: 3166-3174.

Kuphal, K. 2009. Design flaw led to manure spill Pipestone County Star, Minnesota

Kuroda, K., T. Osada, M. Yonaga, A. Kanematu, T. Nitta, S. Mouri, and T. Kojima. 1996. Emissions of malodorous compounds and greenhouse gases from composting swine feces. Bioresource Technol. 56: 265-271.

Lalander, C., S. Diener, M. E. Magri, C. Zurbrugg, A. Lindstrom, and B. Vinneras. 2013. Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*) - From a hygiene aspect. Sci. Total Environ. 458: 312-318.

Lalander, C. H., J. Fidjeland, S. Diener, S. Eriksson, and B. Vinnerås. 2015. High waste-to-biomass conversion and efficient *Salmonella* spp. reduction using black soldier fly for waste recycling. Agron. Sustain. Dev. 35: 261-271.

Lam, K., D. Babor, B. Duthie, E. M. Babor, M. Moore, and G. Gries. 2007. Proliferating bacterial symbionts on house fly eggs affect oviposition behaviour of adult flies. Anim. Behav. 74: 81-92.

Larsen, J. R., R. E. Pfadt, and L. G. Peterson. 1966. Olfactory and oviposition responses of the house fly to domestic manures, with notes on an autogenous strain. J. Econ. Entomol. 59: 610-615.

Le, P. D., A. J. Aarnink, N. W. Ogink, P. M. Becker, and M. W. Verstegen. 2005. Odour from animal production facilities: its relationship to diet. Nutr. Res. Rev. 18: 3-30.

Lechner, M., M. Fille, J. Hausdorfer, M. P. Dierich, and J. Rieder. 2005. Diagnosis of bacteria in vitro by mass spectrometric fingerprinting: a pilot study. Curr. Microbiol. 51: 267-269.

Lindh, J. M., A. Kannaste, B. G. Knols, I. Faye, and A. K. Borg-Karlson. 2008. Oviposition responses of *Anopheles gambiae* ss (Dipera: Culicidae) and identification of volatiles from bacteria-containing solutions. J. Med. Entomol. 45: 1039-1049.

Liu, Q. L., J. K. Tomberlin, J. A. Brady, M. R. Sanford, and Z. N. Yu. 2008. Black soldier fly (Diptera: Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. Environ. Entomol. 37: 1525-1530.

Lusk, P., and A. Wiselogel. 1998. Methane recovery from animal manures: the current opportunities casebook. National Renewable Energy Laboratory Golden, CO.

Macfarlane, S., and G. T. Macfarlane. 1995. Proteolysis and amino acid fermentation. Human Colonic Bacteria: Role in nutrition, physiology and pathology. Scan. J. of Gastroenterol. 32: 75-100.

Mackie, R. I., P. G. Stroot, and V. H. Varel. 1998. Biochemical identification and biological origin of key odor components in livestock waste. J. Anim. Sci. 76: 1331-1342.

Mallin, M. A., and L. B. Cahoon. 2003. Industrialized animal production—a major source of nutrient and microbial pollution to aquatic ecosystems. Pop. Environ. 24: 369-385.

Mawdsley, J. L., R. D. Bardgett, R. J. Merry, B. F. Pain, and M. K. Theodorou. 1995. Pathogens in livestock waste, their potential for movement through soil and environmental-pollution. Appl. Soil Ecol. 2: 1-15.

May, B. 1961. The occurrence in New Zealand and the life-history of the soldier fly *Hermetia illucens* (L.)(Diptera: Stratiomyidae). NZJ Sci. 4. 13-23.

Mayrhofer, S., T. Mikoviny, S. Waldhuber, A. O. Wagner, G. Innerebner, I. H. Franke‐Whittle, T. D. Märk, A. Hansel, and H. Insam. 2006. Microbial community related to volatile organic compound (VOC) emission in household biowaste. Environ. Microbiol. 8: 1960-1974.

McConnell, J. C., K. M. Barth and S. A. Griffin. 1972. Nitrogen metabolism at three stages of development and its relationship to measurements of carcass composition in fat and lean type swine. J. Anim. Sci. 35: 556-560.

McCune, B., J. B. Grace, and D. L. Urban. 2002. Analysis of ecological communities, vol. 28, MjM software design Gleneden Beach, OR.

McGill, A. E., and N. Jackson. 1977. Changes in the short‐chain carboxylic acid content and chemical oxygen demand of stored pig slurry. J. Sci. Food Agri. 28: 424-430.

Mihok, S., and K. Lange. 2012. Synergism between ammonia and phenols for *Hybomitra* tabanids in northern and temperate Canada. Med. Vet. Entomol. 26: 282-290.

Miller, B., J. Teotia, and T. Thatcher. 1974. Digestion of poultry manure by *Musca domestica*. Br. Poul. Sci. 15: 231-234.

Miner, J. R. 1977. Characterization of Odors and Other Volatile Emissions. Agr. Environ. 3: 129-137.

Mitloehner, F., and M. Calvo. 2008. Worker health and safety in concentrated animal feeding operations. J. Agri. Safety Health 14: 163-187.

Moore, P., T. Daniel, A. Sharpley, and C. Wood. 1995. Poultry manure management: Environmentally sound options. J. Soil Water Conser. 50: 321-327.

Mortensen, P., J. Andersen, S. Arffmann, and E. Krag. 1987. Short-chain fatty acids and the irritable bowel syndrome: the effect of wheat bran. Scan. J. Gastroentero. 22: 185-192.

Msangi, S., M. Rosegrant, and P. Conforti. 2011. World agriculture in a dynamically changing environment: IFPRI's long-term outlook for food and agriculture. Looking ahead in world food and agriculture. FAO. 2050: 57-93.

Mulla, M. S., Y.-S. Hwang, and H. Axelrod. 1977. Attractants for synanthropic flies: chemical attractants for domestic flies. J. Econ. Entomol. 70: 644-648.

Müller, H., and M. Kirchgessner. 1985. Energetische verwertung von pektin bei sauen. Z. Tierphysiol. Tierer. 54: 14-20.

Myers, H. M., J. K. Tomberlin, B. D. Lambert, and D. Kattes. 2008. Development of black soldier fly (Diptera : Stratiomyidae) larvae fed dairy manure. Environ. Entomol. 37: 11-15.

Nartshuk, E., and G. Y. Bei-Benko. 1988. Family Stratiomyidae. Keys to the Insects of the European Part of the USSR 5: 701-738.

NASS. 2007. Census of agriculture. US Department of Agriculture, National Agricultural Statistics Service, Washington, DC.

NASS. 2012. Cenus of Agriculture. US Department of Agriculture, National Agricultural Statistics Service, Washington, DC.

National Research Council. 1988. Nutrient Requirements of Swine. Washington, DC. 93.

Newton, G., D. Sheppard, D. Watson, G. Burtle, C. Dove, J. Tomberlin, and E. Thelen. 2005. The black soldier fly, *Hermetia illucens*, as a manure management/resource recovery tool. Sympos. Sci. Anim. Waste Manage. 3: 253-260.

Newton, L., C. Sheppard, D. W. Watson, G. Burtle, and R. Dove. 2005. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. Animal and Poultry Waste Management Center, North Carolina State University, Raleigh, NC 17.

North Carolina General Assembly. 2007. Senate Bill 1465: Performance standards for animal waste management systems that serve swine farms; lagoon and sprayfield systems prohibited. Session 2007. 523.

Nguyen, T. T. X., J. K. Tomberlin, and S. Vanlaerhoven. 2013. Influence of resources on *Hermetia illucens* (Diptera: Stratiomyidae) larval development. J. Med. Entomol. 50: 898-906.

Ni, J.-Q., W. P. Robarge, C. Xiao, and A. J. Heber. 2012. Volatile organic compounds at swine facilities: a critical review. Chemosphere 89: 769-788.

Nowak, P., R. Shepard, F. Madison, J. L. Hatfield, and B. A. Stewart. 1998. Farmers and manure management: a critical analysis. Anim. Wast. 3: 1-32.

NRCS. 2008. Agricultural waste management field handbook. Natural Resource Conservation Service. Washington, DC. 345.

O'Neill, D., and V. Phillips. 1992. A review of the control of odour nuisance from livestock buildings: part 3, properties of the odorous substances which have been identified in livestock wastes or in the air around them. J. Agri. Eng. Res. 53: 23-50.

Odam, E. M., J. M. Page, M. G. Townsend and J. G. Wilkins. 1986. Identification of volatile components in headspace from animal slurries. Waste Manage. 34: 284-295.

Oonincx, D., A. van Huis, and J. J. A. van Loon. 2015. Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. J. Insects Food Feed 1: 131-139.

Parker, D. B., J. Gilley, B. Woodbury, K.-H. Kim, G. Galvin, S. L. Bartelt-Hunt, X. Li, and D. D. Snow. 2013. Odorous VOC emission following land application of swine manure slurry. Atmos. Environ. 66: 91-100.

Parliament, T. H., M. G. Kolor, and D. J. Rizzo. 1982. Volatile components of Limburger cheese. J. Agr. Food Chem. 30: 1006-1008.

Perry, A. S., R. W. Fay, and N. Johnson. 1967. Correlation of chemical constitution and physical properties of fatty acid esters with oviposition response of *Aedes aegypti*. Mosq. News 27: 175-183.

Pew Commission on Industrial Farm Animal Production. 2008. Putting meat on the table / Industrial farm animal production in America / industrial farm animal production in America.

Powers, W. J., and S. Bastyr. 2004. Downwind air quality measurements from poultry and livestock facilities. Anim. Indust. Rep. 650: 86.

Price, P. W. 2011. Insect Ecology: Behavior, Populations and Communities. Cambridge University Press. Cambridge. 828.

Rabalais, N. N. 2002. Nitrogen in aquatic ecosystems. J. Human Environ. 31: 102-112.

Rasmussen, H., K. Holtug, and P. Mortensen. 1988. Degradation of amino acids to short-chain fatty acids in humans: an in vitro study. Scand. J. Gastroenterol. 23: 178-182.

Ribaudo, M., and N. Gollehon. 2006. Chapter 4.5: Animal Agriculture and the Environment. US Department of Agriculture. Washington, DC. 124

Ribaudo, M., N. Gollehon, and J. Agapoff. 2003. Land application of manure by animal feeding operations: Is more land needed? J. Soil Water Conserv. 58: 30-38.

Ritz, C. W., and W. C. Merka. 2009. Maximizing poultry manure use through nutrient management planning. Univ. Georg. Coop. Ext. 6.

Robacker, D. C., and D. S. Moreno. 1995. Protein feeding attenuates attraction of Mexican fruit flies (Diptera: Tephritidae) to volatile bacterial metabolites. Florida Entomologist. 1: 62-69.

Roustan, J., A. Aumaitre, and E. Salmon-Legagneur. 1977. Characterization of malodours during anaerobic storage of pig wastes. Agr. Environ. 3: 147-157.

Rushton, L. 2007. Occupational causes of chronic obstructive pulmonary disease. Rev. Environ. Health 22: 195-212.

Russell, E. G. 1979. Types and distribution of anaerobic bacteria in the large intestine of pigs. Appl. Environ. Microbiol. 37: 187-193.

Russell, J. B., C. Sniffen, and P. Van Soest. 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. J. Dairy Sci. 66: 763-775.

Ruth, J. H. 1986. Odor thresholds and irritation levels of several chemical substances: a review. Am. Indust. Hyg. Assoc. J. 47: A-142-A-151.

Schenker, M. B., and J. Jacobs. 1996. Respiratory effects of organic solvent exposure. Tuber. Lung Diseas. 77: 4-18.

Schiffman, S. S., J. L. Bennett, and J. H. Raymer. 2001. Quantification of odors and odorants from swine operations in North Carolina. Agri. Forest Meteorol. 108: 213-240.

Sealey, W. M., T. G. Gaylord, F. T. Barrows, J. K. Tomberlin, M. A. McGuire, C. Ross, and S. St - Hilaire. 2011. Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched black soldier fly prepupae, *Hermetia illucens*. J. World Aquacult. Soc. 42: 34-45.

Seddon, H. R. 1931. Conditions which predispose sheep to blowfly attack. Agri. Gaz. New S. Wales 42: 581-594.

Senecal, A. G., J. Magnone, W. Yeomans, and E. M. Powers. 2002. Rapid detection of pathogenic bacteria by volatile organic compound (VOC) analysis. Environ. Indust. Sens. 34: 656-675.

Sharpley, A. 1999. Agricultural phosphorus, water quality, and poultry production: are they compatible? Poul. Sci. 78: 660-673.

Sheppard, C. 1983. Housefly and lesser fly control utilizing the black soldier fly in manure management-systems for caged laying hens. Environ. Entomol. 12: 1439-1442.

Sheppard, D. C., G. L. Newton, S. A. Thompson, and S. Savage. 1994. A value-added manure management-system using the black soldier fly. Biores. Technol. 50: 275-279.

Sheppard, D. C., J. K. Tomberlin, J. A. Joyce, B. C. Kiser, and S. M. Sumner. 2002. Rearing methods for the black soldier fly (Diptera : Stratiomyidae). J. Med. Entomol. 39: 695-698.

Sokal, R. R., and R. L. Sullivan. 1963. Competition between mutant and wild - type house - fly strains at varying densities. Ecology 44: 314-322.

Spiehs, M., and V. Varel. 2009. Nutrient excretion and odorant production in manure from cattle fed corn wet distillers grains with solubles. J. Anim. Sci. 87: 2977.

Spoelstra, S. 1980. Origin of objectionable odorous components in piggery wastes and the possibility of applying indicator components for studying odour development. Agr. Environ. 5: 241-260.

St-Hilaire, S., C. Sheppard, J. K. Tomberlin, S. Irving, L. Newton, M. A. McGuire, E. E. Mosley, R. W. Hardy, and W. Sealey. 2007. Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykis*s. J. World. Aquacult. Soc. 38: 59-67.

Steinfeld, H., P. Gerber, T. D. Wassenaar, V. Castel, M. Rosales M, and C. d. Haan. 2006. Livestock's long shadow : environmental issues and options, Rome : Food and Agriculture Organization of the United Nations.

Sullivan, R. L. and R. R. Sokal. 1963. The effects of larval density on several strains of house fly. Ecology. 44: 120-130.

Sun, H. W., S. L. Trabue, K. Scoggin, W. A. Jackson, Y. Pan, Y. J. Zhao, I. L. Malkina, J. A. Koziel, and F. M. Mitioehner. 2008. Alcohol, volatile fatty acid, phenol, and methane emissions from dairy cows and fresh manure. J. Environ. Qual. 37: 615-622.

Sutton, A., K. Kephart, M. Verstegen, T. Canh, and P. Hobbs. 1999. Potential for reduction of odorous compounds in swine manure through diet modification. J. Anim. Sci. 77: 430-439.

Suzuki, K., Y. Benno, T. Mitsuoka, S. Takebe, K. Kobashi, and J. Hase. 1979. Urease-producing species of intestinal anaerobes and their activities. Appl. Environ. Microbiol. 37: 379-382.

Sweeten, J. M., J. Hatfield, and B. Stewart. 1998. Cattle feedlot manure and wastewater management practices. Anim. Waste. Utiliz. 13: 125-155.

Tangtrakulwanich, K., H. Chen, F. Baxendale, G. Brewer, and J. Zhu. 2011. Characterization of olfactory sensilla of *Stomoxys calcitrans* and electrophysiological responses to odorant compounds associated with hosts and oviposition media. Med. Vet. Entomol. 25: 327-336.

Tate, R. L. 1978. Cultural and environmental factors affecting the longevity of *Escherichia coli* in histosols. Appl. Environ. Microbiol. 35: 925-929.

Thu, K., K. Donham, R. Ziegenhorn, S. Reynolds, P. S. Thorne, P. Subramanian, P. Whitten, and J. Stookesberry. 1997. A control study of the physical and mental health of residents living near a large-scale swine operation. J. Agri. Safety Health 3: 13-26.

Tingle, F., E. Mitchell, and W. Copeland. 1975. soldier fly, *Hermetia illucens* in poultry in north central Florida. J. Georg. Entomol. Soc. 23: 44-54.

Tomberlin, J. K., and D. C. Sheppard. 2001. Lekking behavior of the black soldier fly (Diptera: Stratiomyidae). Florida Entomol. 84: 729-730.

Tomberlin, J. K., and D. C. Sheppard. 2002. Factors influencing mating and oviposition of black soldier flies (Diptera : stratiomyidae) in a colony. J. Entomol. Sci. 37: 345-352.

Tomberlin, J. K., D. C. Sheppard, and J. A. Joyce. 2002. Selected life-history traits of black soldier flies (Diptera : Stratiomyidae) reared on three artificial diets. Ann. Entomol. Soc. Am. 95: 379-386.

Tomberlin, J. K., P. H. Adler, and H. M. Myers. 2009. Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. Environ. Entomol. 38: 930-934.

Trexler, J. D., C. S. Apperson, L. Zurek, C. Gemeno, C. Schal, M. Kaufman, E. Walker, D. W. Watson, and L. Wallace. 2003. Role of bacteria in mediating the oviposition responses of *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 40: 841-848.

Trusts, C., and J. Hopkins. 2008. Putting meat on the table: Industrial farm animal production in America. NCIFAP. Washington: DC.

Ullyett, G. 1950. Competition for food and allied phenomena in sheep-blowfly populations. Biol. Sciences 234: 77-174.

Urech, R., P. E. Green, M. J. Rice, G. W. Brown, F. Duncalfe, and P. Webb. 2004. Composition of chemical attractants affects trap catches of the Australian sheep blowfly, *Lucilia cuprina*, and other blowflies. J. Chem. Ecol. 30: 851-866.

USDA. 2000. Manure and Wastewater Handling and Storage Costs USDA.Washington, DC.

USDA. 2007. Dairy 2007 Part I: Reference of Dairy Cattle Health and Management Practices in the United States, 2007, Dairy 2007. USDA, Washington, DC.

USDA. 2014. Layers 2013 Part I: Reference of Health and Management Practices on Table-Egg Farms in the United States, 2013. USDA Washinton, DC.

Vale, G. 1980. Field studies of the responses of tsetse flies (Glossinidae) and other Diptera to carbon dioxide, acetone and other chemicals. Bull. Entomol. Res. 70: 563-570.

Van Horn, H., A. Wilkie, W. Powers, and R. Nordstedt. 1994. Components of dairy manure management systems. J. Dairy Sci. 77: 2008-2030.

Waldbauer, G. 1968. The consumption and utilization of food by insects. Adv. Insect Physiol. 5: 229-288.

Wang, G., T. Zhao, and M. P. Doyle. 1996. Fate of enterohemorrhagic *Escherichia coli* O157: H7 in bovine feces. Appl. Environ. Microbiol. 62: 2567-2570.

Wang, L., K. Mankin, and G. Marchin. 2004. Survival of fecal bacteria in dairy cow manure. T. ASAE 47: 1239.

Ward, M. H., T. M. DeKok, P. Levallois, J. Brender, G. Gulis, B. T. Nolan, and J. VanDerslice. 2005. Workgroup report: Drinking-water nitrate and health-recent findings and research needs. Environ. Health Persp. 45: 1607-1614.

Warnke, G. 1931. Experimentelle Untersuchungen über den Geruchssinn von *Geotrupes silvaticus* Panz. und *Geotrupes vernalis* Lin. J. Compar. Physiol. 14: 121-199.

Wilkinson, S. 1979. Plant Nutrient and Economic Valus of Animal Manures. J. Anim. Sci. 48: 121-133.

Wilkton, T. F. 2004. Biological assessment of Iowa's wadeable streams: project report. Iowa Dept. Nat. Res.

Woodbury, B. L., J. E. Gilley, D. B. Parker, D. B. Marx, D. N. Miller, and R. A. Eigenberg. 2014. Emission of volatile organic compounds after land application of cattle manure. J. Environ. Qual. 43: 1207-1218.

Wright, R. 1998. Agricultural uses of municipal, animal, and industrial byproducts. FAO AGRIS 44. 130.

Yasuhara, A. 1987. Identification of volatile compounds in poultry manure by gas-chromatography mass-spectrometry. J. Chromatogr. 387: 371-378.

Yasuhara, A., and K.-i. Fuwa. 1977. Odor and volatile compounds in liquid swine manure. I. Carboxylic acids and phenols. Bull. Chem. Soc. Jap. 50: 731-733.

Yasuhara, A., and K. Fuwa. 1983. Isolation and analysis of odorous components in swine manure. J. Chromatogr. 281: 225-236.

Yokoyama, M., C. Tabori, E. Miller, and M. Hogberg. 1982. The effects of antibiotics in the weanling pig diet on growth and the excretion of volatile phenolic and aromatic bacterial metabolites. Am. J. Clin. Nutri. 35: 1417-1424.

Yu, G., P. Cheng, Y. Chen, Y. Li, Z. Yang, Y. Chen, and J. K. Tomberlin. 2011. Inoculating poultry manure with companion bacteria influences growth and development of black soldier fly (Diptera: Stratiomyidae) larvae. Environ. Entomol. 40: 30-35.

Zheng, L. Y., T. L. Crippen, L. Holmes, B. Singh, M. L. Pimsler, M. E. Benbow, A. M. Tarone, S. Dowd, Z. N. Yu, S. L. Vanlaerhoven, T. K. Wood, and J. K. Tomberlin. 2013. Bacteria mediate oviposition by the black soldier fly, *Hermetia illucens* (L.), (Diptera: Stratiomyidae). Sci. Rep. 3: 1-8.

Zhou, F., J. K. Tomberlin, L. Y. Zheng, Z. N. Yu, and J. B. Zhang. 2013. Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures. J. Med. Entomol. 50: 1224-1230.

Zhu, J. 2000. A review of microbiology in swine manure odor control. Agri. Ecosys. Environ. 78: 93-106.

APPENDIX A

A.1 STRESS PLOTS AND NMDS ORDINATIONS FROM VOLATILES FROM MANURE WITH AND WITHOUT *Hermetia illucens* (L.) LARVAE COMPARED TO CONTROL MANURE

Poultry Manure

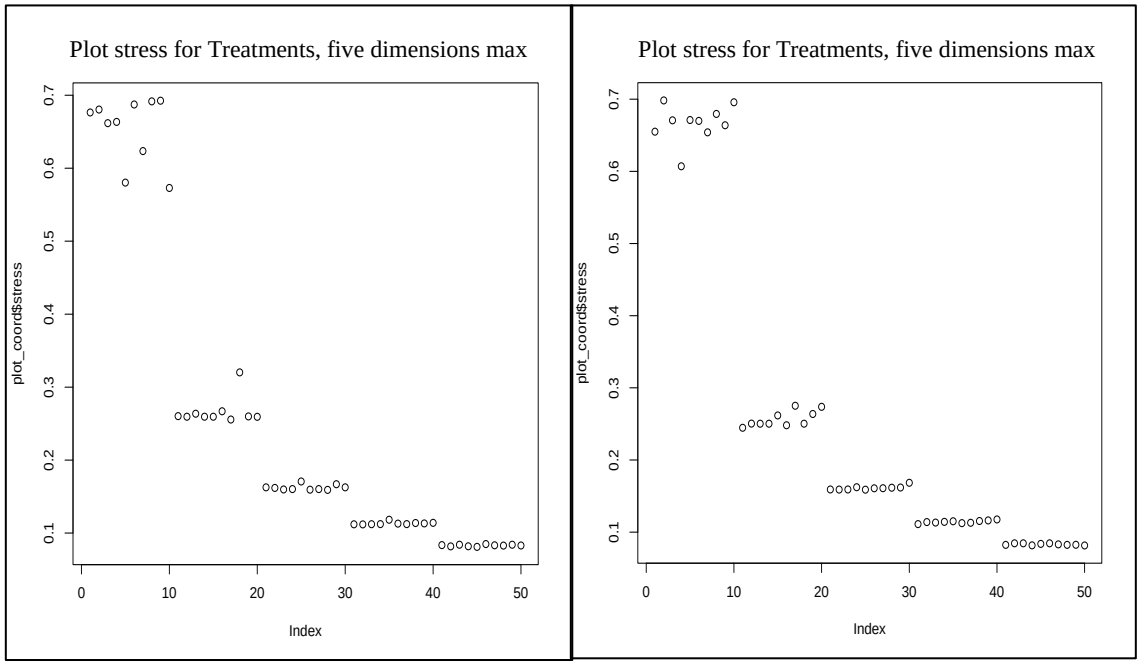

Figure A.1.1. NMDS stress plots for all volatiles emitted from poultry manure (n = 3) from Trial 1 (left) and Trial 2 (right) with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1: Stress: 0.1588, r^2 = 0. 8064; Trial 2: Stress: 0.1590, r^2 = 0. 8068). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

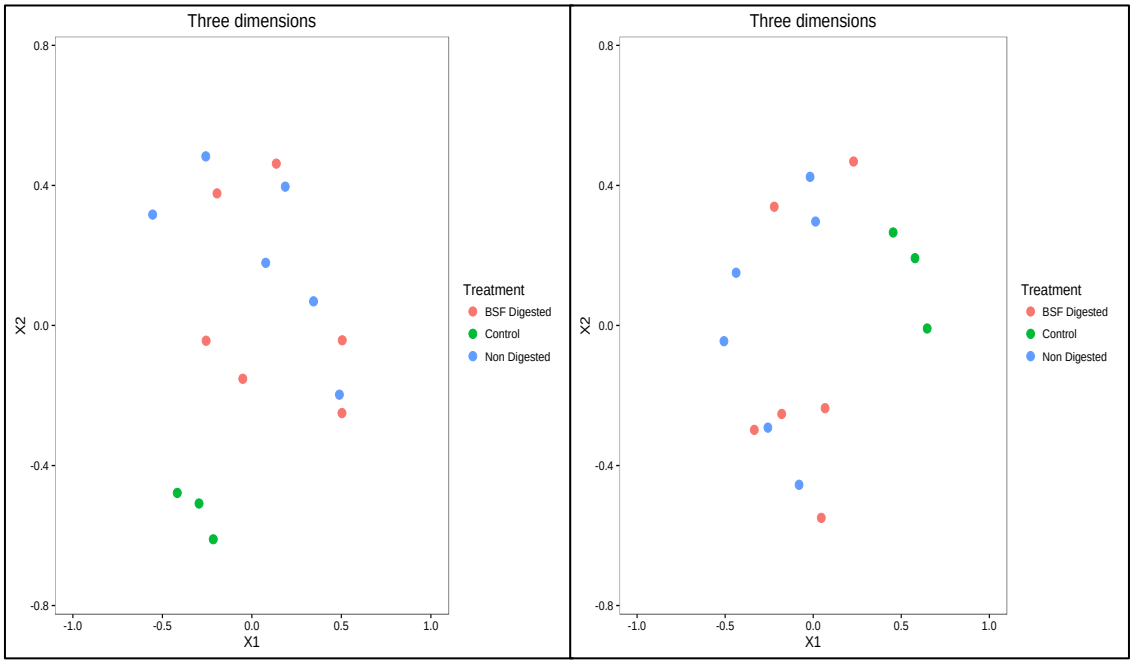

Figure A.1.2. NMDS ordinations of the community of volatile organic compounds from Trial 1 (left) and Trial 2 (right) of control, non-digested and digested poultry manure (n = 3) by *Hermetia illucens* (L.). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1%RH and14:10 L:D cycle ($P < 0.05$).

Swine Manure

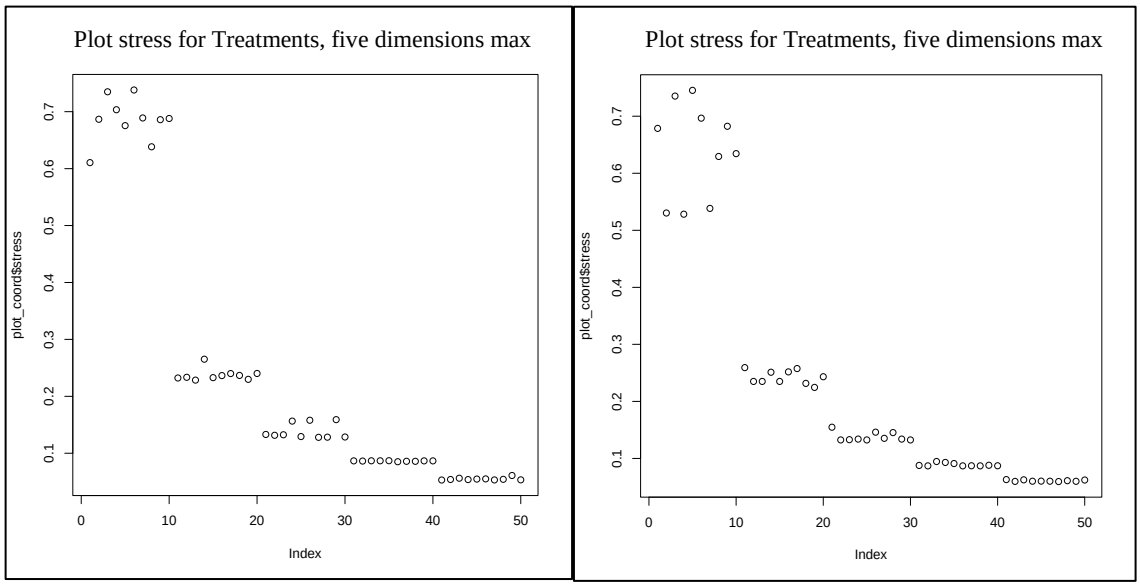

Figure A.1.3. NMDS stress plots for all volatiles emitted from swine manure (n = 3) from Trial 1(left) and Trial 2 (right) with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1: Stress: 0.1280, r^2 = 0. 8849; Trial 2: Stress: 0.1323, r^2 = 0. 8858). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

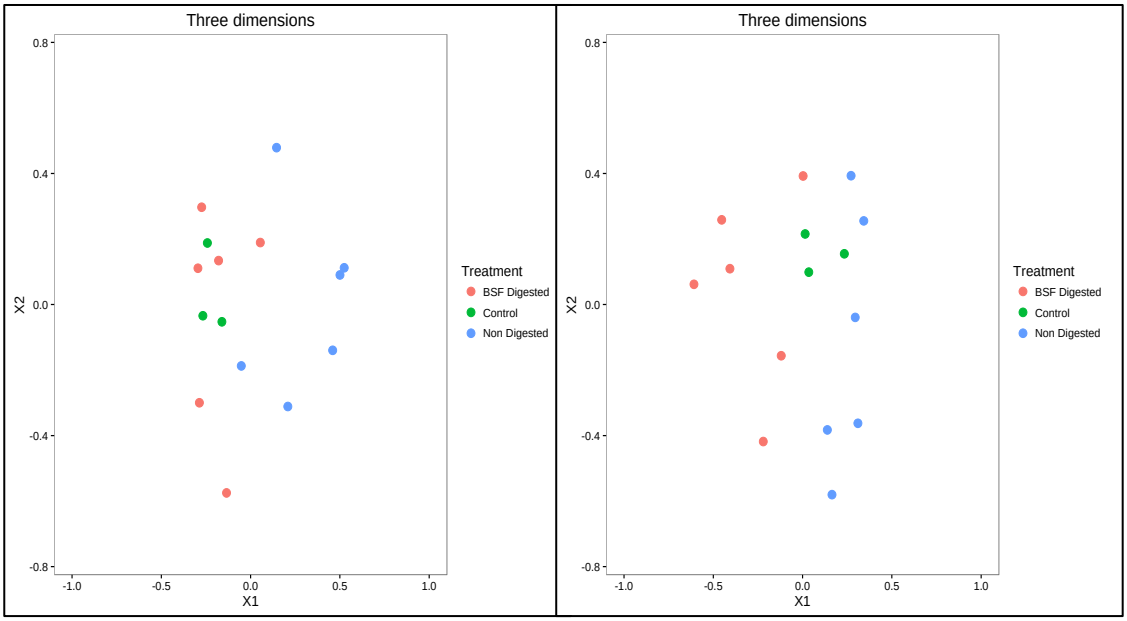

Figure A.1.4. NMDS ordinations of the community of volatile organic compounds from Trial 1 (left) and Trial 2 (right) of control, non-digested and digested swine manure (n = 3) by *Hermetia illucens* (L.). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and14:10 L:D cycle ($P < 0.05$).

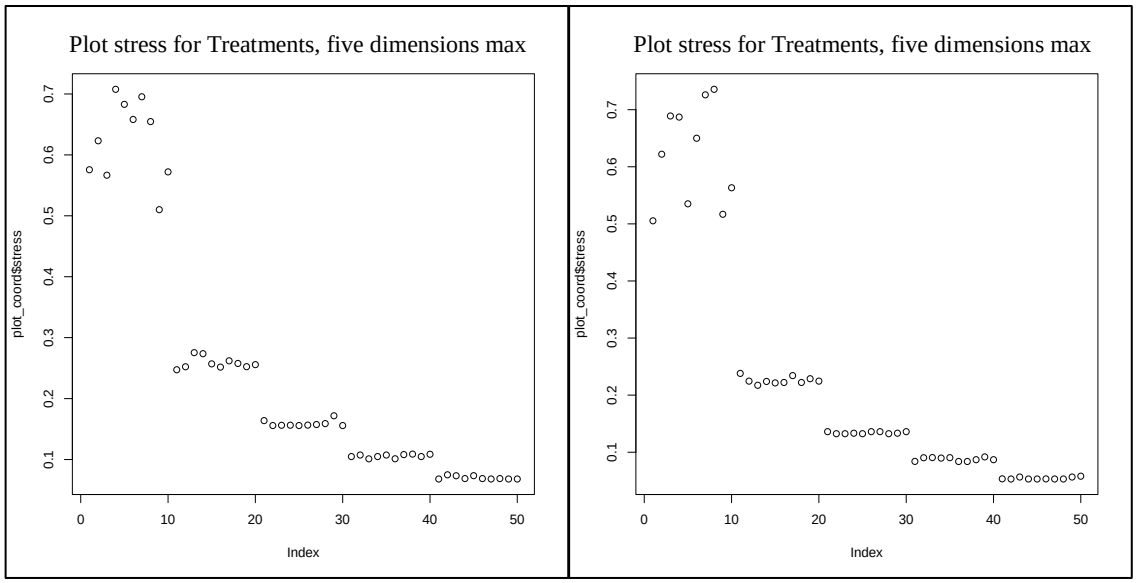

Figure A.1.5. NMDS stress plots for all volatiles emitted from dairy manure (n = 3) from Trial 1 (left) and Trial 2 (right) with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1: Stress: 0.1557, r^2 = 0. 8146; Trial 2: Stress: 0.1325, r^2 = 0. 8829). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

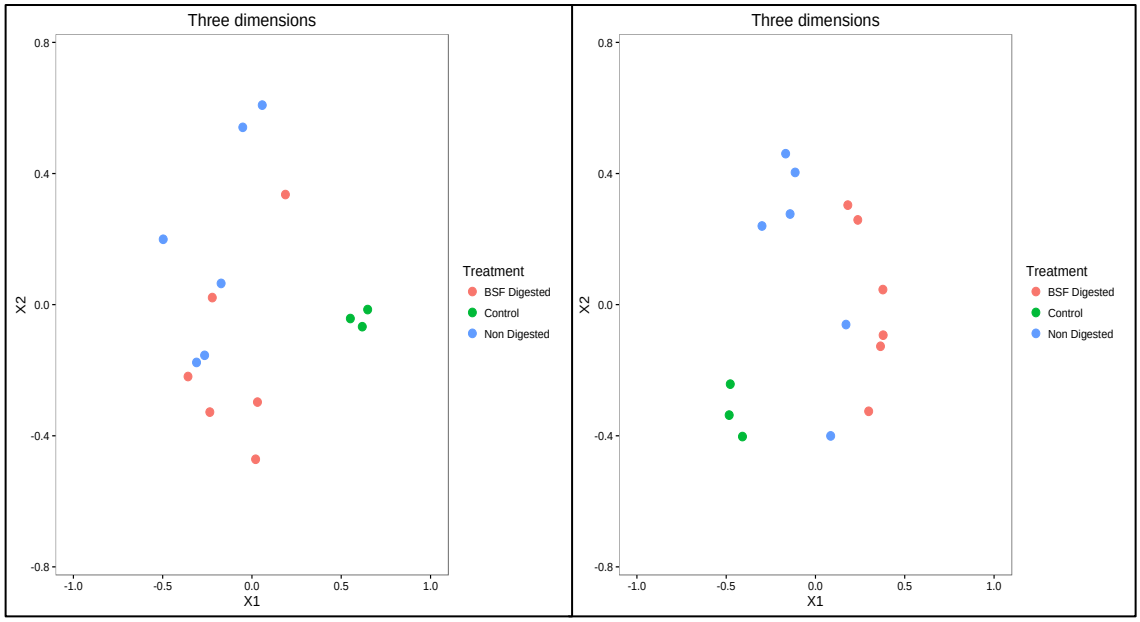

Figure A.1.6. NMDS ordinations of the community of volatile organic compounds from Trial 1 (left) and Trial 2 (right) of control, non-digested and digested dairy manure (n = 3) by *Hermetia illucens* (L.). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and14:10 L:D cycle ($P < 0.05$).

A.2 STRESS PLOTS AND NMDS ORDINATIONS FROM REDUCED VOLATILES FROM MANURE WITH AND WITHOUT *Hermetia illucens* (L.) LARVAE COMPARED TO CONTROL MANURE

.

Volatile profile was reduced by eliminating compounds, which were only present in one technical replicate from the analyses.

Poultry Manure

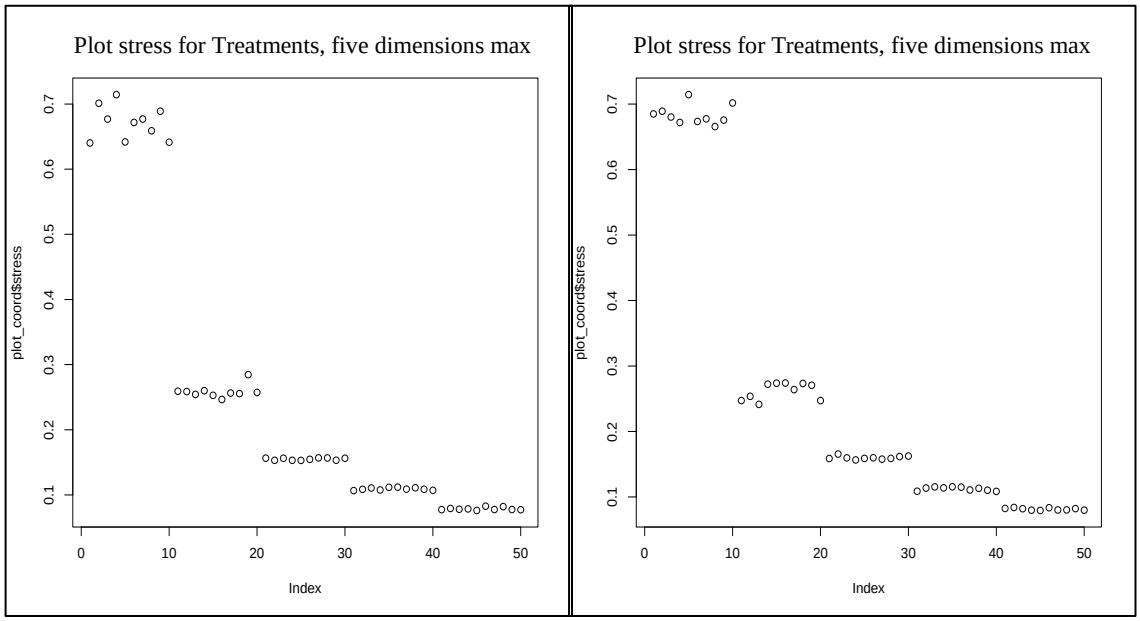

Figure A.2.1. NMDS stress plots for reduced volatiles emitted from poultry manure (n = 3) from Trial 1 (left) and Trial 2 (right) with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1: Stress: 0.1530, r^2 = 0. 8262; Trial 2: Stress: 0.1565, r^2 = 0. 8074). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

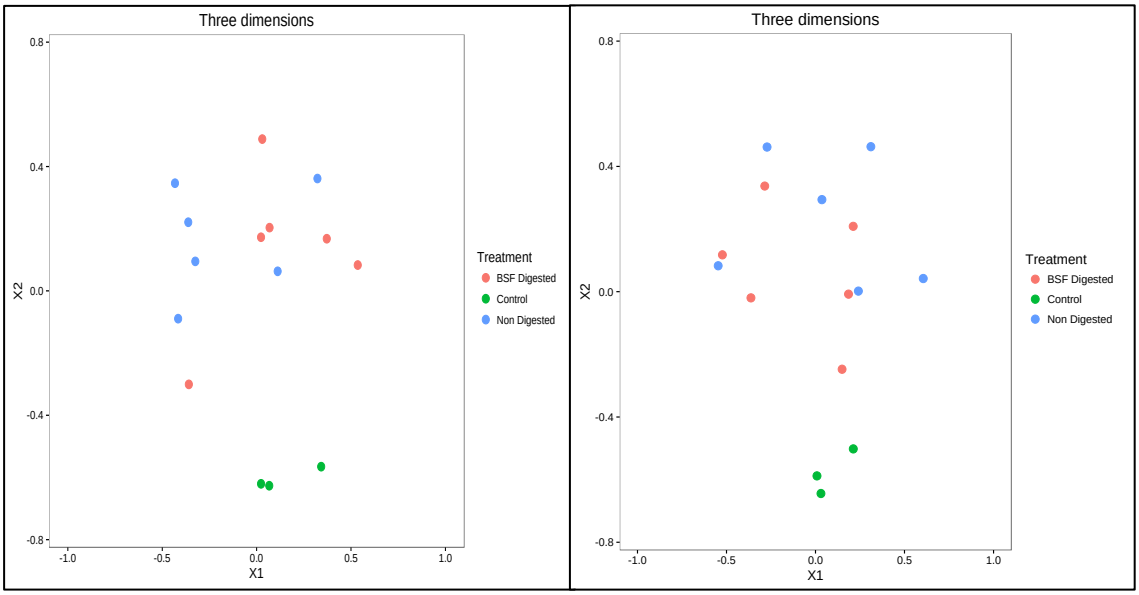

Figure A.2.2. NMDS ordinations of the reduced community of volatile organic compounds from Trial 1 (left) and Trial 2 (right) of control, non-digested and digested poultry manure (n = 3) by *Hermetia illucens* (L.). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Swine Manure

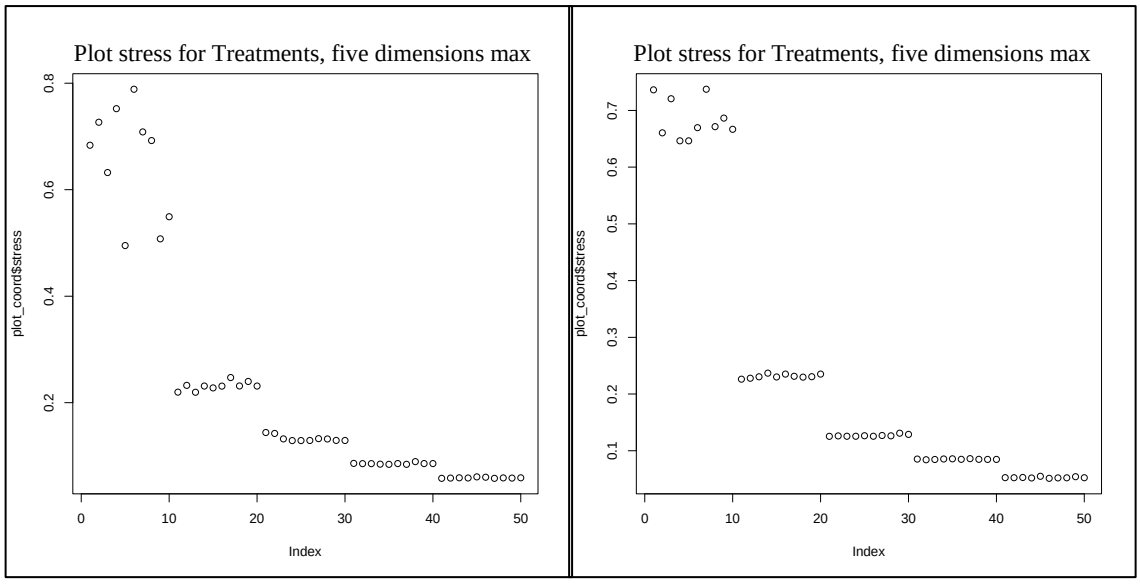

Figure A.2.3. NMDS stress plot for reduced volatiles emitted from swine manure (n = 3) from Trial 1 (left) and Trial 2 (right) with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1: Stress: 0.1288, r^2 = 0. 8939; Trial 2: Stress: 0.1255, r^2 = 0. 8890). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

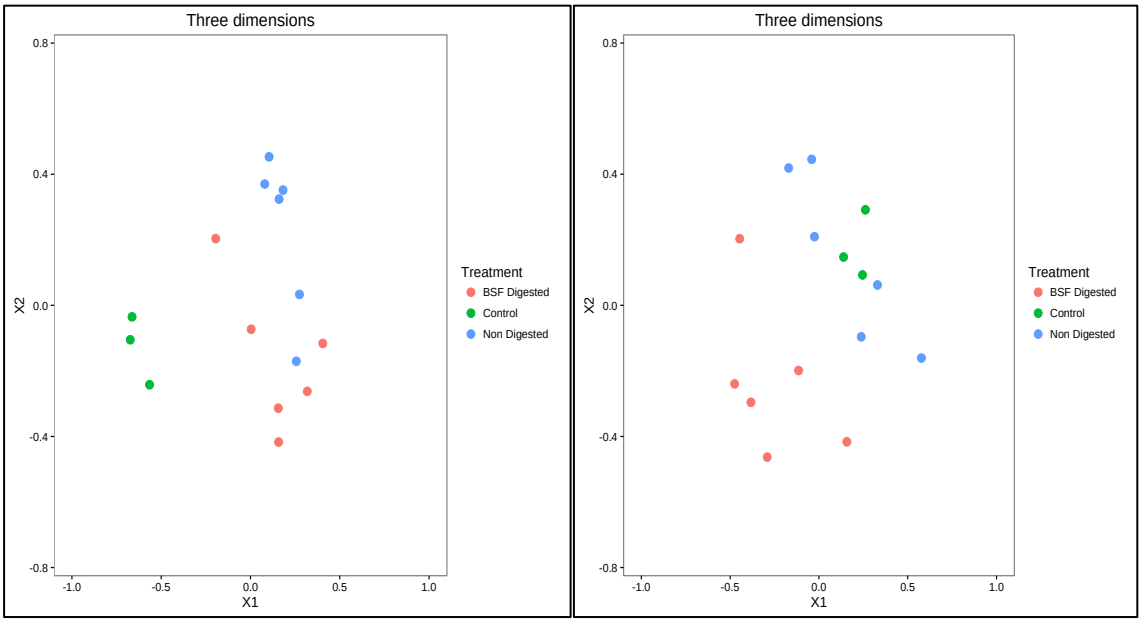

Figure A.2.4 NMDS ordinations of the reduced community of volatile organic compounds from Trial 1 (left) and Trial 2 (right) of control, non-digested and digested swine manure (n = 3) by *Hermetia illucens* (L.). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and14:10 L:D cycle ($P < 0.05$).

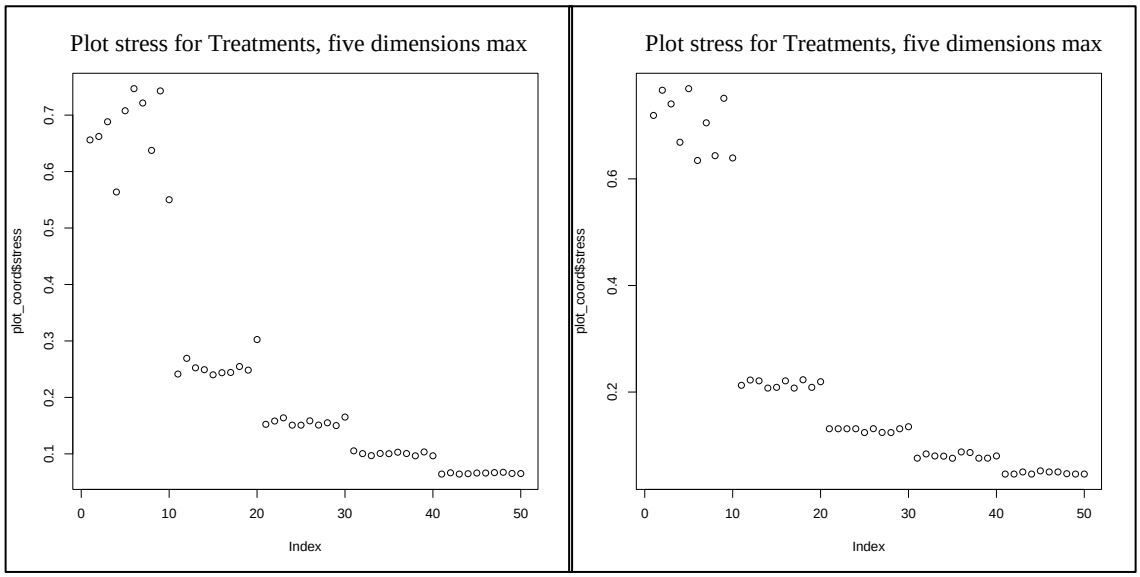

Figure A.2.5. NMDS stress plot for reduced volatiles emitted from dairy manure (n = 3) from Trial 1 (left) and Trial 2 (right) with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1: Stress: 0.1502, r^2 = 0. 8340; Trial 2: Stress: 0.1240, r^2 = 0. 9008). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

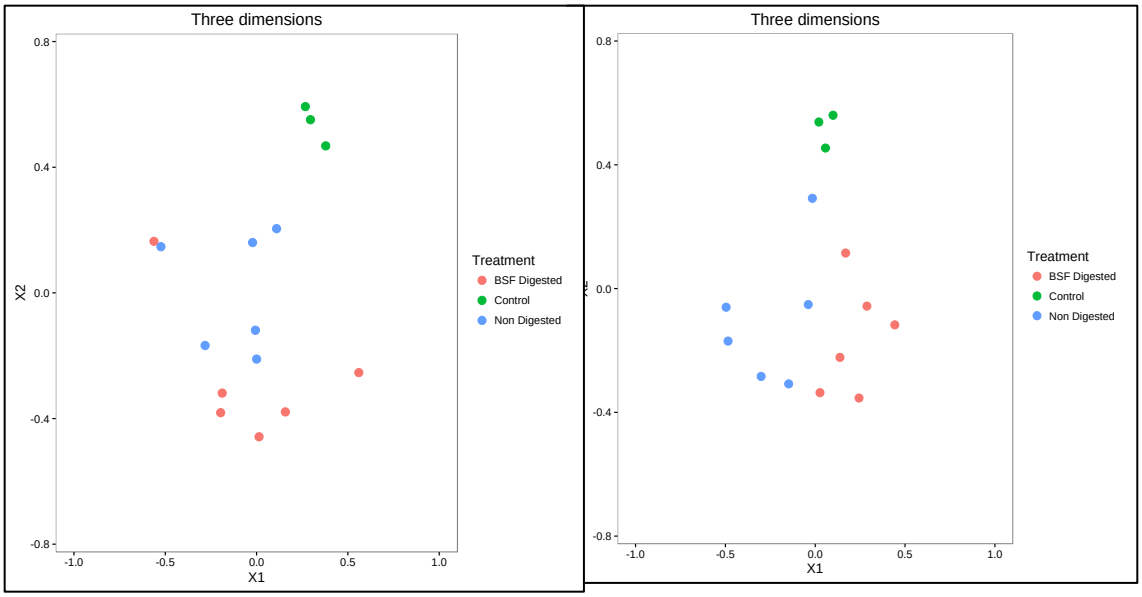

Figure A.2.6 NMDS ordinations of the reduced community of volatile organic compounds from Trial 1 (left) and Trial 2 (right) of control, non-digested and digested dairy manure (n = 3) by *Hermetia illucens* (L.). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and14:10 L:D cycle ($P < 0.05$).

A.3 STRESS PLOTS AND NMDS ORDINATIONS FROM REDUCED VOLATILES FROM MANURE WITH AND WITHOUT *Hermetia illucens* (L.) LARVAE COMPARED TO CONTROL MANURE

.

Volatile profile was reduced by grouping compounds into chemical classes.

Poultry Manure

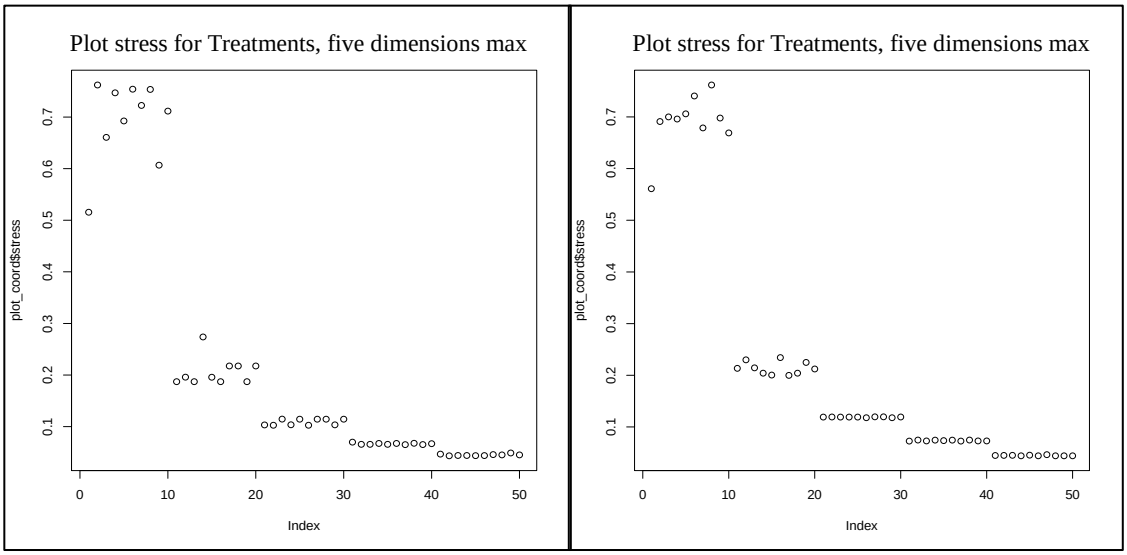

Figure A.3.1. NMDS stress plots for reduced volatiles emitted from poultry manure (n = 3) from Trial 1(left) and Trial 2 (right) with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1: Stress: 0.1027, $r^2 = 0.9320$; Trial 2: Stress: 0.1178, $r^2 = 0.9020$). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

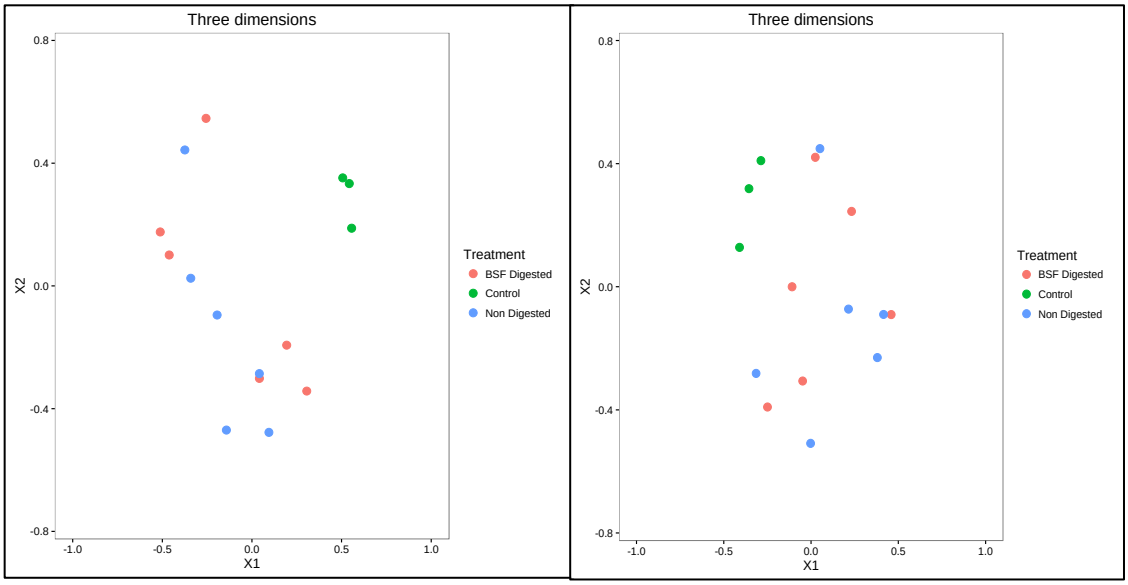

Figure A.3.2. NMDS ordinations of the reduced community of volatile organic compounds from Trial 1 (left) and Trial 2 (right) of control, non-digested and digested poultry manure (n = 3) by *Hermetia illucens* (L.). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and14:10 L:D cycle ($P < 0.05$).

Swine Manure

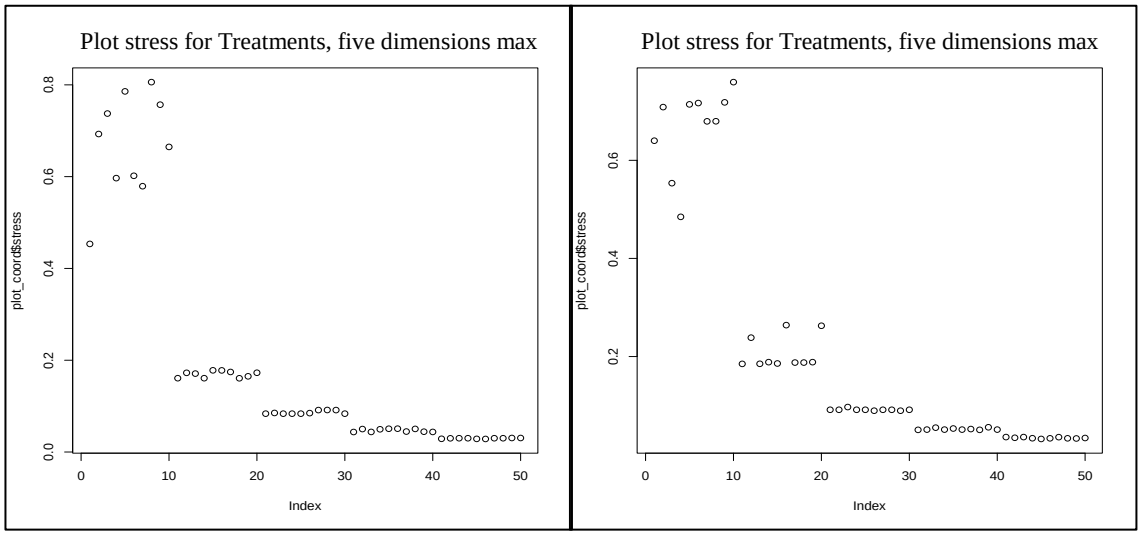

Figure A.3.3. NMDS stress plots for reduced volatiles emitted from swine manure (n = 3) from Trial 1 (left) and Trial 2 (right) with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1: Stress: 0.0836, r^2 = 0. 9644; Trial 2: Stress: 0.0895, r^2 = 0. 9473). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

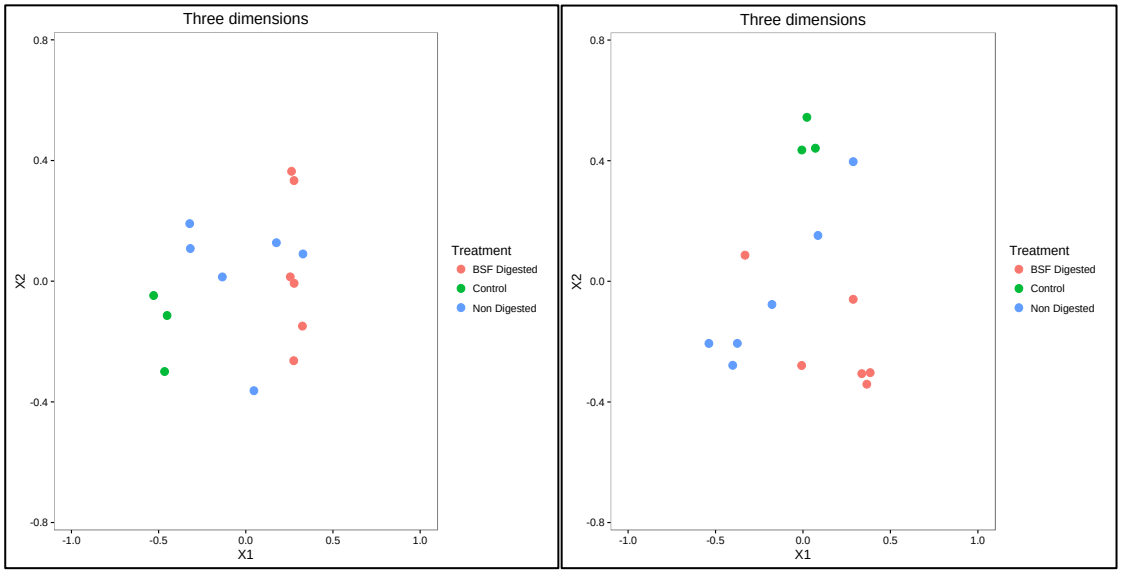

Figure A.3.4. NMDS ordinations of the reduced community of volatile organic compounds from Trial 1 (left) and Trial 2 (right) of control, non-digested and digested swine manure (n = 3) by *Hermetia illucens* (L.). Trials were conducted at 29 ± 0.3 °C with 60 ± 5.1% RH and14:10 L:D cycle ($P < 0.05$).

Dairy Manure

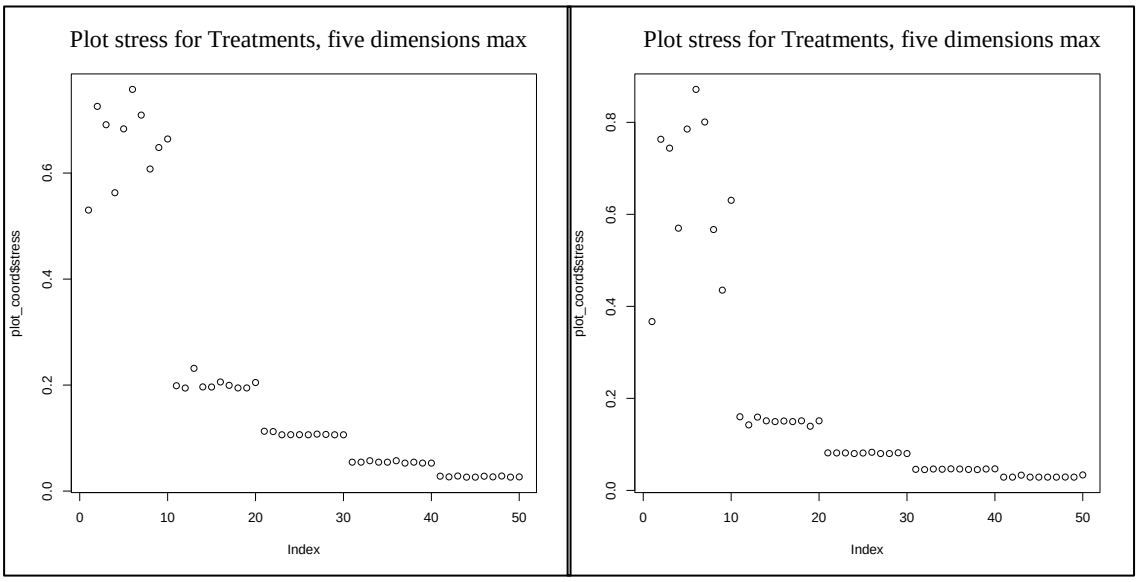

Figure A.3.5. NMDS stress plots for reduced volatiles emitted from dairy manure (n = 3) from Trial 1 (left) and Trial 2 (right) with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1: Stress: 0.1062, r^2 = 0. 9313; Stress: 0.0801, r^2 = 0. 9707). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle (P < 0.05).

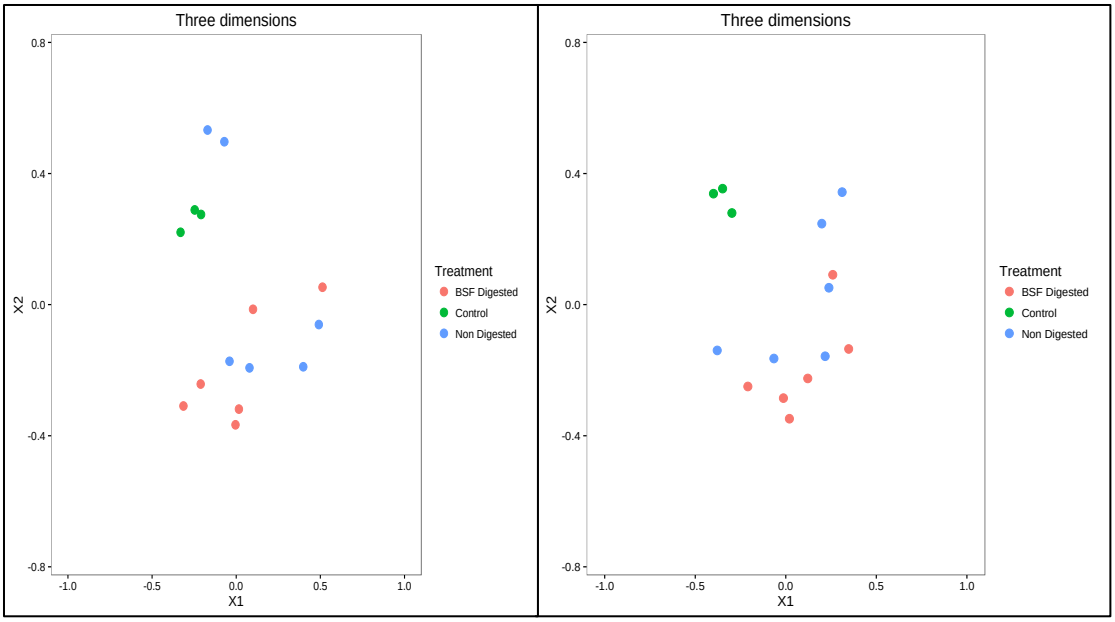

Figure A.3.6. NMDS ordinations of the reduced community of volatile organic compounds from Trial 1 (left) and Trial 2 (right) of control, non-digested and digested dairy manure (n = 3) by *Hermetia illucens* (L.). Trials were conducted at 29 ± 0.3°C with 60 ± 5.1% RH and14:10 L:D cycle ($P < 0.05$).

APPENDIX B

PERMANOVA AND MULTIPLE COMPARISON TABLES

B.1 PERMANOVA AND MULTIPLE COMPARISON TABLES FOR VOLATILES FROM MANURE WITH AND WITHOUT *Hermetia illucens* (L.) LARVAE COMPARED TO CONTROL MANURE

Poultry Manure

Table B.1.1. Analysis of volatiles emitted from poultry manure with and without *Hermetia illucens* (L.) larvae using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	1	6.522	**0.002**
Feed Rate	1	1.223	0.232
Trial	1	2.883	**0.002**
Treatment x Feed Rate	1	1.732	0.064
Treatment x Trial	1	1.627	0.076
Feed Rate x Trial	1	0.648	0.844
Treatment x Feed Rate x Trial	1	0.753	0.705
Total	7		

Table B.1.2. Analysis of volatiles emitted from poultry manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	6.689	**0.001**
Feed Rate	1	1.399	0.202
Treatment x Feed Rate	1	1.347	0.189
Total	4		

Table B.1.3. Pairwise comparisons of volatiles emitted among treatments of poultry manure (Trial 1) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.269	1.269	6.688	0.488	0.026
	Residual	7	1.328	0.189		0.512	
	Total	8	2.597			1.000	
BSF Digested x Control	Treatment	1	1.430	1.430	6.807	0.494	0.030
	Residual	7	1.470	0.210		0.506	
	Total	8	2.901			1.000	
Non-Digested x BSF Digested	Treatment	1	1.428	1.428	5.794	0.366	**0.004**
	Residual	10	2.465	0.246		0.634	
	Total	11	3.893			1.000	

Table B.1.4. Analysis of volatiles emitted from poultry manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 2) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	3.912	**0.001**
Feed Rate	1	0.803	0.571
Treatment x Feed Rate	1	1.513	0.152
Total	4		

Table B.1.5. Pairwise comparisons of volatiles emitted among treatments of poultry manure (Trial 2) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	0.984	0.984	4.189	0.374	**0.022**
l	Residual	7	1.645	0.235		0.626	
	Total	8	2.630			1.000	
BSF Digested x Control x	Treatment	1	1.251	1.251	6.156	0.467	0.028
l	Residual	7	1.423	0.203		0.533	
	Total	8	2.675			1.000	
Non-Digested x BSF Digested x	Treatment	1	0.653	0.653	2.389	0.192	0.028
	Residual	10	2.734	0.273		0.808	
	Total	11	3.388			1.000	

Swine Manure

Table B.1.6. Analysis of volatiles emitted from swine manure with and without *Hermetia illucens* (L.) larvae using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	1	2.283	**0.018**
Feed Rate	1	2.994	**0.003**
Trial	1	5.411	**0.000**
Treatment x Feed Rate	1	1.738	0.069
Treatment x Trial	1	0.712	0.732
Feed Rate x Trial	1	3.512	**0.000**
Treatment x Feed Rate x Trial	1	0.841	0.589
Total	7		

Table B.1.7. Analysis of volatiles emitted from swine manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	5.726	**0.001**
Feed Rate	1	3.707	**0.006**
Treatment x Feed Rate	1	1.015	0.401
Total	4		

Table B.1.8. Pairwise comparisons of volatiles emitted among treatments of swine manure (Trial 1) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.374	1.374	6.950	0.498	**0.024**
	Residual	7	1.384	0.197		0.502	
	Total	8	2.759			1.000	
BSF Digested x Control x	Treatment	1	1.462	1.461	9.579	0.577	0.026
	Residual	7	1.068	0.152		0.423	
	Total	8	2.530			1.000	
Non-Digested x BSF Digested	Treatment	1	0.178	0.178	0.779	0.072	1.252
	Residual	10	2.290	0.229		0.928	
	Total	11	2.468			1.000	

Table B.1.9. Analysis of volatiles emitted from swine manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 2) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	5.316	**0.001**
Feed Rate	1	4.017	0.003*
Treatment x Feed Rate	1	2.027	0.066
Total	4		

Table B.1.10. Pairwise comparisons of volatiles emitted among treatments of swine manure (Trial 2) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.103	1.103	5.393	0.435	0.036
	Residual	7	1.431	0.204		0.564	
	Total	8	2.534			1.000	
BSF Digested x Control	Treatment	1	1.342	1.342	6.926	0.497	**0.024**
	Residual	7	1.356	0.194		0.502	
	Total	8	2.698			1.000	
Non-Digested x BSF Digested x	Treatment	1	0.409	0.409	1.556	0.134	0.300
	Residual	10	2.625	0.263		0.865	
	Total	11	3.034			1.000	

Dairy Manure

Table B.1.11. Analysis of volatiles emitted from dairy manure with and without *Hermetia illucens* (L.) larvae using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	1	2.561	**0.007**
Feed Rate	1	1.864	0.050
Trial	1	5.054	**0.000**
Treatment x Feed Rate	1	1.527	0.121
Treatment x Trial	1	0.601	0.837
Feed Rate x Trial	1	1.863	**0.045**
Treatment x Feed Rate x Trial	1	0.864	0.552
Total	7		

Table B.1.12. Analysis of volatiles emitted from dairy manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	5.065	**0.001**
Feed Rate	1	1.603	0.139
Treatment x Feed Rate	1	2.167	0.068
Total	4		

Table B.1.13. Pairwise comparisons of volatiles emitted among treatments of dairy manure (Trial 1) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.234	1.234	5.111	0.422	0.042
	Residual	7	1.691	0.241		0.578	
	Total	8	2.925			1.000	
BSF Digested x Control	Treatment	1	1.427	1.427	10.175	0.5925	0.032
	Residual	7	0.981	0.140		0.4075	
	Total	8	2.409			1.000	
Non-Digested x BSF Digested	Treatment	1	0.428	0.428	1.647	0.141	0.238
	Residual	10	2.600	0.260		0.859	
	Total	11	3.028			1.000	

Table.B.1.14. Analysis of volatiles emitted from dairy manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 2) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	5.840	**0.001**
Feed Rate	1	3.141	0.015
Treatment x Feed Rate	1	0.568	0.763
Total	4		

Table B.1.15. Pairwise comparisons of volatiles emitted among treatments of dairy manure (Trial 2) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.291	1.291	7.348	0.512	**0.024**
l	Residual	7	1.229	0.175		0.488	
	Total	8	2.530			1.000	
BSF Digested x Control	Treatment	1	1.328	1.328	10.684	0.604	**0.024**
l	Residual	7	0.879	0.124		0.396	
	Total	8	2.199			1.000	
Non-Digested x BSF Digested	Treatment	1	0.233	0.233	1.151	0.103	0.614
	Residual	10	2.027	0.202		0.897	
	Total	11	2.261			1.000	

B.2 PERMANOVA AND MULTIPLE COMPARISON TABLES FOR REDUCED VOLATILES FROM MANURE WITH AND WITHOUT *Hermetia illucens* (L.) LARVAE COMPARED TO CONTROL MANURE

Volatile profile was reduced by eliminating compounds, which were only present in one technical replicate from the analyses.

Poultry Manure

Table B.2.1. Analysis of reduced volatiles emitted from poultry manure with and without *Hermetia illucens* (L.) larvae using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	1	6.852	**0.00**0
Feed Rate	1	1.217	0.239
Trial	1	2.020	**0.002**
Treatment x Feed Rate	1	1.728	0.067
Treatment x Trial	1	1.695	0.073
Feed Rate x Trial	1	0.623	0.855
Treatment x Feed Rate x Trial	1	0.731	0.732
Total	7		

Table B.2.2. Analysis of reduced volatiles emitted from poultry manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	7.072	**0.001**
Feed Rate	1	1.375	0.192
Treatment x Feed Rate	1	1.310	0.242
Total	4		

Table B.2.3. Pairwise comparisons of reduced volatiles emitted among treatments of poultry manure (Trial 1) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.281	1.281	6.968	0.499	0.026
l	Residual	7	1.287	0.183		0.501	
	Total	8	2.569			1.000	
BSF Digested x Control	Treatment	1	1.453	1.453	7.248	0.509	**0.018**
	Residual	7	1.403	0.200		0.491	
	Total	8	2.856			1.000	
Non-Digested x BSF Digested	Treatment	1	1.467	1.467	6.222	0.384	**0.060**
	Residual	10	2.357	0.235		0.616	
	Total	11	3.824			1.000	

Table B.2.4. Analysis of reduced volatiles emitted from poultry manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 2) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	4.046	**0.001**
Feed Rate	1	0.792	0.616
Treatment x Feed Rate	1	1.510	0.152
Total	4		

Table B.2.5. Pairwise comparisons of reduced volatiles emitted among treatments of poultry manure (Trial 2) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	0.992	0.992	4.298	0.380	0.028
l	Residual	7	1.616	0.230		0.620	
	Total	8	2.609			1.000	
BSF Digested x Control	Treatment	1	1.258	1.258	6.288	0.473	**0.022**
	Residual	7	1.401	0.200		0.527	
	Total	8	2.660			1.000	
Non-Digested x BSF Digested	Treatment	1	0.656	0.656	2.443	0.196	0.038
	Residual	10	2.684	0.268		0.804	
	Total	11	3.341			1.000	

Swine Manure

Table B.2.6. Analysis of reduced volatiles emitted from swine manure with and without *Hermetia illucens* (L.) larvae using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	1	2.334	**0.014**
Feed Rate	1	3.077	**0.002**
Trial	1	5.604	**0.000**
Treatment x Feed Rate	1	1.762	0.071
Treatment x Trial	1	0.726	0.695
Feed Rate x Trial	1	3.658	**0.000**
Treatment x Feed Rate x Trial	1	0.826	0.589
Total	7		

Table B.2.7. Analysis of reduced volatiles emitted from swine manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	5.941	**0.002**
Feed Rate	1	3.878	**0.003**
Treatment x Feed Rate	1	0.995	0.404
Total	4		

Table B.2.8. Pairwise comparisons of reduced volatiles emitted among treatments of swine manure (Trial 1) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.382	1.382	7.111	0.504	0.032
	Residual	7	1.360	0.194		0.496	
	Total	8	2.743			1.000	
BSF Digested x Control	Treatment	1	1.470	1.470	9.891	0.586	0.037
	Residual	7	1.041	0.148		0.414	
	Total	8	2.511			1.000	
Non-Digested x BSF Digested	Treatment	1	0.178	0.178	0.797	0.074	1.170
	Residual	10	2.242	0.224		0.926	
	Total	11	2.420			1.000	

Table B.2.9. Analysis of reduced volatiles emitted from swine manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 2) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	4.454	**0.001**
Feed Rate	1	4.116	**0.002**
Treatment x Feed Rate	1	2.051	0.075
Total	4		

Table B.2.10. Pairwise comparisons of reduced volatiles emitted among treatments of swine manure (Trial 2) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.111	1.111	5.516	0.441	**0.024**
	Residual	7	1.410	0.201		0.559	
	Total	8	2.521			1.000	
BSF Digested x Control	Treatment	1	1.349	1.349	7.060	0.502	0.034
	Residual	7	1.337	0.191		0.498	
	Total	8	2.686			1.000	
Non-Digested x BSF Digested	Treatment	1	0.405	0.405	1.565	0.136	0.306
	Residual	10	2.588	0.258		0.864	
	Total	11	2.993			1.000	

Dairy Manure

Table B.2.11. Analysis of reduced volatiles emitted from dairy manure with and without *Hermetia illucens* (L.) larvae using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	1	2.661	**0.008**
Feed Rate	1	1.916	**0.048**
Trial	1	5.292	**0.000**
Treatment x Feed Rate	1	1.613	0.108
Treatment x Trial	1	0.604	0.807
Feed Rate x Trial	1	1.927	**0.046**
Treatment x Feed Rate x Trial	1	0.854	0.562
Total	7		

Table B.2.12. Analysis of reduced volatiles emitted from dairy manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	5.213	**0.001**
Feed Rate	1	1.613	0.144
Treatment x Feed Rate	1	2.179	0.049
Total	4		

Table B.2.13. Pairwise comparisons of reduced volatiles emitted among treatments of dairy manure (Trial 1) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non Digested x Control	Treatment	1	1.241	1.241	5.216	0.427	0.028
	Residual	7	1.666	0.238		0.573	
	Total	8	2.908			1.000	
BSF Digested x Control	Treatment	1	0.416	0.416	1.644	0.141	0.298
	Residual	7	2.535	0.253		0.859	
	Total	8	2.952			1.000	
Non-Digested x BSF Digested	Treatment	1	1.441	1.441	10.735	0.605	0.040
	Residual	10	0.940	0.134		0.394	
	Total	11	2.381			1.000	

Table B.2.14. Analysis of reduced volatiles emitted from dairy manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 2) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	6.319	**0.001**
Feed Rate	1	3.327	**0.017**
Treatment x Feed Rate	1	0.629	0.678
Total	4		

Table B.2.15. Pairwise comparisons of reduced volatiles emitted among treatments of dairy manure (Trial 2) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control l	Treatment	1	1.303	1.303	7.701	0.524	**0.020**
	Residual	7	1.185	0.169		0.476	
	Total	8	2.488			1.000	
BSF Digested x Control l	Treatment	1	1.344	1.344	11.587	0.623	**0.018**
	Residual	7	0.812	0.116		0.377	
	Total	8	2.156			1.000	
Non-Digested x BSF Digested	Treatment	1	0.236	0.236	1.226	0.109	0.500
	Residual	10	1.926	0.192		0.891	
	Total	11	2.162			1.000	

B.3 PERMANOVA AND MULTIPLE COMPARISON TABLES FOR REDUCED VOLATILES FROM MANURE WITH AND WITHOUT *Hermetia illucens* (L.) LARVAE COMPARED TO CONTROL MANURE

.

Volatile profile was reduced by grouping compounds into chemical classes.

Poultry Manure

Table B.3.1. Analysis of reduced volatiles emitted from poultry manure with and without *Hermetia illucens* (L.) larvae using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	1	9.709	**0.000**
Feed Rate	1	1.016	0.393
Trial	1	2.615	**0.022**
Treatment x Feed Rate	1	1.229	0.268
Treatment x Trial	1	1.991	0.066
Feed Rate x Trial	1	0.605	0.767
Treatment x Feed Rate x Trial	1	0.609	0.756
Total	7		

Table B.3.2. Analysis of reduced volatiles emitted from poultry manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	10.530	**0.001**
Feed Rate	1	1.232	0.260
Treatment x Feed Rate	1	0.823	0.507
Total	4		

Table B.3.3. Pairwise comparisons of reduced volatiles emitted among treatments of poultry manure (Trial 1) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.244	1.244	11.852	0.629	0.034
l	Residual	7	0.734	0.105		0.371	
	Total	8	1.979			1.000	
BSF Digested x Control	Treatment	1	1.547	1.547	10.215	0.593	**0.014**
	Residual	7	1.060	0.151		0.407	
	Total	8	2.607			1.000	
Non-Digested x BSF Digested	Treatment	1	1.562	1.562	10.059	0.502	**0.010**
	Residual	10	1.553	0.155		0.498	
	Total	11	3.116			1.000	

Table B.3.4. Analysis of reduced volatiles emitted from poultry manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 2) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	5.078	**0.001**
Feed Rate	1	0.712	0.594
Treatment x Feed Rate	1	1.246	0.260
Total	4		

Table B.3.5. Pairwise comparisons of reduced volatiles emitted among treatments of poultry manure (Trial 2) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	0.994	0.994	5.920	0.458	0.036
	Residual	7	1.175	0.167		0.542	
	Total	8	2.170			1.000	
BSF Digested x Control	Treatment	1	1.273	1.273	7.980	0.533	**0.020**
	Residual	7	1.117	0.159		0.467	
	Total	8	2.391			1.000	
Non-Digested x BSF Digested	Treatment	1	0.605	0.605	2.949	0.228	0.042
	Residual	10	0.051	0.205		0.772	
	Total	11	2.656			1.000	

Swine Manure

Table B.3.6. Analysis of reduced volatiles emitted from swine manure with and without *Hermetia illucens* (L.) larvae using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	1	2.394	**0.039**
Feed Rate	1	3.069	**0.011**
Trial	1	5.520	**0.000**
Treatment x Feed Rate	1	1.788	0.104
Treatment x Trial	1	1.156	0.305
Feed Rate x Trial	1	4.212	**0.002**
Treatment x Feed Rate x Trial	1	0.673	0.659
Total	7		

Table B.3.7. Analysis of reduced volatiles emitted from swine manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	8.181	**0.002**
Feed Rate	1	3.894	**0.017**
Treatment x Feed Rate	1	0.773	0.504
Total	4		

Table B.3.8. Pairwise comparisons of reduced volatiles emitted among treatments of swine manure (Trial 1) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.379	1.3790	10.362	0.596	0.032
	Residual	7	0.935	0.134		0.404	
	Total	8	2.317			1.000	
BSF Digested x Control	Treatment	1	1.450	1.450	13.742	0.663	0.037
	Residual	7	0.738	0.105		0.337	
	Total	8	2.189			1.000	
Non-Digested x BSF Digested	Treatment	1	0.118	0.118	0.768	0.071	1.014
	Residual	10	1.539	0.153		0.929	
	Total	11	1.657			1.000	

Table B.3.9. Analysis of reduced volatiles emitted from swine manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 2) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	6.379	**0.001**
Feed Rate	1	4.632	**0.004**
Treatment x Feed Rate	1	2.008	0.091
Total	4		

Table B.3.10. Pairwise comparisons of reduced volatiles emitted among treatments of swine manure (Trial 2) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.052	1.052	6.804	0.493	**0.018**
	Residual	7	1.082	0.154		0.507	
	Total	8	2.135			1.000	
BSF Digested x Control	Treatment	1	1.280	1.280	7.344	0.512	0.036
	Residual	7	1.219	0.174		0.488	
	Total	8	2.499			1.000	
Non-Digested x BSF Digested	Treatment	1	0.393	0.393	1.814	0.154	0.230
	Residual	10	2.168	0.216		0.846	
	Total	11	3.034			1.000	

Dairy Manure

Table B.3.11. Analysis of reduced volatiles emitted from dairy manure with and without *Hermetia illucens* (L.) larvae using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	1	2.054	0.080
Feed Rate	1	1.795	0.122
Trial	1	5.871	**0.000**
Treatment x Feed Rate	1	1.766	0.129
Treatment x Trial	1	0.278	0.919
Feed Rate x Trial	1	0.841	0.522
Treatment x Feed Rate x Trial	1	0.549	0.736
Total	7		

Table B.3.12. Analysis of reduced volatiles emitted from dairy manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 1) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	6.557	**0.001**
Feed Rate	1	1.853	0.133
Treatment x Feed Rate	1	2.263	0.083
Total	4		

Table B.3.13. Pairwise comparisons of reduced volatiles emitted among treatments of dairy manure (Trial 1) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.204	1.204	6.079	0.465	0.034
	Residual	7	1.386	0.198		0.535	
	Total	8	2.591			1.000	
BSF Digested x Control	Treatment	1	1.451	1.451	16.994	0.7083	0.026
	Residual	7	0.597	0.085		0.292	
	Total	8	2.049			1.000	
Non-Digested x BSF Digested	Treatment	1	0.288	0.288	1.484	0.129	0.438
	Residual	10	1.941	0.194		0.871	
	Total	11	2.230			1.000	

Table B.3.14. Analysis of reduced volatiles emitted from dairy manure with and without *Hermetia illucens* (L.) larvae compared to control (Trial 2) using Permutational analysis of variance (PERMANOVA). Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	df	F Model	*P* Value
Treatment	2	7.687	0.001
Feed Rate	1	1.314	0.261
Treatment x Feed Rate	1	0.325	0.888
Total	4		

Table B.3.15. Pairwise comparisons of reduced volatiles emitted among treatments of dairy manure (Trial 2) after Bonferroni's correction ($P < 0.025$).

Factor		df	SS	MS	F Model	R^2	*P* value
Non-Digested x Control	Treatment	1	1.320	1.320	12.821	0.647	**0.022**
	Residual	7	0.720	0.102		0.353	
	Total	8	2.041			1.000	
BSF Digested x Control	Treatment	1	1.329	1.329	17.138	0.710	0.034
	Residual	7	0.542	0.077		0.290	
	Total	8	1.872			1.000	
Non-Digested x BSF Digested	Treatment	1	0.064	0.064	0.524	0.050	1.476
	Residual	10	1.221	0.122		0.950	
	Total	11	1.285			1.000	

APPENDIX C

SUMMARY OF INDICATOR SPECIAL ANALYSIS (ISA)

C.1 ISA SUMMARY FROM VOLATILES EMITTED FROM MANURE WITH AND WITHOUT *Hermetia illucens* (L.) LARVAE COMPARED TO CONTROL MANURE

Poultry Manure

Table C.1.1.1. Indicator compound analysis based on volatiles emitted from poultry manure (n = 3) (Trial 1) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound	Indicator Value	*P* Value
Control	2(3H)-Furanone, dihydro-5-pently	1.000	**0.010**
	2(3H)-Furanone, 5-ethylhydro-5-methyl	0.946	**0.010**
	Benzene, 1,4-dichloro	0.779	**0.015**
	Benezenepropanoic acid, ethyl ester	1.000	**0.010**
	Benzenepropanoic acid, methyl ester	1.000	**0.010**
	Benzoic, 2-hydroxy-, methyl ester	1.000	**0.010**
	Butanoic acid	0.995	**0.010**
	Butanoic acid, 2-methyl	0.985	**0.010**
	Butanoic acid, 3-methyl	0.986	**0.010**
	Butanoic acid, propyl ester	1.000	**0.010**
	Heptanoic acid	1.000	**0.010**
	Hexanoic acid	1.000	**0.010**
	Indole	0.881	**0.015**
	Indole, 3-methyl	0.816	**0.025**
	Isobornyl acetate	1.00	**0.010**
	Maltol	0.816	**0.035**
	Oleic acid	0.816	**0.050**
	Pentanoic acid	0.989	**0.010**
	Pentanoic acid, 4-methyl	0.993	**0.010**
	Pentanoic acid, propyl ester	1.000	**0.010**
	Phenol	0.964	**0.010**
	Phenol, 2-methoxy	1.000	**0.010**
	Phenol, 2-methoxy-4-methyl	0.931	**0.010**
	Phenol, 4-ethyl	0.978	**0.010**
	Phenol, 4-methyl	0.986	**0.010**
	Propanoic acid	0.976	**0.010**
	Propanoic acid, 2-methyl	0.816	**0.035**
BSF Digested	2-Undecanone	0.913	**0.015**
	3-Octanone	1.000	**0.005**
	3,4-Dimethoxytoluene	1.000	**0.005**

Table C.1.1 (Continued)

	Acetophenone	0.985	**0.010**
	Cyclohept-4-enone	0.913	**0.020**
	Dimethyl sulfone	0.991	**0.005**
	Hexadecane	0.898	**0.035**
	Pentadecane	0.972	**0.005**
	Phenol, 2-(1-methylethyl)	0.907	**0.020**
	Pyrazine, tetramethyl-	0.786	**0.050**
	Pyrazine, trimethyl-	0.838	**0.030**
Non-Digested	2-Nonanone	0.986	**0.005**

Table C.1.2. Indicator compound analysis based on volatiles emitted from poultry manure (n = 3) (Trial 2) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound	Indicator Value	*P* Value
Control	Benezenepropanoic acid, ethyl ester	1.000	**0.005**
	Benzenepropanoic acid, methyl ester	1.000	**0.005**
	Benzoic, 2-hydroxy-, methyl ester	1.000	**0.005**
	Butanoic acid	0.968	**0.040**
	Butanoic acid, 2-methyl	0.863	**0.035**
	Butanoic acid, 3-methyl	0.858	**0.030**
	Butanoic acid, propyl ester	1.000	**0.005**
	Heptanoic acid	0.896	**0.015**
	Hexanoic acid	0.848	**0.040**
	Indole	0.962	**0.005**
	Isobornyl acetate	1.000	**0.005**
	Maltol	0.816	**0.045**
	Pentanoic acid	0.941	**0.020**
	Pentanoic acid, 4-methyl	0.937	**0.005**
	Pentanoic acid, propyl ester	1.000	**0.005**
	Phenol	0.984	**0.005**
	Phenol, 2-methoxy	1.000	**0.005**
	Phenol, 2-methoxy-4-methyl	0.952	**0.005**
	Phenol, 4-ethyl	0.968	**0.010**
	Phenol, 4-methyl	0.979	**0.005**
	Propanoic acid	0.863	**0.035**
BSF Digested	Benzoic acid, 4-ethoxy-ethyl ester	0.732	**0.025**
Non-Digested	2-Nonanone	0.988	**0.005**
	2-Undecanone	0.959	**0.005**
	3,4-Dimethoxytoluene	0.913	**0.030**
	Acetophenone	0.981	**0.005**
	Dimethyl sulfone	0.859	**0.015**

Swine Manure

Table C.1.3 Indicator compound analysis based on volatiles emitted from swine manure (n = 3) (Trial 1) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound	Indicator Value	*P* Value
Control	2-Decanone	1.000	**0.005**
	Acetophenone	0.855	**0.005**
	Benzaldehyde	0.761	**0.010**
	Butanoic acid	1.000	**0.005**
	Butanoic acid, 3-methyl	1.000	**0.005**
	Decane	0.816	**0.015**
	Diphenyl sulfide	1.000	**0.005**
	Indole	1.000	**0.005**
	Indole, 3-methyl	1.000	**0.005**
	Isobornyl acetate	1.000	**0.005**
	Pentanoic acid, 4-methyl	0.977	**0.005**
	Phenol	1.000	**0.005**
	Phenol, 4-ethyl	0.994	**0.005**
	Phenol, 4-methyl	1.000	**0.005**
	Propanoic acid	1.000	**0.005**
	Propanoic acid, 2-methyl	1.000	**0.005**
	Toluene	1.000	**0.005**
	Undecane	0.955	**0.005**
Non-Digested	3-Octanone	0.816	**0.035**

Table C.1.4. Indicator compound analysis based on volatiles emitted from swine manure (n = 3) (Trial 2) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound	Indicator Value	*P* Value
Control	2-Decanone	1.000	**0.010**
	Acetophenone	0.821	**0.040**
	Butanoic acid	1.000	**0.010**
	Butanoic acid, 3-methyl	1.000	**0.010**
	Decanal	0.725	**0.050**
	Diphenyl sulfide	1.000	**0.010**
	Indole	0.905	**0.015**
	Indole, 3-methyl	0.956	**0.010**
	Pentanoic acid, 4-methyl	1.000	**0.010**
	Phenol	0.964	**0.010**
	Phenol, 4-ethyl	0.982	**0.010**
	Phenol, 4-methyl	0.963	**0.010**
	Propanoic acid	1.000	**0.010**
	Propanoic acid, 2-methyl	1.000	**0.010**
	Toluene	1.000	**0.010**
Non-Digested	3-Octanone	0.816	**0.020**
	Benzoic acid, 4-ethoxy-ethyl ester	0.735	**0.050**

Dairy Manure

Table C.1.5. Indicator compound analysis based on volatiles emitted from dairy manure (n = 3) (Trial 1) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound	Indicator Value	*P* Value
Control	Butanoic acid	1.000	**0.010**
	Butanoic acid, 3-methyl	1.000	**0.010**
	Decane	0.816	**0.035**
	Indole	0.784	**0.045**
	Indole, 3-methyl	0.999	**0.010**
	Isobornyl acetate	0.816	**0.040**
	Pentanoic acid	1.000	**0.010**
	Phenol	0.992	**0.010**
	Phenol, 2-ethyl	0.816	**0.045**
	Phenol, 4-ethyl	0.957	**0.015**
	Phenol, 4-methyl	0.998	**0.010**
	Propanoic acid	1.000	**0.010**
	Propanoic acid, 2-methyl	1.000	**0.010**
	Undecane	0.968	**0.010**

Table C.1.6. Indicator compound analysis based on volatiles emitted from dairy manure (n = 3) (Trial 2) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound	Indicator Value	*P* Value
Control	Butanoic acid	1.000	**0.005**
	Butanoic acid, 3-methyl	1.000	**0.005**
	Indole	0.816	**0.015**
	Indole, 3-methyl	1.000	**0.005**
	Pentanoic acid	1.000	**0.005**
	Phenol	0.997	**0.005**
	Phenol, 2-ethyl	0.816	**0.015**
	Phenol, 4-ethyl	1.000	**0.005**
	Phenol, 4-methyl	1.000	**0.005**
	Pinene	0.816	**0.035**
	Propanoic acid	1.000	**0.005**
	Propanoic acid, 2-methyl	1.000	**0.005**
BSF Digested	Naphthalene	0.913	**0.020**
Non-Digested	3-Octanone	0.816	**0.035**
	Pentadecane	0.967	**0.005**

C.2 ISA SUMMARY BASED OFF REDUCED VOLATILES EMITTED FROM MANURE WITH AND WITHOUT *Hermetia illucens* (L.) LARVAE COMPARED TO CONTROL MANURE

Volatile profile was reduced by eliminating compounds, which were only present in one technical replicate from the analyses.

Poultry Manure

Table C.2.1. Indicator compound analysis based on reduced volatiles emitted from poultry manure (n = 3) (Trial 1) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound	Indicator Value	*P* Value
Control	2(3H)-Furanone, dihydro-5-pently	1.000	**0.005**
	2(3H)-Furanone, 5-ethylhydro-5-methyl	0.945	**0.005**
	Benzene, 1,4-dichloro	0.779	**0.005**
	Benezenepropanoic acid, ethyl ester	1.000	**0.005**
	Benzenepropanoic acid, methyl ester	1.000	**0.005**
	Benzoic acid, 2-hydroxy-, methyl ester	1.000	**0.005**
	Butanoic acid	0.995	**0.005**
	Butanoic acid, 2-methyl	0.985	**0.005**
	Butanoic acid, 3-methyl	0.986	**0.005**
	Butanoic acid, propyl ester	1.000	**0.005**
	Heptanoic acid	1.000	**0.005**
	Hexanoic acid	1.000	**0.005**
	Indole	0.881	**0.030**
	Indole, 3-methyl	0.816	**0.030**
	Isobornyl acetate	1.00	**0.005**
	Maltol	0.816	**0.030**
	Oleic acid	0.816	**0.035**
	Pentanoic acid	0.989	**0.005**
	Pentanoic acid, 4-methyl	0.993	**0.005**
	Pentanoic acid, propyl ester	1.000	**0.005**
	Phenol	0.964	**0.005**
	Phenol, 2-methoxy	1.000	**0.005**
	Phenol, 2-methoxy-4-methyl	0.931	**0.010**
	Phenol, 4-ethyl	0.977	**0.010**
	Phenol, 4-methyl	0.987	**0.010**
	Propanoic acid	0.976	**0.005**
	Propanoic acid, 2-methyl	0.816	**0.030**
Non-Digested	2-Butanone, 3-hydroxy	0.816	**0.035**
	2-Nonanone	0.986	**0.005**
	2-Undecanone	0.912	**0.010**
	3,4-Dimethoxytoluene	1.000	**0.005**
	3-Octanone	1.000	**0.005**
	Acetophenone	0.985	**0.005**
	Cyclohept-4-enone	0.913	**0.015**
	Dimethyl sulfone	0.991	**0.005**
	Hexadecane	0.898	**0.035**
	Pentadecane	0.972	**0.005**
	Phenol, 2-(1-methylethyl)	0.907	**0.015**
	Pyrazine, trimethyl-	0.838	**0.020**

Table C.2.2. Indicator compound analysis based on reduced volatiles emitted from poultry manure (n = 3) (Trial 2) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$)**.**

Factor	Compound	Indicator Value	*P* Value
Control	2(3H)-Furanone, dihydro-5-pently	0.799	**0.040**
	Benezenepropanoic acid, ethyl ester	1.000	**0.005**
	Benzenepropanoic acid, methyl ester	1.000	**0.005**
	Benzoic acid, 2-hydroxy-, methyl ester	1.000	**0.005**
	Butanoic acid	0.968	**0.020**
	Butanoic acid, 2-methyl	0.863	**0.025**
	Butanoic acid, 3-methyl	0.858	**0.025**
	Butanoic acid, propyl ester	1.000	**0.005**
	Heptanoic acid	0.896	**0.020**
	Hexanoic acid	0.848	**0.040**
	Indole	0.962	**0.005**
	Isobornyl acetate	1.000	**0.005**
	Maltol	0.816	**0.025**
	Pentanoic acid	0.941	**0.015**
	Pentanoic acid, 4-methyl	0.937	**0.010**
	Pentanoic acid, propyl ester	1.000	**0.005**
	Phenol	0.984	**0.005**
	Phenol, 2-methoxy	1.000	**0.005**
	Phenol, 2-methoxy-4-methyl	0.952	**0.005**
	Phenol, 4-ethyl	0.968	**0.010**
	Phenol, 4-methyl	0.979	**0.005**
	Propanoic acid	0.863	**0.030**
BSF Digested	Benzoic acid, 4-ethoxy-ethyl ester	0.732	**0.025**
Non-Digested	2-Nonanone	0.988	**0.005**
	2-Undecanone	0.959	**0.005**
	3,4-Dimethoxytoluene	0.913	**0.010**
	3-Octanone	0.816	**0.045**
	Acetophenone	0.981	**0.005**
	Dimethyl sulfone	0.859	**0.020**
	Dimethyl trisulfide	0.816	**0.045**
	Pentadecane	0.782	**0.050**

Swine Manure

Table C.2.3 Indicator compound analysis based on reduced volatiles emitted from swine manure (n = 3) (Trial 1) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound	Indicator Value	*P* Value
Control	2(3H)-Furanone, dihydro-5-pently	1.000	**0.010**
	2(3H)-Furanone, 5-ethylhydro-5-methyl	0.945	**0.010**
	Acetic acid, phenylmethyl ester	0.700	**0.020**
	Benzene, 1,4-dichloro	0.779	**0.010**
	Benezenepropanoic acid, ethyl ester	1.000	**0.010**
	Benzenepropanoic acid, methyl ester	1.000	**0.010**
	Benzoic acid, 2-hydroxy-, methyl ester	1.000	**0.010**
	Butanoic acid	0.995	**0.010**
	Butanoic acid, 2-methyl	0.982	**0.010**
	Butanoic acid, 3-methyl	0.986	**0.010**
	Butanoic acid, propyl ester	1.000	**0.010**
	Heptanoic acid	1.000	**0.010**
	Hexanoic acid	1.000	**0.010**
	Indole	0.881	**0.025**
	Indole, 3-methyl	0.817	**0.010**
	Isobornyl acetate	1.000	**0.030**
	Maltol	0.816	**0.015**
	Pentanoic acid	0.989	**0.010**
	Pentanoic acid, 4-methyl	0.993	**0.010**
	Pentanoic acid, propyl ester	1.000	**0.010**
	Phenol	0.964	**0.015**
	Phenol, 2-methoxy	1.000	**0.010**
	Phenol, 2-methoxy-4-methyl	0.931	**0.010**
	Phenol, 4-ethyl	0.978	**0.010**
	Phenol, 4-methyl	0.907	**0.010**
	Propanoic acid	0.976	**0.010**
	Propanoic acid, 2-methyl	0.816	**0.015**
Non-Digested	2-Nonanone	0.986	**0.005**
	2-Undecanone	0.912	**0.020**
	3,4-Dimethoxytoluene	1.000	**0.005**
	3-Octanone	1.000	**0.005**
	Acetophenone	0.985	**0.005**
	Cyclohept-4-enone	0.913	**0.010**
	Dimethyl sulfone	0.991	**0.005**
	Pentadecane	0.972	**0.005**
	Phenol, 2-(1-methylethyl)	0.907	**0.005**
	Pyrazine, tetramethyl	0.786	**0.400**
	Pyrazine, trimethyl	0.838	**0.035**

Table C.2.4. Indicator compound analysis based on reduced volatiles emitted from swine manure (n = 3) (Trial 2) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound	Indicator Value	*P* Value
Control	2(3H)-Furanone, dihydro-5-pently	0.799	**0.020**
	Benezenepropanoic acid, ethyl ester	1.000	**0.005**
	Benzenepropanoic acid, methyl ester	1.000	**0.005**
	Benzoic acid, 2-hydroxy-, methyl ester	1.000	**0.005**
	Butanoic acid	0.968	**0.015**
	Butanoic acid, 2-methyl	0.862	**0.015**
	Butanoic acid, 3-methyl	0.858	**0.015**
	Butanoic acid, propyl ester	1.000	**0.005**
	Heptanoic acid	0.896	**0.010**
	Hexanoic acid	0.848	**0.015**
	Indole	0.962	**0.010**
	Isobornyl acetate	1.000	**0.005**
	Maltol	0.816	**0.025**
	Oleic acid	0.766	**0.040**
	Pentanoic acid	0.941	**0.005**
	Pentanoic acid, 4-methyl	0.937	**0.005**
	Pentanoic acid, propyl ester	1.000	**0.005**
	Phenol	0.984	**0.010**
	Phenol, 2-methoxy	1.000	**0.005**
	Phenol, 2-methoxy-4-methyl	0.952	**0.005**
	Phenol, 4-ethyl	0.968	**0.005**
	Phenol, 4-methyl	0.979	**0.005**
	Propanoic acid	0.863	**0.020**
BSF Digested	Benzoic acid, 4-ethoxy-ethyl ester	0.732	**0.040**
Non-Digested	2-Nonanone	0.988	**0.005**
	2-Undecanone	0.959	**0.005**
	3,4-Dimethoxytoluene	0.913	**0.010**
	3-Octanone	0.816	**0.040**
	Acetophenone	0.981	**0.005**
	Dimethyl sulfone	0.859	**0.035**
	Dimethyl trisulfide	0.816	**0.035**
	Pentadecane	0.783	**0.045**

Dairy Manure

Table C.2.5. Indicator compound analysis based on reduced volatiles emitted from dairy manure (n = 3) (Trial 1) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound	Indicator Value	*P* Value
Control	Butanoic acid	1.000	**0.010**
	Butanoic acid, 3-methyl	1.000	**0.010**
	Decane	0.816	**0.015**
	Indole	0.784	**0.035**
	Indole, 3-methyl	0.999	**0.010**
	Isobornyl acetate	0.816	**0.025**
	Pentanoic acid	1.000	**0.010**
	Phenol	0.992	**0.010**
	Phenol, 2-ethyl	0.816	**0.035**
	Phenol, 4-ethyl	0.957	**0.010**
	Phenol, 4-methyl	0.998	**0.010**
	Propanoic acid	1.000	**0.010**
	Propanoic acid, 2-methyl	1.000	**0.010**
	Undecane	0.968	**0.010**

Table C.2.6 Indicator compound analysis based on reduced volatiles emitted from dairy manure (n = 3) (Trial 2) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound	Indicator Value	*P* Value
Control	Butanoic acid	1.000	**0.005**
	Butanoic acid, 3-methyl	1.000	**0.005**
	Indole	0.816	**0.015**
	Indole, 3-methyl	1.000	**0.005**
	Pentanoic acid	1.000	**0.005**
	Phenol	0.997	**0.005**
	Phenol, 2-ethyl	0.816	**0.015**
	Phenol, 4-ethyl	1.000	**0.005**
	Phenol, 4-methyl	1.000	**0.005**
	Pinene	0.816	**0.035**
	Propanoic acid	1.000	**0.005**
	Propanoic acid, 2-methyl	1.000	**0.005**
BSF Digested	Naphthalene	0.912	**0.020**
Non-Digested	3-Octanone	0.816	**0.035**
	Pentadecane	0.967	**0.005**

C.3 ISA SUMMARY BASED OFF REDUCED VOLATILES EMITTED FROM MANURE WITH AND WITHOUT *Hermetia illucens* (L.) LARVAE COMPARED TO CONTROL MANURE

Volatile profile was reduced by grouping compounds into chemical classes.

Poultry Manure

Table C.3.1 Indicator compound class analysis based on reduced volatiles emitted from poultry manure (n = 3) (Trial 1) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound Class	Indicator Value	*P* Value
Control	Carboxylic acids	1.000	**0.005**
	Esters	0.957	**0.005**
	Fatty acids	0.991	**0.005**
	Indoles	0.817	**0.020**
	Phenols	0.943	**0.010**
Non-Digested	Hydrocarbons	0.851	**0.010**
	Ketones	0.923	**0.005**
	Phenols	0.943	**0.010**
	N-Containing	0.885	**0.015**

Table C.3.2. Indicator compound class analysis based on reduced volatiles emitted from poultry manure (n = 3) (Trial 2) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound Class	Indicator Value	*P* Value
Control	Carboxylic acids	0.855	**0.025**
	Esters	0.922	**0.010**
	Fatty acids	0.932	**0.010**
Non-Digested	Ketones	0.947	**0.005**
	S-Containing	0.867	**0.005**

Swine Manure

Table C.3.3. Indicator compound class analysis based on reduced volatiles emitted from swine manure (n = 3) (Trial 1) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound Class	Indicator Value	*P* Value
Control	Esters	0.743	**0.015**
	Ethers	0.767	**0.050**
	Fatty acids	0.998	**0.005**
	Hydrocarbons	0.695	**0.040**
	Indoles	1.000	**0.005**
	Phenols	0.998	**0.005**
	S-Containing	1.000	**0.005**

Table C.3.4. Indicator compound class analysis based on reduced volatiles emitted from swine manure (n = 3) (Trial 2) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound Class	Indicator Value	*P* Value
Control	Fatty acids	1.000	**0.010**
	Indoles	0.956	**0.010**
	Phenols	0.963	**0.010**
	S-Containing	0.777	**0.030**
Non-Digested	Ketones	0.943	**0.035**

Dairy Manure

Table C.3.5. Indicator compound class analysis based on reduced volatiles emitted from dairy manure (n = 3) (Trial 1) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound Class	Indicator Value	*P* Value
Control	Fatty acids	1.000	**0.005**
	Indoles	0.999	**0.005**
	Phenols	0.995	**0.005**

Table C.3.6. Indicator compound class analysis based on reduced volatiles emitted from dairy manure (n = 3) (Trial 2) with and without *Hermetia illucens* (L.) larvae compared to control. Trial was conducted at 29 ± 0.3°C with 60 ± 5.1% RH and 14:10 L:D cycle ($P < 0.05$).

Factor	Compound Class	Indicator Value	*P* Value
Control	Fatty acids	1.000	**0.005**
	Indoles	1.000	**0.005**
	Phenols	0.999	**0.005**
Non-Digested	Ketones	0.977	**0.030**