THE FORENSIC APPLICATION OF SOIL:

CLANDESTINE GRAVES AND HUMAN REMAINS DETECTION DOGS

A Dissertation

by

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ABSTRACT

The use of soil in forensic applications is widespread from mud left on tires and shoes to the examination of soil for pollens endemic to specific areas. The research presented examined 1) the role of soil texture in clandestine grave detection, 2) residual scent of human remains in cadaver decomposition islands (CDI) through identification by human remains detection (HRD) dogs, 3) the chemistry profile of the CDI and its relationship to the post mortem interval and 4) the chemistry profile of plants near CDI's and potential identification by HRD dogs.

Results indicate that 1) soil texture determines gas release potential and therefore has the potential to affect clandestine grave detection by HRD dogs, 2) residual odor of human remains in the CDI can be viable to HRD dogs up to 915 days PMI or 667 days after the body has been removed 3) chemistry profiles between control reference soils and CDI soils can show significant differences between DOC, DON, NO_3-N, NH_4-N, and PO_4-P. Ammonium-N shows a strong relationship with PMI at $R^2 = 0.45$ and DOC with $R^2 = 0.424$ values, and 4) plant chemistry retrieved from by CDI's show strong relationships to HRD dog alert accuracy. The research in this study indicated the importance of further research into each of these elements which may yield better understanding of soil decomposition interactions as well as presumptive tools for law enforcement for criminal investigations.

DEDICATION

I would like to dedicate this work to the countless victims and families who have lost loved ones to acts of violence; to those who are never found, left buried, unknown to those who pass by, no longer able to speak for themselves.

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Thanks to my late aunt and uncle for believing in me and to my grandmother for her unwavering faith in me and to my fiancé Tiffanie Turner, for her patience, love and support through all of it.

Finally, to K9 Pete, without whom I would have never taken a step into what has now become my passion and my life.

NOMENCLATURE

CDI	Cadaver Decomposition Island
COB	Change of Behavior
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
HRD	Human Remains Detection
NASAR	National Association for Search and Rescue
NNDDA	National Narcotic Detector Dog Association
NAPWDA	North American Police Work Dog Association
RCDI	Residual Cadaver Decomposition Island
TFR	Trained Final Response
TN	Total Nitrogen
TPWD	Texas Parks and Wildlife
VOC	Volatile Organic Compound

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Importance of soil in forensic sciences

Soil can be an extremely important component in forensic science. The heterogeneity and diversity among soils enables forensic soil scientists to distinguish between soil samples presenting an additional way for forensic scientists to use them as trace evidence in criminal and environmental investigations (Fitzpatrick 2008; Fitzpatrick et al. 2009). While police and crime scene investigators routinely encounter soil as trace evidence, most forensic laboratories are unable to adequately characterize soil materials presented as trace evidence (Fitzpatrick 2009). Many cases have been solved using soil as trace evidence and most of these are the result of transfer of soil from the crime scene to the perpetrator or their belongings (13th Interpol Forensic Science Symposium 2001; Fitzpatrick and Raven 2012). Unfortunately there is a shortage of well trained soil scientists in forensic laboratories throughout the world (13th Interpol Forensic Science Symposium 2001). Luckily the interest in soil forensic science has increased exponentially in the USA over the last five years with many universities offering courses in the subject area.

Soil is also important in other areas of forensics. For example, soil texture may be important when trying to detect clandestine graves (Alexander et al. submitted). Soil also has the ability to encapsulate the environmental conditions under which a cadaver lived in life based on some of the persistent organic pollutants (POPs) found in soil beneath decomposing cadavers (Vass 2012). This is because many of these POPs are only slightly water soluble but highly lipid soluble so they tend to bioaccumulate in animal tissue (Geyer et al. 2000) and after death, are readily released to soil when the cadaver decomposes. Some soils may carry a high metal load from a decomposing cadaver giving an indication of the cadaver's age and the environment in which they lived. This area of forensics has not been developed at all and has the potential to help identify human remains. The subject area of soil taphonomy, which is defined as the study of processes that affect the decomposition, dispersal, erosion, and burial, after, at, and even before death (Nawrocki 1996) has been useful. For example, more effective methods of locating clandestine graves have resulted from a more detailed understanding of the effects that a cadaver has on the soil environment (Tibbett and Carter 2009). Soil beneath decomposing cadavers has also been used in an attempt to predict post mortem interval of the deceased (Vass et al. 1992; Aitkenhead-Peterson et al. 2015).

Clandestine graves

There are over 15,000 homicides annually in the United States (Federal Bureau of investigation 2010) and more than 100,000 active missing person cases (National Crime Information Center 2009). The number of reported missing persons that are missing due to a homicide is undetermined and up to 25% of case investigations end up

shelved as cold cases (Larson et al. 2011). Many unrecovered victims may be due to the use of a clandestine grave as the choice of disposal of the body and evidence. Clandestine graves have been defined as unrecorded burials, often dug by hand, approximately 0.46 meters to 0.76 meters in depth, and in close proximity to an infrequently traveled road or path (Hoffman et al. 2009; Pringle et al. 2008). Because clandestine graves are not intended to be found, establishing an approximate location of the grave is one of the first challenges for investigators.

Clandestine grave detection

Various methods have been used to locate clandestine graves with varying degrees of success, including changes in vegetation, chemical analysis of volatile organic compounds (VOC's) (Hoffman et al. 2009; Lovestead and Bruno 2011; Statheropolous et al. 2007; Vass et al. 2004; Vass et al. 2008), human remains detection (HRD) dogs (Alexander and Turner 2010; Bulanda 2010; Furton and Myers 2001; Furton 2010; Hammond and Morris 2010; Komar 1999; Lasseter et al. 2003; Oesterhelweg et al. 2008; Rebmann et al. 2000; SWGDOG 2013) and ground penetrating radar (GPR) (Bevan 1991). Each of these methods has their own set of limitations and advantages (Furton 2010; Hammond and Morris 2010; Komar 1999; Lasseter et al. 2003; Bevan 1991).

Locating clandestine graves - vegetation

Vegetation can be invaluable markers of clandestine grave sites (Caccianiga et al. 2012; Tibbett and Carter 2003; Watson and Forbes 2008). Digging a clandestine grave will disturb and flatten vegetation at the site. Initially as the cadaver decomposes, purge fluids released into the soil will kill the surrounding vegetation. As decomposition of the purge fluids progresses releasing carbon, nitrogen and phosphorus to the grave soil, vegetation at the clandestine grave site may be more abundant than other vegetation in the area. However, for buried cadavers, the disturbance of the soil itself tends to have a greater effect on plant succession sequence compared to non grave sites (Caccianiga et al. 2012). Burials with about 40 cm soil covering the cadaver tend to have no significant effect on nutrient balance of herbaceous vegetation (Caccianiga et al. 2012). Surface grave sites tend to have a greater effect on surrounding vegetation because of the increased fertility at plant root depths (Towne 2000).

Many handlers of HRD dogs have noted their dogs have alerted on trees in close proximity of a clandestine grave, often more strongly than on the ground directly above the grave (Alexander and Turner 2010; Shaffer 2011). Additionally, some handlers have reported their dogs lick the leaves of the trees at clandestine grave sites. Some of the vegetation species noted to have elicited this behavior are the American sweetgum *Liquidambar styraciflua* (Alexander and Turner 2010), eastern red cedar *Juniperus virginiana* (Shaffer 2011) and winged elm *Elmus alata* (Alexander and Turner 2010). One possible explanation for this phenomenon is the phytovolatilization of organic compounds by the plants where the components which are water soluble pass through

the plant or are modified by the plant and transpired into the atmosphere. This has been shown to occur with light organics (Davis and Erickson 2002; Kakkar et al. 1998). Currently there is no scientific support for what HRD dogs may be detecting in vegetation at clandestine grave sites.

Humans have a C:N ratio of 6:1 allowing for fast decomposition under adequate temperature and moisture conditions. As the muscle tissue breaks down, nitrogen from amino acids is transformed to ammonium which is then immobilized by microbial biomass. While some forest plant species residing on low pH soils will also utilize the ammonium (Tischner 2000), nitrate is the most commonly nitrogen product by taken up by vegetation and it has been suggested that plant growth may be enhanced by shallow buried remains nearby (Carter et al. 2007; Dent et al. 2004). The plant roots take up nitrate through H^+/nitrate co-transporters (Ullrich 1987) which then reduce, store or transport the nitrate via the xylem to the shoot for reduction or storage (Tischner 2000). Carbon and nitrogen isotopes of cadaver decomposition products may be influenced through diet or environment and further investigation may assist in isolating markers.

Because vegetation utilizes the nutrients in the soil released from decomposition products, examining the chemical components of plants near CDI's may solve the question of the phenomenon of HRD dogs which alert on vegetation. All tree roots possess an apical meristem which generates growth and a protective root cap. Housed within the root is the vascular tissue which contains the xylem responsible for transport of water and organic solutes from the soil. The rate of root growth is variable throughout a growing season. Roots usually begin to grow before the tree canopy does, although

root growth is cyclic and responds to environmental changes such as soil depth, water supply, aeration, mineral supply and temperature. Studies have shown root spread to be 4 to 7 times the drip-line distance (radius) of the tree canopy (Fahey et al. 1988). The shallow portions of the root (top 15 to 30 cm of the soil) are responsible for the majority of water absorption. The future potential for identifying mass grave sites in forests using remote sensing techniques (Asner et al. 2011; Martin et al. 2008) to identify areas of high N uptake may be possible as a successor to evidence of plant uptake of human decomposition products.

Detecting clandestine graves – volatile organic carbons

Numerous volatile organic compounds (VOC's) have been identified at clandestine grave sites. Vass et al. (2004; 2008), Vass (2012), Hoffman (2009) and Stratheropolous (2007) have identified more than thirty compounds commonly detected by a mass spectrometer. In a study examining VOC emissions from multiple mammal species, Vass (2012) noted that there were certain VOC's only found in human and dog decomposition suggesting there may be cohabitation factors from sharing home environments.

Most active odor signature research also used for determining post mortem interval (PMI) has focused on VOC's produced during decomposition (Hoffman et al. 2009; Lovestead and Bruno 2011; Statheropoulos et al. 2007; Vass et al. 2008). The sulfhydryl's, putriscine and cadaverine are familiar agents for contributing to the odor emanating from decaying surface corpses although they volatilize rapidly (Dent et al., 2004). Vass et al. (2008) found neither putriscine nor cadaverine present in the 478

separate volatile compounds emitted from the graves they tested, and no single specific VOC signature specific to humans was found. A common core of 30+ components was noted by Cablk et al. (2012) and Vass et al. (2008), whereas Statheropoulos et al. (2007) found only 11 core odors. Cablk et al. (2012) concluded that chicken and human decomposition were most closely related with 60% of the chicken VOC's in common with human VOC's, whereas cow had only 40% in common and pig (*Sus domesticus*) even less with only 23% of VOC's in common with human decomposition. While Cablk et al. (2012) found chicken VOC's more similar to human, only small portions of chicken tissue were utilized instead of the entire chicken, therefore not necessarily comparable to an entire human, cow, or pig. Vass (2012) pointed out that using only portions or pieces of mammal or bird organs in decomposition studies can lead to an incomplete odor representation in the VOC signature. Carbon tetrachloride, pentane, undecane and decane were detected in human whole body decomposition (Vass, 2012) leading to speculation that some VOC's may be anthropogenic and POP's related to modern chemical exposures. There is however no scientific evidence that these particular compounds are bioaccumulative which suggests that they may be degradation products of other compounds during the decomposition process.

Mechanical instrumentation known as an "electronic nose" was developed recently to detect clandestine graves (Rock et al. 2008; Vass et al. 2010). Electronic nose is a broad term encompassing several types of sensors from gaseous compound sensors to optical sensor systems, mass spectrometry, ion mobility spectrometry, gas chromatography, and infrared spectroscopy (Rock et al. 2008). All of these methods

measure specific features analyzed with algorithms which compare samples with quantified known chemicals or compound concentrations. Like the biological systems they attempt to mimic, the purpose is to distinguish what the sample is and verifies variations such as fresh or spoiled. One major problem with constructed sensors with our current technology is mobility versus specificity. Mammalian noses can detect thousands of odors and the receptors which provide this bio-sensing are housed in relatively tiny areas (the nose) compared to man-made devices. A constructed sensor must have a sampling system, sensor array components, a reference data set and data evaluation algorithms (Rock et al. 2008). Selectivity increases the number of sensors, which increases size, so the more odors or odor nuances the device must detect, the larger it becomes. Currently devices are unable to duplicate the mammalian nose's ability for selectivity and sensitivity. Vass developed an odor detector at the Oak Ridge National Laboratory called the LABRADOR (light weight analyzer for buried remains and decomposition odor recognition) which is a twelve sensor array chemical vapor detector sensitive to chemical compounds relevant to human remains decomposition. Units will cost approximately $1500 and sensitivity is comparable to gas chromatography. Limitations of this device is that it is unable to locate and follow odor plumes or low odor concentrations of which both might be detected by an HRD dog. It is also designed to work on shallow burials in low barometric pressure settings such as early or mid-morning. Performance in other conditions is currently unknown.

Detecting clandestine graves – HRD dogs

Training HRD dogs to locate clandestine graves through odor diffusion

Despite technological advances, HRD dogs remain one of the most efficient, accurate, portable methods available for locating clandestine graves (Furton 2010; Komar 1999; Lorenzo et al. 2003; Oesterhelweg et al. 2008; Rebmann et al. 2000). Countries such as Great Britain do not allow possession of human remains of any kind for training HRD dogs, thus domestic swine has been used for several decades successfully as a surrogate training odor. Pseudo-scents manufactured for HRD dog training consist primarily of cadaverine and putrescine are also available but as discussed earlier these volatile compounds have a short lifetime before degradation (SOURCE). Furthermore, both cadaverine and putrescine evolve from all decaying animal tissue and are not unique to humans, thus training on these scents may lead to odor discrimination issues with HRD dogs in the field (Mondor et al. 2012). This may also be the case in training HRD dogs with small portions of tissue or organs, causing a distorted odor "signature" for the HRD dog's training resulting in less than successful field performance. In the early seventies, Rebmann, a Connecticut state trooper began utilizing methods similar to those employed to train bomb and narcotic dogs to train dogs to detect the odor of human remains (Rebmann et al. 2000). Initially referred to as cadaver dogs, trained HRD dogs have been shown to locate as little as a single tooth to an entire human body (Alexander and Turner 2010; Hammond and Morris 2010; Komar 1999; Lasseter et al. 2003; Rebmann et al. 2000; Dotson 2012). HRD dogs are typically trained and handled by civilians (Alexander and Turner 2010; Hammond and Morris

2010; Komar 1999; Lasseter et al. 2003; Rebmann et al. 2000; Dotson 2012). The reliability of HRD dog obtained evidence is however increasingly challenged in courts of law due to the lack of peer reviewed research on detector dog capabilities and the lack of a national standardized test for credentialing (Scientific Working Group on Dog and Orthogonal Detector Guidelines 2013).

HRD dogs communicate to their handlers that they have located the source of human remains odor through a trained behavior referred to by the court system as a trained final response (TFR). The TFR that the dog offers can be of a passive or aggressive nature, consisting of a sit or down for a passive TFR or a bark, dig and/or re-find for an aggressive TFR. Re-find refers to a behavior where the dog indicates the location of the target odor by returning to the handler, giving a specific trained indication behavior such as a bark or sit and then leads the handler to the source of the target odor. TFR's are essential not only for communication to the handler of the location of human remains but also verification of the location of evidence in a court of law. A trained final response on a location, building, or vehicle by an HRD dog is not recognized by the court for probable cause, but does constitute reasonable suspicion taken in hand with other collaborating evidence such as witnesses, confessions, blood stains and the like.

Domestic swine is substituted as the target odor for training HRD dogs in many countries, particularly Great Britain however, in the USA, this would not be acceptable in the court system (Fleck, 2010). Although largely unpublished to date, HRD dogs have been demonstrated to differentiate between pig and human remains (Lorenzo et al. 2003). This demonstrated ability to differentiate between humans and other animals

indicates that there may be an odor marker that is unique to human beings and detectible by canines. The recent finding by Cablk et al. (2012) that determined chicken VOC's were more like human VOC's than were pigs, belays the importance of the use of animal remains during training sessions as distracters or non-target odors.

Properly training HRD dogs can often be problematic due to the inability to obtain adequate training aids. While aged human bone (20 – 100 yrs. old) can presently be purchased online, supplies are dwindling due to exportation bans in countries which previously supplied the USA. Fresh bone and soft tissue are almost impossible for most handlers to obtain. Maintaining the few obtained samples can also be problematic. Handlers must then choose whether to freeze and re-thaw their training aids for each training session, or allow their sample or training aid to decompose over time. The latter may allow for a more complete array of decomposition odors available to the dog, but samples must then be monitored for mold which can interfere with odor specificity. Due to various laws or vagueness of laws at state levels, most handlers have difficulty in obtaining adequate training aids.

SWGDOG was established by the Federal Bureau of Investigation in 2003. SWDDOG's purpose was to establish best practices guidelines for all types of detector dogs to improve performance and reliability. These guidelines include recommendations for consensus based best practices in training, testing, documentation, and research (SWGDOG 2013) to enhance the performance of detector dogs. Some areas of recommended research include: finding complementary instrumentation for application with canines, determining the effectiveness of training aids and the relationship between

experience with an aid and subsequent detection of the real odor, generalization of odors, and determining whether odor quantity affects detection.

Explosives and narcotic dogs can be considered analogous with HRD dogs and are actually referenced by the court when dealing with cases utilizing HRD dogs; therefore, studies conducted on bomb and drug detector dog capabilities are valuable tools that can also be applied to HRD dogs (Fleck 2010; Gazit and Terkel 2003; Rebmann et al. 2000) to some extent. Recent published research efforts have focused on determining active odor signatures to assist in designing better training aids, new methods of odor release, and better quantification of minimal odor thresholds (Furton and Myers 2001; Harper 2004; Rock et al. 2008; Walker et al. 2006). Walker et al. (2006) determined odor sensitivity in dogs as low as 1.14 ppt. Rains et al. (2009) found that the dog's sensitivity for minute thresholds was equal to that of the parasitic hympenopteran wasp species *Microplitis croceipes* (Rains et al. 2010). *Hymenoptera:braconidae* has been used in the Wasp Hound to sniff out scents from explosives to cancer.

Few empirical studies have been performed specifically on HRD dog capabilities or limitations. Komar (1999) investigated the use of HRD dogs in locating surface-scattered human remains in Canada. Weather conditions for this study were cold, ranging from -30 to 10˚ C. Eight HRD dogs: one law enforcement RCMP certified dog, five civilian owned, certified (certifications not listed) dogs, and two civilian owned, uncertified dogs were utilized in the study. Dog teams were tested after a two month training period with the testing paradigm with ten blind field tests simulating mission

conditions in varied field conditions and a diverse set of human remains training aids. The ten field test trails yielded an overall recovery rate of 81% and individual dog performance rates ranging between 57% and 95%. Komar (1999) noted that the most consistent performance was found on human bone, which was also noted as the most familiar training material to this group of dogs. It was also observed that several dogs did not react to animal bone. Increasing the age of the bone resulted in decreased performance which was also seen with decomposition fluids. Komar (1999) also reported contextual issues where the dog teams did not recover bone in unfamiliar settings such as bone placed in water puddles. However after repeated exposure and reward, the dogs performance increased in subsequent trials in the same context. A similar decrease in proficiency was found when the dogs were tested in a wooded environment dissimilar to where they normally trained. This information supports the importance of training dogs in different environments and under different circumstances to be proficient for mission deployments in all situations. Lasseter et al. (2003) evaluated the performance of four HRD dogs: three experienced and certified and one un-experienced and un-certified ranging in age from 20 months to 10 years of age (certifications not listed). Trials were field searches for buried human remains in forested areas of the southeastern United States in summertime conditions. Five trials were performed with human remains and distraction animal remains buried at varying depths and stages of decomposition. The dog's performance were scored as alert, alert not recognized, narrowed area, no alert, and false alert. Temperatures ranging between 27.8 and 33.9˚ C and humidity between 55% and 74% were recorded which resulted in

frequent breaks. Heat and humidity can result in heavy panting which may have inhibited the dog's ability to smell and thereby yielded poor results. This resulted in 50% of trials yielding no alerts, with 20% in the fifth trial held during the heat of the day. The results varied with performance ranging with only two alerts, four unrecognized alerts, six narrowed areas, 22 no alerts and six false alerts. Results from this study were also inconclusive on distinction between animal and human remains due to the proximity of buried animal remains in some of the "narrowed area" results.

Oesterhelweg et al. (2008) tested the performance of 3 law enforcement HRD dogs on 354 trials. Dogs were tested on carpet squares which were collected from under a sheet where a cadaver of less than three hours post mortem was placed for two minutes and 10 minutes. Carpets did not come into direct contact with the bodies and because of the post-mortem age and preservation of the bodies; it is highly unlikely that any body fluid transfer between the body, sheet and carpet square occurred. Dogs were scored for correct, false positive and false negative. Further analysis determined false alerts were largely caused due to "over-runs" where the dog alerted on a sample just beyond the actual HRD sample. Dogs were tested on both exposures times up to 65 d post non-direct exposure for 10 min (98% accuracy), and up to 35 d for non-direct exposure for two minutes (94% accuracy). This study provided evidence that dogs are capable of detecting residual odor of human remains on items that did not even directly contact the corpse.

HRD dogs are often called in as a last resort in both contemporary and cold cases. HRD dogs have been reported to detect gravesites that are many years old; long

after the expected *"volatile scent"* has diminished. Often in cases of scattered remains or homicides where the body has been moved from the primary decomposition site a HRD dog can still correctly identify decomposition products in the soil. No study to date has evaluated the period of time post decomposition that a HRD dog can correctly identify a human remains decomposition site. This has important implications on the use of HRD dogs in cold cases and in cases where unproductive TFR's from HRD dogs occur. There has also been no research on how the soil texture affects the HRD dog's ability to locate clandestine graves. Since soil series type effects the aeration, soil solution contents, movement of decomposition fluids, and even the speed of decomposition, these factors likely also affect the amount of odor available for detection by the HRD dog.

Detecting clandestine graves – ground penetrating radar

Alongside the increasing interest in soil forensics and soil taphonomy there has been an increased interest in the field of forensic geosciences specifically in criminal investigations (Billinger 2009; Ruffell et al. 2009; Schultz 2007; Schultz and Martin 2012). The most recent study by Schultz and Martin (2012) used pigs buried in a Spodosol in Florida. Pigs were placed naked (one shallow grave and one deep grave), wrapped in tarpaulin, wrapped in blanket, covered with rock or covered with dolomitic lime prior to covering with soil. Fake graves (one shallow and one deep) were also used in the study; these are graves excavated and refilled with no animal content. The graves were monitored for 12 months. Visibility scores defined by the researchers based on information from the GPR showed that the shallow burial and shallow fake grave were not detectable at all during the 12 month period. For the other grave scenario's there was

no detection of a buried pig in the first two months of the study and often no detection of a buried pig the last two months of the study. Only the grave where the pig was covered in rock and a deep fake grave showed visibility in the last two months of the study (Schultz and Martin 2012).

Clandestine graves can be detected using various methods but no one method is perfect and it is suggested that a single method be used such as HRD dogs with a confirmatory method such as soil chemistry (conductivity, ninhydrin-nitrogen or volatiles) or GPR be used.

Decomposition processes

The processes of cadaver decomposition have been well defined (Carter et al. 2007; Dent et al. 2004; Forbes et al. 2005; Jaffe 1983; Rodriguez and Bass 1985). The cadaver progresses from autolysis to putrefaction, liquefaction, and finally skeletonization, also termed dry remains, over the course of time (Carter et al. 2007). Microbes and enzymes within the body begin the process of autolysis within moments after death (Campobasso et al. 2001; Carter et al. 2007; Dent et al. 2004; Fiedler and Graw 2003). Dent (2004) noted that during autolysis, hydrolytic enzymes begin the breakdown of carbohydrates, fats, and proteins which is followed by putrefaction generally no earlier than 48 hours post mortem. Carbohydrates are broken down into sugars by microorganisms in the soil while fats are broken down into fatty acids which under specific conditions will convert to adipocere. Adipocere is generally formed under anaerobic conditions (Section 1.6.3). Products of the protein breakdown include skatole and indole, carbon dioxide, hydrogen sulfide, ammonia, and methane (Dent et al. 2004).

Eventually, liquefaction of tissues and organs result in complete disintegration, leaving only the dry skeletal remains.

Factors affecting rate of decomposition may be corpse specific, such as cause of death, age, body build, clothing type and method of burial (Fiedler and Graw 2003). Individuals who die of natural causes and are not autopsied tend to decompose slower than those who die from violence or trauma. Trauma such as stab or bullet wounds have additional entrances made readily available for both insect and microbial colonization. Wounds can also increase interest by scavengers resulting in more tissue removed more quickly and less left for microbes to feed on. Wounds to the abdomen release body cavity fluids and result in faster microbial decomposition within the cavity if intestinal structures are punctured in the wound. Body composition such as leanness or body fat can also affect how the person decomposes and how quickly. Leaner individuals tend to mummify more readily whereas individuals with large amounts of body fat tend to decompose more slowly with significantly larger decomposition areas in the soil than thin bodies (Simmons et al. 2010; Matuszewski et al. 2014). Putrefaction onset was earlier and had a longer duration in larger cadavers although active decay was slower resulting in slower mass loss and later onset of advanced decay compared to smaller cadavers (Matuszewski et al. 2014). Smaller body mass has been shown to release ninhydrin-nitrogen to the CDI more slowly compared to larger body mass (Spicka et al. 2011) which led to the conclusion that post mortem interval estimation may be compromised.

Clothing can offer some protection from scavengers and insects and thereby may slow decomposition although overall, clothing is not as important as size of the cadaver (Matuszewski et al. 2014). Very few studies have examined the effect of clothing on decomposition rates (Gonzales et al. 1954; Haglund 1989; Galloway et al. 1989; Cahoon 1992; Miller 2002) yet more than half of all forensic cases comprise clothed individuals (e.g. Komar 1998). Gonzales et al. (1954) and Galloway et al. (1989) reported that decomposition was retarded in the advanced stages of decomposition in clothed individuals. Clothing as a protection against temperature fluctuations may also play an important part in decomposition rates. Cahoon (1992) reported that during a winter study in Tennessee a clothed individual reached bloating stage quicker than a naked individual. Inversely Miller (2002) found that there was no significant difference in estimated post mortem interval using accumulated degree days (ADD) between clothed and naked individuals.

Medications and treatments may also have an effect on decomposition (Zhou et al. 2011). Cadavers that have received chemotherapy or antibiotics or those whose death resulted from drug overdose may not be colonized readily by bacteria nor do they tend to attract scavengers readily. Antibiotics and cocaine will impede decomposition rates (Vass, 1991) as will poisons such as arsenic, antimony and mercury (Watkins 1983). Relatively little research has been conducted on the effect of drugs on cadaver decomposition rates. This is mainly because most decomposition studies have used surrogates for humans due to the low number of body farms in the USA and indeed

globally, and the greater interest in body mass and clothing on rates of cadaver decomposition.

Burial method also can affect decomposition. Shallow graves are more prone to predation disturbance and cadavers more readily decompose in warmer temperatures than a body buried with instrumentation such as a backhoe, six feet or deeper within the ground. Post-skeletonization estimation of PMI or PBI can be intensive in the man hours required to exhume and examine the skeleton and therefore expensive and have focused on bone biochemistry and skeletal microstructure requiring trained forensic anthropologists (Mendonca et al., 2008). For example, Jaggers and Rogers (2009) reported that bone mass was lost over time, indicative that decomposition processes continues as chemical components from the bone are weathered and leach into the soil.

Cadaver decomposition island

The physical area in which a cadaver decomposes is termed a cadaver decomposition island (CDI) (Carter et al. 2007). Soil chemistry within the CDI has been examined to provide a chemical "fingerprint" that could be used to estimate PMI for investigators. Some studies using mammal decomposition have also been used in an ecological framework (Carter et al., 2007) but are, nevertheless important indicators of changes to soil chemistry in the CDI.

Aitkenhead-Peterson et al. (2012) examined two CDI's at the Southeast Texas Applied Forensic Science (STAFS) Facility, Huntsville, Texas, USA and showed the spatial extent of the CDI from two human cadavers for several nutrients. The expected spread of nutrients was far larger for some nutrients than others. For example, the spread

of dissolved organic carbon and organic nitrogen was much larger than the expected 101 x 254 cm area sampled. Hotspots of nitrate-N and sulfate which were lower than control soils were also apparent suggesting that at 248 and 288 d post mortem that the CDI in some places was still anaerobic.

Benninger et al. (2008) utilized the CDI of decomposing domestic swine and focused on carbon, nitrogen, and phosphorus decomposition. Soil samples were extracted weekly for six weeks, then monthly up to 100 days. Benninger et al. (2008) concluded that the trends of significant increases in total nitrogen (72 days), soil extractable phosphorus (160 days) and lipid phosphorus (43 days) could be used for early PMI estimation.

Ninhydrin reactive nitrogen (NRN) is a measure of organic nitrogen plus ammonium-N which has recently been acclaimed as the answer to determining PMI in grave soil. Van Belle et al. (2009) examined NRN concentrations in the soil solutions from the CDI beneath domestic swine with results indicating that trends in NRN concentrations may also be useful in PMI determination during the first two months of burial or the first 3 months of a surface decomposition (Van Belle et al. 2009). Carter et al. (2008) used juvenile rat (*Rattus rattus*) cadavers to evaluate NRN concentrations in extracted soil solution retrieved from their CDI and concluded that tests for NRN might be used as a good presumptive test for gravesoils.

Post mortem interval

PMI refers to the time interval spanning from time of death to discovery (Pringle et al. 2010; Tibbet and Carter 2009). A universal PMI estimation for deceased victims

does not currently exist but depends upon interpretations from forensic anthropology, entomology, and chemical changes within the body and soil. Vass (2011) however recently developed two formulas; one for surface decomposition and one for burial decomposition, which were based on temperature, moisture, the partial pressure of oxygen and extent of soft tissue remaining, as being the primary drivers for human decomposition. He has called for researchers to use the formula so that it can be modified according to climatic conditions. To date there have been no publications using this method to validate it in regions other than Tennessee.

Establishment of the PMI is important once a clandestine grave is located (Pringle et al. 2004). Jaffe (1983) stated that the most researched problem of forensic medicine was determining accurate methods for establishing PMI. Thirty-one years later, this is still a leading area of research. The success or failure of criminal investigations often relies on accurate estimations of time since death (TSD) (Marks and Love 2001). Estimated PMI is essential to forensic investigators as an aide in identifying the victim and thereby potentially the perpetrator. However, a uniformly reliable method for accurately estimating PMI that meets Daubert standards remains elusive.

Clandestine graves present additional problems when trying to determine PMI compared to exposed surface remains (Wilson et al. 2007). For example, insect colonization, which has had great success determining PMI of surface remains, is of little use in determining PMI due to the lack of insects present in a buried environment (Bevan 1991). Various methods have been developed to estimate PMI in surface decomposition cases. Examples include colonization intervals for insects (Anderson

2004; Marchenko 2001; Mendonca et al. 2008; Richards et al. 2007; Riebe and Burkhard 2010; Honda et al. 2008) accumulated degree days (Campobasso et al. 2001; Vass et al. 1992) and soil solution and soil extract chemistry (Pringle et al. 2010, Vass et al. 1992; Aitkenhead-Peterson et al. 2015) including whole CDI soil NIR spectroscopy (Aitkenhead-Peterson et al. 2015).

Estimating PMI - insects

PMI estimations from insect colonization on decomposing bodies have been highly studied and are of benefit in early post-mortem stages (< 365 d PMI) if conditions that support insect activity are present (SOURCE). Blow flies lay eggs on a cadaver within minutes of death in the right conditions (SOURCE). Forensic entomology is most useful after 72 hours and in most cases only if the body is located while eggs or larvae are still present on the body so they can be collected and identified (Anderson 2004; Marchenko 2001; Mendonca et al. 2008; Richards et al. 2007; Riebe and Burkhard 2010; Honda et al. 2008). Photographs of the deceased rarely have enough detail or scale reference to be of use, so retrospective analysis is difficult to impossible in most cases (Marchenko 2001; Mendonca et al. 2008). Limitations of forensic entomology include weather, available species and species-specific behavior determined by time of year or weather.

Estimating PMI - climate

Environmental factors such as temperature and humidity also affect the speed at which a cadaver decomposes and therefore estimation of a PMI. Humidity and wind conditions can lead to rapid drying. Temperature may well be the most important

extrinsic variable affecting decomposition (Campobasso et al. 2001). Extremes in temperatures, both hot and cold, inhibit decomposition due to reduction of bacterial activity. This variance in decomposition can also greatly skew PMI estimations. Accumulated degree days (ADD) was formulated to account for temperature effects on decomposition and is calculated by taking the average daily temperature and summing it for the estimated time interval the body has been in the environment (Vass et al. 1992). Limitations of ADD are lack of accounting for rain events or relative humidity, which may have a strong influence in some environments.

Estimating PMI – soil chemistry

Soil chemistry as a means to determine PMI or ADD has received much less attention. Vass et al. (1992) examined soil extract solution chemistry from under bodies at the University of Tennessee's Forensic Anthropology Center in Knoxville, TN. Attention was given to volatile fatty acids, melanin, and specific cations and anions common to decomposition. During the spring and summer three soils samples were obtained from under the torso region of a cadaver every three days and then weekly thereafter. The frequent sampling may have led to aeration of the CDI which is generally thought to be anaerobic based on the lower concentrations of nitrate and sulfate in the CDI aged 248 and d 288 PMI due to the use of the oxygen in these compounds as an electron acceptor by soil microbes (Aitkenhead-Peterson et al. 2012, 2015). Thus, the soil chemistry may be changed in a CDI that is repeatedly sampled compared to one that is sampled for the first time. Anaerobic conditions in a CDI may persist for up to a year or more resulting in minimal nutrient cycling and minimal use of substrate carbon

(Aitkenhead-Peterson et al. 2012). Therefore sampling the soil frequently may result in aeration and quicker return to aerobic conditions, leading to earlier nitrate peaks than generally expected in the CDI. This may be why it has been challenging to determine PMI using soil chemistry (Aitkenhead-Peterson et al. 2015).

UV-Vis-near infrared spectroscopy was used to examine change in soil chemistry in a cadaver decomposition island of a single cadaver over a period of 580 to 1269 d PMI. Changes in soil chemistry in the near infrared (NIR) region allowed PMI to be estimated to within 13-16 d of known PMI (Aitkenhead-Peterson et al. 2015). More research needs is needed using soil and NIR spectroscopy on grave soil as this may prove to be a useful tool in combination with entomology to predict PMI in the future.

Conductivity in extracted soil solutions from the grave soil of burials has also been shown to be promising for PMI determination (Pringle et al. 2010). Pringle et al. (2010) used domestic swine in an attempt to utilize soil solution obtained from lysimeters inserted beneath the buried cadavers to examine conductivity for two years. They found conductivity increased rapidly during the first year and continue to slowly increase thereafter for the remaining year. During the first 307 days after burial the conductivity in the collected soil solution was highly correlated to the ADD. A limiting factor of this study was sample size, consisting of only one swine burial and one control as well as only one soil type.

A limiting factor of many of these studies is the variety of soil's utilized and lack of replication in other soil types. Because of the perceived issues of repeated sampling of CDI's and its potential effect on PMI estimation, Aitkenhead-Peterson et al. (2015)

examined the water extractable soil solution of previously undisturbed CDI's beneath 14 cadavers with known PMI's at two sites with different soil orders in Texas. The PMI's ranged from 18 to 580 d and ADD from 1199 to 36,902. Control soils were sampled on the same date that the CDI was sampled and its chemistry deducted from the chemistry of the CDI leaving decomposition products only thus negating any seasonal differences in soil nutrient status. DOC:DON ratio divided by the initial mass of the cadaver which showed an exponential decline with time provided the best model for predicting PMI. Using partial least squares regression analysis with a full cross validation the relationship between predicted and observed PMI was relatively strong but tended to over predict estimated PMI (Aitkenhead-Peterson et al. 2015).

To date, despite the efforts of all types of forensic disciplines, no PMI estimation method has been found which works conclusively in all situations and for all time frames. Furthermore, while studies with other mammals assist in the knowledge base for soil extract solutions and decomposition products in general, it is also essential to examine soil chemistry beneath decomposing human remains to ensure valid extrapolations have been made that will assist law enforcement in determining PMI.

Expectations in most of the research discussed in this chapter are that a whole body will remain and decompose within the CDI. Recent findings from Spradley et al. (2012) documented vulture scavenger activity that could lead to inaccurate estimations of PMI based on observed decomposition alone. Vultures were captured on video over a period of six hours taking a fully fleshed corpse to skeletal remains. Had these remains been found in an investigation the PMI would have been over-estimated. Prior to this

finding, it was assumed that scavengers could not so quickly or completely reduce a cadaver to bone. The vultures however did not attack the cadaver until after the purge and creation of the initial CDI which suggests that soil chemistry could still be used to estimate PMI from the CDI.

Estimations of post burial interval (PBI) for clandestine graves are even more problematic as they also require an understanding of how the decomposition events occur underground. Soil texture and its associated properties porosity, aeration, and water holding capacity affect the decomposition of the buried body (Wilson et al. 2007). Soil texture can be separated into sand, silt, and clay. Because burial environment can affect decomposition rate, PMI estimates can become skewed. Soil type such as clay which tends to be more poorly drained and holds more moisture for longer periods of time due to micropores may limit microbial activity and keep soil temperatures cooler thereby slowing decomposition; whereas well drained sandy soils with larger pore space have better access for aerobic microbial activity thereby increasing decomposition. Soil moisture contents exceeding 50% of pore space may retard decomposition thereby preserving the corpse in a much better condition than expected, whereas lower soil moisture may expedite decomposition with either high or low soil moisture conditions resulting in skewed PBI estimates (Rodriguez and Bass 1985, Wilson et al. 2007). The flush of nutrients into grave soil has been reported to lower soil pH, creating a less suitable environment for most bacteria but encouraging fungal growth. Soils which are more neutral or slightly acidic in nature promote microbial decomposition. Soil pH is one of these factors, as bacteria are inhibited in soils with either high acidic or alkaline

composition (Forbes et al. 2005; Wilson et al. 2007). Time of year may affect temperature and precipitation volumes, while depth of a grave may also effect decomposition (Wilson et al. 2007). Deeper graves typically preserve the corpse better due to lower soil temperatures and increased clay content. Soil temperatures are cyclical with the seasons however changes in soil temperature are less obvious with depth, cooling slowly in the fall from the surface down, with the drop in ambient temperatures and shorter days. Longer days and rising temperatures slowly warms the soil in the spring, surface first and initiating microbial activity. Most soils show a significant decrease in soil respiration during the lower winter and early spring temperatures due to inactive microbes. Likewise excessive heat and low soil moisture in drought years may increase soil temperature and decrease microbial activity, likely slowing the processes of decay. The difference being that in the summer, bodies are more likely to mummify due to the moisture being pulled from the cadaver into the adjoining soil thereby desiccating the tissue faster than the microbes can decompose it.

Adipocere formation also greatly reduces the speed of decomposition (Forbes et al. 2005; Fiedler and Graw 2003). Adipocere formation is noted to resist decay and occurs in soils that favor anaerobic, moist conditions; due to their inherent qualities, clay soils may be better for adipocere formation and preservation of the corpse (Forbes et al. 2005, Fieldler et al. 2009) because of their porosity and poor drainage characteristics.

Objectives

Due to the immense range of fields in forensics in which soil can play a role, the major objectives of my PhD research selected those in which soil or vegetation is

important and has not been fully researched previously. These include how soil texture may affect transmission of odor for HRD dogs, the use of whole body decomposition products in soil as a training aid for HRD dogs, use of water extractable CDI soil in estimating PMI and uptake of human decomposition products by plants. The objectives of the research with their testable hypotheses were as follows:

Objective 1: Determine the effect of soil texture on HRD dogs' ability to find clandestine graves (Chapter II)

Hypotheses to be tested:

- Ho1-1: There is no difference in final response accuracy of HRD dogs due to differences in soil textures in which the training aid is buried.

- Ha1-1: The final response accuracy of HRD dogs will be statistically stronger for samples buried in sand due to their larger pore size and greater aeration.

Objective 2: Examine the potential of HRD dogs to respond to contemporary and residual decomposition scent (Chapter III)

Hypotheses to be tested:

- Ho2-1: There is no significant detectable residual scent available in a CDI post remains removal or grave soil solution for identification by a HRD dog.

- Ha2-1: There is significant detectable residual scent available in a CDI post remains removal for identification by a HRD dog.
- Ha2-2: There is a significant detectable residual scent available in grave soil solution for identification by an HRD dog.

Objective 3: Determine if water extractable soil solution can be used to determine PMI at 1) individual sites and 2) across sites with different soil classes (Chapter IV)

Hypotheses to be tested

- Ho3-1: There is no significant difference between pH and cold water extractable DOC, DON, NH_4-N, NO_3-N and PO_4-P collected from CDI and reference soils.
- Ha3-1: There is a significant increase in extractable DOC, DON, NH_4-N, NO_3-N, PO_4-P collected from CDI's compared to reference soils.
- Ha3-2: A decrease in pH to a more acidic state is expected in CDI soils when compared to reference soils.

Objective 4: Determine if human decomposition products increase concentrations of N and P in herbaceous and woody plants at gravesites and determine if HRD dogs can differentiate between gravesite and non-gravesite plants (Chapter V).

Hypotheses to be tested

- Ho4-1: Plants near gravesites are not significantly different in water extractable chemical constituents relative to plants obtained from non-gravesite locations.
- Ha4-2: Ammonium-N and dissolved organic carbon is significantly higher in water extractable vegetation obtained near grave sites relative to vegetation retrieved from non-gravesite locations.
- Ho5-1: HRD dogs will not be able to differentiate between gravesite and non-gravesite vegetation.
- Ha5-1: HRD dogs will have greater than 60% success rate detecting and identifying vegetation retrieved from gravesites with a correct trained final response.

CHAPTER II

THE EFFECTS OF SOIL TEXTURE ON THE ABILITY OF HUMAN REMAINS DETECTION DOGS TO DETECT BURIED HUMAN REMAINS

Introduction

Clandestine graves

Human remains detection (HRD) dogs are frequently used to locate clandestine graves (Alexander 2009; Alexander and Turner 2010; Lasseter et al. 2003; Pringle et al. 2008; Rebmann et al. 2005). Clandestine graves have been defined as unrecorded burials, often dug by hand, on average 0.46 meters to 0.76 meters in depth, and in close proximity to an infrequently traveled road or path (Hoffman et al. 2009; Rodriguez and Bass 1985). The obscurity of the grave location, available tools and time the perpetrator can spare often govern the depth of these graves. Soil texture, moisture content (Carter et al. 2010) and vegetation type (tree roots vs grass roots) will also affect the depth of the clandestine grave. Dry conditions will greatly impede burials in clayey soils because the soil is harder to dig, whereas moist soil conditions are conducive to burials in many soils. Soil texture and soil water content will affect the escape of decomposition gases (Van Belle et al. 2009; Vass et al. 2008; Wilson et al. 2007) and, therefore, affect the amount of odor available for the HRD dogs to detect.

Soil texture is one of the basic properties of soil that should be determined before searching specific locations for a clandestine grave. Soil texture is the mineral proportions of sand, silt and clay and how these minerals are apportioned determines some of the basic characteristics of the soil such as porosity, aeration, drainage,

compactability, shrink swell potential and water holding capacity (Lowe et al. 2013). Soils are comprised of a mixture of these minerals plus organic matter. Many of the properties of soil texture may contribute to, or hinder, the detection of decomposition odors by HRD dogs. Properties that may affect olfactory detection include soil aeration, water holding capacity, available pore space and sealing capacity such clays used to line ponds (Al-Quinna et al. 2013). Pore space is filled with air, water or a mixture of the two depending upon the soil moisture content. The proportion of water to air in the soil pore space affects the aeration of the soil. Clayey soils generally have high soil porosity including macro and micro pores resulting in a high water holding capacity with generally a greater percentage of pore space allocated to water than air. Sandy soils generally have lower soil porosity but due to larger pore size more space is allotted to air than water. The pore size also allows for better drainage (Skyortsova and Utkaeva 2011). Diffusion occurs in soil air to exchange gases between the atmosphere and the soil (Fukikawa and Miyazaki 2005). Pores filled with water impede the flow of these gases because clayey soils have higher water holding capacity than sand (higher porosity), diffusion of gases in clayey soils is often restricted. In other words, in clayey soils the escape of decomposition gases from decomposing human remains (HR) may be hampered (Lowe et al. 2013; Fukikawa and Miyazaki 2005). For example, oxygen diffuses rapidly into air filled pores but up to ten thousand times slower in water filled pores (Fukikawa and Miyazaki 2005; Skyortsova and Utkaeva 2011).

In springtime, clayey soils tend to warm more slowly than sandy soils, which may also retard gas production. Lower soil temperatures contribute to slower

decomposition of organic matter (Archer 2004; Carter et al. 2008), therefore, a buried body, may be slower to decompose during cooler temperatures. Prangnell and McGowan (2009) reported an association between soil temperature and decomposition rates, where higher temperatures in shallow graves lead to faster degradation. Soil moisture content is important for soil microbes in the degradation process, yet Pragnell and McGowen (2009) did not include this in their analyses. Soil properties that affect decomposition gas escape from clandestine graves may also affect the efficiency of HRD dogs to locate and pinpoint the source of the odor in a buried environment. Soil texture properties may be an important factor affecting HRD dog detection of potential clandestine grave missions. Master trainers and evaluators who perform certifications for various organizations would also benefit from basic understanding of a soils effect on gas escape when testing HRD dog's capabilities on buried scenarios.

Human remains detection dogs

Performance expectations for the narcotic dog industry are also applied to HRD dogs by the court systems which expect annual credentialing (Fleck 2013; 2014). Credentialing evaluations for HRD dogs generally comprises testing the dog's single blind in different scenarios such as elevated, surface, and buried human remains as well as blank areas with no remains (Christensen 2014; NAPWDA 2014; NASAR 2014). Ann Christensen, K9 committee chair for the National Association for Search and Rescue (NASAR) reported that buried human remains or blank areas with no human remains represent the area of credentialing with the least amount of success (Christensen 2014). Herein the factors which may lower success rates for buried remains are

hypothesized to be a function of the effect of soil texture. No study has examined how soil texture correlates to the effectiveness of HRD dog searches on buried remains.

The objective of this study was to investigate the effects of soil texture and its subsequent properties on the HRD dog's ability to detect the odor of human remains in buried scenarios. I hypothesized that the properties associated with soil texture would make buried human remains easier for HRD dogs to detect in sandy soils than in clayey soils. The design of the study was comparable to buried testing scenarios utilized by many national organizations credentialing standards (Christensen 2014; NAPWDA 2014; NNDDA 2014).

Methods and Materials

Locations

Two locations on private properties were utilized for testing. The clayey soil was located at Renfroe Ranch in Robertson County, TX, USA. The soil was classified as a Hearne Fine Sandy Loam with a profile of 0- 25.4 cm Fine Sandy Loam, and 25.4-63.5 cm Clay (WSS 2014). This site has been used as a cattle pasture for over 100 years (coastal Bermuda grass); it also had stands of timber. The sandy soil, was located at Carl Catropia Ranch in Robertson County, TX, USA and was classified as Padina Loamy Fine Sand with a profile of 0-12.7 cm loamy fine sand, 12.7-142.2 cm loamy fine sand and 142.2 – 203.2 cm sandy clay loam (WSS 2014). This site has been used as a cattle pasture (coastal Bermuda grass) for over 63 years and has stands of timber. The two soils differed in texture with one being primarily fine sand and the other having a B horizon of clay (25.4 – 63.5 cm) making it satisfactory for the experimental design. Soils

which had both the A and B horizons of contiguous clay or sand profiles were preferred but unavailable. The HRD dog testing areas had < 10% slope in open fields' void of trees to prevent any scent bias due to convection or shade.

Soil texture verification

Soil texture for both the sandy and clayey soil was verified through six randomly selected replicate samples taken from both sites by means of a drop hammer soil corer with an inner removable sleeve (AMS Inc., American Falls, ID, USA). The corer sleeve was 4.6 cm diameter and 15.3 cm in length). Samples were collected from the clayey soil with horizons A and B collected separately. Compaction was standard between sites with the top of the corer driven even with soil surface resulting in a 2.5 cm above the sleeve for a uniform compaction factor for all samples. The sandy soil consisted of the same soil texture for both the A and B horizons and one core was taken representing the 15.3 cm sample and no distinct separation between horizons. Samples were oven dried for 48 h at 105° C (Blake and Hartge 1986). The dry soil samples were sieved through a 2 mm mesh sieve and the percent of clay, silt and sand was determined using the Bouyoucos hydrometer particle size analysis standard method (Gee and Buader 1986).

Burial method

Human remains tissue consisting of skin, fat, and muscle sections from the thigh and calf of a human cadaver at 4 days post mortem was used for burial. Tissue was provided by the Forensic Anthropology Center at Texas State (FACTS), Texas State University, San Marcos, Texas, USA. Tissue was apportioned to individual samples weighing around 0.91 kg. Tissue samples were placed inside cotton soil bags for burial.

The HR tissue was maintained in a freezer prior to burial in the field and thawed in a cooler the night before the experimental procedure. The HR tissue was refrigerated overnight (approximately 4˚ C) between field tests.

To bury the HR tissue sample, a circular plug, approximately 7 cm deep and 18 cm in diameter of the top vegetation was removed with a knife and set aside. The remainder of the hole was dug to a depth of 46 cm and diameter of 18 cm with a standard post-hole digger. Each hole had the soil removed, sorted by relevant horizon and placed into a labeled bag so that the soil could be placed back into the hole after tissue burial in the appropriate order, maintaining the soil profile and bulk density as closely as possible (Figure 1).

Figure 1. Soil is removed from hole and placed into plastic garbage bags in order of removal so that horizons can be replaced in order.

All holes were dug 48 hours prior to burials to prevent any inadvertent contamination of holes. Blank holes (burials of cotton bags with no tissue) were filled in first prior to any HR burial to further prevent contamination. Soils were replaced in the holes from which they were excavated without compacting to maintain a similar bulk density to that prior to removal. Time constraints and potential rain events required buried HR to 'set' a minimum of 30 minutes prior to execution of the experiment to allow diffusion of gases. The removed vegetation plug was replaced into the top of the hole in a manner to prevent little visual difference from the surrounding vegetation and ground (Figure 2).

Figure 2. A vegetation plug which was removed with a knife the diameter of the desired hole was set aside so that when the blank or target material was placed in the hole, the plug could be replaced on top in a manner to minimize visual detection by the canine handler.

Subjects: Nationally certified HRD dog teams

A HRD dog team comprises the HRD dog and its handler. Six nationally certified HRD dog teams were utilized for the experiment. The dog teams were certified annually and each dog had achieved certifications previously through multiple agencies including the National Association for Search and Rescue (NASAR) (Christensen 2014), the National Narcotic Detector Dog Association (NNDDA) (2014) and the North American Police Work Dog Association (NAPWDA) (2014). Both male and female dogs participated and ranged in age from 3 to 13 yrs. old. Three of the dogs used were trained solely for HRD, while three of the dogs used were cross-trained to locate live subjects as well. Four of the six dogs had passive trained final responses (3 'down' and 1 'sit'), while two had 'active dig' trained final responses. A trained final response (TFR), as defined by the Scientific Working Group on Dog and Orthogonal detector Guidelines (2013) is a trained behavior that has been associated with the presence of a target odor source. Trained final responses may be passive: sits, downs, stares or active: bark, scratch, dig (SWGDOG 2013). All six dogs had previous recoveries on real world missions. All dogs were border collies or border collie mixes and were trained by members of the same local specialized canine search team (CTSAR 2014).

Experimental design

At each site, five square plots (7.62 x 7.62 m squares) arranged in a linear fashion (Plots 1 through 5). Each row of plots was searched by one HRD dog team (Figure 3). Six rows of five square plots were marked off with construction flags on every plot corner. One hole, 46 cm deep by ~ 18cm in diameter was placed roughly in

the center area of each plot. Three of the five holes were randomly assigned to receive HR, while two holes were left blank. The plots were run in sequential order either from Plot 1 to Plot 5 or from Plot 5 to Plot 1. The rows of plots were arranged so that the HR materials were upwind of the dog upon the start of the search.

	Plot 1	**Plot 2**	**Plot 3**	**Plot 4**	**Plot 5**	
Row 6	5	⇦4	⇦3	⇦2	⇦1	Dog 6
Row 5	1⇨	2⇨	3⇨	4⇨	5↑	Dog 5
Row 4	↑ 5	⇦4	⇦3	⇦2	⇦1	Dog 4
Row 3	1⇨	2⇨	3⇨	4⇨	5↑	Dog 3
Row 2	↑ 5	⇦4	⇦3	⇦2	⇦1	Dog 2
Row 1	1⇨	2⇨	3⇨	4⇨	5↑	Dog 1
TRIALS Wind Direction ↑						

Figure 3. A diagram of the layout of the plots and the associated wind direction, each plot measuring 7.62 m x 7.62m (25”x25”). Based on the wind direction in this example dog number six ran the first row of five plots left to right, then dog five ran the next row of five plots right to left.

Each plot to be searched was surrounded on three sides with a temporary 1.21 m barrier fence and held in place with 7 Fi-Shock 1.2 m plastic black step-in fence posts (Figure 4).

Figure 4. A temporary fence was placed around each plot prior to the dog working it. When finished it was moved to the subsequent plot.

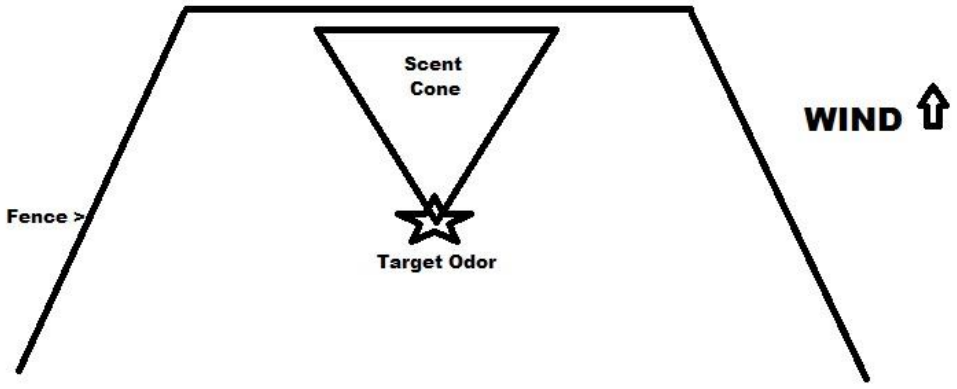

Figure 5. Illustration of theoretical scent cone radiating downwind from target odor within the fenced search plot. Dogs were started on open side and had to cross behind the buried target in order to cross within the area of the odor plum.

Prior to the start of the trial, the handler was asked to identify their dog's TFR. Each HRD dog team was allowed 60 s to search each plot. The handler was instructed to stand roughly in the middle of the open side of the fencing but not allowed to enter the search plot (Figure 5).

The handler was instructed to verbally call a TFR when it occurred. Alternatively, at the conclusion of the 60 s period, if the dog had not alerted, they were asked to indicate if the dog had given a TFR and were then instructed to enter the plot and indicate the exact location their dog was offering the TFR with a flag. Handlers were instructed to call an area blank if their dog did not offer a TFR. Upon the completion of each plot, the fencing was moved to the next plot successively until the five plots were completed. Each HRD team searched one row of five successive plots once for a completion of five trials per soil type, resulting in N=30 for each soil for a total N=60. Each plot search was timed using a stopwatch. All observational data, such as the dog's behavior and handler interference, was video recorded. The handlers had no knowledge of location of buried HR. Testers recording data had knowledge of the exact locations of buried HR but were obscured from sight of the dogs and trainers to eliminate cues to dogs and their handlers.

Soil:							Date:			
Dog	Plot 1	R/T	Plot 2	R/T	Plot 3	R/T	Plot 4	R/T	Plot 5	R/T
6	B		HR		B		HR		HR	
5	B		HR		HR		B		HR	
4	HR		B		HR		B		HR	
3	B		HR		HR		HR		B	
2	B		HR		HR		B		HR	
1	HR		B		HR		HR		B	

Figure 6. Example scoring sheet. This is an example scoring sheet used to determine differences between sandy and clayey soils. Each dog ran a row of five plots on each soil site. Plot boxes indicate whether the plot had a hide or was blank. R/T box was to record the response. C for correct, P for false positive and N for false negative and the length of time till the first trained response. Each dog ran five trials successively, either Plot 1 to 5 or Plot 5 to 1.

Response accuracy was scored on each trial with one of three responses: 1) correct, 2) false positive or 3) false negative and results were recorded (e.g. Figure 6). Testers recorded 1) the dog's first actual TFR on both buried human remains or blanks, 2) any attempts to leave the area on blanks, 3) the response accuracy (correct, false positive, false negative) and 4) the length of time required for the initial response. The time required for the handler to call the TFR or a clear (dog displayed no TFR) and accuracy of the call (correct, false positive, false negative) were recorded separately (Komar 1999; Lasseter et al. 2003, Oesterhelweg et al. 2008).

Experiments were run on two consecutive days on a Saturday and Sunday morning September 28 and 29, 2013 in an attempt to control for major seasonal temperature fluxes in weather. Temperature, humidity, barometric pressure, wind speed and direction were verified through online weather archives for the location of each site (Wunderground 2013).

Statistical analysis

Data were tested for normal distribution prior to statistical analyses. Response accuracy was calculated through chi-square analysis between soil textures and between dog responses and handler called responses. Response times were evaluated using a 1-tailed independent T-Test with an α=0.05 between textures clay and sand and between dog responses versus handler called responses. Differences between dog response times and handler called response times were evaluated through a paired, two tailed t-test with α=0.05. Observational data pertaining to how fluently the dogs covered the area and scenting behavior were also recorded for further evaluation for any difference between the two sites. SPSS V16 was used for statistical analysis.

Results

Soil texture percentage

The two horizons of the clayey soil were evaluated separately. The A horizon consisting of Hearne Fine Sandy Loam at a depth of 0 – 25.4 cm was comprised 70% sand, 10% silt and 20% clay. The B horizon was the clay fragment of the soil at a depth of 25.4 - 63.5 cm and comprised 40% sand, 8% silt and 52% clay. The sandy soil

(Padina Loamy Fine Sand) to a depth of 142 cm comprised 85% sand, 6% silt and 9% clay.

Weather conditions

Table 1. Hourly Weather Conditions for Hearne, Robertson County TX, USA on Sept 12 and 13, 2013[a]. The Clay site was used on day 1 and the Sand site was used on day 2.

	Temperature		Humidity		Barometric Pressure		Wind Speed in mph		Wind Direction	
Time[b]	Clay	Sand	Clay	Sand	Clay	Sand	Clay	Sand	Clay	Sand
8 am	77.9	68.4	86%	94%	Rising	Rising	11.5	5.8	SE	W/NW
9 am	77.7	68.2	89%	94%	Rising	Rising	5.8	8.1	SE	NW
10 am	79.7	70.0	86%	91%	Rising	Steady	6.9	5.8	SE	NW
11 am	82.6	70.3	75%	89%	Rising	Steady	8.1	8.1	S	NW

[a]Data collected at Easterly Airport. Both clayey and sandy sites were within a 16 kilometer distance from downtown Hearne where weather conditions were collected.
[b] All tests were completed prior to 11:30 am.

The temperature did not exceed 29°C (85°F) on either day of the trial (Table 1). Previous work has identified 29˚C as the critical temperature point when humidity must be taken into account to assist in heat stress prevention for working dogs (LAFB 1985). Air temperature was lower on the Sunday testing but humidity was higher making the average working conditions for both days approximately the same. Both days consisted of light drizzle and overcast skies. The barometric pressure was rising or steady on both days. Wind speeds and direction were fairly consistent throughout the two testing periods (Table 1). Each dog's five plots took approximately 30 minutes to run through all the stations.

Response accuracy

Human remains detection dog responses were 98.33% accurate; whereas handler called responses were 91.67%, accurate. The first trained final response based on the handler's identification of the dog's TFR or attempt to leave a blank area was recorded. Handler interference was also recorded. Handlers called responses were subjective and based on their experience with their dog and generally occurred within a 15-20 second latency period. Collectively, five incorrect responses were called by handlers (Table 2).

Table 2. Correct Final Responses by Handlers Call and Dog Trained Final Response. Correct response for each dog ID for both clay and sand sites is 5. Therefore for Dog 1 at the Clay site the result was 3 out of a possible 5.

	Correct Responses			
	Handler calls		Dog TFR	
Dog ID	Clay	Sand	Clay	Sand
1	3	5	4	5
2	5	5	5	5
3	4	4	5	5
4	5	5	5	5
5	5	5	5	5
6	5	4	5	5

There was no significant difference between the percentages of correct responses for dog responses versus handler called responses. Chi-square analysis showed that the correct response rate by both handler and dog were significantly above chance ($p < 0.0001$). Dog correct responses had a value $X^2=56.067(DF=1; p < 0.0001)$. Handler

called correct responses had a value X^2=41.667 (DF=1; $p < 0.0001$). Soil texture had no effect on accuracy of correct response with a value X^2=.067 (DF=1; $p = 0.80$). No significant difference was detected between the accuracy of the HRD only dogs and the cross trained dogs.

Time difference measures

The amount of time spent searching before coming to a final response was measured to determine any difference in difficulty in obtaining odor (Table 3). There was a significant difference between the time it took for dogs to come to TFR on sand versus clay ($p <0.0001$) as well as a significant difference between the time of the dog's TFR and the handler's called TFR ($p < 0.0001$). Time differences between clay and sand were not significant with handler called TFRs but dog called TFRs showed a significant difference between clay and sand with a one tailed T-Test ($p < 0.001$; Table 3).

Table 3. Response Time for Dog TFR and Handler Call by Soil Type.

T-Test	Texture Mean Response Time			
	Clay	Sand	Std. Error	Significance
Dog TFR's	37.9s	20.5s	2.46	0.000
Handler Calls	45.5	37.6	4.02	0.173

There was also a significant difference between handler called TFR's and dog TFR's (paired T-test $p < 0.001$; Table 4) verifying the latency between the dog's response and the handlers call of the response.

Table 4. Paired T-test results between Dog TFR and Handler Called TFR.

	Mean Response Time Differences			
Paired T-Test	Dog TFR	Handler Call	Std. Error	Significance
Dog TFR's versus Handler Calls	29.2s	41.5s	2.05	0.000

Observational data

Dogs' search behavior on the clayey soil consisted of slow methodical sweeps quartering back and forth across the predominant wind. Dogs on the clayey soil carried their nose much closer to the ground than on the sandy site. All dogs had to cross directly over the hole to show a change of behavior (COB). COB or alert behavior is defined as a change in behavior which is recognized by the handler to indicate the detection of the trained odor by the dog (SWGDOG 2014). Dog search behavior on the sandy soil was considerably faster and consisted of generally taking one sweep across the wind close to the fence and then turning, head up into the area which contained the target scent. Initial changes in behavior on the sandy soil were generally much faster than on the clayey soil however, dogs appeared to have more difficulty pinpointing the exact location of the scent.

Discussion

This is the first study to examine the effect of soil properties on a HRD dog's performance in locating and alerting on buried human remains. A perfectly controlled

environment cannot be achieved in field conditions. However, several controls were implemented to make the testing as controlled as possible. Controls included: 1) both sites were used for cattle grazing with minimal till and fertilizers over their history, 2) both sites had good ground cover of coastal Bermuda grass, 3) holes were dug for each site 48 hours prior to testing, 4) neither field had been used previously for training or testing and lacked any kind of previous burial or knowledge of any animal or human remains and, 5) control for weather conditions were attempted by having the tests occur within one weekend on back to back days. Weather conditions were similar between the two test days and common conditions for the time of year the tests were run. Since weather conditions affect the movement and location of available odor and are often governed by time of day and location of the sun, both test series were run during the morning hours.

Soil texture

My hypothesis that odor detection would be faster for dogs in sandy soil was supported. The clayey soil site was approximately 0.81 km^2 (200 acres) and contained approximately 100 head of cattle, whereas the sandy site was approximately 3.04 km^2 (750 acres) and at the time of testing there were no cattle present on the property although there was a history of cattle using the property for grazing. The significantly slower response time of the dogs on clay supports the hypothesis that HRD dogs to will encounter more problems on clay as gases associated with decomposition will diffuse through such soils more slowly. Land use of grazing cattle for 100 years could have caused some compaction and contributed to the difficulty the dogs exhibited in locating

the odor origin. This coupled with the B horizon layer of clay and the sealing properties of clay contributed to the lack of odor escape and thereby available odor for detection. Differences in the aeration between clayey soils and sandy soils have important implications for actual mission deployments of HRD dogs searching for clandestine graves. This information can assist search managers and HRD dog teams when planning strategies and apportioning search area sizes and approximate sweep widths to cover the area efficiently but with best precision by HRD dog teams. The more clayey the soil, the poorer aeration, which will require slower more methodical searching with decreased sweep width on grids. It is recommended that soil texture be taken into account when apportioning the size of search sectors and the time allotted to search the area. Soil texture can be obtained through either field hand texturing with minimal training or through online formats such as the USDA web soil survey website or the smart phone application "Soil Web" which operates with the GPS function of the phone and gives the soil series and other pertinent information such as soil texture at each soil horizon accurate up to one meter of the location of the user.

Weather conditions

Though somewhat counter intuitive, greater pore space in clayey soils is mainly due to a larger number of micropores where a closer proximity of water to the clay particle surface allows water to be held with more force than exerted by gravitational pull, resulting in a greater potential to hold water than in sandy soils where the majority of water is lost to gravitational drainage. This high water retention by clayey soils limits aeration and escape of gases (Fujikawa and Miyasaki 2005; Skyortsova and Utkaeva

2008). Micropores also provide for slower diffusion due to tortuosity of flow paths. Light rains on the Saturday trial on clayey soil did not result in water standing in the pre-dug holes but did result in a damp surface soil. Removed soil was slightly damp at the mouth of the plastic heavy duty garbage bags prior to replacement. The light rain however, to cause substantial changes in the field water retention. Despite heavy rain Saturday night, the permeability and good drainage of the sandy soil site also resulted in slightly damp surface soil and holes that were not standing in water. The prior removed soil was wet prior to replacement however due to storage in the field overnight in plastic heavy duty garbage bags which were not tied shut, allowing rainwater to enter; this wetness may have contributed to odor moving laterally instead of straight up through the soil. This may have made it more diffuse and harder to pinpoint, which was consistent with the dogs' search behaviors once they acquired odor.

Visualization of the potential movement of odor due to wind effects can be accomplished through the use of smoke bombs and scent detection dog handlers are generally trained in "scent theory" or the study of the transport of odor (Alexander and Turner 2010). Temperature, humidity, and wind speed and direction are generally accepted conditions tracked by most handlers in their training logs. Temperature and humidity are of special consideration in terms of heat conditions and efficiency of scenting by the detector dog. Lackland Air Force military working dog division devised a formula for temperature and humidity correlations to safely work a dog without causing excessive heat stress (LAFB 1985). Critical temperatures generally begin at 29°C (85°F). The formula is an inverse of temperature listed in Fahrenheit and humidity

whereby for every degree rise in temperature above 29°C (85°F), humidity must fall from 90% humidity four percentage points for optimal safety. Barometric pressure is regularly tracked by tracking/trailing dog handlers but often neglected by other disciplines. However, it may potentially be a very important component of odor detection from ground, water or buried remains. Rising or steady pressure allows the scent to rise and spread out above the surface with the wind, whereas, falling pressure tends to push gases back towards the earth (Syrotuck 1972). Smoke bombs utilized during different barometric pressure conditions can easily demonstrate visually the effects of wind and barometric pressure. Dogs that naturally work with a high head may have more difficulty obtaining odor on days where the barometric pressure is falling. More attention to research on the effect of barometric pressure conditions would enhance deployment capabilities of search dogs in general.

Accuracy

Case law for minimal narcotic dog accuracy has ranged between 54% and 67% depending upon the state (Fleck 2013; 2014) for the last several decades. Because evidence located by HRD dogs can render the handler in court for testimony, this accuracy expectation is also applied to HRD dogs. However, SWGDOG (2014) best practice guidelines recommend a positive alert rate of 90%. A positive alert is the correct identification with a trained final response (TFR) of the target odor when present. A false alert rate of no more than 10% is acceptable (Fleck 2013; 2014; SWGDOG 2014). False alerts are TFRs in the verified absence of the target odor. This requires controlled environments to achieve a positive alert rate of 90% and a false alert rate of < 10%.

Uncontrolled environments in real world scenarios do sometimes lead to non-productive alerts (NPAs), which are alerts or TFRs that are unable to be verified through some type of physical evidence. Residual odors, detectable by dogs but undetectable by the human nose may be the culprit of NPAs (Chapter III).

Recent court cases (Fleck 2013; 2014) have better clarified accuracy, training and certification expectations of the court. Florida vs. Harris was recently reviewed by the U. S. Supreme court that concluded that *"if a bona fide organization has certified a dog after testing his reliability in a controlled setting"* (or *"if the dog has recently and successfully completed a training program"*), *"a court can presume (subject to any conflicting evidence offered) that the dog's alert provides probable cause to search"*. Courts have deemed that detector dogs must show they are trained, certified and reliable (Fleck 2013). Training records should be maintained for all detector dogs which include a reliability percentage. Reliability is determined through controlled environment single-blind certifications and proficiency assessments (Fleck 2013, 2014). Proficiency assessments are generally weekly for bomb detector dogs and bi-weekly or monthly for narcotic detector dogs. HRD dog teams should hold their dogs to the same standard, as case law pertaining to narcotic dogs is generally accepted for use with HRD cases (Fleck 2013, 2014). United States v Cedano-Arellano (332 F. 3d 568 (2003) Ninth Circuit) determined that a detection dog's records, both training and certifications are discoverable by the defense (Fleck 2013). This allows for assessment of the dog's reliability by the defense. It is also important to note that industry standards for narcotic dogs do not endorse fielding un-certified dog teams (Fleck 2013; SWGDOG 2014),

whereas, this practice is still done with some volunteer search dog teams. Further, most law enforcement agencies are moving towards outside third party certifications from recognized organizations due to pressures from court cases, however, this practice is still not uniform among volunteer search dog teams. Many volunteer search dog teams still test with in-house certifications which may or may not be set up in a manner which truly challenges the team. Furthermore, most in-house tests evaluate the dog teams' ability to locate the same aids which the dogs routinely train on, rather than appropriate but novel aids of human remains. Our study utilized novel human remains which the dog teams had not previously trained with but would be consistent with potential real world cases. Assessment using aids that dogs regularly train with may be more indicative of the dog's capabilities to find their own training aids rather than novel human remains odors as would occur in real world scenarios.

HRD dog accuracy within this study was established to be well within acceptable ranges associated within the SWGDOG best practices guidelines (SWGDOG 2014). It is important to note that on proficiency testing and certifications 100% accuracy is not uncommon or unrealistic **(**Fleck 2013, 2014). In this study, dogs gave correct responses 100% of the time on HR buried targets and 91.7% on blank targets. It is also important to note that no significant differences were seen in performance between the HRD only trained dogs and the cross trained dogs verifying that cross trained dogs are equally able to detect clandestine graves as HRD trained only dogs. One of the five incorrect responses was a true false alert on a blank hole indicated by the dog prior to any handler interference. Two incorrect responses were called as TFRs by the handlers after calling

their dogs back into the blank plots multiple times resulting in two false alerts. The other two incorrect responses were made by the handlers based on changes of their dog's behavior, one being called as a false alert and one being called as a false negative. The false negative was called on one of the sand plots which had fire ants on the burial area of human remains which impeded the dog from offering its full TFR at the location of the hole.

Time difference measure

Time was used as the measure of difficulty for the dog to obtain odor. The dogs in this study found buried remains significantly faster in sandy soil (Table 2.3). Sandy soils typically have less overall pore space compared to clayey soil due to micropores found within clay aggregates (Lowe et al. 2013; Al-Qinna et al. 2013). Pore spaces in sandy soils are larger and connected; they are generally better drained thereby providing better conditions for air and gas diffusion.

Observational data

Handlers were on average 15-20 seconds latent in calling dog TFR's. Handlers called the dog's responses correctly 94% of the time for HR buried targets but only 58.3% of the time on blanks. The discrepancy on blanks may be attributed to handlers repeatedly sending the dogs back into the search area when the dog attempted to leave on offering no TFR. A HRD dog leaving the area after thoroughly checking it, including sniffing the blank hole, should have been an indication of no response and therefore a called as a blank area. In 3 of the 5 false alerts called, the dogs never gave a full trained final response. Handlers called the area as an HR hole due to interest and repeated

returns to a specific location which turned out to be the negative hole. This suggests that the handler assumed that "some" interest but no alert meant the dog had failed to offer a TFR. This indicates that more time should be invested by teams in working both negative scenarios where no HR is present and scenarios with non-target odors present to build handler confidence in their dogs assessment when no odor is present.

Many master dog trainers point out changes in inhalation cycles and intensity of sniffing as an indication of the detection of a target odor (Alexander and Turner 2010; Dotson 2012). This change in inhalation cycles is most readily heard in buildings but can also be heard outdoors in low wind conditions. Both locations elicited similar reactions from the dogs on both the HR and blank holes. Deep sniffing, forceful exhalation and termination of interest in the hole was observed for blank holes, whereas deep sniffing followed by movement of the grassy plug (passive alert dogs) or deep sniffing followed by aggressive digging or continual deep sniffing (aggressive alert dogs) were observed for HR holes on the clayey site. This emphasizes the need for inclusion of blank or negative areas which do not contain any HR materials within the normal training regime both known, blind, and double blind (Dotson 2012). Dogs are not trained to specifically give a trained response indicating no target odor found, so it is imperative that handlers learn to read the body language that their dogs exhibit when searching an area void of a target odor. Behavioral differences are also often noted with non-target odors such as dead animals or food (Alexander and Turner 2010; Christensen 2014; Dotson 2012). Handlers must also train with various distractor odors to verify dead animal odors will not elicit a TFR and also to learn their dog's body language with non-target but odors of

interest to the dog. Achieving true independence of work also requires the handlers to pressure the dogs in training in the same manner they would on an actual mission or test to ensure the dogs learn that even with handler pressure, in the lack of the target odor, the only correct response is no response (Alexander and Turner 2010; Dotson 2012).

The clayey site produced slower, tighter and more methodical searching whereas the sandy site produced faster and larger sweep widths across the search area. Dogs in the clayey site worked with their head low to the ground, whereas, at the sandy site, dogs worked with their heads up. This may be indicative of the lack of scent escape at the clayey soil site. Further support of this was indicated by observable changes in behavior only when the dog passed directly over the burial hole in the clayey site, whereas, change of behavior was noted as soon as the dog was downwind of the hole at the sandy site. This suggests that the sandy site had enough odor emanating from the burial to develop a significant scent cone which is a zone of odor resembling a triangle (Figure 2.5) due to gas escape whereas the clayey site, the gas escape and therefore scent cone was significantly smaller and fainter. Changes in behavior (COB) can vary between dogs but generally accepted COB's are sudden changes in direction when passing through a scent cone by turning back into the odor zone, changes in tail carriage, head carriage, ear position, body position such as erectness or crouching closer to the ground. Handlers generally know through prior training experience, those COB's that indicate their dogs have entered scent cones and are working to the origin of the odor, however, dogs can also indicate COB's with non-target odors. Because handlers are visual, even if the dog is just checking an anomaly that may not be a target odor, handlers may interpret the

COB as the detection of a target odor. This may cause handler interference which results the handler pressuring the dog until a non-productive TFR is offered. Despite handlers maintaining their position out of the search area, verbal cues and pointing gestures to check areas were allowed and may have contributed to the incorrect TFR's that were offered (Hare and Tomasello 1999).

Further implications

The quantitative concentration of odor emanating from either site is not known, nor is it known how it would compare to contemporary or cold case graves in terms of the concentrations of odor diffused as it was not within the scope of this study. The current practice that many trainers and evaluators use for buried training consists of utilizing small size (14-30 g) source material, holes dug in a manner which allows for a cylinder of soil to be removed like a cork from a wine bottle then replaced back into the ground by stomping and compacting the cylinder back into place which further alters the porosity and aeration of the original condition of the soil, or utilizing glass jars to contain the human remains to limit odor diffusion with the philosophy that this will simulate older graves (Alexander and Turner 2010). There is no scientific verification that any of these methods successfully mimic gases diffused by full body burials of any age. The importance of training HRD dogs on adequate source materials and realistic scenarios needs more scientific study and verification, as do the various effects that the environment and soil types and textures have upon the diffusion of odor to HRD dogs. There is no mandatory national standard to become and maintain proficiency as a search dog handler. Volunteer search dog teams, unless existing in one of the few states having

state standards such as Maine, California and Virginia, have no oversight and consequently can be led by trainers with years of field experience as well as inexperienced first time handlers. It should be emphasized that trainers are often also handlers or retired handlers; however in contrast, not all handlers are trainers. Handlers range from individuals with various levels of dog training experiences to novice individuals. It is becoming common practice for a group, for example, of firefighters to select someone from a squad to become a canine handler due to their other trained skills yet they may lack any kind of dog training experience. These handlers may then be sent to short training courses where they are paired with pre-trained canine partners (SDF 2014). This may be efficient in terms of utilizing already trained human resources; however the lack of understanding of dog behavior and training has a high potential to erode the team's efficiency and accuracy over time.

Despite the lack of a national certification standard or law mandating such, HRD dogs deployed on criminal cases should be certified with a reputable organization (Fleck 2013, 2014; SWGDOG 2014). Many such national, regional and state organizations exist governed by both civilian and law enforcement (Christensen 2014, NNDDA 2014; NAPWDA 2014; SWGDOG 2014). Testing standards vary from organization to organization; however, most HRD evaluations include a buried search scenario as part of the team's certification. This variability among testing also includes variability in methods used to conduct the test. For example, varying amounts and types of tissue, 14 grams up to 60lbs (Christensen 2014, NAPWDA 2014; NASAR 2014, SWGDOG 2014) or other substances such hair, nail clippings, teeth, and pseudo scent or rags infused with

pseudo scent, permeated with indirect contact with human remains or impregnated with blood or decomposition fluids, are buried in the ground (OSSA 2014). The viability of these various target odors as accurate substitutions for full bodies necessitates further research. Depth requirements generally range from as minimal as 15 cm up to 76 cm (6-30 inches). Actual depth utilized on any given test depends on the organizations standards, the experiences of the evaluator, availability and size of materials to bury, instruments to dig with and the texture, profile and moisture content of the soil. Methods for placement of the HR also vary greatly between evaluators. Some evaluators utilize leaves, rocks, sticks and other materials to increase aeration of the hole whereas others utilize cylinder core plugs. Core plugs are analogous to a cork in a bottle, some remove only a section of vegetation and top soil while others pull a plug as deep as the soil will allow. The entire plug is removed generally with a post hole digger and set aside, HR placed at the bottom of the hole and the soil plug is replaced and compacted in a manner to decrease chances of handlers seeing the location of the burial. Clayey soils, which already have poor aeration and less gas diffusion and have been shown to be more difficult to detect odor, work best for this method due to clay soils stickiness and tighter structure. This method typically compacts the soil more, contributing to an even more difficult detection scenario. The size of the HR typically used (14-30 grams) is not comparable to a human body nor has decomposition occurred in the area so the soil is void of the typical cadaver decomposition island (CDI) and the accompanying gaseous diffusion and nutrient dispersal associated with an actual decomposing corpse (Carter et al. 2010; Van Belle et al. 2009; Vass et al. 2008; Wilson et al. 2007). Organizations also

vary on the length of time required for the burial to 'set' prior to testing, anywhere between a half hour to twelve hours (NNDDA 2014; NAPWDA 2014, NASAR 2014). Short deposition or set times may result in a lower release of decomposition gases. Soils which are packed down into the hole compact the soil after burial of the HR and may also greatly reduce the amount of gases that can escape as the natural structure and pore space of the soil has been disturbed and adequate time to return to normal structure cannot be obtained in the given time frame of the test. The results of the trials conducted in this study indicated that the method utilized resulted in adequate escape of odor, while still impairing visual identification of the target holes by the handlers. This more correctly mimics visual conditions found in most real world searches on older cases.

Summary

This study provided verification that the texture of soils can be a useful tool for estimating difficulty of detection levels for HRD dogs. Recent events, such as the mudslide in Oso, WA, USA on March 22, 2014 killing 42 people where search dogs were brought in to locate the buried victims demonstrates how knowledge of the effects of soil texture could be beneficial in searches beyond clandestine graves. Soil texture is an important aspect that can be assessed in the field with minimal experience through hand texturing, feeling for the gritty feel of sand, the sticky feel of clay or the smooth silky feel of silt. This may also help in quickly getting a sense of the soil type in the absence of a soil survey report. Soil information can then be utilized for training, testing and mission deployments of buried remains. Law enforcement, search managers and

HRD dog teams can benefit from researching soil profiles of designated areas prior to activities to best determine strategies that will lead to successful outcomes for HRD dogs.

CHAPTER III

RESIDUAL ODOR AND HUMAN REMAINS DETECTION DOGS

Introduction

Human decomposition

The rate of soft tissue decomposition can be dramatically affected by factors that impact the body such as cause of death, animal scavenging, environmental conditions (temperature, rainfall, humidity, soil type), presence or absence of clothing, body mass, mummification and adipocere formation (Campobasso et al. 2001; Komar 1998; Mant 1987; Micozzi 1986; Rodriguez and Bass 1985; Vass et al. 1992). Decomposition progresses from autolysis to putrefaction, liquefaction, and finally skeletonization over the course of time (Dent et al. 2004). Microbes and enzymes within the body begin the process of autolysis within minutes after death (Fiedler and Graw 2003; Rodriguez and Bass 1985; Vass et al. 1992; Vass et al. 2002; Vass et al. 2004; Vass et al. 2008). However, Dent (2004) noted that during autolysis, hydrolytic enzymes began the breakdown of carbohydrates, fats, and proteins, followed by putrefaction generally no earlier than 48 hours post mortem. Carbohydrates are broken down into sugars by microorganisms in the soil while fats are broken down into fatty acids which under specific conditions will convert to adipocere (Fiedler et al. 2003; 2009; Forbes et al. 2005). Adipocere is a waxy soap like substance formed from the decomposition of fats in warm, moist anaerobic environments (Fiedler et al. 2003; 2009; Forbes et al. 2005). Some products of the protein breakdown include skatole and indole, carbon dioxide,

hydrogen sulfide, ammonia, and methane (Dent et al. 2004). This large purge of nutrients into the soil results in a noticeable cadaver decomposition island (CDI) which may benefit insects, microbes and plants (Aitkenhead-Peterson et al. 2015; Paczkowski and Schutz 2011). Eventually, liquefaction of tissues and organs result in complete disintegration, leaving only the dry skeletal remains. After the body has purged and created the CDI this will remain as trace evidence of human decomposition, even if the body has been disarticulated and scattered by scavengers or moved by criminals to a different 'hiding place'. On some very rare occasions a 'body burn' may be observed (Figure 7).

Figure 7. Body stain from Soil G at the STAFS facility in Hunstville, TX. USA Source: with permission from Kevin Derr, STAFS, Huntsville, TX, USA.

Human remains detection dogs

Human remains detection (HRD) dogs are trained to search for and pinpoint the strongest concentration of the odor of human remains and thus can be used as tools to locate trace evidence. HRD dogs are trained to communicate to their handlers they have located human remains (HR) through a trained final response (TFR) such as a 'down', 'sit', or 'bark'. HRD dogs are often trained to locate everything from a single drop of blood to an entire body (Alexander 2009; Alexander and Turner 2010; Christensen 2014; Dotson 2012; Hammond and Morris 2010; Rebmann et al 2000). There are currently no instruments that can detect the minimal amount of human decomposition product that a dog can detect (Myers, 2009), however, Furton and Myers (2004) estimated a dog's sensitivity to odors at least as low as 1 ppt. Most HRD dog handlers can only obtain small amounts of training materials that will provide the odor of human decomposition. Common training materials include teeth, blood, body fluids, and placenta. Bone of 20+ years old can be purchased on the internet from Skulls Unlimited (2014) and the Bone Room (2014). Use of these materials may result in dogs with low sensitivity thresholds and as a consequence, HRD dogs that are very sensitive to small amounts of decomposing human tissue, bone or blood in the natural environment.

The validity of HRD dogs are often called into question when an unproductive final trained response occurs in the field. One reason for this may be that water soluble nutrients from the CDI will move off site during rain events if the topography has a slope (Aitkenhead-Peterson et al. 2012). Law enforcement often assumes these responses to be "false alerts" or "mistakes" if a body is not recovered.

Residual odor from human remains may be the culprit of non-productive alerts where nothing visible can be recovered. When a properly trained and credentialed dog offers a trained final response (TFR) in a location where no visible remains are present the handler or law enforcement may interpret this as a mistake or a clandestine grave burial, when in fact, it may be residual scent from a body previously decomposing in the location. The body may have been moved, or disarticulated by animal scavengers. It is currently unknown how long a HRD dog can detect residual scent in the soil after the body has been removed. While there are many anecdotal stories of HRD dogs detecting residual scent months to years after a body was removed, there have been no studies that have examined if HRD dogs can recognize and offer a TFR on soil of a range of post mortem intervals (PMI) from residual human decomposition within the residual cadaver decomposition island (RCDI). While all instruments, mechanical or biological have an error rate, residual odor may be a justified explanation in many cases.

Residual scent and VOCs

Residual odor is defined as odor originating from a "target substance that may or may not be physically recoverable or detectable by other means*"* (SWGDOG 2014). Prior to Osterhelweg et al. (2010), no peer reviewed published scientific evidence existed to support the concept of residual odor. Osterhelweg et al. (2010) reported that three trained HRD dogs could detect the odor of human remains when there had not even been direct contact between the cadaver and the target object. Carpet squares were placed on a table then the corpse, wrapped in a sheet was placed on top of the carpet squares. Squares were left in place for two minutes and 10 minutes. The bodies used for

the sample acquisition were less than 3 hours post mortem thereby substantially decreasing the possibility of fluid contamination of the carpets from decomposition. Oesterhelweg et al. (2010) showed that HRD dogs correctly identified carpet squares (92% – 100% accuracy) up to 65 days post- exposure for 10 min (98% accuracy), and up to 35 days for post-exposure for 2 min (86% accuracy).

Residual odors are most likely emitted in the form of volatile organic compounds (VOCs). Vass et al. (2004; 2008) noted that over 478 VOCs were emitted from buried whole human remains. Sample sizes utilized for training HRD dogs vary in size but are generally small (> 2 g). There are different chemical signatures between a large sample and a smaller subsample of the same (Furton 2010), suggesting that small samples may smell differently to dogs than larger samples, even if it is the same type of tissue. The National Incident Management System (NIMS 2014) categorizes HRD dog types by source size, with Type I dogs being certified on human remains in amounts less than 15 g and Type II dogs being certified on human remains 30 g or above. Most handlers routinely train on amounts that are 30 g or less and few have any training materials that weigh more than 500 g (~1lb).

The Department of Justice Bureau of Alcohol, Tobacco and Firearms (ATF 1997) utilizes a two part test, with the first consisting of an odor recognition proficiency test established to assess the canine's ability to recognize target odor. This method calls for the use of clean unused perforated containers holding the target odor housed within a larger external container such as paint cans. The cans are placed in a line and the dogs are allowed to sample each can up to two times working on lead with their handler. This

is often referred to as a scent-line up; however, this line up contains actual known target odors. This differs from scent line-ups used for matching articles and suspects.

Canine handlers have been aware for many decades that handler gestures and body language can hinder canine performance in inadvertent cueing. Cueing occurs when their canine partners react to specific body language that is usually ritually repeated that indicates the location of the target odors the canines are searching for. This can occur through pointing, eye gaze, or body positioning. Extensive research performed by Brian Hare at the Duke Canine Cognition Center has concluded that dogs do read and act upon human pointing gestures (Hare and Tomasello 1999; 2006). Furthermore and more importantly, Lit et al. (2012) showed that handler's beliefs could influence the detector dog's trained final response, specifically resulting in alerts in the absence of the target odor.

The objective of this study was to examine the use of human remains grave soil as a training aid on the sensitivity and accuracy of eight nationally credentialed HRD dogs. I hypothesized that HRD dogs will be able to correctly identify the scent of grave soils from CDI (with human remains) and the residual scent of grave soils from CDIs without human remains (RCDI)for up to one year after body placement. Solutions from soil water extracts of grave soil or grave soils that have undergone water extraction will not be identified.

Materials and Methods

Soil samples

Soils for this research were obtained from The Southeast Texas Applied Forensic Science Facility (STAFS) which is located within the Sam Houston State University, Walker County, TX, USA. The soils at the facility are loamy, silicious, semiactive, thermic arenic Plinthic Paleudalfs and fine, mixed, semiactive, thermic Aquic Paleudalfs of the Depcor and Huntsburg series (USDA 2012). Vegetation at the site is primarily loblolly (*Pinus taeda*) and short leaf pine (*Pinus echinata*) (TPWD 2014).

Soil cores (7 cm depth) were collected with a soil probe (2 cm diameter) from under the torso region of 1) decomposing cadavers and 2) body stains where human remains had been removed (Table 5).

Table 5. List of all grave soils that were tested with a 30 g sample. PMI = days post body placement, RCDI = days post body removal, CHR* human remains contaminated soil which was used as a baseline throughout the trials. Na = not applicable.

Soil ID	Date Body Placed Out	PMI	RCDI
C3	8/19/2009	915	361
A	3/3/2009	907	667
C2	8/19/2009	893	339
C1	8/19/2009	804	252
E2	1/26/2010	753	494
E1	1/26/2010	731	453
D3	12/17/2009	684	407
D2	12/17/2009	618	na
B	8/17/2009	572	452
CHR*	8/19/2009	572	18
F2	3/8/2011	347	240
F1	3/8/2011	291	na
D1	12/17/2009	288	na
G3	3/8/2011	113	na
G2	3/8/2011	53	na
G1	3/8/2011	18	na

Control soils were taken from upslope and outside the fence of the STAFS facility, but within the same soil series. Soil samples were placed in new clean ziplock bags for transport. Samples contaminated with human remains (grave soils: CDI or RCDI) and control soil samples were transported in separate sealed containers to avoid cross contamination. Soil samples (grave soil CDI/RCDI and control) were air dried in separate locations for approximately 1 week prior to gently breaking up the soil peds for passing through a 2 mm sieve to homogenize. Soils were stored in brown paper bags inserted in ziplock bags in sealed containers to prevent moisture access to lower the potential for any further microbial activity. Sixteen grave soils with post mortem

intervals ranging from 18 – 915 d (CDI) and 18 – 667 RCDI (days after body removal) were utilized in line up trials with 8 nationally certified human remains detection dogs. Soils were separated into four categories: 1) grave soils from under decomposing bodies (CDI), 2) grave soils from body stains only where the remains had been removed (RCDI), 3) grave soils which had been extracted at a 1:10 soil: water ratio and 4) the water extracted soil solutions placed on commercially available latex free sterile pads. Soils used for testing HRD dog response were placed in cotton bags for trials to allow for odor diffusion.

Extracted residual soil

Five 3 g soil samples, which had been extracted at a 1:10 soil:water ratio were air dried for seven days under a hood. These soils were then placed in ziplock bags and saved for testing against non-extracted duplicates.

Soil solution samples

For residual scent, some of the soils were extracted at a 1:10 soil: water ratio and the solution only was used for testing HRD dogs. Here 3 g was combined with 30 mL of ultra-pure water in 50 mL high density polyethylene (HDPE) centrifuge tubes and shaken for 23 h prior to centrifugation at 19,500 g-force. The supernatant was removed using a cannula and syringe. Twenty mL of the supernatant was then placed on a commercially available latex free sterile pad (ULTA Beauty Inc., Bolingbrook, Illinois, USA) and placed in the oven at 50° C for 48 h to dry and then stored in ziplock bags and refrigerated until testing.

To test the HRD dogs on residual scent of water extracted from CDI, three training aids were made: 1) Clean: Latex free sterile pads with no addition and oven dried for 48 h at 50˚ C, 2) Control soil: Latex free sterile pads impregnated with 20 mL control soil water extract and oven dried for 48 h at 50˚ C, 3) DDW: Latex free sterile pads impregnated with 20 ml ultra-pure water only and oven dried for 48 h at 50˚ C and 3) Grave soil: Latex free sterile pads impregnated with 20 mL CDI soil extract and oven dried for 48 h at 50˚ C.

HRD dog teams

Eight nationally certified HRD dog teams were used to detect residual scent of human remains in soil and soil extracts. A dog team is considered to be a dog and handler. Each HRD dog team had achieved repeated certifications from one or more of the following national organizations: the National Association for Search and Rescue (NASAR), The North American Police Work Dog Association (NAPWDA), and the National Narcotic Detector Dog Association (NNDDA) and had documented recoveries on real world missions. Participating HRD dogs ranged in age from 3 y to 12 y and comprised three males and five females. Trained final responses (TFR) of the 8 dogs included: 2 dogs that gave an aggressive scratch TFR, 1 dog with a sit TFR, and 5 dogs with a down TFR. The HRD dogs included in the study were referenced by number to protect anonymity of the team. Prior to data collection trials, handlers were allowed three training sessions utilizing their own training materials and aids to condition the dogs to the "off lead line-up" testing procedure to minimize learning bias once the soil trials began and eliminate error due to lack of understanding procedural expectations.

Experiment trials

The testing model used was consistent with generally accepted methods utilized by agencies around the world for training and testing detector dogs and consisted of five new metal paint cans lined up in a row, 46 cm apart which contained a smaller aerated container holding odor samples. This is normally performed with the dog working on lead accompanied by the handler. These trials were modified to have no handler accompaniment on the line-up (ATF 1997; Oesterhelweg et al. 2008; Rebmann et al. 2000). Based on the findings of Hare and Tomasello (1999; 2006) and Lit et al. (2012), the "no handler accompaniment" on the line-up was initiated to minimize if not avoid any handler-dog cues. Each test was quasi double-blind; neither handlers nor dogs knew the correct answer (location of grave soil) prior to each trial. Testers who placed the human remains source materials were present but obscured from view of the dogs and handlers during each trial to prevent any subsequent cueing. Newly purchased clean one gallon metal paint cans were used for the standard line-up tests. Cans were cleaned between use with a 10% bleach solution, rinsed with DDW water and Acetone and air dried. All samples and containers were handled with nitrile gloves which were discarded between each trial. Trials consisted of a line-up of five metal cans (Figure 8 A), each containing one box holding one of either: 1) one uncontaminated cotton soil bag, 2) one cotton soil bag contaminated with handler scent, 3) one cotton soil bag containing control soil, 4) one cotton soil bag containing grave soil, and 5) one empty box. Soil samples of either 30 g or 3 g were contained inside 8.9 x 12.7cm cotton soil sample bags (Figure 8 B).

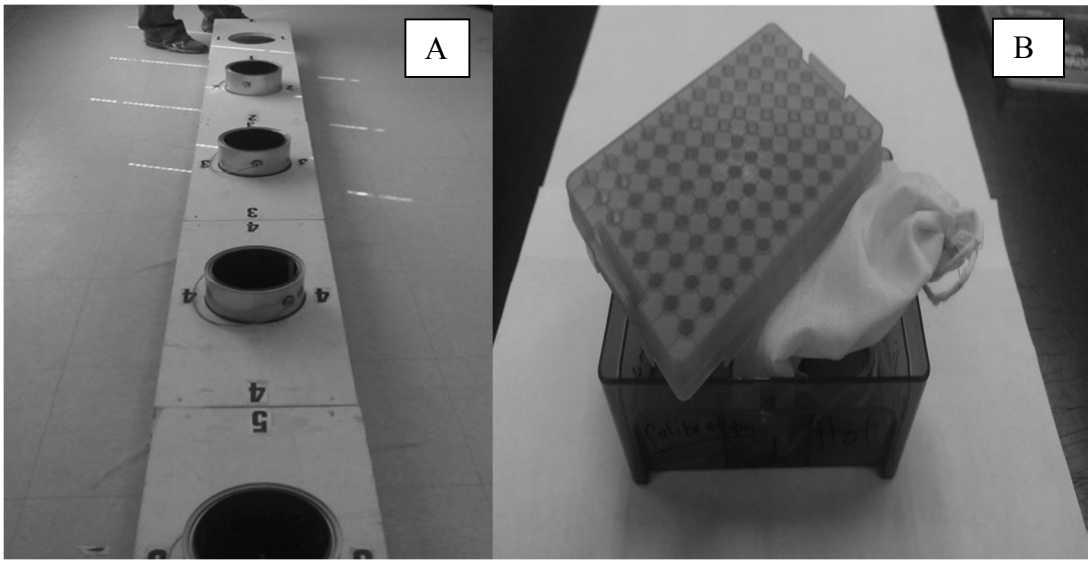

Figure 8. A) Five can line-up and B) Sterile pipette tip box with cotton soil bag.

The boxes used to house the cotton soil bags with sample were new, sterile pipette tip boxes which are aerated with 96 tip well holes which allowed diffusion of gases (Figure 8 B). Each dog was allotted its own set of boxes for each session that were then disposed of after the session. Cans were numbered 1-5 and assigned a position in the line-up. The metal cans containing boxes with or without cotton bags were placed in a numbered, enamel painted wooden platform to prevent the dogs from disturbing or moving the cans (Figure 8 A). Line-up tests were conducted as follows:

1) An independent post-doc assigned, conducted, directed and scored the trials accompanied by trained volunteer undergraduate student assistants.
2) Each dog of the 8 dogs in the trials had its own set of sample boxes to prevent any inadvertent scent cueing from teammate's dogs.
3) Ten trials were run for each dog in random order during each experiment testing session for N=80 trials per session. Ten testing sessions were completed for

N=800, however, one session set for one canine had to be excluded due to the canine's refusal to work, for a final total N=790.

4) Each session included a baseline grave soil (CHR) that was used in all tests as a as a representative of a "training" material that would typically be utilized over and over again at trainings.

An example of one session's scoring sheet for one canine is shown (Figure 9). The baseline grave soil (CHR; 1b) was replicated in each session within 3 trials randomly (Figure 9). Each session of ten trials also included one blank trial where no grave soil was included, instead a can with an empty box (Figure 3.3; Trial 5) was placed in the line-up in place of the grave soil (Figure 9; Trial 6). The eight dogs were run in random order at each experimental session and each dog had its own random sample order for each of its 10 trials. Dogs worked off lead and were allowed a maximum of 60 s per trial. Handlers were not allowed to enter the trial area and could not accompany dogs on the line up. Handlers were instructed to indicate verbally which, if any, elicited a trained final response within the 60 s timeframe. If the TFR was correct, the handler was instructed to reward their dog. Handlers were not allowed to reward their dogs for false positives. All HRD dogs used in the trials were toy reward dogs.

TRIAL SHEET								
TRIAL	Platform 1	Platform 2	Platform 3	Platform 4	Platform 5	Time	Score	LEGEND
1	1a	2	3	4	5			Cans
2	2	5	1b	3	4			1 Grave Soil
3	5	1c	3	2	4			2 Control Soil
4	4	3	2	1b	5			3 Handler Scent
5	3	2	1a	4	5			4 Empty bag
6 Blank	2	5	3	5	4			5 Empty box - Vacant
7	1c	4	5	3	2			Score
8	3	1b	2	5	4			C – correct
9	5	2	4	1c	3			F+ incorrect
10	3	2	4	5	1a			F- incorrect

Figure 9. Example score sheet for an experiment testing session for one canine. 1a would be the baseline grave soil, whereas 1b and 1c would be "test" hr soils that were novel to the dog. One Blank trial was run with no grave soil present in each session of ten trials. Responses recorded were C for correct, F+ for a false positive and F- for a false negative.

Figure 10. Participating HRD dog checks cans for the appropriate HR odor signature during testing trials.

Responses were recorded for each trial as either C = correct, F+ = false positive or F- = false negative. A false positive was categorized as the dog coming to a TFR at any sample other than human remains. A false negative was categorized as the dog failing to offer a TFR on a human remains target when present within the line-up. Figure 10 shows an example of how experiment participant dogs worked off lead during the identification trials.

Statistical analysis

Soil samples (30g), soil samples extracted and non-extracted (3g) and soil solution (20g) on latex-free pads were evaluated as separate groups with chi square analysis in

SPSS V. 16.0. Chi-Square analysis was run for response, no response against soil sample, soil age, and paired non-extracted/extracted soils with $\alpha<0.05$; significance was determined at $p<0.05$.

Results

Soil samples

Table 6. Soil samples, (30g) with responses and cumulative accuracy for the 8 HRD dogs over 790 trials. Letter ID's were assigned to each soil and if used for more than one PMI, the sample was also assigned a number to differentiate the samples (see table 5).

Soil ID	Correct Responses	False Positive Responses	False Negative Responses	Session Accuracy
C3	24	0	0	100.0%
A	18	3	0	85.7%
C2	22	2	0	91.7%
C1	23	1	0	97.8%
E2	18	2	4	75.0%
E1	23	0	1	97.8%
D3	23	1	0	97.8%
D2	17	3	1	81.0%
B	23	1	0	97.8%
CHR*	225	3	2	97.8%
F2	20	1	3	83.3%
F1	23	1	0	97.8%
D1	21	0	3	87.5%
G3	24	0	0	100.0%
G2	24	0	0	100.0%
G1	23	0	1	97.8%

Runs with grave soils were available for choice 711 times and runs without grave soils and 2 vacant boxes were available 79 times (Table 6). Correct responses were

recorded 660 times out of 711 for an overall accuracy of 92.8% on grave soils (Table 6). The results indicated that a significant detectable residual scent is available in a RCDI post remains removal for identification by a HRD dog. Team errors were the results of either dog error or handler error. Dog errors consisted of over-runs which resulted in some of the false positives when the dog alerted on the can just beyond the correct target and misses when the dog did not put their nose into each can. Handler errors consisted of miss-calls or failure to call. The most significant errors in the trials were false indications on handler scent and false negatives or misses. Over-runs, defined to be incorrect alerts either just before or just after the correct can (Oesterhelweg et al. 2008) accounted for 15 of the 27 false positives. Handler error on false negatives accounted for 8 of the 24 incorrect responses and handler error on blank trials accounted for 11 of the 21 incorrect responses (Table 7).

Table 7. A total of 790 trials were run, 711 with grave soil available for choice and 79 Null (no grave soil) to check for sensitivity. Blank trials with no grave soil and two empty boxes elicited the largest margin of error with the teams. HR Trials (χ^2=104.0, DF=5, Probability = 0.000), Null Trials (χ^2=33.7, DF=4, Probability = 0.0001).

N=711 HR Trials	HR Soil	Control Soil	Empty Bag	Handler Scent	Empty box	False Negative
Correct Response	660	705	707	696	709	687
Incorrect Response	51	6	4	15	2	24
N= 79 Blank Trials	Blank	Control Soil	Empty Bag	Handler Scent	Empty box	
Correct Response	58	72	69	75	79	58
Incorrect Response	21	7	10	4	0	21

A significant difference was found in the ability of the dog to make the correct response when HR soil was present and the correct no response when no HR soil was present.

Soil HR odor detectability

HRD dog teams correctly identified HR soil samples and RCDI soil samples ranging in age from 18 days PMI to 915 days PMI with no less than 75% accuracy up to 100% accuracy (Figure 11).

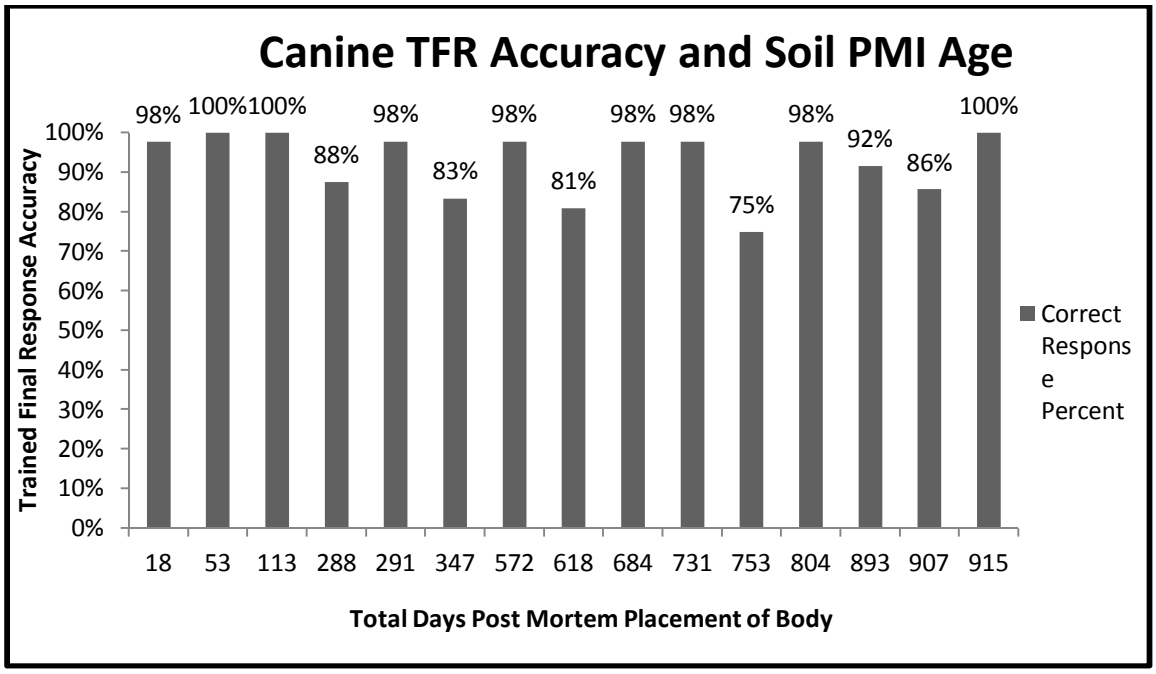

Figure 11. HRD dog correct trained final response percent for each trial in order from youngest to oldest PMI for 30 g of sample.

RCDI soils ranged from 240 days post body removal to 667 days post body removal. HRD dogs correctly identified RCDI soil samples with an accuracy ranging from 75% to 100%, with the 100% score occurring at almost 1 year post remains removal (361 days) (Figure 12).

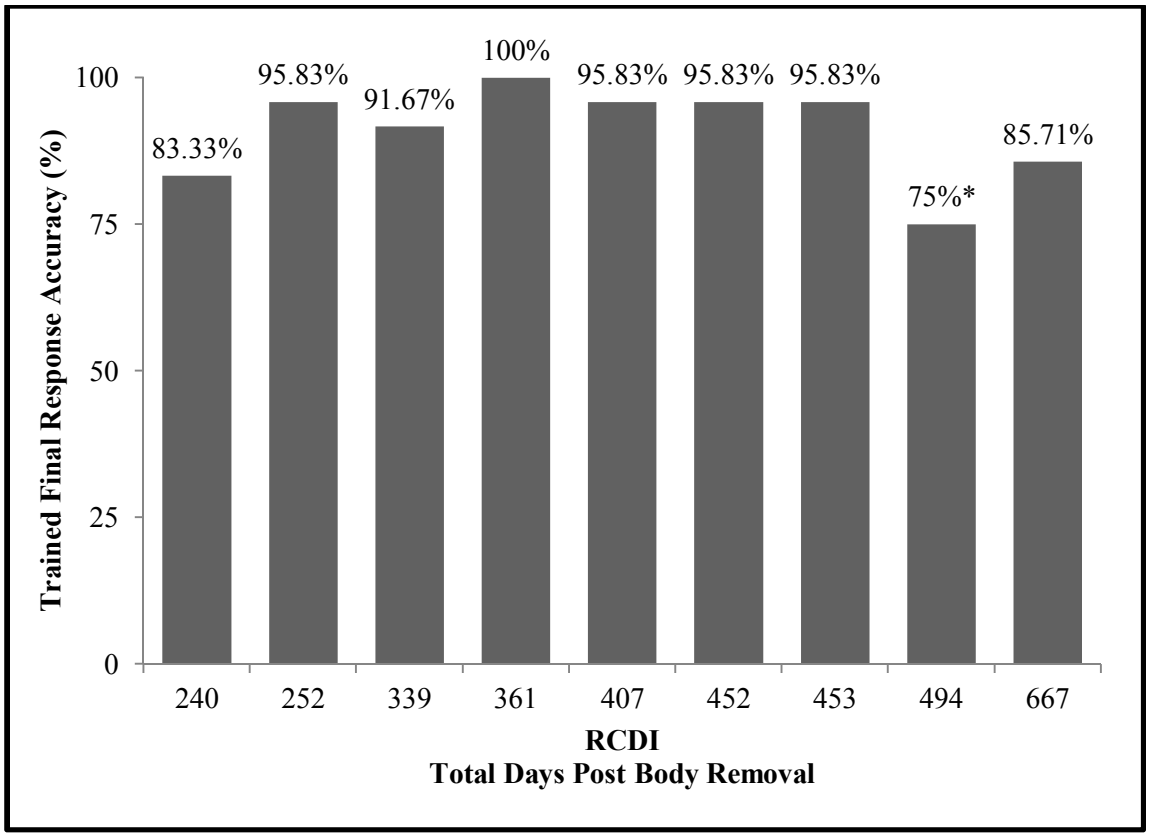

Figure 12. RCDI ranging from 240 days post body removal to 667 days post body removal with an accuracy rate of 75% to 100%. *One handler with multiple HRD dog partners was ill during trial and scoring is based on the handler's identification of their dog's TFR, consequently the handler had several runs with their dogs which they failed to accurately call their dog's TFR, resulting in a particularly low correct response rate for the session.

Extracted soil

Further testing for RCDI was performed by testing a grave soil against the same soil after water extraction (1:10 soil:water extraction). Five paired soils were tested (5 un-extracted soils [3g] and 5 extracted soils [3g]). Each HRD dog ran 3 random trials on the non-extracted sample and 3 random trials on the extracted sample for a total of 6 trials per dog per sample set. Seven HRD dogs completed this experiment for total trials of N=210. No significant difference was found between three of the five soils in terms of odor recognition. In G1 (18 d PMI) and F2 (347 d PMI), there was a significant difference found between responses in trials. Overall results indicate that the residual HR odor in water extracted soils is as detectible as unprocessed soils (Table 8).

Table 8. Results of a paired 3 g unprocessed non-extracted sample with a 3 g extracted sample against the 3g non-extracted sample with 7 HRD dogs over a total of 210 trials. *The delineation na under RCDI means that the soil utilized still had a body present on it when the sample was taken which included G1, G2, and F1, whereas F2 and B had the bodies already removed at sample collection.

			Team Accuracy		
ID	PMI	RCDI	Not extracted	Extracted	Significance
G1	18	na*	100.0%	83.3%	0.037
G2	53	na	100.0%	95.8%	0.312
F1	291	na	95.8%	91.7%	0.551
F2	347	240	83.3%	100.0%	0.037
B	572	452	95.8%	100.0%	0.312

Soil solution

Three HR soil solution samples were tested with 7 dogs in three experiment testing sessions with 9 trials per dog per session for N=189. Dogs were successful in accurately identifying the oven dried grave soil solution on sterile latex-free pads in 183 trials for an overall accuracy of 96.8% (Table 9).

Table 9. Soil solution pad accuracy shown for solution extracted from indicated soils for the 189 trials performed by 7 dogs.

Soil Solution Sample Accuracy				
Pad	ID	Correct	Incorrect	Percent Accuracy
1-3	G2	58	5	88.9%
4-6	F2	62	1	96.3%
7-9	CHR	63	0	100%

Canine performance on 30g soil samples

Canine individual performance ranged from 87% accuracy to 94% accuracy (Figure 13). Performance ratings did not account for handler error or influence (Figure 13). Overall group performance did show an increase in proficiency over the ten sessions (Figure 14), however, the $R^2 = 0.0885$ value indicated no significant learning effect was shown.

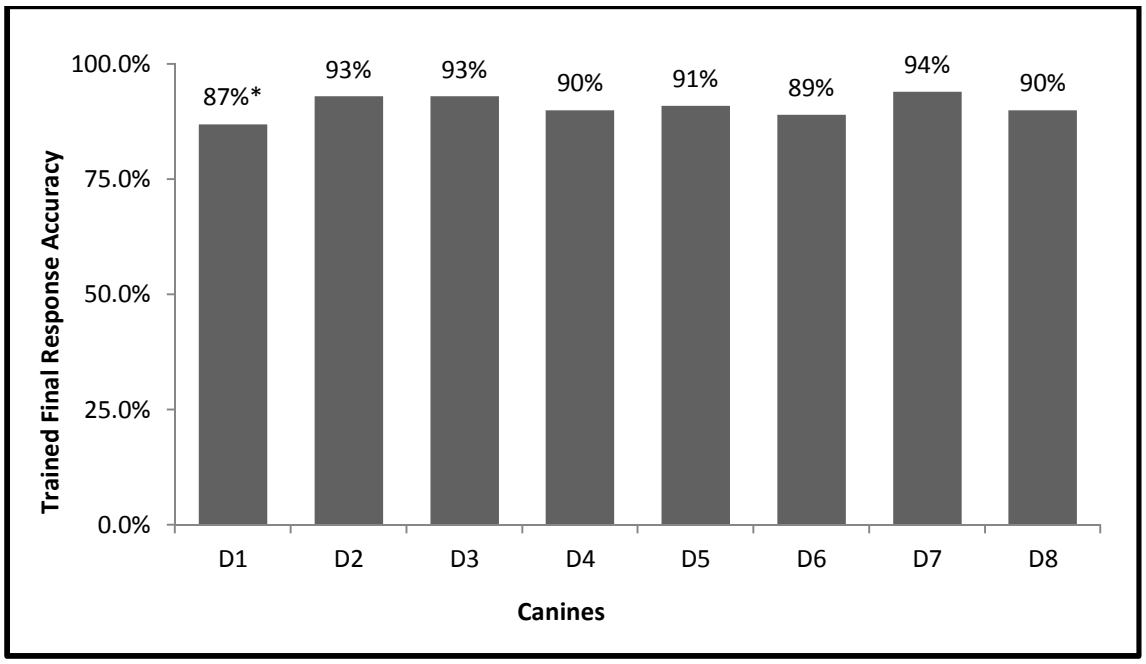

Figure 13. The performance average for each dog (D1-D8) at the conclusion of the ten sessions on 30 g samples. No significant difference in performance was found between dual trained live find and HRD dogs and HRD only dogs. *D1 session three was excluded from statistical analysis due to canine's refusal to work (no trials performed) thereby resulting in only 90 trials instead of 100 as with the other canines.

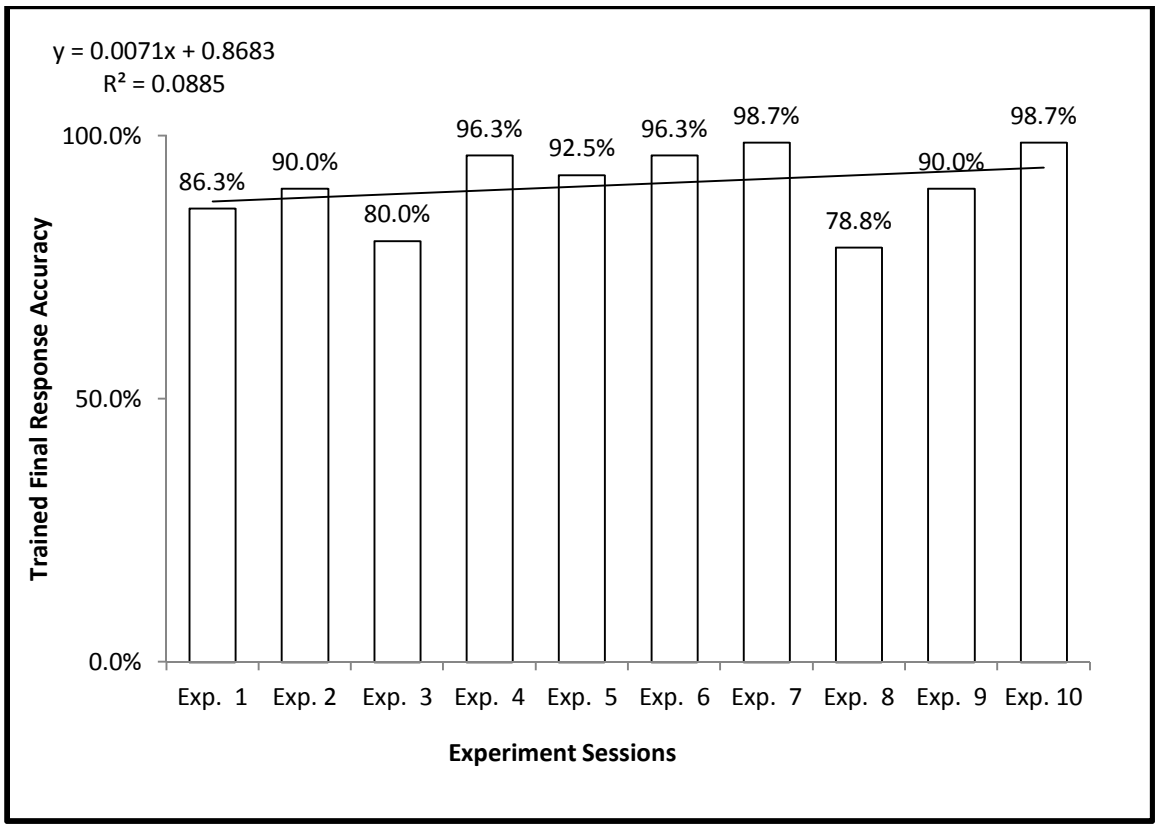

Figure 14. The percent of correct trained final responses from canines over the course of the ten sessions on 30 g samples. Linear regression with $R^2 = 0.0885$ indicated no significant learning effect over time despite a higher accuracy on the final trial.

Discussion

Source of residual scent

Decomposition of an adult human body results in a large purge of nutrients into the soil which is termed the cadaver decomposition island (CDI). The recalcitrance and likely aromacity of the compounds purged into the soil is currently unknown. It is generally assumed that most carbon compounds in the CDI are mineralized fairly quickly (Putman 1978) although no studies to date have quantified CO_2-C evolution

from human produced CDI's. Yet organic carbon in the CDI and grave soil is still significantly higher than control soil after 288 d post mortem in the CDI (Aitkenhead-Peterson et al. 2012) and 27 years in exhumed grave soil (Fiedler et al. 2004). The assumed increase in microbial activity as a response to new substrate utilizes all available oxygen in the CDI, which means that nitrate and sulfate are used as an oxygen source. This reduces the nitrate to nitrous oxide or di-nitrogen and sulfate to hydrogen sulfide; which are released as gases (Vass 2012). In the anaerobic CDI environment after the purge NH_4-N may show a measure of volatilization but this has not been researched in the CDI; Kirchmann and Witter (1989) reported that less than 1% of manure nitrogen was volatilized as ammonia in anaerobic conditions. Organic-N however tends to be quite recalcitrant in the CDI at 288 d post mortem (Aitkenhead-Peterson et al. 2012). Phosphorus does not have a gaseous phase except in the case of phosphine gas which has been observed under natural conditions specifically under acidic, anaerobic conditions such as those that occur in an initial CDI.

This study provided evidence that residual human decomposition compounds retrieved from CDI's remain identifiable by HRD dogs under controlled experimental conditions for at least 667 d post body removal. While it is not known how long HRD dogs can detect grave soil under controlled experimental conditions, documented recoveries of cold cases between 3 to 20 years have been recorded as well as historical graves from the 1800's (Christensen 2014; Dotson 2012).

This study examined soils ranging from 18 d to 915 d post body placement. HRD dogs were able to correctly identify the oldest post body placement of 915 d with

100% accuracy. Residual cadaver decomposition island soils ranged from 18 d to 667 d post body removal from the CDI. HRD dogs were able to identify the oldest post body removal soil of 667 days with 85.7% accuracy. Not only were dogs able to differentiate control soils from grave soils, dogs were also able to differentiate between control solution and grave soil solution dried onto latex-free pads. This lends further credence to the ability of properly trained HRD dogs to detect trace evidence such as purge fluids in soil. The implications of this are enormous, for example, in a case of scattered human bones, it is vital to determine the location of the CDI which can be used to predict PMI of the deceased through soil chemistry (Vass et al. 1992) or UV-Vis near infrared spectroscopy (Aitkenhead-Peterson et al. 2015)

Much research has focused on the volatile organic compounds (VOC's) emitted from graves and decomposing bodies. Vass et al. (2004) initially found 478 compounds from grave emissions. His 2008 paper indicated that 30 compounds were key markers in buried bodies, whereas 19 of these were found in surface decomposition.

Stratheropolous et al. (2007) identified 32 substances during a 24 hour collection period at 4 days post mortem, with a common core of only11 substances. Vass's (2012) latest research which examined soils from known and potential graves, again found a large number of compounds present which varied over time due to soil type, weather, age and depth of burial. Vass indicated that only four appeared to be human specific when compared to other animal decompositions. These were carbon tetrachloride, pentane, decane and undecane. While these compounds may contribute to the HRD dog's identification of soils tested in this study, further evaluation is warranted. A

volatile substance infers that the substance will be lost to the atmosphere at normal air temperatures. One would expect that if the substance the HRD dogs were sensing were volatile then many of these VOC's would be lost from the CDI over long periods of time exposed to the environment or kept as training aids at room temperatures. It is suggested then that more research be conducted into non-volatile, aromatic compounds in the CDI. More recent work suggests that scent may be due to pheromones released by insects and bacteria during cadaver decomposition. For example, it is well known that many of the putrefactive bacteria can release volatile compounds, particularly the obligate fermenter *Clostridium* spp. But it has only recently been observed that bacteria can directly mediate oviposition of carrion insects (Ma et al. 2012; Zheng et al. 2013). Thus chemistry plays a crucial role because many chemicals attract insects to cadavers and some of these chemicals are the products of microbial metabolism.

Soils tested were collected both while the cadaver lay on the soil surface and after it had been removed. Soils were also tested before and after the solution extraction process with the hypothesis that since rain has been shown to displace and transport elements down slope (Aitkenhead-Peterson et al. 2012) extracted soils would lose key elements essential to HRD dog detection thereby reducing the dog's accurate identification of extracted HR soils. This did not occur. HRD dogs were able to correctly identify extracted soils with accuracy rates ranging between 83 – 100%. Soils for these tests were comprised of 3 g soil pre- and post-extraction. The results are counter intuitive as the water extraction did not remove enough elements to render them undetectable by

the HRD dogs yet well enough for chemistry analysis. This again suggests the recalcitrance of the compounds that elicit HRD dog responses.

Training materials for HRD dogs

The current state of training for HRD dogs allows handlers to train their dogs on everything from a single drop of blood to whatever is available to them. Typically a 1 to 2 kg placenta is the largest aid obtainable. Most trainers can obtain placenta, teeth and blood. Most can purchase human bone that is 20 years or older from online sites such as the Bone Room or Skulls Unlimited. Very few are able to obtain fresh tissue, contemporary bone, body parts such as arms or legs or whole cadavers. Therefore, many times HRD dogs are trained and certified without ever having been exposed to a full cadaver, yet with the expectation that the dog can locate an entire body. Hoffman et al. (2009) reported that placenta is significantly different in composition from muscle and fat tissue, being closer to blood and internal organs, which is different from the vast majority of a cadaver. It is unknown the level to which HRD dogs will generalize skin, muscle and fat tissue from exposure only to blood, teeth and placenta, however, given the compositional differences it is a safe estimate that generalization may not occur leading to credentialed dogs who cannot locate an entire body. While law enforcement agencies could be encouraged to assist HRD trainers in obtaining or accessing large intact training aids such as arms, legs or whole bodies this is often not feasible. Instead access to CDI soil which has the full complement of chemical compounds from a whole human body at a certain time post-mortem has been shown through my research to be a suitable training aid.

In locations such as Great Britain, where obtaining or possessing actual human remains may pose a legal problem to handlers, another viable alternative is to utilize soils or extracted soil solutions from under surface or buried human remains. The fluids that purge into the soil are the decomposition products the dogs will be looking for in real recovery missions. These products can be kept in large amounts and control soils from the area can be utilized to ensure the dogs are only reacting to the decomposition products and not any of the local soil constituents. The importance of exposing HRD dogs to whole body decomposition products in the soil cannot be overstated.

The use of dogs as a confirmatory tool for clandestine graves may be suited for use beyond field work. Soil cores which have been extracted from suspected grave areas may be run in a controlled indoor setting utilizing the method from this study, and with HRD dog confirmation constitute further chemical analysis and investigation to corroborate the findings.

Training HRD dogs

The method utilized for line-up discernment of HR embedded soils was modeled from methods utilized by ATF and other legal entities. The protocols for these agencies require the dog to generally work on lead and with the handler's direction (ATF 1997). Generally the handler walks in front of the dog and presents the area with their hand where they want the dog to smell for their target odor. It is well known among detection dog trainers that handlers can have a high degree of influence on a dog's performance. Pressure by the handler can elicit false positives in the absence of the target odor (Lit et al. 2012). This effect may be due to the trainability of dogs by man or as theorized by

Hare and Tomasello (1999; 2006) a consequence of an innate response to the pointing gesture that has evolved during the domestication of the dog. Unlike the programs that were used as a model in this research study, handlers were not allowed to accompany the dogs in an effort to limit handler influence. While not completely absent, this method did minimize handler influence.

Handler scent is an odor that is common to individual training and can become a problem depending upon how often a dog team can train with others setting up the problems and in how the training aids are handled and maintained (Christensen, 2014; Dotson, 2012). Based on anecdotal knowledge and the error results within this test, handler scent should be one of the main areas of concentration for proofing of HRD dogs. Dogs become accustomed to handler scent accompanying any target odor, thereby; the lack of handler scent in a real situation may result in a miss or false negative.

Even the most sensitive mechanical instruments have error rates. False negatives or false positives offered by a HRD dog are considered biologic sensor error rates. SWGDOG (2014) recommends no more than a 10% error rate for scent detection dogs. False negatives occurred when the handler wrongly called the run a blank when an HR soil was available for a response. False negatives occurred 24 times with 8 being incorrectly called by handlers despite their dogs correctly indicating on the correct target. The majority of these false negative mistakes occurred during trial session 8 with one of the handlers with multiple dogs suffered from an illness which may have impaired their performance. Adjustment for handler error resulted in an accuracy rate of 96% for HR soils or 683 correct responses out of 711 HR soil presentations. Even on

correct responses from the dogs, there was an average of 5-15 seconds latency for handlers to indicate their dogs TFR.

The majority of missions is often performed due to crime tips and search sites are often void of any human remains. Therefore, training the dogs to work negative or blank areas is equally important in the HRD dog's proficiency. There were N=79 blank trial presentations resulting in 58 correct responses for a team accuracy of 73.4%. Blank trials were also biased by handler error. The 21 incorrect responses recorded for blank trials included 11 from handler error. Adjustment for handler error resulted in an accuracy rate of 87%. Errors on blanks resulted from dogs sniffing on one can longer than another one and the handler then calling that platform as a TFR whether the dog gave a false response or not, or in asking the dogs to return and check the line multiple times after the dog had already given no response due to the lack of a target odor. Blanks are customarily the least practiced search scenario but are usually what makes up the bulk of the dogs actual search mission career (Alexander and Turner 2010; Christensen 2014; Dotson 2012).

Experimental designs using HRD dogs often do not take into account the effects of previous experience upon the performance of dogs. The experimental design of this study along with the selection of HRD dogs to take part in the experimental procedure is unique compared to other studies examining HRD dog capabilities and limitations. This study took three weeks to properly train the HRD dogs on the procedure that was to be used for testing. Negligence to this detail would have possibly resulted in skewed results due to confusion on the dog's part in what the procedure was and what was expected of

them and possibly increases in accuracy due to learning. The major purpose of this study was to examine the potential for using CDI soil as a training aid for HRD teams. The model used to test HRD teams required the dog to be trained to the method was new to the HRD teams and thus training in the method was needed to provide accurate results to the questions posed. Dogs selected for this study were proven HRD dogs with multiple national certifications from more than one agency, thereby, lowering the possibility of skewed certification qualifications. Dogs utilized in this study had achieved certifications through the National Association for Search and Rescue (NASAR), the North American Police Work Dog Association (NAPWDA), and the National Narcotic Detector Dog Association (NNDDA); each an independent organization, the latter two with law enforcement officials, the former, NASAR, the oldest inclusive national civilian SAR organization, all of which have precedence in court cases with recognized certifications. Each dog used in this study also had real world recoveries and previous exposure to full corpses. None of the dogs in this study had ever been trained with pseudo scents or exposed to them.

Actual search missions for HRD teams are double blind scenarios. Typically there is no one or manner by which any previous events resulting in human remains odor would be known. Cases in which HRD dogs alert and offer TFR's on what appears as nothing are presumed to be false or unproductive final responses which often result in the entire area discounted and the focus of the investigation shifted to another area. This study has shown that credentialed HRD dogs can detect and correctly identify residual from a CDI 667 days, almost two years, post body removal. This raises questions as to

whether some false or unproductive final responses on missions are truly incorrect. Aitkenhead-Peterson et al. (2012) reported significant movement of decomposition products, specifically DOC, DON and potassium, down slope from the decomposition site. This is most likely due to rain events and the diffusion of the liquids into the soil. Consequently, in searches with what appears to be unproductive final responses, further investigation upslope may be warranted. This is consistent with many field cases where HRD dogs are able to locate and identify the primary CDI site which has led to further bone recovery. Depending upon the environment, time of year, nature of disposal and native scavengers, bodies may be disarticulated at varying stages of decomposition leaving multiple small secondary CDI's and potential information behind. If an area results in multiple TFR's from competent credentialed dog teams, close examination is warranted for potential trace evidence even if nothing visible is observed. The soil itself may hold evidence of decomposing human remains and may lead to further evidence that can be used to close the case, give the family closure and prosecute the perpetrator.

Summary

This study tested soils that were up to 667 days post body removal with 85.7% accuracy maintained, therefore the length of time a dog can detect the odor of human remains in soil from the CDI is still unknown based on this study. Soils (3g) which had been extracted and even soil solution (20g) itself were readily identifiable by the HRD dogs. This study clearly demonstrated that competent credentialed HRD dogs are capable of identifying residual or trace amounts of human remains left in the soil. CDI

soil could potentially be a good training material with proper handling, storage and negative comparative soil samples. Further investigation of the soils from CDI's and RCDI's is warranted in search for methods for verifying evidence human remains as well as identifying the component odors recognized by HRD dogs to improve the exposure training and thereby performance of the HRD dog.

CHAPTER IV

SOIL CHEMISTRY POST MORTEM INTERVAL ESTIMATION

Introduction

PMI

Post mortem interval (PMI) refers to the time elapsed since death and is a crucial component in a criminal investigation. The cadaver decomposition island (CDI) is formed from the rich pulse of nutrients released into the surrounding soil when the cadaver decomposes (Carter et al. 2007). The CDI of various mammals has been evaluated for the nutritional value of decomposition products entering the soil (Carter et al. 2007; Forbes et al. 2005). The CDI is not only a rich source of nutrients but may also provide a significant source of information to forensic investigators. The chemical composition of the CDI may also be a valuable tool for determining PMI. Various studies have examined a variety of methods for estimating PMI such as anthropologic examination of remains (Dent et al. 2004; Marchenko 2001; Micozzi 1986; Rodriguez and Bass 1985), insect succession (Anderson 2004; Campobassa et al. 2001; Honda et al. 2008; Mendonca et al. 2008; Reibe and Burkhard 2010), volatile organic compound emissions (Cablk et al. 2012; Hoffman et al. 2009; Lovestead and Bruno 2010; Paczkowski and Schutz 2011; Stratheropoulos et al. 2007; Vass et al. 2004; Vass et al. 2008; Vass 2010; Vass 2012) and chemical analysis of CDI soils (Aitkenhead-Peterson et al. 2012, 2015; Jervis et al. 2009; Pringle et al. 2010; Vass et al. 1992). Studies however have often been performed using animals as surrogates for humans which may result in some inconsistencies. This has resulted in multiple methods applicable to

specific situations such as: soil types (Forbes et al. 2005; Jaggers and Rogers 2009; Tibbett and Carter 2009; Turner et al. 2013; Wilson et al. 2007), weather conditions (Carter et al. 2008, Carter et al. 2010; Jaggers and Rogers 2009), and animal species (Benninger et al. 2008; Carter et al. 2008; Pringle, et al. 2010) but there has been no single efficient method applicable in all settings for human PMI estimations. The CDI is an extremely important pool of nutrients whose nutrient concentrations change over time; this is important in cases of scavengers that disarticulate and scatter the remains. For example, Spradley et al. (2012) recently showed that vulture activity could dramatically alter anthropologic PMI estimations after capturing a five hour scavenging window with time lapse photography which showed a fully intact corpse reduced to skeletal remains within the five hour window. Oftentimes, particularly during the summertime this scavenging can initiate the purge release into the soil forming the CDI.

Temperature and moisture have major influences on decomposition (Carter et al. 2008; Carter et al. 2010; Jaggers and Rogers 2009) and can therefore greatly affect PMI. One method used to compensate for temperature affects is accumulated degree days (ADD) where the average daily temperature is added together for the estimated time interval the body has been in the environment.

Soil chemistry under decomposing mammals has been greatly ignored until recent years. Vass et al. (1992) examined soil water extracts beneath decomposing human cadavers for fatty acids, anions and cations in an attempt to develop a method for determining PMI. The study concluded that volatile fatty acid trends could be used in early decomposition to estimate PMI. The majority of studies have utilized non-human

mammals such as domestic swine (*Sus domesticus*) and rat (*Rattus* sp.) carcasses rather than human cadavers (Carter et al. 2008; Pringle et al. 2010). Benninger et al. (2008) focused on soil CDI carbon, nitrogen, and phosphorus based compounds beneath decomposing domestic swine and found significant increases in soil pH and total nitrogen at72 days, soil extractable phosphorus at 100 days and lipid phosphorus at 43 days post mortem which could be used to assist in early stages of PMI determination. Pringle et al. (2010) examined water extractable soil conductivity under domestic swine carcasses, which increased rapidly during the first year and continued to slowly decrease thereafter until the conclusion of the study at two years. Ninhydrin reactive nitrogen (NRN) is a measure of organic nitrogen plus ammonium-N. It has been hailed as a useful tool for locating clandestine graves (Carter et al. 2008; Lovestead and Bruno 2010; Van Belle et al. 2009). Studies have also indicated that NRN may be useful in PMI determination during the first two months of burial or the first 3 months of a surface decomposition. Research over longer periods of time (i.e. > 2 years) and with larger sample groups of human remains are lacking; therefore long term trends for PMI estimation from soil chemistry currently does not exist.

Recently published research examined water extractable soil chemistry beneath two human remains from the Southeast Texas Applied Forensic Science Facility (STAFS) for dissolved organic carbon (DOC), organic nitrogen (DON), pH, electrical conductivity and various cations and anions (Aitkenhead-Peterson et al. 2012). Significant differences were observed between up-slope and down-slope control soils for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), soil pH and EC.

Most compelling in this study was the significant differences between control soils as well as the evidence of movement of DOC, DON and orthophosphate-P downslope from the decomposition sites.

Objectives

Because robust studies examining soil chemistry from more than a few human graves are lacking. The objectives of this study were to:

1) Compare the C, N and P chemistry of control soil and CDI soil at two cadaver donor facilities in Texas, USA.
2) Create a model to determine PMI using soil chemistry
3) Examine the viability of utilizing small soil bags constructed of landscape cloth filled with native soil to test soil chemistry placed under the torso of a body to alleviate inadvertent over-aeration of the soil due to frequent pulling of soil cores.

Materials and Methods

Site descriptions

Two of the five national body farms were utilized in this study.

FACTS

The Forensic Anthropology Center of Texas State (FACTS) in San Marcos, TX, USA is located within 17 km^2 of the Freeman ranch owned by Texas State University in Hays County TX, USA. The United States Geological Survey soils maps for San Marcos, Hays County, TX, USA define this area as rocky outcrops with dolomitic

limestone with shallow soils serving as rangeland and habitat for wildlife (USDA 2012). Vegetation consists of grasslands and tree clusters consisting of cedar, juniper, live oak, mesquite, and Texas persimmon (TPWD 2014). Rooting depth is limited and there is very low available water for plants (USDA 2012). The mean annual temperature is 15.6˚ C (60˚ F) with an average high of 32.2˚ C (90˚ F) in the summer and a low of 4.4˚ C (40˚ F) in the winter (USDA 2012). Annual precipitation ranges from 254 – 914 mm (10 – 36 inches) (USDA 2012). Hays County lies on the Edward plateau with soils developed from sedimentary deposits and rocks from the Recent and Pleistocene age. The FACTS facility is dominated by two well drained stony soils that were formed by weathering dolomitic limestone and indurated fractured limestone (USDA 2012). The Comfort – Rock outcrop complex with 1 – 8 percent slopes is composed of extremely stony clay at 0-33 cm (0 to 13 inches), with a restrictive feature of bedrock as shallow as 0 - 5 cm (0 - 2 inches) and lithic bedrock averaging 50.8 -101.6 cm (20 - 40 inches) and a water table deeper than 203.2 cm (80 inches). The Rumple-Comfort soil association with 1 – 8 percent slopes comprise the rest of the facility with gravelly clay loam from 0 - 25.4 cm (0 – 10 inches) followed by very gravelly clay from 25.4 - 1.1 cm (10 – 28 inches) and bedrock depth averaging 71.1 - 91.4cm (28 - 36 inches). The soil is mildly alkaline and non-calcareous. The lithic bedrock restrictive feature typically occurs between 508 - 1016 mm (20 - 40 inches), with a water table deeper than 203.2 cm (80 inches) (USDA 2012).

STAFS

The Southeast Texas Applied Forensic Science Facility (STAFS) facility is located within the 1 km^2 area of the Center for Biological Field Studies at Sam Houston State University, Walker County, TX, USA. The United States Geological Survey soils maps for Huntsville, Walker County, TX, USA define this area as deciduous woodland primarily consisting of loblolly and short leaf pine (TPWD 2014). Soil at the facility is of the Depcor-Huntsburg association, gently undulating (USDA 2012). Walker County soil was deposited during the Tertiary and Quaternary periods with the largest being Pliocene and Pleistocene sands of the Willis, Bently, and Beaumont Formations. The soil is moderately well drained with a 60.96 - 106.7cm (24 – 42 inch) depth to the water table and over 203.2 cm (80 inches) to restrictive features. The typical soil profile for this series consists of loamy fine sand from 0 to 66 cm, sandy clay loam from 66 cm to 165 cm, and sandy clay loam from 165 cm to 200 cm. with clay subsoil (USDA 2012). The parent material is formed from clayey marine deposits and the soil is moderately well drained. The slope ranges from 1 to 5%. Depcor soils tend to be 10YR with hues and chroma ranging from 4-8, low organic matter content, and slightly-to-very strongly acidic (USDA 2012). The mean annual temperature is 18.9 to 21.1˚ C (66 to 70˚ F) with an average high of 25˚ C (77˚ F) and a low of 13.9˚ C (57˚ F). Annual precipitation ranges from 1016 - 1219 mm (40 to 48 inches) per year (USDA 2012).

Soil collection

Plots containing human cadavers and plots which previously contained human cadavers were sampled in 2012 (Table 1). Multiple sites were sampled when donated

bodies became available for sampling. Soils were collected from STAFS and FACTS at approximately monthly intervals. A soil probe (2 cm diameter) was used to extract a soil core 7 cm in depth from the CDI beneath human remains (Torso region) (HR) and from upslope control plots. Following its use after each CDI, the soil probe was cleaned with acetone and rinsed with DDW before reuse to minimize cross-contamination. After each field trip, soil probes were cleaned with a 10% bleach solution followed by acetone and heated for 24 h at 200˚ C to dissipate any remaining volatiles from the samples. Soils were air-dried under a hood for an average of 1 week and processed by sieving (2mm) to homogeneity prior to extraction.

Soil extractions

Three grams of soil was combined with 30 mL of DDW (1:10 ratio) in 50 mL high density polyethylene (HDPE) centrifuge tubes and shaken for 23 h prior to centrifugation at 19,500 *g-force*. The supernatant was removed using a cannula and syringe and pH and conductivity (EC) was recorded. Supernatant was then syringe filtered through ashed (500˚ C for 4 h) Whatman GF/F filters (nominal pore size 0.7 µm). Solutions were analyzed immediately when possible or frozen for future analysis.

All samples were handled with gloves. Cross contamination was controlled for by collecting control samples first at each time point. Control samples were stored separately from treatment samples.

Table 10. Details of cadavers sampled at STAFS and FACTS facilities that were used for analysis. If sampled date is after the removed date then the CDI with no cadaver was sampled. If sampled date is before the removed date then the CDI was sampled beneath the torso of the cadaver.

Site	Weight (kg)	Sex	PMI (d)	Placed Date	Removed Date	Sampling Dates
FACTS	122.02	M	348-672	2/17/2011	9/13/2011	3/9/12 to 12/19/12
FACTS	98.88	M	196-551	7/19/2011	1/2/2012	1/30/2012 to 12/19/12
FACTS	92.97	F	176-500	8/8/2011	‡	1/30/2012 to 12/19/12
FACTS	90.72	M	407-732	12/20/2010	‡	1/30/2012 to 12/19/12
FACTS	47.63	M	90-445	11/2/2011	‡	1/30/2012 to 12/19/12
FACTS	61.24	F	96-412	11/2/2011	‡	1/30/2012 to 12/19/12
FACTS	65.77	F	43-398	12/19/2011	‡	1/30/2012 to 12/19/12
STAFS	131.54	M	570-1213	8/19/2009	2/22/2011	3/11/2011 to 12/13/12
STAFS	72.57	F	357-684	12/17/2009	9/20/2010	12/8/2010 to 1/28/12
STAFS	181.44	M	317-1099	1/26/2010	11/22/2010	12/8/2010 to 1/18/12
STAFS	72.57	M	18-693	3/8/2011	‡	3/25/2011 to 1/28/12

‡Cadaver was in place at the time of sampling.

Chemical analyses

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using high temperature Pt-catalyzed combustion with a Shimadzu TOC-VCSH and Shimadzu total measuring unit TNM-1 (Shimadzu Corp. Houston, TX, USA).

Dissolved organic carbon was measured as non-purgeable carbon using USEPA method 415.1 which entails acidifying the sample and sparging for 4 min with C-free air. Ammonium-N was analyzed using the phenate hypochlorite method with sodium nitroprusside enhancement (USEPA method 350.1) and nitrate-N was analyzed using Cd–Cu reduction (USEPA method 353.3). All colorimetric methods were performed using a Westco Scientific Smartchem Discrete Analyzer (Westco Scientific Instruments Inc. Brookfield, CT, USA). DON was calculated as: TDN - (NH_4-N + NO_3-N). Sample replicates, blanks, NIST traceable and check standards were used every 12th sample to monitor instrument precision and co-efficient of variance among replicate samples.

Statistical analyses

Four CDI's from STAFS and 7 CDI's from FACTS were used in the final analysis (Table 4.1). The number of CDI's available to sample was larger from the two body donor facilities, STAFS and FACTS (8 and 14 respectively) but some of the CDI soils had to be eliminated from analysis due to prior contamination due to: a) a prior cadaver placed on the plot and unrecorded, b) early removal of the cadaver, or c) co-contamination by placing another body too close prior to cessation of the study.

Two sample one tail t-tests were used to test the hypothesis that CDI soils had significantly higher nutrient concentrations when compared to control soils (Table 4.2). Post mortem interval (PMI), the number of days since death, or in this case, since placement, was calculated as well as accumulated degree days (ADD). ADD is the mean daily temperature which was calculated for each body for each location.

Regression analyses between each decomposition product chemistry and PMI were performed. Here the mean concentration of each of the nutrients examined in control soils was subtracted from grave soils prior to regression analysis with PMI. This represented the decomposition products only and normalized the data by removing seasonal and site influences. Regression analyses was used and the best fit chosen to determine if a simple predictive model could be constructed to describe PMI based on changes in soil chemistry. In addition a stepwise backward multiple regression analysis was performed using PMI as the dependant variable and DOC, ammonium-N, nitrate-N and phosphate-P chemistry as independent variables.

Soil bags

Soil bags (8cm x 8cm) were constructed from retail available landscape cloth to allow for the movement of liquid through the bag. Each bag was folded on one side and sewn on two sides with the fourth side left open to allow it to be filled with approximately 20 g of soil native to the STAFS facility. The open side was then stapled shut. A 100% polyester yarn string approximately 91cm long was attached to allow it to be pulled from under the body.

Results

Chemistry values

Not all CDI's analyzed had significantly higher soil chemistry (Table 11). Soil pH from the CDI was significantly different when compared to control soil for three CDI's at FACTS but none of the CDI's tested at STAFS (Table 11). Electrical conductivity was significantly different in the CDI soil of all CDI's examined with the

exception of one CDI at STAFS. There was only a significant difference in nitrate-N concentrations for one CDI at FACTS and one CDI at STAFS (Table 11). Ammonium-N concentrations were significantly higher in all CDI's when compared to control soil with the exception of one CDI at STAFS (Table 11). Phosphate-P concentrations were significantly higher or around $p = 0.05$ in all CDI's with the exception of one CDI at STAFS. Dissolved organic carbon was significantly higher in all CDI's examined compared to control soils (Table 12) and DON was significantly higher in all CDI's with the exception of one CDI at STAFS (Table 12).

Table 11. Result of the two sample, 1-tailed t-test comparing control soil and CDI soil from the STAFS and FACTS sites. Bold italicized values are significantly different from control soil at $p < 0.05$).

			NO_3-N	NH_4-N	PO_4-P	DOC	TN	DON
	pH	EC	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
FACTS								
1	***0.004***	***0.000***	0.163	***0.000***	***0.002***	***0.001***	***0.000***	***0.000***
2	***0.007***	***0.001***	0.116	***0.000***	***0.000***	***0.000***	***0.000***	***0.015***
3	0.124	***0.000***	***0.028***	***0.002***	0.057	***0.000***	***0.004***	***0.000***
4	0.081	***0.000***	0.050	***0.013***	***0.002***	***0.000***	***0.002***	***0.012***
5	0.079	***0.000***	0.098	***0.000***	***0.008***	***0.004***	***0.000***	***0.040***
6	0.308	***0.000***	0.056	***0.000***	***0.002***	***0.000***	***0.000***	***0.000***
7	***0.005***	***0.000***	0.142	***0.006***	***0.023***	***0.001***	***0.002***	***0.009***
STAFS								
1	0.225	***0.042***	0.126	***0.024***	0.050	***0.033***	***0.018***	***0.020***
2	0.282	***0.001***	0.054	***0.001***	***0.005***	***0.000***	***0.000***	***0.001***
3	0.148	0.399	0.130	0.118	0.163	***0.035***	0.101	0.476
4	0.084	***0.049***	***0.010***	***0.003***	***0.037***	***0.002***	***0.000***	***0.000***

Table 12. Chemistry values for mean, standard deviation and ranges of human decomposition products only (CDI soil minus Control Soil) at STAFS. Negative values in the range indicate that concentrations were lower in the CDI compared to control soils. Values for Control soils are for reference only.

		STAFS						
		pH	EC	NO_3-N	NH_4-N	PO_4-P	DOC	DON
			$\mu S\ cm^{-1}$			$mg\ kg^{-1}$		
Control	Mean	6.1	19	4	5	1.61	181	9
	Std Dev	1.07	15	1	3	1.65	93.44	14
	Range	4.8 to 7.8	10 to 60	3 to 8	2 to 8	0.3 to 5	75 to 401	0.0 to 49
Cadaver 1	Mean	6.3	28	2	8	2	111	10
PMI = 570 - 1213	Std Dev	0.6	11	5	13	3	186	11
N=18	Range	5.5 to 7.5	20/60	-3 to 19	2 to 42	-0.5 to 9	-56 to 730	-2 to 33
Cadaver 2	Mean	6.4	79	15	32	5	497	30
PMI = 357 - 684	Std Dev	1.0	51	31	31	6	206	24
N =9	Range	5.4 to 8.6	40 to 160	-2 to 89	0.8 to 88	0.4 to 21	160 to 746	7 to 84
Cadaver 3	Mean	6.1	59	7	15	5	432	18
PMI = 317 - 1099	Std Dev	0.7	41	15	27	13	605	14
N = 21	Range	4.2 to 7.4	20 to 170	-4 to45	-3 to 103	-2 to 48	-87 to 2165	-0.7 to 65
Cadaver 4	Mean	6.3	95	10	17	7	614	17
PMI = 18 - 693	Std Dev	0.6	135	38	36	13	1590	34
N = 19	Range	4.6 to 7.0	30 to 560	-3 to 161	-3 to 118	-0.6 to 53	-40 to 5524	-7 to 140

Table 13. Chemistry values for mean, standard deviation and ranges of human decomposition products only (CDI soil minus Control Soil) at FACTS. Negative values indicate that the concentration of nutrient was lower in the CDI compared to the control soil. Values for Control are for reference only.

		Soil pH	Soil EC	NO_3-N	NH_4-N	PO_4-P	DOC	DON
			µS cm^{-1}	Human decomposition product (mg kg^{-1})				
Control	Mean	6.8	102	11	24	2	373	0.4
	Std Dev	0.5	88	8	72	2	1119	0.5
	Range	5.5 to 8.5	20 to 465	0 to 27	2 to 382	0 to 9	8 to 523	-0.3 to 1
Cadaver 1	Mean	6.1	476	-2	223	60	3225	100
PMI = 348 - 672	Std Dev	0.7	237	14	152	54	2979	103
N= 7	Range	5.4 to 6.7	200 to 870	-9 to 29	18 to 465	-1 to 156	1123 to 9072	25 to 249
Cadaver 2	Mean	6.4	349	42	270	21	2673	113
PMI = 196 - 551	Std Dev	0.3	169	98	210	14	1857	179
N= 9	Range	6.0 to 7.1	150 to 523	-8 to 286	6 to 559	-2 to 47	1116 to 5770	3 to 488
Cadaver 3	Mean	6.7	692	-4	523	237	5058	155
PMI = 176 - 500	Std Dev	0.7	432	4	494	425	3578	125
N= 9	Range	6.4 to 7.4	170 to 1640	-9 to 2	0.9 to 1421	-0.2 to 1330	1165 to 9289	25 to 345
Cadaver 4	Mean	6.6	483	97	204	31	1401	51
PMI = 407 - 732	Std Dev	0.7	176	169	285	32	652	71
N= 9	Range	5.7 to 7.9	200 to 690	-11 to 500	-9 to 783	-2 to 86	677 to 2790	6 to 204
Cadaver 5	Mean	7.1	494	56	272	37	1177	90
PMI = 90 - 445	Std Dev	1.1	108	108	156	33	644	144
N= 10	Range	5.8 to 9	350 to 680	-11 to 334	6 to 461	2 to 92	84 to 2700	-10 to 339
Cadaver 6	Mean	6.8	1219	-8	938	104	6907	528
PMI = 96 - 412	Std Dev	0.5	619	2	471	105	6681	160
N= 9	Range	5.8 to 7.5	820 to 2710	-11 to -4	68 to 1705	-2 to 336	1116 to 19203	244 to 664
Cadaver 7	Mean	6.0	557	-6	691	65	5689	208
PMI= 43 - 398	Std Dev	0.7	245	4	799	101	7428	213
N= 10	Range	4.8 to 6.7	250 to 960	-9 to 4	18 to 2779	-2 to 313	1116 to 25432	24 to 465

Human decompositions products retrieved from CDI's at STAFs were extremely high in DOC, DON, ammonium-N and PO_4-P at both STAFS and FACTS sites (Tables 12 and 13). Nitrate-N concentrations of human decomposition products ranged from negative values indicative of lower nitrate-N concentrations in the CDI's at both sites compared to control soils to high concentrations indicative of nitrification as the soils became aerated.

Average concentrations of human decomposition products alone among the four CDI's sampled at STAFS ranged from 111 – 614 mg kg $soil^{-1}$ for DOC, 10 - 30 mg kg $soil^{-1}$ for DON, 8 – 32 mg kg $soil^{-1}$ for ammonium-N and 2 – 7 mg kg $soil^{-1}$ for PO_4-P. Average Nitrate-N concentrations in human decomposition products ranged from 2 – 15 mg kg $soil^{-1}$ at STAFS (Table 12). Human decomposition product concentrations in the seven CDI's sampled at FACTS tended to be higher than observed at STAFS (Tables 12 and 13). At FACTS average DOC concentrations in human decomposition products retrieved from individual CDI's ranged from mg kg 1177 - 6907 $soil^{-1}$. Average ammonium-N ranged between 204 – 938 mg kg $soil^{-1}$. Average Nitrate-N concentrations in human decomposition products ranged from -8 - 97 mg kg $soil^{-1}$ and 31 – 237 mg kg $soil^{-1}$ for PO_4-P.

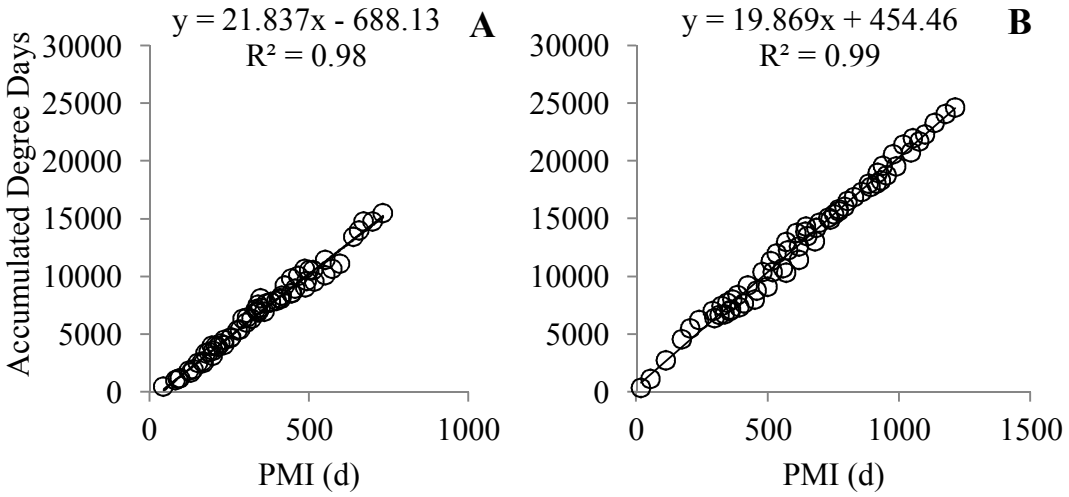

Figure 15. A) Accumulated degree days (ADD) plotted against days of the post mortem interval at FACTS. B) Accumulated degree days (ADD) plotted against days of the post mortem interval at STAFS. Both show significant R^2 values of above 0.98, indicating a strong relationship between the ADD calculations and PMI.

Accumulated degree days (ADD) (Figure 15 A) were shown to be effective for both outdoor decomposition facilities and 98% of the variance in ADD was described by days PMI (Figure 15 B). ADD showed a strong relationship to PMI and therefore both PMI and ADD are valuable confirmatory tools for estimations of time since placement for constructing models to estimate PMI from soil CDI chemistry and due to this strong relationship either can be used for accurate reporting.

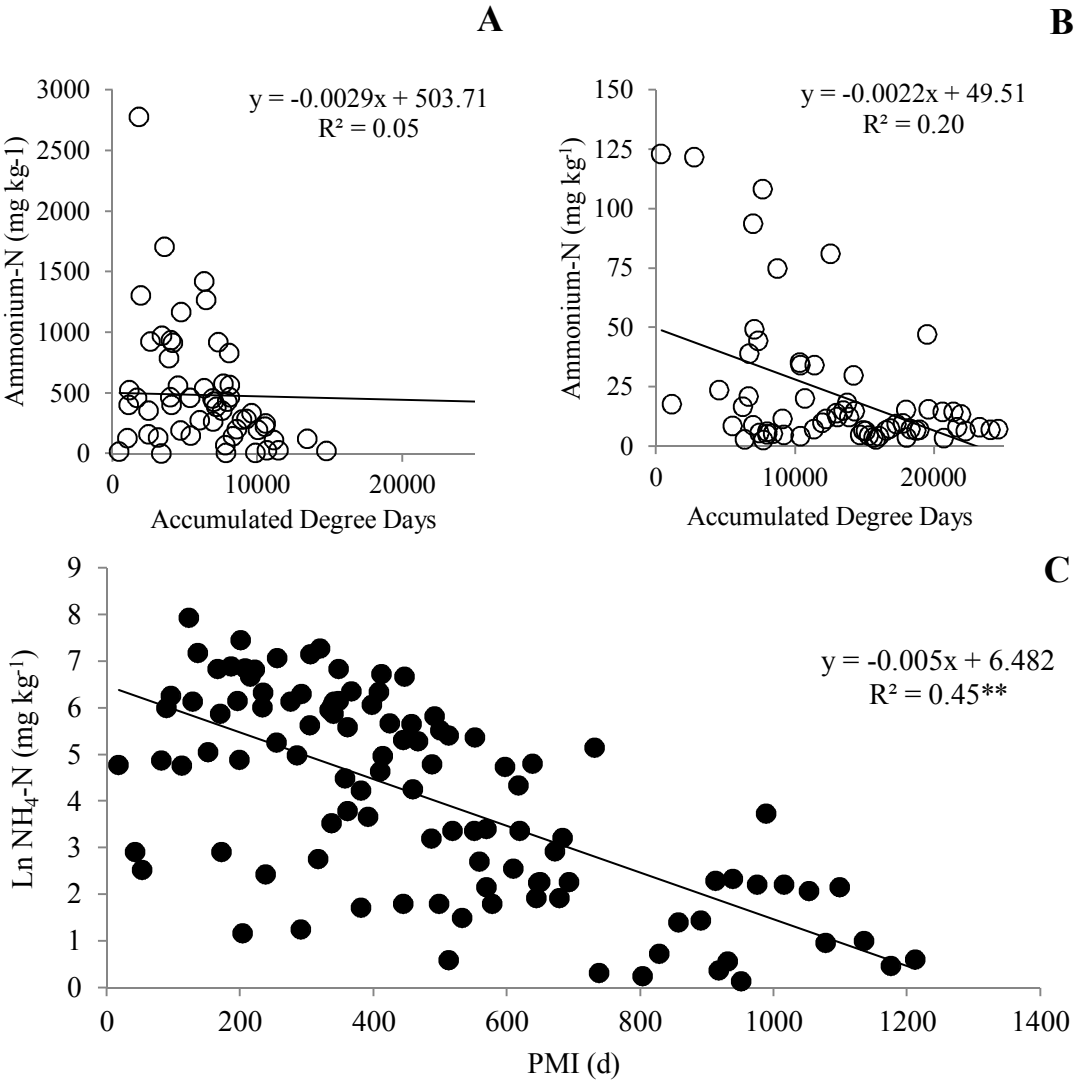

Figure 16. A) Decomposition product ammonium-N concentrations (mg kg^{-1} soil) collected from the CDI's from outdoor decomposition facilities in A) San Marcos, TX (FACTS) and B) Huntsville, TX (STAFS) and C) Both sites combined and transforming decomposition product ammonium-N to a natural logarithm. Zero on the y-axis represents mean ambient soil conditions. Note differences in y-axis between the two sites to better show concentrations.

Decomposition product ammonium-N concentrations were elevated above baseline (zero on y-axis) from shortly after ADD = 297 to ADD = at both FACTS (Figure 16 A) and STAFS (Figure 16 B) sites. Only five percent of the variance in decomposition product ammonium-N concentrations was described by ADD at FACTS and 20% of the variance at STAFS (Figures 16 A and 16 B). The general pattern was a decrease in decomposition product ammonium-N over time. A strong relationship between ADD and decomposition product was not observed at either site but when combining the two sites and transforming the decomposition product ammonium-N concentrations to their natural logarithm 45% of the variance of ammonium-N was explained by PMI (Figure 16 C).

Decomposition product nitrate-N concentrations remained at or below soil ambient concentrations for most of the study at both facilities, though a slight peak around ADD 5,000 and 10,000 was noted at FACTS, with random high peaks observed (Figure 17 A). Lower concentrations of decomposition product nitrate-N which were at or below soil ambient conditions (y-axis zero) were also observed at the STAFS site although a small peaks between day 5,000 and 10,000 ADD were observed (Figure 17 B). Less than 1% of the variance in decomposition product nitrate-N concentrations were described by ADD at FACTS and around 12% of the variance at STAFS (Figures 17 A and 17 B). A strong relationship between ADD and decomposition product was not observed at either site. Based on the low amount of variance explained in nitrate-N by ADD at the individual sites I did not combine the data for both sites.

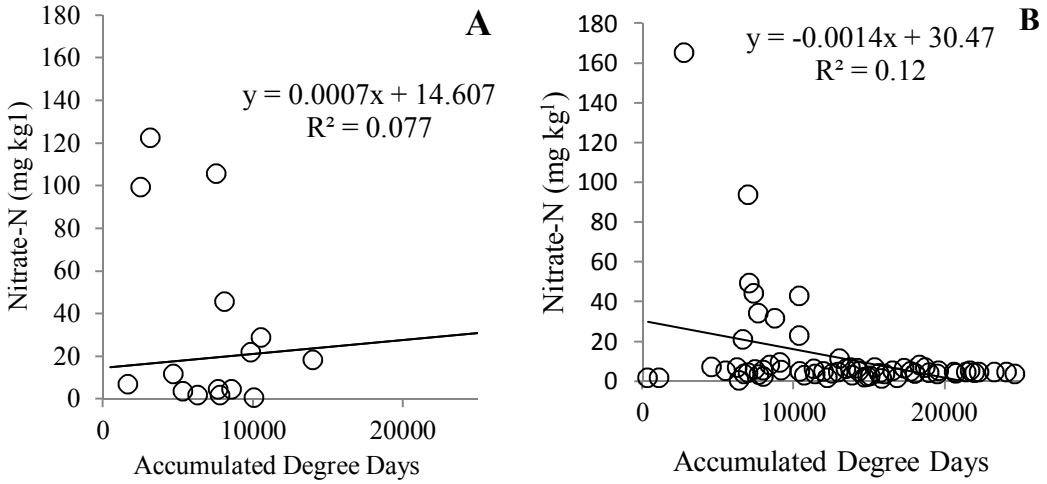

Figure 17. A) Decomposition product nitrate-N concentrations (mg kg^{-1} soil) collected from the CDI's from outdoor decomposition facilities in A) San Marcos, TX (FACTS) and B) Huntsville, TX (STAFS). Zero on the y-axis represents mean ambient soil conditions. Note differences in y-axis between the two sites to better show concentrations.

Phosphate-P

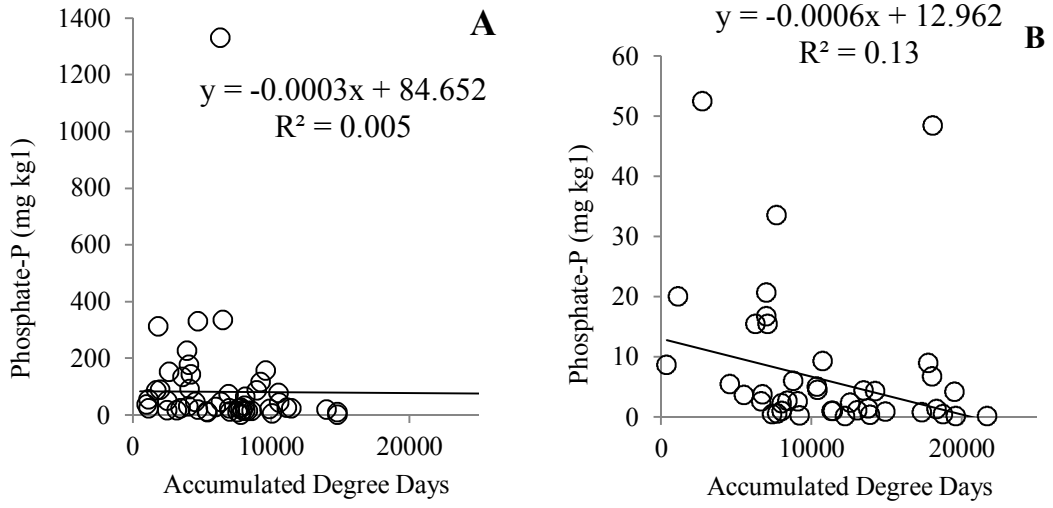

Figure 18. A) Decomposition product phosphate-P concentrations (mg kg^{-1} soil) collected from the CDI's from outdoor decomposition facilities in A) San Marcos, TX (FACTS) and B) Huntsville, TX (STAFS). Zero on the y-axis represents mean ambient soil conditions. Note differences in y-axis between the two sites to better show concentrations.

Decomposition product phosphate-P concentrations showed some samples with elevated concentrations between 2,500 and 10,000 ADD at FACTS (Figure 18 A). Generally decomposition product phosphate-P was increased above ambient soil (y-axis zero) between 5,000 and 15,000 ADD at FACTS (Figure 18 B). Less than 1% of the variance in decomposition product phosphate-P concentrations was described by ADD at FACTS and 13% of the variance at STAFS (Figures 18 A and 18 B). Based on the low percentage of variance in phosphate-P explained by ADD I did not combine data for the two sites.

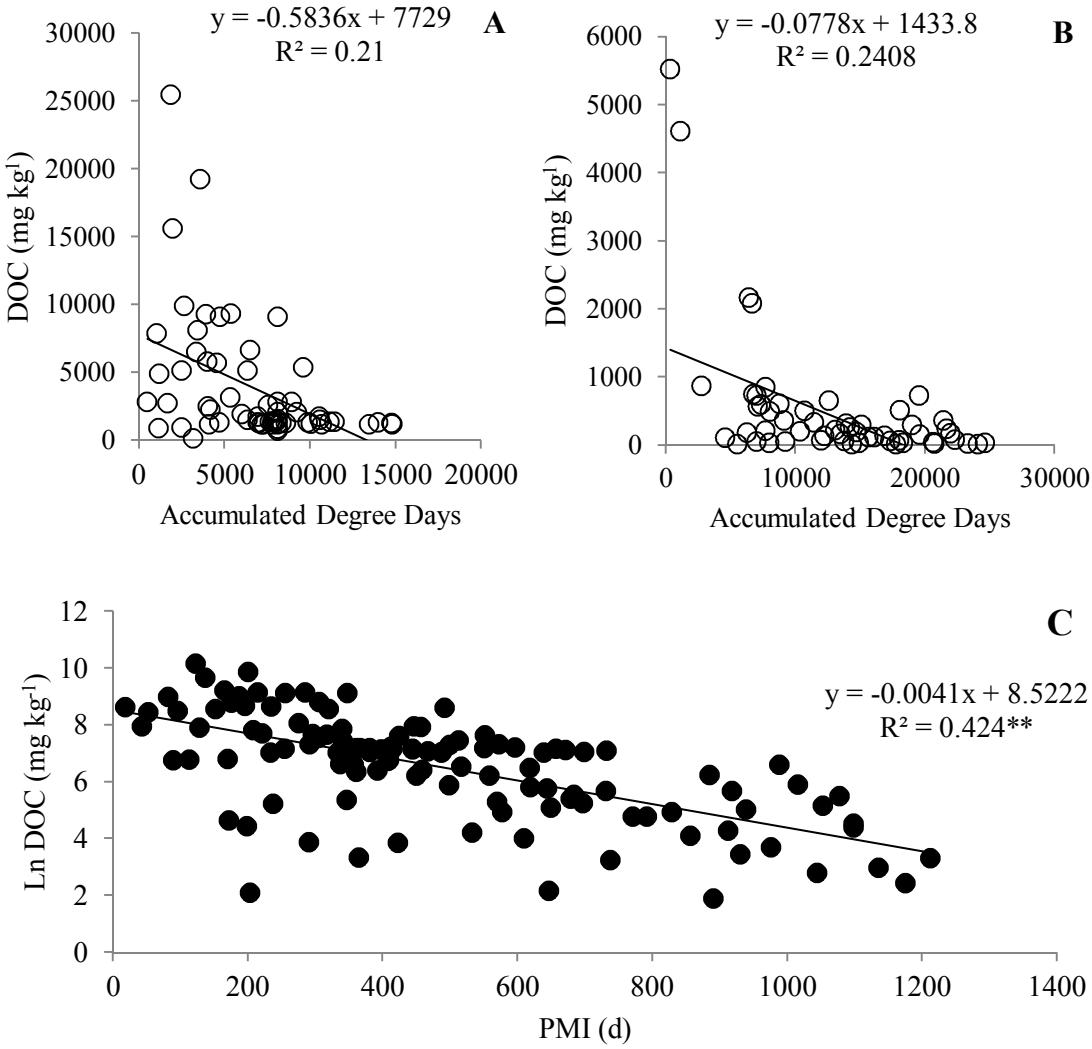

Figure 19. A) Decomposition product DOC concentrations (mg kg^{-1} soil) collected from the CDI's from outdoor decomposition facilities in A) San Marcos, TX (FACTS), B) Huntsville, TX (STAFS) and C) Both sites combined and transforming decomposition product DOC to a natural logarithm. Zero on the y-axis represents mean ambient soil conditions. Note differences in y-axis between the two sites to better show concentrations.

Decomposition product dissolved organic carbon (DOC) was elevated from ADD 346 to 10,000 at FACTS (Figure 19 A). The decomposition product DOC at STAFS was overall an order of magnitude lower than observed at FACTS but was higher than ambient soil DOC up to 25,000 ADD (Figure 19 B). A low to moderate relationship between decomposition DOC and ADD (R^2 = 0.21-.24) was seen at both facilities suggesting that bulk DOC or carbon compounds may be a useful predictor of ADD in soils. When both sites were combined and decomposition DOC transformed to its natural logarithm (Figure 19 C), forty-two percent of the variance in Ln DOC was described by PMI ($p < 0.01$).

Individual relationships

Although both DOC and ammonium-N showed some relationship to ADD at individual sites and when sites were combined, a reason why individual chemistries are relatively unrelated to ADD is due to the different stages of decomposition in which the sample sets were initiated in relation to the seasonal differences of when the body was placed and purged. Decomposition products tend to be absent until the cadaver undergoes purge forming the CDI, as time continues individual chemistries peak at different times (Figure 20).

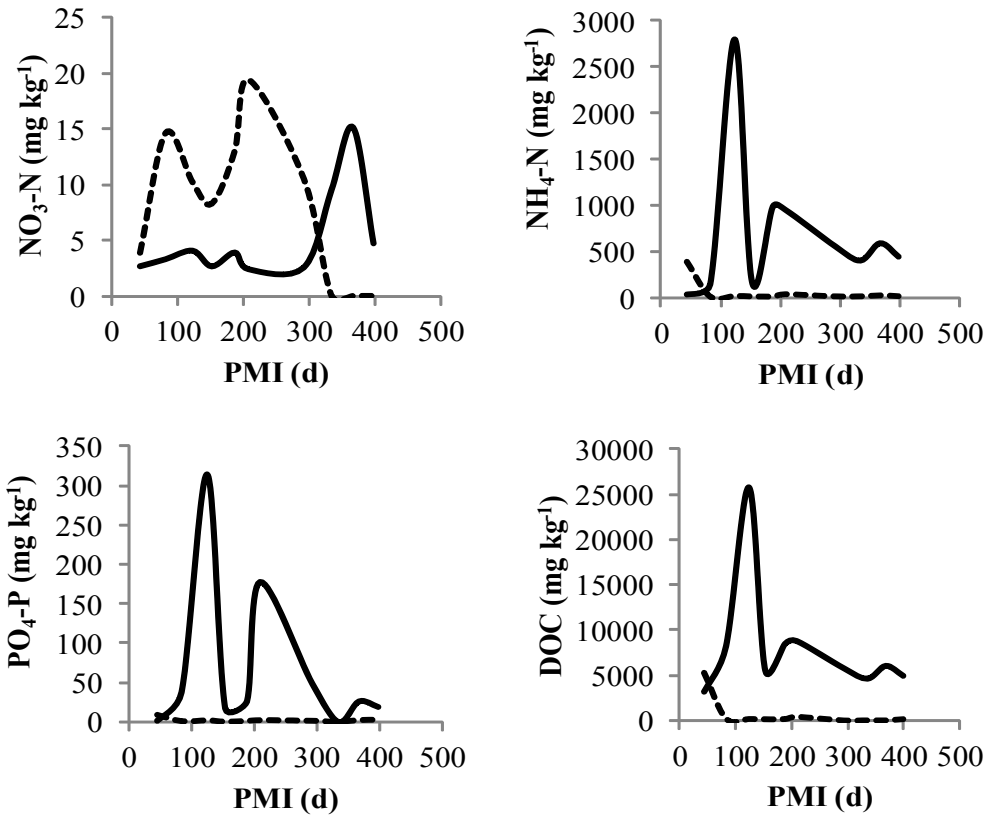

Figure 20. Example of progression of NO_3-N, NH_4-N, PO_4-P and DOC concentrations over one year in one CDI at FACTS. Time at start is 43 d post mortem and time at last day of sampling is 398 d post mortem. Solid lines are concentrations in CDI and dashed lines are concentrations in control soils.

As expected nitrate-N was decreased relative to control until approximately 300 d PMI (Figure 20). Ammonium-N displayed two peaks at approximately 120 and 200 d PMI (Figure 20). Because of the increase or peak and decrease in decomposition products over time it is impossible to determine if a certain concentration occurred at 100, 150, 200 or 350 d PMI in the example of PO_4-P (Figure 18), or 100, 200 or 300 d

PMI in the example of DOC (Figure 19). Hence it is virtually impossible to determine PMI from a single CDI chemistry.

To model PMI using ADD soil chemistry it is possible the multiple chemistries over time using a backward multiple regression analysis may be the best option. Using the decomposition only data from both sites and a total of 11 CDI's I used all the chemistries in a backward linear regression analysis. The best suite of predictors for PMI were non-transformed DOC, nitrate-N and ammonium-N concentrations ($R^2 = 0.25$ $p < 0.001$). This still did not produce a PMI model that could be used successfully and is perhaps due to low decomposition products at the front and back end of decomposition in the CDI as illustrated in Figure 6.

Soil bags

The soil bags resulted in significant differences between the chemistries with the bag often having twice or more the concentrations of conductance, and higher NH_4-N and NO_3-N than the regular grave soil core extracted. It was determined to be an ineffective method for sampling soil. Adipocere formation was noted in the soil bags from FACTS however none was present on the soil bags from STAFS.

Discussion

The relationship between ADD and PMI were both explained with above 98% variance accounted for by post mortem days supporting previous research (Vass et al. 1992) that both PMI and ADD can be equally useful in determining time since death.

Water Extracted Soil Chemistry

The value that PMI holds for forensic investigations cannot be understated. Determining an accurate PMI may allow for identification of the victim, setting a time line of events for the forensic investigator, and help with the placement of the perpetrator and victim within the same time line for potential prosecution. Thus far no model using soil chemistry within the CDI has been determined to be effective in any and all situations. The purpose of this study was to evaluate the use of decomposition product chemistry concentrations to estimate post mortem intervals across two very different sites in Texas for developing a model to determine PMI from grave soil chemistry. Typically whole soil chemistry from the CDI has been used in past studies (Vass et al. 1992). This study however represents one of the first studies to examine the chemistry of decomposition products only to enable model use across multiple sites (Aitkenhead-Peterson et al. 2015). The deduction of ammonium-N, nitrate-N, orthophosphate-P, total nitrogen, dissolved and organic carbon, control soil values were subtracted from CDI soils prior to regression analysis for discerning patterns or relationships with PMI. What was evident from this study was that one individual decomposition chemistry cannot be used to predict PMI. This is likely due to the hyperbolic relationships observed in chemical concentrations in CDI's over time which makes it virtually impossible to conclude that at a given concentration that PMI is 'X' days. Using a suite of decomposition product chemistries from the two donor facilities in Texas in multiple regression analysis did not improve the model for predicting PMI using soil chemistry

and may have been due to lower concentrations of nutrients at the beginning and end of the PMI period used.

More success in determining PMI has been achieved using near-infra-red spectroscopy (Aitkenhead-Peterson et al. 2015) which likely picks up subtle changes in organic carbon and nitrogen compounds better than water extractable bulk DOC and DON products. The model developed by Aitkenhead-Peterson et al. (2015) only examined decomposition over time of one CDI and is not transportable to other sites with cadavers that may be of a different size or potentially different causes of death. Indigenous invertebrates and microbes at the two sites may also have had a significant effect on the rate of decomposition in the CDI.

Sampling at both facilities began after the purge stage, with the earliest sampling occurring at 18 days PMI at STAFS and 43 days PMI at FACTS, however, the rest of the sample collections did not begin until between 90 and 570 days PMI. The only soil collected shortly after purge was STAFS (Cadaver 4 starting at PMI 18 d). Several of the cadavers at FACTS were in the rapid decay stage, with large amounts of liquefied fat tissue seeping into the soil.

Decomposition has five generally accepted stages of decay beginning with fresh, bloat, rapid decay, advanced decay and skeletonization (Carter et al. 2008). During the initial stages of decomposition cells lyse and rupture and an anaerobic environment inside the body dominates, causing microbial decomposition of organ tissues. Gases from the decomposition and microbial respiration build in the abdominal cavity causing the body to bloat until purge occurs, where there cavity ruptures and releases cadaveric

fluids into the surrounding soil from various orifices. Bodies placed during the summer decompose more rapidly, with bloat occurring more quickly and the subsequent tissues liquefying and entering the soil (*personal observation, 2012*). Cobaugh (2013) showed that as the body decomposes, enteric bacteria from the body itself will also enter the soil matrix during the purge and blend with the microbial community already there likely resulting in a very different microbial community composition in the CDI compared to control soils. Ambient temperature determines the rate at which the decomposition occurs, scavengers aside, and warmer temperatures (30-60°C optimal) support rapid decomposition (Dent et al. 2004). Organs tissues break down first followed by fat then muscle. During this stage of decomposition ammonium ions are the dominant decomposition product. It was expected that ammonium concentrations would rise sharply after bloat caused the rupture of the body cavity and elevated ammonium-N concentrations were observed between day 43 and day 600 PMI at the FACTS facility, with the largest number of elevated samples occurring between days 200 and day 400. Some samples remained elevated above control soil concentrations till day 600 with most falling back to control concentrations after day 600. STAFS showed similar elevations between day 18 and 600. Clear patterns or relationships between NH_4-N concentrations and PMI were not however evident. Once purge has occurred, there are numerous possible fates for the NH_4-N. It can be immobilized by microbes, nitrified, taken up by plants, held on soil exchange sites, fixed within inner layers of clays, or volatilized as NH_3-N, though only a few pH values rose high enough (7.5+) to support volatilization at either site. Generally NH_4-N is not nitrified under the anaerobic

conditions observed in CDI's (Aitkenhead-Peterson et al. 2012) and this is unlikely to occur until the CDI becomes aerobic due to scavenger activity (insect or mammal) that disturb the soil. Cobaugh (2013) observed similar results and theorized that low nitrate-N may be due to the lack of nitrification occurring; but that it could also be due to immobilization of the products by the microbes and uptake by plants.

The pH was variable throughout decomposition with no clear patterns observed at either facility. Cobaugh (2013) also found no consistent pattern with pH, with some values rising above the control baseline, while others fell at the donor facility in Tennessee. Data from my study at the two donor facilities in Texas supported Cobaugh's findings. Other studies have reported increases in soil pH (Carter et al. 2010; Hopkins et al. 2000; Rodriguez and Bass 1985; Wilson et al. 2007) whereas Benninger et al. (2008) reported a decrease in pH values in CDI's examined. The average control soil pH for FACTS was 6.8 and FACTS pH values ranged from just above 4.5 to as high as 8 in CDI soils, while, the STAFS average control soil pH was 6.1 with values ranging from just above 4 to as high as 7.5 in CDI soils. STAFS CDI soils had 60% (N= 68, 41 above, 27 below) of samples with pH higher the control soil, whereas FACTS only had 35% (N=63, 22 above, 41 below) of the samples with the pH higher than the control soil. The pH values from each facility were opposite in terms of distribution above and below the control soil value, implying that pH is highly variable and not a good predictor of PMI or a confirmatory tool for human decomposition.

Conductivity values showed significant differences between control soil values and CDI soil values with $p<0.0001$ (Table 16) for both facilities. Conductivity has been

used in several studies as a presumptive indicator of clandestine gravesites (Jervis et al. 2009; Pringle et al. 2010). My findings support the use of conductivity in this manner however, future research should evaluate if human decomposition values can be distinguished from animal decomposition values. Conductivity values were highly variable at each site with no pattern between concentrations therefore a reliable PMI could not be discerned.

CDI soils show promise as confirmatory or presumptive tests for decomposition, however further research is needed to determine if differences in control and CDI soils can be useful in PMI estimation.

Soil bags

Repeated coring of soil under a decomposing body may increase aeration and thereby alter ammonium concentrations (Aitkenhead-Peterson et al. 2014). This study also attempted to compensate for this issue through the construction of soil bags to eliminate the need for coring. Unfortunately this was not successful. The soil within the soil bags consisted of approximately 30g with a depth of 3cm within the bag of natural soil previously collected from each location. Landscape cloth was utilized to allow for flow of liquids through the material. The theoretical use of the bags was to allow liquids to move through the bag and have an accurate model of what seeped into the soils as a body decomposed to be used for analysis without severely altering the soil through aeration. However, the soils inside the bags were found to be two to three times higher in decomposition products than the surrounding soil. The bags appeared to trap liquid for

longer periods of time within the bag, causing the make-up of the soil bag soil to be very different from the free soil under the body and rendering the method non-productive.

Adipocere formation was noted at FACTS but not at STAFFS in conjunction with the soil bags. It was noted that the bags collected from under the body at FACTS had adipocere on the exterior of the cloth as well as within the soil inside the bag. Adipocere is a waxy soap like substance. Adipocere formation occurs in anaerobic environments which are damp and warm (Fiedler and Graw 2003; Fiedler et al. 2009; Forbes et al. 2005). Adipocere forms through the hydrogenation of decomposing fat tissue; the fat being transformed into a mix of saturated fats, unsaturated fats, calcium salts and hydroxyl- and oxo-fatty acids (Forbes et al. 2005) which have been found to comprise adipocere. This indicates there was an anaerobic environment beneath the cadaver and within the bag which retarded decomposition. The environment could have been created by the placement of the bags (under the torso), the cloth barrier used or possibly limited soil bacteria for decomposition. Regardless, the method did not produce the desired results and was discontinued early on after observing values that were doubled or tripled within the bag versus core samples taken at 0 – 2.5 cm.

Summary

No significant patterns were seen between soil chemistry and PMI although significant differences were observed between control soils and CDI soils and further investigation is recommended for development of a presumptive test for human decomposition versus other animals.

Currently the most effective method for collecting chemistry samples is through soil core samples from within the CDI. Care however, should be given to ensure so many samples are not collected as to alter the soil environment through aeration and thereby distorting the chemistry results.

CHAPTER V

ANALYSIS OF HUMAN DECOMPOSITION PRODUCT UPTAKE BY PLANTS

Introduction

Many methods are utilized by law enforcement to locate clandestine graves with varying degrees of success including ground penetrating radar (GPR) (Bevan 1991; Davenport et al. 1992; Pringle et al. 2008), thermal imagery (Davenport et al. 1992; Ruffell 2005) or digging by hand or with back hoe's (Bevan 1991; Jaffe 1983; Rodriguez and Bass 1985) and human remains detection dog (Lasseter et al. 2003). Once located, the potential gravesite must be dug by hand or with heavy equipment and may entail intensive man hours. There are few confirmatory tools presently available to be used with the human remains detection (HRD) dog's trained final responses (TFR) prior to digging for human remains. We propose that changes in the C, N and P concentrations in leaves of vegetation surrounding the cadaver dog's TFR may be valuable in confirming the presence of a cadaver decomposition island (CDI) thereby saving labor and money for the agency having jurisdiction.

Many HRD dog handlers have reported that their HRD dogs offer their trained final response on trees near gravesites (Alexander and Turner 2010; Christensen 2014; Shaffer 2010). HRD dogs can detect gravesites that are many years old; long after the expected *"volatile scent"* has diminished (Alexander and Turner 2010; Christensen 2014; Dotson 2012; Hammond and Morris 2009; Shaffer 2010). Furthermore, HRD dogs frequently lick the vegetation in the vicinity of gravesites (Alexander and Turner 2010;

Christensen 2014; Hammond and Morris 2009; Shaffer 2010). Some of the vegetation species noted to have elicited this behavior are the American sweetgum (*Liquidambar styraciflua*), eastern red cedar (*Juniperus virginiana*) and winged elm (*Elmus alata*) (Alexander and Turner 2010; Christensen 2014; Hammond and Morris 2009; Shaffer 2010). This has been observed on actual cases as well as training in historic cemeteries. Whether this behavior is to elicit scent from the vegetation or lick volatiles from the vegetation is unknown. Investigating this phenomenon to determine if and what chemical agents are being taken up through the root system, incorporated into the cambium and other plant tissue, and potentially stored within or transpired through the stomata of the leaves may provide a confirmatory tool for clandestine graves. Historical human remains detector dogs have been documented with successfully locating buried human remains as old as 4300 BC (ICF, 2013). This supports that whatever this marker is, it is long lived and distinct enough to survive millennia. Although there is no scientific support on what compounds HRD dogs might be detecting in the vegetation, it is known that as cadavers decompose, fluids and nutrients are purged into the soil forming the CDI (Carter et al. 2007; Dent et al. 2004; Rodriguez and Bass 1985; Vass et al. 1992; Wilson et al. 2007). Amino acids are transformed to ammonium which is then available to higher plants and it has been suggested that plant growth may be enhanced by shallow buried remains nearby (Bohun et al. 2010; Carter et al. 2007; Rodriguez and Bass 1985; Wilson et al. 2007). One possible explanation for the phenomenon of HRD dogs alerting on vegetation is phytovolatilization. Phytovolatilization is the uptake and transpiration of primarily organic compounds present in the water taken up by the plant

(Davis and Erickson 2002; Masayuki et al. 2010). Theoretically, contaminants or components which are water soluble pass through the plant or are modified by the plant and transpired into the atmosphere through evaporation or vaporization. This has been shown to occur with light organic molecules (Davis and Erickson 2002; Masayuki et al. 2010).

It is known that plant roots uptake nutrients and water. All tree roots possess an apical meristem which generates growth and a protective root cap (Harris-Haller 2008). Housed within the root is the vascular tissue which contains the xylem responsible for transport of water and organic solutes from the soil. The rate of root growth is variable throughout a growing season. Roots usually begin to grow before the tree canopy, although root growth is cyclic and responds to environmental changes such as soil depth, water supply, aeration, mineral supply and temperature (Espinoza et al. 2005; Plomion et al. 2001). Studies have shown root spread to be 4 to 7 times the drip-line distance (canopy radius) of the tree (Fahey et al. 1988). The shallow portions of the root system in some plants (located in the top 15 to 30 cm of the soil) are assumed to be responsible for the majority of water absorption. Trees also possess vascular cambium, a secondary meristem that grows between the xylem and the phloem. The vascular cambium results in increased annual girth and is commonly known as growth rings (Fahey et al. 1988; Plomion et al. 2001). Nitrogen and phosphorus are essential elements required by plants for growth and yield (Cleveland et al. 2004; Espinoza et al. 2005; Neff and Asner 2001). Nitrate and ammonium are utilized by plants to build proteins and amino acids for strong growth and foliage. Phosphorus is a highly limiting nutrient due to unavailability in most

soils but is essential for the conversion of light energy to chemical energy (ATP) during photosynthesis needed for root and flower growth (Espinoza et al. 2005).

The processes of decomposition have been well defined (Carter et al. 2007; Dent et al. 2004; Wilson et al. 2007). The cadaver progresses from autolysis to purification, liquefaction, and finally skeletonization over the course of time (Dent et al. 2004; Wilson et al. 2007). Vass et al. (1992) suggested that these stages can be organized into two basic categories of pre-skeletonization and post-skeletonization. When bloat gives way to active decay the skin and orifices rupture resulting in a purge of nutrient rich fluids into the soil. This occurs over a period of time starting with the eyes and mouth and the anus (Bohum et al. 2010; Dent et al. 2004). Body areas and organs with high moisture content such as the brain and liver also break down more rapidly (Bohum et al. 2010). Cadavers are approximately 20% carbon and the depth and extent of the CDI are dependent upon the size and body composition of the cadaver (Bohum et al. 2010; Carter et al. 2007; Dent et al. 2004). The initial purge results in death of plants immediately under a body as well as on top of the grave (Benninger et al. 2008; Carter et al. 2007; Dent et al. 2004). This is coupled with disturbance and disruption of the plant community during the act of burial (Fiedler and Graw 2003; Rodriguez and Bass 1985). Localized decreases in nitrate-N, and increases in ammonium-N, dissolved organic nitrogen (DON), dissolved organic carbon (DOC) and phosphate-P in the soil may be associated with the CDI (Aitkenhead-Peterson et al. 2012).

The purpose of this study was to explore the potential for development of a confirmatory tool that could be used to corroborate HRD dog TFR's at suspected

gravesites. Objectives were to determine: a) if there was a significant difference in the DOC, DON, NO_3-N, NH_4-N and PO_4-P concentrations, and, b) if trained credentialed HRD dogs could accurately identify plants from grave CDI's versus matching plants from control soil outside of the CDI. I hypothesized that a) plants within the CDI take up more N and P and are able to fix more DOC than control plants taken from away from decomposition sites, and, b) that HRD dogs will be able to differentiate between trees from the CDI versus control areas, but will not be able to differentiate between woody weeds such as goat weed. These confirmatory tools coupled with observation of surrounding topography (slope) will avoid unnecessary manpower and hours excavating a site that contains no human remains.

Materials and Methods

Site descriptions

Vegetation samples for this study were collected at the Southeast Texas Applied Forensic Science Facility (STAFS) and the Forensic Anthropology Center at Texas State (FACTS).

The STAFS facility is located within the 1 km^2 area of the Center for Biological Field Studies at Sam Houston State University, Walker County, TX, USA. The STAFS facility sits within the Walker County portion of the Sam Houston National Forest Wildlife Management Area. The United States Geological Survey soils maps for Huntsville, Texas define this area as woodland primarily consisting of loblolly and short leaf pine, with soil of the Depcor-Huntsburg association Annual precipitation ranges from 1016 to 1219 mm (USDA 2014).

The Forensic Anthropology Center of Texas State (FACTS) in San Marcos TX, USA facility is located within 17 km^2 of the Freeman ranch owned by Texas State University in Hays County TX, USA. Vegetation consists of grasslands and tree clusters consisting of cedar, juniper, live oak, mesquite, and Texas persimmon, with soils of the Comfort – Rock outcrop complex and Rumple-Comfort association. Annual precipitation ranges from 25.4 – 91.4 cm (USDA 2014).

Plant collection and analyses

STAFS

Grave soil cadaver decomposition islands (CDI) were dominated by two plant species, *Pinus taeda* and *Croton capitatus* that were also present outside of the STAFS facility. Mulitple branches containing needles and stems of both mature growth and new growth were collected from *Pinus taeda*, which was situated in the center of the STAFS facility and next to CDI. Whole plants (6) of the *Croton capitatus* were cut just above the soil on the CDI. Samples from *Pinus taeda* and *Croton capitatus* (3) were also obtained from outside the STAFS facility but of the same soil association.

FACTS

Multiple branches were collected from both *Juniperus ashei* and *Ulmus alata* at CDI soils as well as control locations within the facility with no graves nearby. Whole plants of *Croton monanthogynus (6)* were cut just above the soil on the CDI. Control plants (3) of *Croton monanthogynus* were collected away from graves but within facility and of the same soil association, as well as multiple branches of *Juniperus ashei* and *Ulmus alata* from trees (3) away from graves but of the same soil association.

Plant processing

All plant cut ends were then wrapped in water saturated paper towels as collected and placed into plastic containers containing water. CDI plants were placed in one large plastic tote and control plants in a separate tote for transport which took two to three hours. Upon arrival at the lab plants were rinsed independently with DDW water and were refrigerated prior to testing with HRD dogs and then dried afterwards.

All plant samples were oven dried (60° C, 3 d) prior to separation into stem, leaf or needle and flower and passed through a Wiley mill to fit through a 2 mm sieve. Three separate samples per plant of 2.5 g of tissue sample was combined with 100 mL of ultra-pure water and shaken at 60 rpm at room temperature for 20 h. Aliquots of the extract solution were taken from each sample and centrifuged at room temperature at 15,000 g-force for 15 min. pH and electrical conductivity was recorded on unfiltered supernatant. Supernatant was then filtered through an ashed (500˚ C 4 h) Whatman GF/F filter. Tissue extracts were analyzed immediately or frozen (at -4°C) for later analysis.

Chemical analysis

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using high temperature Pt-catalyzed combustion with a Shimadzu TOC-VCSH and Shimadzu total measuring unit TNM-1 (Shimadzu Corp. Houston, TX, USA). Dissolved organic carbon was measured as non-purgeable carbon using USEPA method 415.1 which entails acidifying the sample and sparging for 4 min with C-free air. Ammonium-N was analyzed using the phenate hypochlorite method with sodium nitroprusside enhancement (USEPA method 350.1) and nitrate-N was analyzed using

Cd–Cu reduction (USEPA method 353.3). All colorimetric methods used a Westco Scientific Smartchem Discrete Analyzer (Westco Scientific Instruments Inc. Brookfield, CT, USA). DON is the product of TDN - (NH_4-N + NO_3-N). Sample replicates, blanks, NIST traceable and check standards were analyzed every 12th sample to monitor instrument precision and co-efficient of variance among replicate samples was set at a maximum of 4% CV or the sample was re-run. Water extractions of vegetation were chosen over any chemical extractions in an attempt to replicate what occurs naturally in the field with rain water (Aitkenhead-Peterson et al. 2006; McCrary et al. 2013).

HRD dog teams

Seven nationally certified HRD dog teams were used to test for HRD odor detection in plants. Participating HRD dogs ranged in age from 4 y to 13 y and comprised two males and five females. Trained final responses (TFR) of the seven dogs included: two dogs that gave an aggressive scratch TFR, one dog with a sit TFR, and four dogs with a down TFR. The HRD dogs included in the study were referenced by number to protect anonymity of the team. Dog teams were familiar with the procedure due to previous training and testing on soils in the same manner (chapter III).

HRD dog testing

The testing model used was consistent with previous studies performed for research with residual soils and are generally accepted methods utilized by military and law enforcement agencies around the world for proficiency testing with the slight modification of no handler accompaniment (ATF 1997; Oesterhelweg et al. 2008; Rebmann et al. 2000).

Trials consisted of a line-up of five metal paint cans, each containing either: 1) Clippings of a grave CDI plant 2) clippings of control plant 3) clippings of control plant 4) clippings of control plant, and 5) one empty can. Trials were run in the same fashion as those in chapter 3 without the removal of the plants between trials with an independent post-doc that assigned, conducted, directed and scored the trials accompanied by volunteer undergraduate student assistants. Nine trials were run for each dog in random order during each experiment testing session for N= 63 trials per session. Two testing sessions were completed for N=126. Mesh screens were replaced per dog to prevent cross contamination and cueing. Paint cans were covered with screen mesh to prevent direct contact with the dogs (Figure 21).

Figure 21. A new 1 gallon paint can purchased solely for use in line-up experiment. Plant was placed inside can then fine mesh screen was used to cover the can held in place with a rubber band to prevent dogs from physically contacting the plants.

Results

STAFS site – Huntsville, TX

Two vegetation types were examined at this site: a) goat weed (*Croton capitatus*), leaves and stem and b) loblolly pine (*Pinus taeda*) needles, stem and wood.

Goat Weed Chemistry

Extractable DOC was significantly higher in the goat weed leaves and stems from the grave site when compared to the control site ($p = 0.04$ and 0.003 respectively) (Figure 22 A). There was no significant difference in extractable DON when comparing

goat weed leaves from control and grave sites (Figure 22 B) but DON in the stems of goat weed from the grave site was a three times higher compared to DON in the stems of goat weed from the control site (Figure 22 B) and was significantly higher ($p = 0.0003$). The ratio of DOC: DON was significantly higher in the grave site goat weed leaves ($p = 0.005$) and significantly lower in the grave site goat weed stems ($p = 0.002$) (Figure 22 C). For the inorganic chemistry, ammonium-N was significantly lower in the grave site leaves ($p = 0.003$) but there was no significant difference in ammonium-N concentrations in the grave and control site goat weed stems (Figure 22 D). Nitrate-N concentrations were significantly higher in both the leaves and stems of goat weed at the control site when compared to the grave site ($p = 0.002$-0.004; Figure 22 E). There was no significant difference in PO_4-P concentrations in either the leaves of stem of goat weed when comparing the control and grave sites (Figure 22 F).

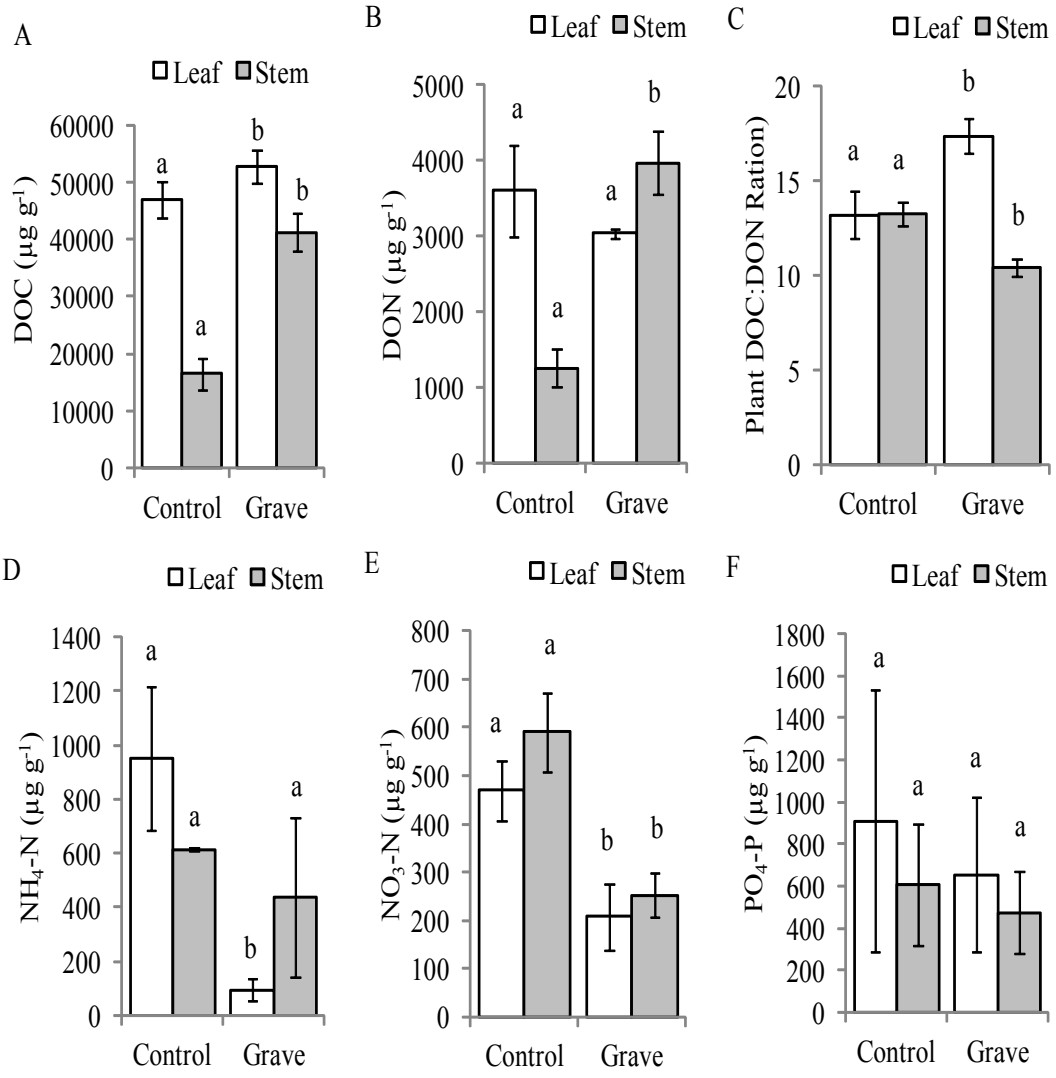

Figure 22. Water extractable C, N and P chemistry of goat weed leaves and stems at the STAFS site in Huntsville, TX. Error bars are standard deviation. Differences in lower case letters indicate a significant difference between control and grave vegetation.

Loblolly pine chemistry

Extractable DOC was significantly higher in the pine needles and wood from the control site ($p = 0.04$ and $p = 0.001$ respectively) compared to the grave site (Figure 23 A) and DOC was significantly higher in the grave site stems when compared to the control site ($p = 0.0003$). There was no significant difference in extractable DON in pine needles when comparing control and grave sites (Figure 23 B), but DON in the stems of pine at grave site was significantly higher compared to DON concentrations in the stems of pine from the control site ($p = 0.03$). The wood retrieved from pine at the control and grave sites had significantly higher concentrations of extractable DON at the control sites when compared to the grave sites ($p = 0.02$). There was no significant difference in DOC: DON ratios when comparing control sites and grave sites for the needles, stems or wood of the Loblolly pine (Figure 23 C). For the inorganic chemistry, ammonium-N was not significantly different in the pine needles and wood when comparing control and grave sites (Figure 23 D), however significantly higher ammonium-N was observed in the pine stems at the grave sites when compared to the control sites ($p = 0.0001$). Nitrate-N concentrations were not significantly higher in the needles, stems or wood of Loblolly pine when comparing control and grave sites (Figure 23 E). There was no significant difference in PO_4-P concentrations in either the needles or wood of Loblolly pine when comparing the control and grave sites (Figure 23 F), however PO_4-P was significantly higher in the stems of Loblolly pine of the grave site when compared to the control site ($p = 0.0001$).

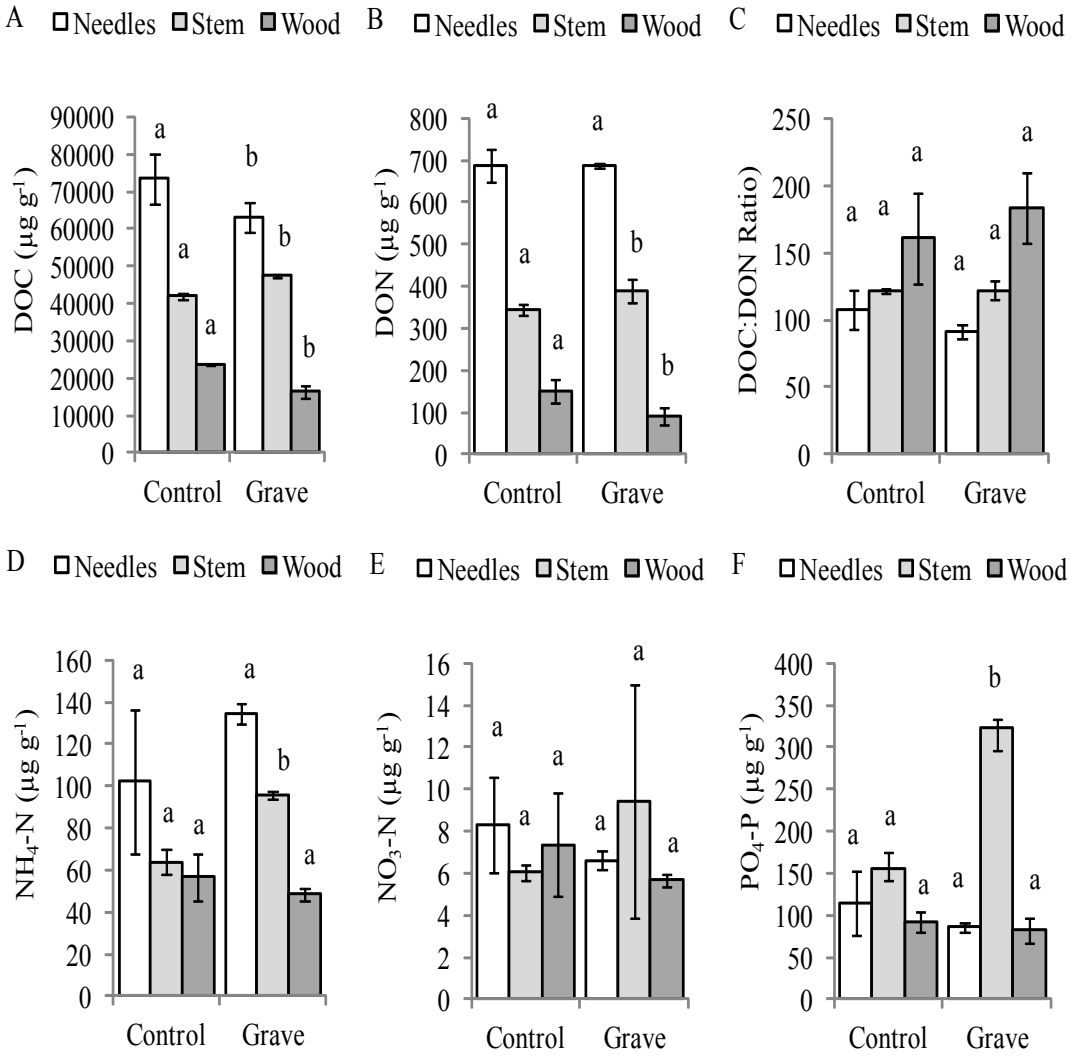

Figure 23. Water extractable C, N and P chemistry of Loblolly Pine tree needles, stems and wood at the STAFS site in Huntsville, TX. Error bars are standard deviation. Differences in lower case letters indicate a significant difference between control and grave vegetation.

FACTS site – San Marcos, TX

Three vegetation types were examined from grave and control sites at FACTS: goat weed (*Croton monanthogynus*) leaves and stems, winged elm (*Ulmus alata*) leaves and stems and juniper (*Juniperus ashei*) leaves and stems.

Goat weed chemistry

Extractable DOC concentrations were not significantly different in the goat weed leaves and stems from the grave site when compared to the control site (Figure 24 A). There was no significant difference in extractable DON when comparing goat weed leaves and stems from control and grave sites (Figure 24 B). The ratio of DOC:DON was significantly higher in the grave site goat weed stems ($p = 0.04$) but there was no significant difference between control and grave site obtained goat weed leaves (Figure 24 C). For the inorganic chemistry, ammonium-N was significantly lower in the grave site stems ($p = 0.02$) but there was no significant difference in ammonium-N concentrations in the grave and control site goat weed leaves (Figure 24 D). Nitrate-N concentrations were not significantly different in either the leaves or stems when comparing control and grave site goat weed (Figure 24 E). There was no significant difference in PO_4-P concentrations in either the leaves of stem of goat weed when comparing the control and grave sites (Figure 24 F).

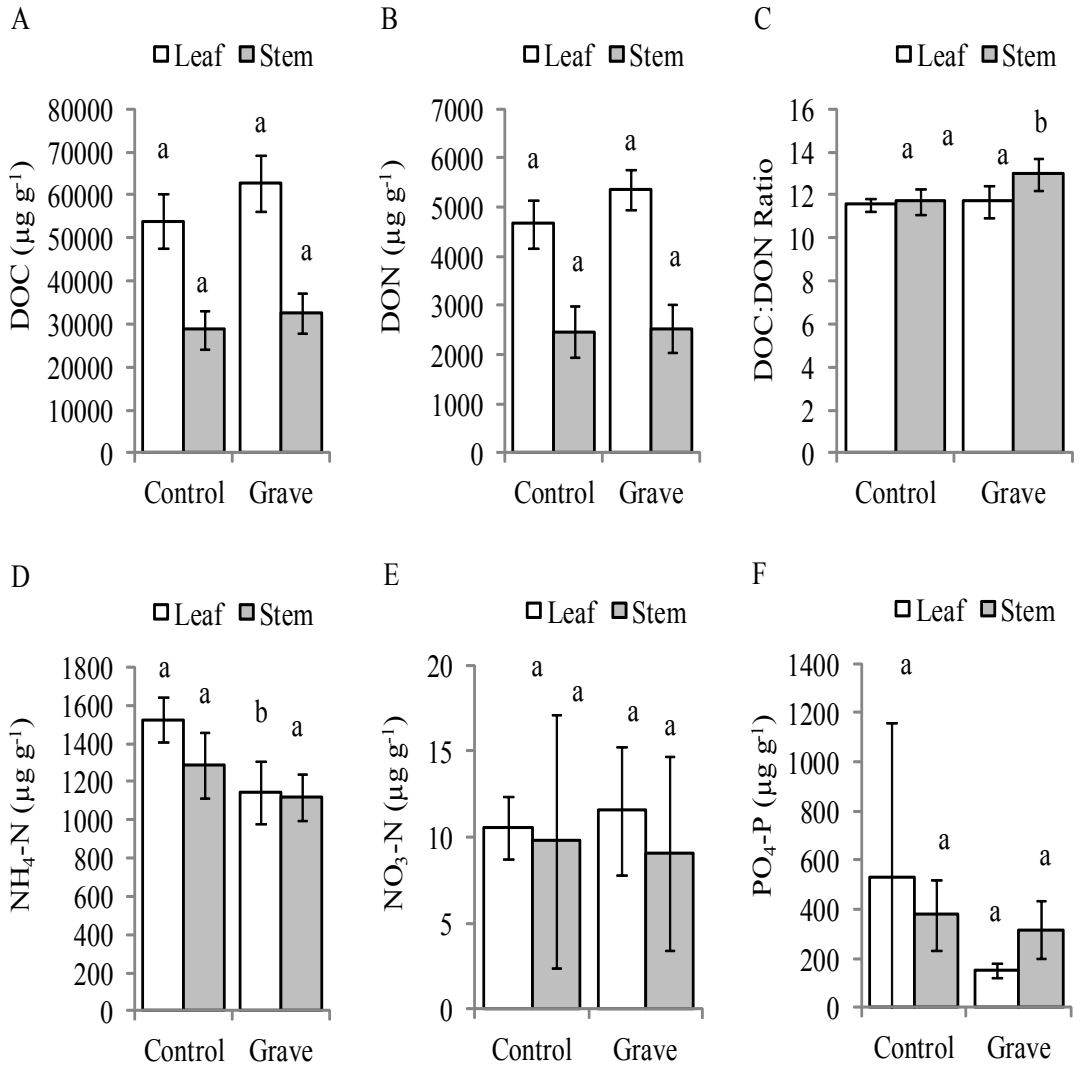

Figure 24. Water extractable C, N and P chemistry of goat weed leaves and stems at the FACTS site in San Marcos, TX. Error bars are standard deviation. Differences in lower case letters indicate a significant difference between control and grave vegetation.

Winged elm tree chemistry

Extractable DOC concentrations were significantly higher in both the leaves and stems of the elm tree at the grave site when compared to the control site ($p = 0.001$ and 0.01 respectively) (Figure 25 A). There was also a significant difference in extractable DON when comparing elm tree leaves and stems from control and grave sites (Figure 25 B), here significantly higher of concentrations of DON were observed in the leaves obtained from the grave site when compared to the control site ($p = 0.0003$). The ratio of DOC:DON was significantly lower in the grave site elm leaves ($p = 0.005$) but there was no significant difference between control and grave site obtained elm stems ($p = 0.055$; Figure 25 C). For the inorganic chemistry, ammonium-N was not significantly different when comparing elm leaves and stems from the grave sites and control sites (Figure 25 D). Nitrate-N concentrations were significantly higher in the grave site leaves ($p = 0.004$) and stems ($p = 0.01$) when compared to nitrate-N concentrations in the control site leaves and stems (Figure 25 E). There was no significant difference in PO_4-P concentrations in the elm leaves when comparing the control and grave sites (Figure 25 F) but PO_4-P concentrations were significantly higher in the grave site elm tree stems when compared to the control site elm tree stems ($p = 0.01$).

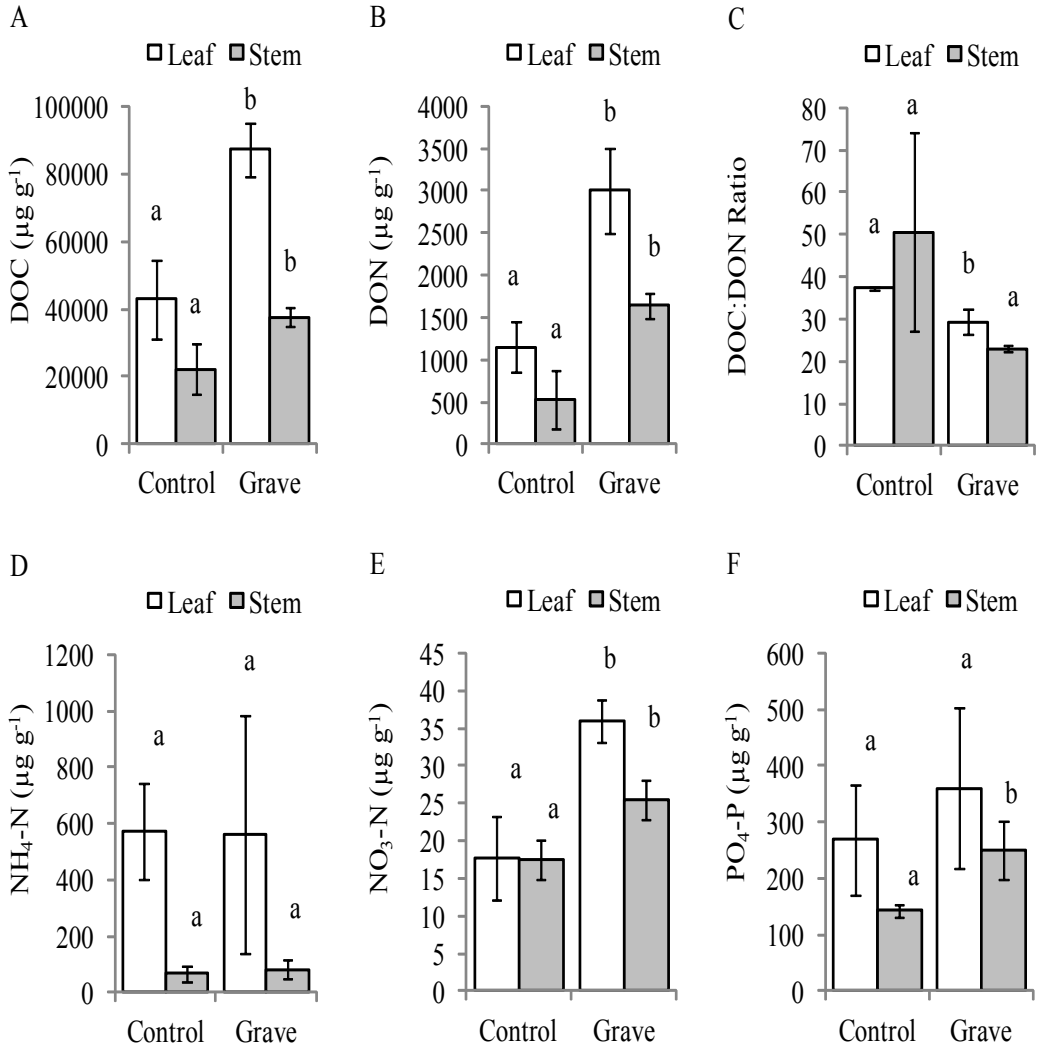

Figure 25. Water extractable C, N and P chemistry of elm tree leaves and stems at the FACTS site in San Marcos, TX. Error bars are standard deviation. Differences in lower case letters indicate a significant difference between control and grave vegetation.

Juniper tree chemistry

Extractable DOC concentrations were not significantly different between grave and control in neither stems nor leaves (Figure 26 A). There was a significant difference in extractable DON when comparing tree leaves from control and grave sites (Figure 26 B), here significantly higher of concentrations of DON were observed in the leaves obtained from the grave site when compared to the control site ($p = 0.003$) but not no significant difference was seen in the stems. The ratio of DOC:DON showed a significant difference between control and grave site obtained leaves ($p=0.006$) and in stems ($p=0.037$) with significantly lower DOC:DON rations in both grave plant stems and leaves (Figure 26 C). For the inorganic chemistry, ammonium-N was significantly different when comparing stems from the grave sites and control sites ($p=0.012$) but not in leaves (Figure 26 D). Nitrate-N concentrations when compared to nitrate-N concentrations in the control site leaves and stems found no significant differences (Figure 26 E). There was no significant difference in PO_4-P concentrations in the leaves or stems when comparing the control and grave sites (Figure 26 F).

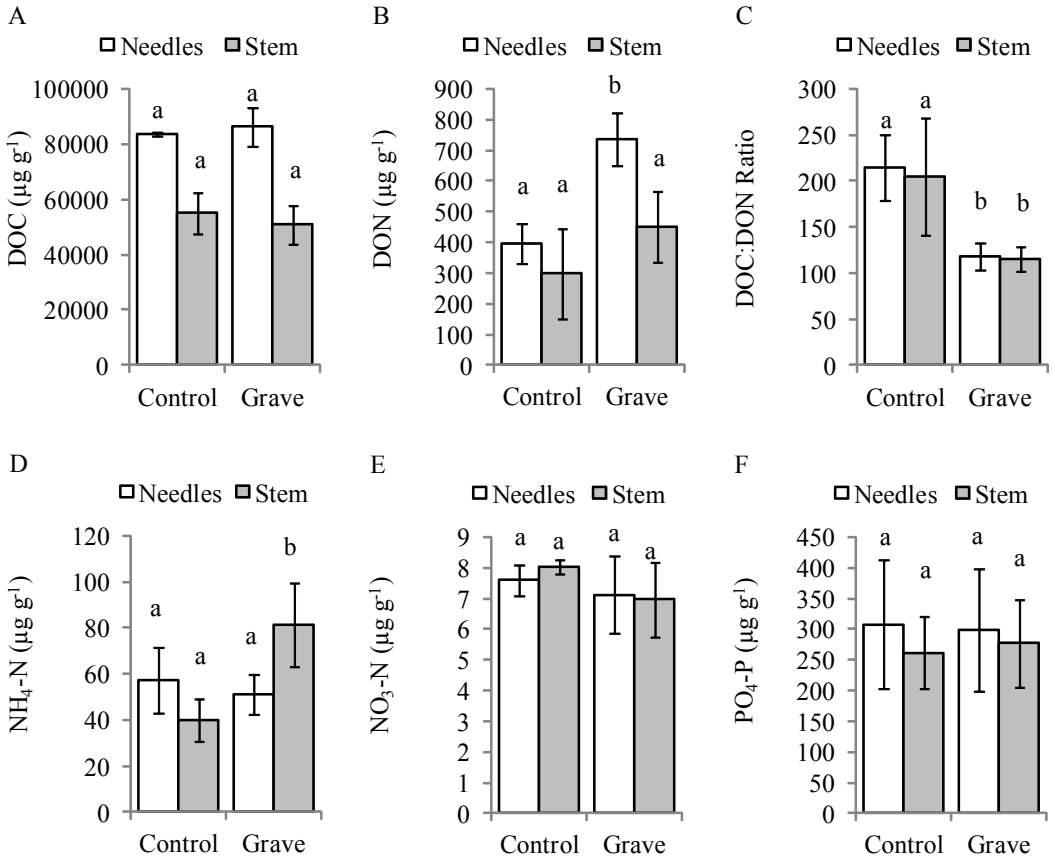

Figure 26. Water extractable C, N and P chemistry of juniper tree needles and stems at the FACTS site in San Marcos, TX. Error bars are standard deviation. Differences in lower case letters indicate a significant difference between control and grave vegetation.

HRD Dog Accuracy

STAFS Plants

HRD dog teams were able to accurately identify plants which had grown on the grave CDI from control plants which had grown in the same area but not next to a grave with an accuracy of 86% for goat weed (*Croton capitatus*), 81% for loblolly pine (*Pinus taeda*) needles and stem and 86% pine wood only (Figure 27).

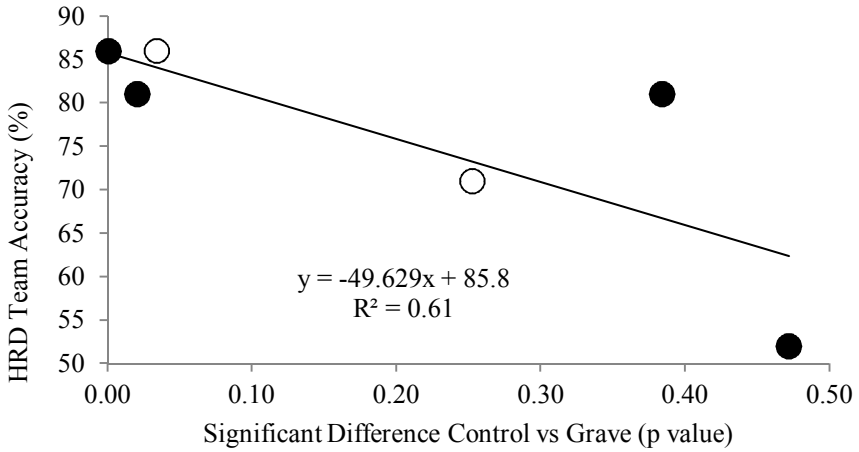

Figure 27. Relationship between HRD team accuracy and amount of significant difference between control and CDI obtained vegetation extractable DOC. White circles = herbaceous plants and Black circles = tree species.

Of the five chemistries examined in the vegetation obtained from CDI and control sites I ranked each vegetation species by the number of significant differences observed between CDI and control vegetation. The higher the number of significant different chemistries in a vegetation type a higher HRD team accuracy was observed (Figure 5.8). For example, *Croton capitatus* collected at STAFS CDI sites was significantly in DOC. NH_4-N, NO_3-N and DON concentrations when compared to control vegetation but not significantly different for PO_4-P thus it was ranked as 4. At the other end of the spectrum, the *Pinus taeda* at STAFS *Croton monanthogynus and Juniperus ashei* collected at FACTS only had one chemistry that was significantly different compared to control; ammonium-N for *Pinus taeda* and *Croton monanthogynus* and DON for *Juniperus ashei* so they were ranked as 1 (Figure 28). It appears that if more than 1 compound chemistry in CDI vegetation is significantly different compared to control then the accuracy of HRD teams' remains above 80% (Figure 28).

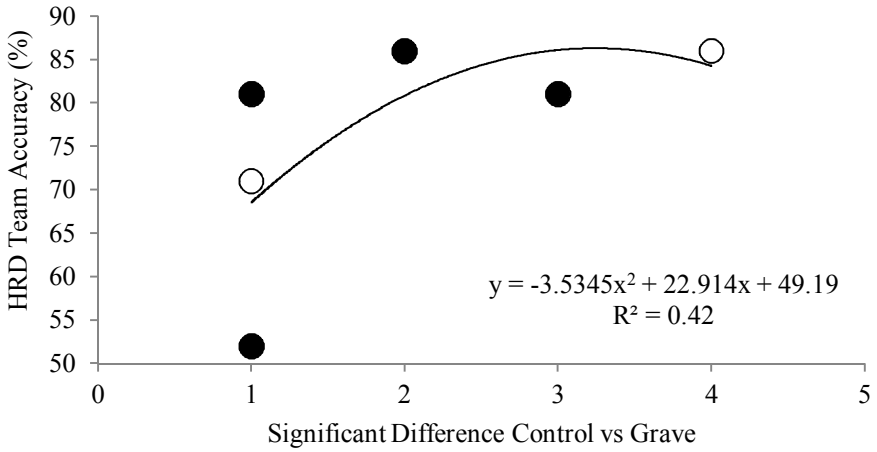

Figure 28. Relationship between HRD team accuracy and number of chemistries that was significantly different when comparing CDI vegetation and control vegetation. White circles = herbaceous plants and Black circles = tree species.

FACTS Plants

HRD dog teams were able to accurately identify plants which had grown on the grave CDI from control plants which had grown in the same area but not next to a grave with an accuracy of 81% for goat weed (*Croton monanthogynus*), 52% for juniper (*Juniperus ashei*) leaves and stem and 71% for winged elm (*Ulmus alata*).

Discussion

South-Central Texas enjoys a relatively long growing season, approximately 253 days a year when rainfall exceeds 33 inches (Land and Hannon 2011). Weather determines daily and seasonal variations in biological activity; however the average date for last frost is March 14 with the growing season extending until the average first frost of November 16 (Land and Hannon 2011). Our samples were gathered in the latter part

of September 2013 as the growing season was diminishing. Therefore, a major portion of plant growth had taken place by the collection date.

Preliminary findings and interpretation

The findings of this preliminary study are encouraging and deserve further investigation into the possibility of utilizing plant chemistry changes coupled with HRD dog TFR's as a confirmatory tool of CDI or clandestine grave. We did find significant differences in many of the chemistry concentrations between CDI plants and control plants and further, HRD dogs were able to accurately identify CDI plants from control plants in 5 of the 6 plant cuttings tested.

Currently there are no HRD dogs trained specifically on identifying human remains decomposition products in plants. The lower percentage accuracy on plants compared to soil (Chapter III) is probably due to absence of training on this particular medium and the concern of handlers to include this medium in training schedules due to the potential to miss-train the dogs and potential legal concerns. Handlers were cooperative but reluctant to run this experiment for fear of training their dogs to alert on plants and agreed with the understanding that it would only consist of two 9 trial sessions. Consequently, handlers did not reward their dogs for TFR's at plants. This portion of the experiment was simply to determine if HRD dogs detected something they recognized from their HRD training within the plant cuttings presented.

Anecdotal observations by some human remains detection dog handlers investigating potential clandestine graves support a relationship between several species of trees and detection by the canines in respect to the location of the grave. No handlers

to date have reported any TFR's elicited by weeds or grasses, only trees. What was apparent was the lower the significant difference in DOC in CDI vegetation compared to control vegetation the greater the accuracy of the HRD team TFR. A recent analogy told to me was that *"while humans smell pizza sauce, dogs smell every ingredient in the pizza sauce"*. Testing this analogy, I found that the greater the number of CDI vegetation chemistries that were significantly different from control vegetation chemistries the greater the accuracy of HRD team response.

Based on the work by Aitkenhead-Peterson et al., (2012) showing decomposition product movement away from the CDI and the results of this study indicating plant uptake of human decomposition products the next step may be developing specialized tools to explore this phenomena. Further research may confirm that HRD dogs that specialize in trace or forensic evidence type searches may benefit from adding these types of training aids to their training protocol. This is specifically important because of the prior observed TFR by HRD dogs on vegetation yet no visual indication of a CDI or grave site. It should be recommended to handlers that based on this type of response that HRD dogs should move upslope to continue searches and employ the use of probe holes to release odor from the soil itself upslope.

Plant use of nutrients

Nitrogen plays an important role in photosynthesis. Plants are unable to convert sunlight and CO_2 into useable energy and nutrients without nitrogen. Nitrogen is an essential component of chlorophyll, DNA, RNA, peptides and proteins in plants. When N is limited, plant growth and production suffers. Nitrogen is acquired from the soil in

the forms of nitrate and ammonium which are then reduced to the amino acids needed. Nitrate uptake was suggested by Ullrich (1987) as H+/nitrate co-transport. Recent studies have also examined the potential for plants to uptake organic forms of N, particularly amino acids (Panungfoo-Lonhienne et al. 2008; Tegeder and Rentsch 2010). Membrane transporters in root cells for Arabidopsis have been identified in root tissues (Tegeder and Rentsch 2010). These transporters uptake amino acids which are then translocated to the shoots and photo-synthetically active leaves via the xylem. Further research shows that plants can also uptake organic N in the forms of small proteins and peptides (Paungfoo-Lonhienne et al. 2008) where root derived proteases break down small proteins and whole proteins were observed to have entered into root hair cells and root cortex cells, possibly through endocytosis. Further research in the area of organic N uptake is currently underway and may yield information that helps to explain the phenomena of HRD dog alerts on trees. Phytovolitilization (Davis and Erickson 2002; Masayuki et al. 2010) research also supports the uptake of small molecular weight organics and may play a role in the transport of these organics through the plant and out of the stomata. Research in these arena's may soon cause many scientists to reassess what plants are capable of utilizing from the environment.

One of the major questions prior to this study was whether volatiles from decomposing human remains released into the atmosphere 'stuck' to vegetation and was responsible for HRD dogs were offering TFR's on vegetation. This study provides evidence that there may be uptake of human decomposition products rather than the

whole plant exposure to volatiles. This study found that leaves or stems of CDI plants were significantly different in some chemistry Further investigation is needed.

Pinus taeda

The tree has a deep root system and is tolerant of acidic soils (TPWD, 2012). This species was sampled from STAFS in Huntsville, TX. HRD dogs were 81% accurate in identifying the grave CDI stem and needle cuttings and 86% accurate on the pine wood. Pine stems from grave CDI were significantly higher in DOC, DON, NH_4-N and PO_4-P whereas CDI needles were lower in DOC. CDI Pine wood was significantly lower in DOC and DON. This appears to indicate that readily available nutrients may have been in transport from the trunk to the needles at the time of collection.

Croton capitatus and Croton monanthogynus Michx.

These species of crotons are also known as woolly croton, dove weed, goat weed and prairie tea, are a woody weed species that begins growth in late April early May and mature with flowers and seeds by August and September. They have shallow root systems and are easily pulled from the soil (TPWD, 2012). HRD teams were 86% accurate for STAFS and 81% accurate for FACTS. STAFS plants had significantly higher concentrations of DOC in grave CDI leaves and DOC:DON ratio, NH_4-N, NO_3-N however were significantly lower in leaves and NO_3-N and DOC:DON ratio in stems. Phosphate concentrations showed no significant difference between vegetation for the stem and leaf and flower concentrations between grave and reference vegetation at either facility. This may indicate that phosphate does not play a role in the odor detection by HRD dogs. FACTS only showed a significant difference in the CDI plant leaves NH_4-N

being lower than control plants and the stem DOC:DON ratio being slightly higher. Nitrogen species did not carry consistency between facilities even though the dogs were fairly consistent in accuracy.

Ulmus alata

Commonly referred to as the winged elm, it is a hardy small to medium sized tree (13 x 13 m) endemic to the southeastern and south-central United States. HRD dogs were able to identify the CDI plant from control plants with 71% accuracy. This particular tree is noted by handlers as eliciting alert responses from trained HRD dogs' downslope of buried human remains (personal observation). DOC was observed significantly higher in both the stems and leaves of the CDI plant compared to control plants as well as DON in the leaves, however, DOC:DON ratio was lower in the leaves. Nitrate-N was significantly higher in both leaves and stems of CDI plants than in control plants, indicating the plant may be transporting and utilizing for photosynthesis.

Juniperus ashei

Commonly referred to as ash juniper or mountain cedar, this drought tolerant shallow rooted evergreen is native to central Texas. This tree is known for its extensive consumption of water. HRD dogs found the CDI plant from control plants with only 52% accuracy. The aromatics of the juniper may have also interfered with odor detection, though of the seven dogs, one dog did identify the correct plant on each presentation for 100% accuracy. Though cedar trees have also been implicated by handlers in involving HRD dog alerts (Shaffer, 2010) they may have been of a different species and the observation was at a cutting at the fresh stump of the tree and at the fresh

cut end of the tree after the tree had been cut and removed from the search area. Wood cuttings of this tree were not available for analysis. The only significant differences seen in chemical analysis were in DON which had significantly higher concentrations in the juniper needles of CDI plants and ammonium-N in the stems which was significantly higher in CDI plants compared to the control plants.

A caveat of this study is that unlike a clandestine grave or body dump site, the skeletons are collected at both facilities once the cadavers have skeletonized to conserve the bones for further study. This means that there is no subsequent leaching of minerals from the bones into the soil as would be the case in a real grave. Phosphate concentrations tended to support that as few differences were found between CDI cuttings and control cuttings. This study was a preliminary investigation to determine if there was any merit in further investigations of plant chemistries as a confirmatory tool for HRD dog trained final response. Consequently, a caveat of these findings is the small sample size. Further research with investigation of other plant species will give a better understanding of the potential of this analysis as a confirmatory tool.

Summary

Further research should focus on replication and establishing well defined patterns of nutrient use by various types of plant within the CDI being studied. Developing easily utilized confirmatory tools that increase the efficiency of law enforcement when investigating a potential clandestine grave is a desired outcome.

HRD dogs were able to detect grave CDI plants from control plants in 5 of the 6 clippings tested with above chance results. This indicates that something being taken up

by the plant is still in a form readily identifiable by the HRD dogs with an above chance accuracy supporting anecdotal findings by handlers of their dogs alerting on trees near gravesites. The phenomenon that makes this detection possible is still unknown.

CHAPTER VI

SUMMARY

The objectives for the dissertation research developed from the core experiment on determining if a method for determining PMI from soil chemistry could be modeled for use on varying soils in varying stages of decomposition. Further the objectives that developed from the PMI research included 1) examining the capabilities of HRD dogs on buried human remains in contrasting soil types, 2) determining the efficacy of HRD dogs on residual odor from body burns and 3) examining the viability of CDI plants for determining potential clandestine grave sites through chemistry analysis and HRD dog testing.

My research verified that soil texture could have use in estimating difficulty of detection levels for HRD dogs. Soil texture may be a useful tool for law enforcement, search managers and HRD dog teams to best determine strategies that will lead to successful outcomes for HRD dogs in conditions where the remains are buried.

My research also showed that properly trained and credentialed HRD dogs could accurately identify soils up to 667 days post body removal with 85.7% accuracy. Further investigation is needed to determine the maximum range of time HRD dogs can detect the odor of human remains in soil from the CDI. Soils (3g) which had been extracted and even soil solution (20g) itself were readily identifiable by the HRD dogs. The use of CDI soil for training materials with proper handling, storage and negative comparative soil samples has future promise especially in states where it is difficult for handlers and trainers to obtain access to human remains.

Unfortunately, no significant and reliable patterns were detected between soil chemistry and PMI although significant differences were observed between control soils and CDI soils. However, there may be promise in development of a presumptive test for human versus animal decomposition sites with further research isolating the differences.

My most interesting finding was the success of the HRD dogs in detecting the grave CDI plants from control plants in 5 of the 6 clippings tested with above chance results. This leads support that something water soluble is being taken up by the plant in a still identifiable form for the HRD dog to identify, however, at this date this phenomenon is still not understood. Further research may produce not only answers to the questions raised but improve the training and thereby performance of HRD dogs everywhere in the future.

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APPENDIX A

Appendix A. Results from two tests on one weekend with 6 HRD dogs with one test on clayey soil and one on sandy soil, sampled for accuracy (C-correct, FP-false positive, FN-false negative) and time. Dog TFR and handler called TFR recorded separately.

	Site:	clay												
	1	Time	TFR	2	Time	TFR	3	Time	TFR	4	Time	TFR	5	TIme	TFR
Dog 1	Blank	33.24	FP	source	17.27	C	source	45.86	FP	blank	2.47	C	source	8.78	C
Dog 2	blank	60	C	source	34.39	C	blank	48.38	C	source	60	C	source	39.78	C
Dog 3	source	60	C	blank	60	C	source	60	C	blank	28.81	FP	source	60	C
Dog 4	source	57.6	C	source	34.54	C	blank	30.09	C	source	60	C	blank	60	C
Dog 5	blank	60	C	blank	60	C	source	31.74	C	source	36.39	C	source	45.02	C
Dog 6	source	47.68	C	blank	42.54	C	source	60	C	blank	60	C	source	60	C
	Site:	sand													
	1	Time	TFR	2	Time	TFR	3	Time	TFR	4	Time	TFR	5	TIme	TFR
Dog 1	blank	53.94	C	source	22.1	C	blank	60	C	source	30.97	C	source	6.4	C
Dog 2	blank	60	C	source	38.89	C	source	60	C	blank	60	C	source	37.55	C
Dog 3	source	36.2	C	blank	20.3	FP	source	60	C	blank	60	C	source	33.92	C
Dog 4	blank	60	C	source	60	C	source	14.82	C	source	12.5	C	blank	60	C
Dog 5	Blank	60	C	source	60	C	source	22.57	C	blank	60	C	source	60	FN
Dog 6	source	34.88	C	blank	37.1	C	source	29.25	C	source	56.39	C	blank	60	C

	Dog 1	clay Time	TFR	2	Time	TFR	3	Time	TFR	4	Time	TFR	5	Time	TFR
Dog 1	Blank	31	FP	source	14.2	C	source	32.5	C	blank	58	C	source	8	C
Dog 2	blank	47	C	source	33.4	C	blank	24	C	source	41.3	C	source	37.6	C
Dog 3	source	57.5	C	blank	60	C	source	46.3	C	blank	26	C	source	44.8	C
Dog 4	source	42.4	C	source	31.4	C	blank	24	C	source	41.8	C	blank	22	C
Dog 5	blank	25	C	blank	24	C	source	29.9	C	source	35.1	C	source	40.7	C
Dog 6	source	45.4	C	blank	37	C	source	48.7	C	blank	20	C	source	51.1	C
	Dog 1	sand Time	TFR	2	Time	TFR	3	Time	TFR	4	Time	TFR	5	Time	TFR
Dog 1	blank	21	C	source	11.1	C	blank	25	C	source	21.2	C	source	6	C
Dog 2	blank	34	C	source	22.3	C	source	28.4	C	blank	28	C	source	18.4	C
Dog 3	source	15.3	C	blank	24	C	source	28.7	C	blank	58	C	source	28.5	C
Dog 4	blank	22	C	source	10.6	C	source	10.8	C	source	11.7	C	blank	56	C
Dog 5	Blank	29	C	source	20.7	C	source	22.2	C	blank	23	C	source	28.5	C
Dog 6	source	23.9	C	blank	16	C	source	23.4	C	source	37.6	C	blank	42	C

APPENDIX B

Appendix B. The results of 800 trials for hrd dog recognition of human remains residual odor in soil samples with time to achieve a trained final response (TFR) and accuracy of response recorded (C-correct, FP-false negative, FP-false positive). *The results from Dog 1, session 3 were not included in the statistical analysis due to dog's refusal to work due to potential injury.

		Dog 1		Dog 2		Dog 3		Dog 4		Dog 5		Dog 6		Dog 7		Dog 8	
Session	Trial	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time
1	1	C	37.5	C	20.3	FP	21.0	C	25.2	C	29.3	C	10.1	C	18.4	C	8.2
1	2	C	28.1	C	21.1	C	29.3	FN	1.00.00	C	7.6	C	8.0	FP	8.0	C	16.2
1	3	C	38.6	C	16.4	FP	25.9	C	26.6	FN	42.0	C	36.0	C	22.4	FN	1.00.00
1	4	C	57.5	C	26.0	C	44.7	C	18.6	C	43.1	C	11.6	C	6.8	C	6.9
1	5	C	41.8	C	28.3	C	48.5	C	16.9	C	8.7	C	36.7	C	7.1	C	1.00.00
1	6	C	20.0	C	31.7	C	17.0	C	21.9	C	8.5	C	14.9	C	18.2	C	40.8
1	7	FN	1.00.00	FN	1.00.00	C	26.8	C	30.1	C	23.6	C	16.9	C	6.1	C	1.00.00
1	8	FN	1.00.00	FP	33.5	C	8.2	C	9.6	C	32.8	C	5.0	FP	12.6	C	28.0
1	9	C	40.9	C	25.1	C	27.6	C	17.7	C	11.6	C	11.7	C	10.5	C	49.3
1	10	C	34.5	C	43.4	C	21.4	C	31.4	C	33.8	C	7.2	C	11.0	C	1.00.00
2	1	C	24.5	C	19.9	C	13.8	C	5.1	FP	12.2	C	25.5	C	6.7	C	19.7
2	2	C	20.7	C	34.6	C	26.0	C	12.4	C	8.3	FN	14.0	C	11.5	C	10.9
2	3	C	59.4	C	12.1	C	15.3	C	6.3	FP	19.0	C	24.9	C	10.6	C	11.4
2	4	C	15.8	C	21.2	C	5.7	C	12.6	C	38.4	C	19.2	C	7.9	FP	10.7
2	5	C	17.4	C	28.5	C	18.0	C	7.1	C	11.1	C	9.8	C	7.7	C	22.0
2	6	C	12.0	C	16.9	C	9.1	C	11.2	C	7.9	C	51.1	FP	21.4	FN	28.4
2	7	C	1.00.00	C	46.1	C	6.7	C	16.5	C	12.2	FP	30.7	C	14.0	C	34.7

		Dog 1		Dog 2		Dog 3		Dog 4		Dog 5		Dog 6		Dog 7		Dog 8	
Session	Trial	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time
2	8	C	36.6	C	17.3	C	16.5	C	20.8	C	13.0	C	27.7	C	11.4	C	17.5
2	9	C	25.4	C	25.0	C	4.3	C	6.5	C	7.5	C	17.0	FP	6.5	C	6.5
2	10	C	39.0	C	16.8	C	9.9	C	8.6	C	9.1	C	16.1	C	50.1	C	6.4
3	1	FP	7.2	C	31.4	C	24.4	FP	1.00.00	C	7.7	C	26.5	FP	8.6	C	22.4
3	2	C	44.2	C	30.9	C	7.3	C	10.3	FP	6.3	C	11.3	C	17.5	C	4.3
3	3	FN	1.00.00	FN	1.00.00	C	18.8	FN	1.00.00	C	11.3	C	23.5	C	15.6	C	50.4
3	4	FN	1.00.00	FP	1.00.00	FP	13.6	FP	38.8	C	11.0	C	8.8	C	26.9	C	18.9
3	5	FN	1.00.00	C	52.0	FP	17.5	C	7.7	C	10.8	C	5.3	C	8.8	C	12.9
3	6	FN	48.3	C	16.1	C	5.7	C	31.1	FP	7.1	C	43.6	C	6.7	FP	13.3
3	7	FN	1.00.00	C	18.0	C	12.1	C	34.9	C	16.3	C	12.2	FP	10.6	C	13.5
3	8	FN	1.00.00	C	16.0	C	25.6	C	26.6	C	37.0	C	13.6	C	16.8	C	20.0
3	9	FN	1.00.00	C	49.4	C	28.4	FP	49.2	C	12.4	C	26.8	C	10.0	C	29.1
3	10	FP	24.1	C	18.3	C	11.3	C	7.0	C	8.0	C	20.0	C	8.2	C	24.3
4	1	C	11.0	FN	1.00.00	C	38.3	FP	26.1	C	8.4	C	19.6	C	8.7	C	7.6
4	2	C	19.2	C	1.00.00	C	30.1	C	52.8	C	14.9	C	21.3	C	10.3	C	5.9
4	3	C	34.8	C	49.9	C	11.9	C	1.00.00	C	17.5	C	11.8	C	7.3	C	8.7
4	4	C	36.1	C	41.8	C	17.5	C	11.0	C	10.5	C	27.3	C	7.3	C	9.7
4	5	C	17.1	C	30.3	C	13.8	C	34.1	C	8.0	C	23.1	C	7.8	C	17.2
4	6	C	39.8	FN	1.00.00	C	5.8	C	15.5	C	5.8	C	13.0	C	8.0	C	8.0
4	7	C	11.0	C	58.8	C	21.8	C	8.0	C	8.5	C	35.5	C	6.8	C	14.9
4	8	C	34.8	C	56.4	C	1.00.00	C	7.1	C	9.8	C	43.3	C	9.3	C	12.6
4	9	C	11.4	C	53.3	C	15.7	C	14.2	C	15.2	C	6.3	C	5.6	C	30.3
4	10	C	29.4	C	45.6	C	30.1	C	20.9	C	14.7	C	21.8	C	5.6	C	21.8
5	1	C	14.1	C	10.2	C	42.7	C	9.0	FP	6.2	C	9.9	C	6.3	C	13.6

		Dog 1		Dog 2		Dog 3		Dog 4		Dog 5		Dog 6		Dog 7		Dog 8	
Session	Trial	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time
5	2	C	17.1	FP	7.1	C	6.3	C	5.1	C	17.7	C	5.6	C	4.5	C	11.6
5	3	FN	37.6	FP	20.2	C	21.1	C	17.2	C	12.1	C	8.8	C	7.5	C	19.5
5	4	C	53.9	C	36.8	C	12.3	C	22.7	C	13.8	C	5.0	C	10.4	C	10.5
5	5	C	28.7	C	17.5	C	17.7	C	5.8	C	8.3	C	5.4	FP	10.7	C	15.6
5	6	C	31.0	C	13.2	C	5.8	C	6.7	C	16.7	C	5.8	C	6.5	C	16.2
5	7	C	56.1	C	36.4	C	18.6	C	12.3	C	25.1	FP	4.7	C	10.0	C	8.3
5	8	C	19.1	C	18.6	C	6.1	C	7.0	C	13.0	C	4.5	C	7.1	C	6.7
5	9	C	25.6	C	15.6	C	11.3	C	7.2	C	13.5	C	7.5	C	8.0	C	18.4
5	10	C	23.5	C	6.4	C	6.2	C	7.9	C	11.8		5.8	C	5.1	C	6.3
6	1	FP	21.7	C	13.8	C	9.1	C	29.5	C	23.7	C	11.2	C	8.2	C	14.7
6	2	C	18.3	C	8.6	C	8.3	C	5.6	C	8.2	C	4.9	C	7.7	C	17.0
6	3	C	26.9	C	10.7	C	7.3	C	6.1	C	11.6	C	6.0	C	7.4	C	26.6
6	4	C	48.1	C	10.2	C	5.5	C	7.8	C	28.1	C	25.5	C	6.6	C	10.9
6	5	C	28.2	C	15.0	C	12.0	C	6.1	C	6.9	C	10.6	C	9.0	C	5.8
6	6	C	12.6	C	20.8	FP	26.6	C	7.8	C	6.5	C	6.6	C	16.9	FP	14.7
6	7	C	38.4	C	11.7	C	16.9	C	13.2	C	9.3	C	4.3	C	6.2	C	26.5
6	8	C	48.3	C	22.6	C	18.3	C	14.1	C	8.9	C	4.8	C	9.3	C	11.1
6	9	C	22.9	C	21.6	C	6.4	C	12.4	C	19.6	C	6.6	C	12.0	C	9.7
6	10	C	30.0	C	14.5	C	4.5	C	26.5	C	4.7	C	20.0	C	5.7	C	9.0
7	1	C	36.3	C	26.3	C	8.7	C	10.7	C	6.7	C	10.8	C	11.6	C	25.9
7	2	C	29.8	C	12.9	C	13.1	C	5.5	C	4.8	C	5.8	C	10.1	C	13.7
7	3	C	41.5	C	12.6	C	8.0	C	5.8	C	11.1	C	9.8	C	8.0	C	14.4
7	4	C	58.8	C	33.2	C	4.2	C	4.5	C	10.9	C	6.1	C	10.9	C	13.1

		Dog 1		Dog 2		Dog 3		Dog 4		Dog 5		Dog 6		Dog 7		Dog 8	
Session	Trial	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time
7	5	C	16.2	C	11.8	C	18.9	C	3.9	C	9.1	C	34.5	C	7.5	C	14.1
7	6	C	23.4	C	18.1	C	2.8	C	14.6	C	8.7	C	5.3	C	5.5	C	17.6
7	7	C	39.7	C	9.4	C	9.7	C	5.6	C	7.3	C	10.1	C	5.5	C	10.9
7	8	C	16.7	C	11.4	C	4.2	C	5.2	C	7.5	C	4.3	C	8.6	C	7.2
7	9	C	37.8	C	11.2	C	5.6	C	6.5	C	7.3	C	3.6	C	11.0	C	11.1
7	10	C	53.8	C	7.9	C	4.0	C	7.8	C	9.8	FP	11.8	C	10.2	C	8.3
8	1	C	19.75	C	13.0	C	13.1	C	7.5	C	11.9	C	3.2	C	7.1	C	7.6
8	2	C	17.84	FN	33.1	FN	18.3	C	42.1	C	39.7	C	7.0	FN	9.8	C	15.8
8	3	FP	1.00.00	C	10.5	FN	13.2	FN	12.4	C	13.9	C	3.2	C	8.8	C	10.3
8	4	C	50.56	C	1.00.00	C	6.8	C	48.3	C	7.4	FP	5.7	C	6.9	C	11.8
8	5	FN	53.9	FP	11.2	FP	16.9	FP	31.8	C	5.3	C	12.7	FP	13.3	FP	10.1
8	6	C	18.22	C	19.8	C	14.5	C	40.4	C	30.0	C	36.6	C	8.7	C	7.9
8	7	C	29.71	C	9.6	C	6.8	C	11.9	C	5.7	C	16.9	FP	7.1	C	6.3
8	8	FP	54.54	C	40.8	C	21.2	FN	1.00.00	C	24.8	C	11.7	C	8.9	C	20.0
8	9	C	39.62	C	12.8	C	9.7	C	16.1	C	23.7	FP	6.8	C	11.0	C	9.2
8	10	C	49.2	C	25.4	FN	20.1	C	1.00.00	C	1.00.00	C	7.8	C	34.7	C	12.8
9	1	C	25.7	C	8.6	C	7.4	C	7.4	C	4.5	C	9.5	C	15.2	C	8.6
9	2	C	18.0	C	26.2	C	24.0	C	24.0	FP	40.5	C	14.5	C	10.2	C	16.8
9	3	C	12.2	C	12.5	C	6.8	C	6.8	C	12.1	C	9.9	C	9.0	C	14.2
9	4	C	14.9	C	23.1	C	25.9	C	25.9	C	14.5	C	16.8	C	5.6	C	18.4
9	5	FP	19.0	C	11.0	C	5.0	C	5.0	C	4.6	C	17.8	C	4.5	C	14.8
9	6	C	55.9	C	9.2	C	5.7	C	5.7	C	6.0	FP	17.9	C	5.3	C	19.9
9	7	C	14.7	C	18.0	C	51.3	C	51.3	C	3.8	C	11.4	FP	25.5	C	17.7
9	8	FP	54.3	C	14.4	C	6.1	C	6.1	C	29.9	C	7.8	C	5.9	C	16.2

		Dog 1		Dog 2		Dog 3		Dog 4		Dog 5		Dog 6		Dog 7		Dog 8	
Session	Trial	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time	TFR	Time
9	9	C	14.0	C	6.5	C	5.4	C	5.4	C	7.1	C	15.3	C	6.3	C	8.6
9	10	FP	1.00.00	C	21.8	C	15.1	C	15.1	C	21.7	C	8.2	C	4.6	C	4.7
10	1	C	15.0	C	7.6	C	3.6	C	45.7	C	10.6	C	11.1	C	5.6	C	8.4
10	2	C	29.4	C	11.4	C	10.4	C	7.4	C	10.5	C	7.7	C	13.8	C	15.9
10	3	C	20.1	C	10.5	C	3.3	C	5.2	C	9.0	C	4.9	C	8.9	C	8.8
10	4	C	41.3	C	13.5	C	4.6	C	4.8	C	19.7	C	13.6	C	10.5	C	20.3
10	5	C	37.0	C	6.1	FP	1.00.00	C	6.8	C	5.9	C	15.2	C	6.4	C	6.9
10	6	C	48.0	C	7.4	C	2.8	C	7.1	C	9.9	C	11.3	C	5.4	C	9.5
10	7	C	48.5	C	13.1	C	4.6	C	9.6	C	23.5	C	12.5	C	11.0	C	7.1
10	8	C	14.9	C	6.7	C	5.0	C	6.4	C	14.8	C	7.5	C	40.0	C	5.7
10	9	C	27.7	C	10.8	C	14.1	C	16.5	C	13.0	C	18.3	C	10.4	C	16.4
10	10	C	18.5	C	6.4	C	3.7	C	17.7	C	7.0	C	7.0	C	6.6	C	6.3

APPENDIX C

Appendix C. Results from soil core samples from beneath decomposing human remains in soil chemistry for ammonium-N, nitrate-N, phosphate-P, dissolved organic carbon, total nitrogen, pH and electrical conductivity according to post mortem interval (PMI) or accumulated degree day (ADD) at both FACTS and STAFS.

SITE	PMI	ADD	NO_3 (mg g-1)	NH_4 (mg g-1)	PO_4 (mg g-1)	DOC (mg g-1)	TN (mg g-1)	EC (dS m-1)	pH
STAFS	570	10368	18.8	29.8	5.1	199.2	73.2	50	5.98
STAFS	619	11441	-0.8	28.7	0.9	332.2	104.6	20	5.91
STAFS	679	13068	6.9	6.8	1.0	218.3	26.4	30	5.62
STAFS	738	14929	-1.9	1.4	0.8	25.5	15.1	30	6.12
STAFS	770	15832	-3.0	-2.4	-0.3	-56.4	0.2	20	6.83
STAFS	804	16569	1.0	1.3	-0.3	-0.8	16.0	20	7.11
STAFS	857	17328	2.3	4.0	0.8	59.9	27.3	20	6.6
STAFS	891	17757	0.6	4.2	8.9	6.5	15.3	20	6.84
STAFS	913	18030	-0.5	9.8	6.7	71.2	15.1	20	7.34
STAFS	931	18326	3.9	1.7	1.3	31.0	16.0	20	6.98
STAFS	952	18741	2.6	1.1	0.5	-3.8	11.5	20	7.49
STAFS	989	19527	-0.6	41.6	4.1	729.9	74.4	29	6.40
STAFS	1045	20735	-0.3	-2.0	-0.5	16.1	-6.6	20	6.07
STAFS	1078	21688	1.1	2.6	0.1	239.9	1.2	60	5.62
STAFS	1099	22296	0.4	0.9	-0.5	79.5	-3.4	30	5.53
STAFS	1136	23292	0.4	2.7	-0.5	19.2	-4.5	30	6
STAFS	1176	24106	0.3	1.6	-0.5	11.2	5.0	30	5.86
STAFS	1213	24658	-0.8	1.8	-0.1	27.0	0.3	30	5.78
STAFS	357	7001	89.4	88.4	20.7	724.8	189.2	160	5.67
STAFS	450	8028	-2.0	0.8	0.9	490.8	11.0	40	8.61
STAFS	499	9101	5.0	6.0	2.6	354.2	39.2	40	5.36
STAFS	559	10728	-1.2	14.8	9.3	500.2	102.3	40	5.91
STAFS	618	12589	-0.3	75.6	2.4	647.6	116.1	90	6.37
STAFS	650	13492	1.7	9.6	4.4	159.8	33.6	160	6.84

SITE	PMI	ADD	NO_3 (mg g-1)	NH_4 (mg g-1)	PO_4 (mg g-1)	DOC (mg g-1)	TN (mg g-1)	EC (dS m-1)	pH
STAFS	684	14229	2.4	24.4	4.3	254.3	58.0	40	6.71
STAFS	337	6733	-0.7	33.5	3.7	746.4	84.3	47	5.64
STAFS	392	7368	39.9	38.9	0.4	594.2	102.1	90	6.2
STAFS	317	6671	16.6	15.6	2.5	2077.1	101.9	170	5.2
STAFS	410	7698	30.0	102.8	33.6	844.6	162.9	120	5.54
STAFS	297	6403	-4.0	-2.6	-1.3	2164.8	57.8	98	4.21
STAFS	361	7103	44.9	43.9	15.4	563.7	107.3	90	5.84
STAFS	459	8771	27.2	69.4	6.0	602.0	123.9	110	5.85
STAFS	519	10398	38.4	28.7	4.5	-86.6	1.8	90	5.93
STAFS	578	12259	-2.5	6.0	0.2	136.9	28.4	50	6.57
STAFS	644	13899	-1.2	6.8	0.4	316.0	44.2	40	6.43
STAFS	697	14685	-2.2	-0.5	-0.6	186.8	23.8	30	6.44
STAFS	731	15087	-1.6	0.9	-1.5	288.3	22.0	20	6.79
STAFS	753	15360	2.7	-0.9	-0.1	-13.2	6.0	20	7.35
STAFS	771	15657	-0.4	-1.7	-0.5	118.0	14.9	20	6.71
STAFS	792	16071	-0.8	-1.3	-0.4	117.9	13.0	20	6.9
STAFS	829	16857	-2.6	2.1	-0.2	137.2	5.8	32	6.35
STAFS	885	18065	-0.5	-1.8	48.4	511.2	12.1	60	5.75
STAFS	918	18997	-0.1	1.4	-1.0	288.8	5.0	70	5.86
STAFS	939	19622	1.0	10.2	0.1	148.8	0.1	30	5.67
STAFS	976	20618	0.4	9.0	-0.5	39.4	-6.1	30	5.79
STAFS	1016	21431	0.4	9.0	-0.5	362.2	14.7	60	6
STAFS	1053	21983	-0.2	7.9	-1.0	173.6	0.2	50	6.03
STAFS	1099	22045	0.2	8.5	-0.7	90.7	-8.3	300	6.04
STAFS	18	346	-2.8	117.7	8.7	5523.9	157.5	180	4.55
STAFS	53	1133	-2.5	12.4	20.0	4612.8	154.4	560	5.15
STAFS	113	2761	160.9	116.2	52.5	869.7	651.6	50	6.82
STAFS	172	4572	2.8	18.2	5.5	102.1	16.7	70	6.65
STAFS	204	5524	0.9	3.2	3.6	8.0	14.5	40	6.63
STAFS	238	6262	2.5	11.2	15.5	183.6	38.5	30	6.42

SITE	PMI	ADD	NO_3 (mg g-1)	NH_4 (mg g-1)	PO_4 (mg g-1)	DOC (mg g-1)	TN (mg g-1)	EC (dS m-1)	pH
STAFS	291	7021	0.0	3.5	16.7	47.6	24.4	20	6.66
STAFS	325	7449	1.7	0.2	-0.4	-12.7	6.0	30	6.65
STAFS	347	7717	-0.9	-2.7	0.6	212.7	8.3	30	6.23
STAFS	365	8013	1.1	-0.2	2.2	27.7	12.7	30	7.03
STAFS	386	8428	3.9	-0.2	2.7	-29.0	11.5	51	6.92
STAFS	423	9214	1.4	-0.5	0.3	46.2	10.7	200	6.18
STAFS	479	10422	0.7	-1.3	-0.6	-40.1	-10.0	40	6.69
STAFS	513	11354	2.0	1.8	0.9	-6.6	-9.3	40	6.2
STAFS	533	11979	0.7	4.4	-0.2	66.2	-4.4	30	5.82
STAFS	570	12974	0.2	8.6	-0.7	-14.7	-13.3	40	6.23
STAFS	610	13788	2.5	12.8	1.4	54.7	-4.4	30	5.36
STAFS	647	14340	0.6	9.4	-0.3	8.6	-5.7	40	6.36
STAFS	693	15279		9.5	-0.2	-3.6	-8.3		6.27
FACTS	348	8129	464.9	-6.4	64.2	9071.6	1270.6	460	6.7
FACTS	457	8943	281.9	-7.8	87.7	2773.2	507.7	680	5.5
FACTS	492	9604	333.2	-7.9	156.2	5345.7	692.1	460	5.4
FACTS	513	10548	220.3	28.7	78.0	1722.6	272.5	430	5.4
FACTS	597	11146	112.4	-5.6	25.8	1326.3	207.2	200	6.6
FACTS	639	13451	121.1	-7.9	-1.0	1122.5	173.7	230	6.5
FACTS	672	14777	18.4	-8.9	10.5	1209.5	188.0	870	6.7
FACTS	196	4001	463.4	-7.5	29.4	5769.3	926.1	523	6.3
FACTS	235	4543	558.7	-7.2	46.8	5687.7	1019.3	520	7.1
FACTS	276	5358	457.9	3.6	12.2	3134.5	647.6	510	6.6
FACTS	305	6019	276.7	-7.8	28.9	1879.9	392.1	430	6.2
FACTS	361	6963	263.4	285.6	22.5	1300.8	203.0	210	6.8
FACTS	340	7560	353.1	105.6	9.9	2568.8	585.2	460	6.2
FACTS	445	9866	6.0	21.8	21.1	1290.0	201.3	170	6.4
FACTS	487	10677	24.2	-6.0	-1.8	1116.1	172.6	170	6.4
FACTS	551	11447	28.7	-7.9	23.9	1312.3	204.9	150	6.0
FACTS	176	3375	0.9	-6.9	-0.2	6463.7	86.4	598	6.9
FACTS	215	3918	785.1	-6.6	227.0	9262.9	1567.4	750	7.4

SITE	PMI	ADD	NO_3 (mg g-1)	NH_4 (mg g-1)	PO_4 (mg g-1)	DOC (mg g-1)	TN (mg g-1)	EC (dS m-1)	pH
FACTS	256	4732	1165.9	-0.7	331.1	9075.5	2792.6	800	7.2
FACTS	285	5393	145.2	-7.4	9.9	9288.8	372.4	820	4.9
FACTS	320	6337	1421.3	1.9	1330.3	5102.2	1795.2	1640	7.3
FACTS	341	6934	455.5	-8.6	72.8	1683.4	266.0	630	6.9
FACTS	425	9240	287.3	-5.8	116.3	2013.5	320.3	170	6.4
FACTS	467	10051	196.3	0.6	4.6	1164.8	180.6	170	6.4
FACTS	500	10566	247.2	-0.7	43.9	1463.7	229.8	650	6.7
FACTS	408	8122	565.9	45.5	15.6	828.0	670.2	661	7.9
FACTS	447	49099	783.0	500.2	85.9	2789.7	1326.4	690	7.1
FACTS	488	90117	119.6	-8.9	30.4	1152.7	253.7	640	6.9
FACTS	517	131164	-0.46	138.7	-1.8	676.5	217.2	320	5.7
FACTS	552	172246	212.0	-7.5	72.9	2045.9	422.9	530	6.5
FACTS	573	213349	-3.2	201.6	50.0	1509.9	237.5	380	5.9
FACTS	657	13987	-2.3	18.3	18.7	1271.9	198.3	200	6.7
FACTS	699	14798	-9.3	-8.6	0.5	1133.5	175.5	350	6.6
FACTS	732	56060	170.2	-10.5	9.5	1202.6	186.8	580	6.3
FACTS	90	1147	398.5	-2.5	55.1	846.8	458.4	500	8.7
FACTS	129	1690	460.9	6.8	87.6	2700.1	692.9	430	8.9
FACTS	170	2504	354.9	99.5	49.5	887.3	506.5	400	7.6
FACTS	199	3165	130.9	122.5	16.8	84.2	226.1	350	6.7
FACTS	234	4109	403.3	333.5	92.2	1122.9	762.6	680	7.2
FACTS	255	4706	189.4	11.6	19.7	1280.1	199.6	460	6.9
FACTS	339	7012	429.1	-8.8	11.8	1219.5	189.7	670	6.9
FACTS	381	7823	5.5	1.7	1.6	1142.4	176.9	440	6.5
FACTS	414	8337	142.6	-10.9	15.1	1244.9	193.8	480	5.8
FACTS	445	8593	200.6	4.5	15.0	1244.2	193.7	530	5.9
FACTS	96	1170	521.5	-4.8	24.4	4860.2	742.9	500	7.1
FACTS	137	1984	1302.7	-8.6	89.7	15583.9	1940.5	960	6.8
FACTS	166	2646	921.7	-8.1	152.8	9881.0	1537.6	1320	5.8
FACTS	201	3589	1705.4	-3.9	135.0	19203.0	2208.4	1240	6.6
FACTS	222	4187	911.7	-7.1	142.5	2212.3	353.1	820	6.8

SITE	PMI	ADD	NO_3 (mg g-1)	NH_4 (mg g-1)	PO_4 (mg g-1)	DOC (mg g-1)	TN (mg g-1)	EC (dS m-1)	pH
FACTS	306	6492	1264.8	-6.0	335.7	6615.0	1872.9	1340	7.5
FACTS	348	7303	916.10	-8.9	-1.8	1116.1	172.6	1120	6.9
FACTS	381	7818	68.05	-10.9	24.8	1318.7	205.9	2710	6.72
FACTS	412	8073	829.2	-9.5	31.6	1369.8	214.4	960	6.69
FACTS	43	486	18.2	-8.2	-0.2	2780.9	64.5	358	6.08
FACTS	82	1029	128.9	-7.6	36.0	7834.8	322.6	370	5.85
FACTS	123	1843	2778.6	-6.8	312.8	25431.6	3219.9	400	5.2
FACTS	152	2504	155.9	-8.2	15.7	5119.4	327.1	450	4.78
FACTS	187	3448	968.8	-6.9	24.4	8076.2	1201.8	250	5.85
FACTS	208	4046	935.6	-8.5	176.1	2468.1	395.1	930	6.74
FACTS	292	6351	537.9	-8.3	46.8	1485.9	233.5	700	6.8
FACTS	334	7162	384.7	-1.3	-1.8	1116.1	172.6	650	6.3
FACTS	367	7677	573.3	4.3	24.4	1315.0	205.4	960	5.85
FACTS	398	7932	427.6	-6.1	17.3	1261.7	196.6	500	6.68

APPENDIX D

Appendix D. Results from plant cold water extractions for dissolved organic carbon, total nitrogen, ammonium-N, nitrate-N, phosphate-P, and dissolved organic nitrogen in leaves and stems from control and grave sites at STAFS (H) and FACTS (SM).

ID	Sample and site	DOC (µg/g)	TN (µg/g)	NH_4-N (µg/g)	NO_3-N (µg/g)	PO_4-P (µg/g)	DON (µg/g)
1	control H - GW leaves	50593	5473	644	526	190	4304
2	Control H - GW stems	19661	2793	610	650	282	1533
3	Control H - Young Pine needles	45208	983	102	20	135	861
4	control H - Mature pine needles	76678	715	64	7	159	644
5	Control H - young pine stems	53063	557	56	7	187	494
6	Control H - Mature Pine stems	42435	415	57	6	150	352
7	Control H- Pine Wood	23557	227	60	6	98	160
8	Control SM - GW leaves	60437	6524	1419	10	187	5096
9	Control SM - GW stems	33831	4209	1121	18	215	3069
10	Control SM - Elm leaves	30126	1200	375	12	157	814
11	Control SM - Elm stems	30671	947	36	20	149	891
12	Control SM - Juniper leaves	83134	511	41	8	187	462
13	Control SM - Juniper stems	63402	525	49	8	195	468
14	SM HR - GW leaves	69128	6466	955	16	188	5495
15	SM HR - GW stems	37545	4134	1075	16	184	3043
16	SM HR - Elm leaves	88972	3505	76	34	195	3395
17	SM HR - Elm stems	40071	1880	43	28	191	1809

ID	Sample and site	DOC (µg/g)	TN (µg/g)	NH_4-N (µg/g)	NO_3-N (µg/g)	PO_4-P (µg/g)	DON (µg/g)
		92893	867	41	8	183	818
18	SM HR - Juniper leaves						
		58394	652	62	8	194	582
19	SM HR- Juniper stems						
		55887	3209	45	129	232	3034
20	H HR - GW leaves						
		44806	4632	97	198	251	4337
21	H HR - GW stems						
		68082	1040	355	6	49	679
22	H HR - young pine needles						
		46242	754	114	5	279	634
23	H HR - young pine stems						
	H HR - mature pine	67332	839	140	6	89	693
24	needles						
		47895	530	94	16	313	420
25	H HR - mature pine stems						
		16885	160	51	5	84	104
26	H HR - pine wood						
		45494	4780	1090	480	1293	3210
27	control H - GW leaves						
		44983	4803	1121	403	1245	3278
28	control H - GW leaves						
		15791	2343	622	498	837	1222
29	Control H - GW stems						
		14287	2253	612	621	700	1020
30	Control H - GW stems						
	Control H - Young Pine	70382	1041	318	16	20	707
31	needles						
	Control H - Young Pine	68742	1015	311	7	16	696
32	needles						
	Control H - young pine	51256	583	75	7	274	501
33	stems						
	Control H - young pine	47508	545	67	7	276	470
34	stems						
	control H - Mature pine	78010	840	130	11	94	699
35	needles						
	control H - Mature pine	65596	839	114	7	91	718
36	needles						
	Control H - Mature Pine	42538	430	69	6	178	355
37	stems						
	Control H - Mature Pine	40923	403	66	6	137	331
38	stems						

ID	Sample and site	DOC (µg/g)	TN (µg/g)	NH_4-N (µg/g)	NO_3-N (µg/g)	PO_4-P (µg/g)	DON (µg/g)
39	Control H- Pine Wood	23546	223	44	6	101	174
40	Control H- Pine Wood	23851	196	66	10	77	119
41	Control SM - GW leaves	47586	5634	1503	9	1259	4122
42	Control SM - GW leaves	53848	6447	1655	13	139	4780
43	Control SM - GW stems	26083	3622	1462	6	450	2154
44	Control SM - GW stems	26137	3459	1283	5	470	2171
45	Control SM - Elm leaves	52806	2087	669	18	310	1400
46	Control SM - Elm leaves	46040	1933	670	23	339	1240
47	Control SM - Elm stems	19100	586	95	15	130	476
48	Control SM - Elm stems	16718	304	70	18	150	216
49	Control SM - Juniper leaves	84277	470	66	8	379	396
50	Control SM - Juniper leaves	83143	403	65	7	356	331
51	Control SM - Juniper stems	49550	273	40	8	302	225
52	Control SM - Juniper stems	52133	240	31	8	288	201
53	SM HR - GW leaves	56108	6141	1223	9	136	4910
54	SM HR - GW leaves	63091	6950	1254	10	133	5685
55	SM HR - GW stems	28152	3077	1025	6	358	2046
56	SM HR - GW stems	32278	3786	1259	6	407	2521
57	SM HR - Elm leaves	78673	3290	811	39	436	2439
58	SM HR - Elm leaves	94172	4018	796	34	448	3187
59	SM HR - Elm stems	37997	1730	101	26	289	1604

ID	Sample and site	DOC (µg/g)	TN (µg/g)	NH_4-N (µg/g)	NO_3-N (µg/g)	PO_4-P (µg/g)	DON (µg/g)
60	SM HR - Elm stems	34576	1630	105	23	271	1502
61	SM HR - Juniper leaves	79043	810	56	8	351	746
62	SM HR - Juniper leaves	86661	705	56	6	361	644
63	SM HR- Juniper stems	49122	476	85	6	318	385
64	SM HR- Juniper stems	44922	486	97	6	319	382
65	H HR - GW leaves	52019	3463	113	253	887	3098
66	H HR - GW leaves	50293	3346	124	240	848	2982
67	H HR - GW stems	40712	4927	585	279	573	4063
68	H HR - GW stems	38286	4423	631	277	601	3515
69	H HR - young pine needles	69103	1153	339	14	56	800
70	H HR - young pine needles	67774	1066	328	7	43	731
71	H HR - young pine stems	42912	666	96	6	264	564
72	H HR - young pine stems	40254	635	86	6	244	543
73	H HR - mature pine needles	62611	825	131	7	89	687
74	H HR - mature pine needles	59178	823	133	7	80	684
75	H HR - mature pine stems	47358	482	96	6	322	379
76	H HR - mature pine stems	47067	473	97	6	335	370
77	H HR - pine wood	17699	156	49	6	96	102
78	H HR - pine wood	14343	119	46	6	66	67