U.S. Department of Justice

Office of Justice Programs

Office of the General Counsel

Washington, D.C. 20531

VIA EMAIL
john@greenewald.com

John Greenwald, Jr.
The Black Vault
[redacted]

JAN 25 2017

Re: OJP FOIA No. 16-00353

Dear Mr. Greenwald:

This letter is in response to your Freedom of Information Act/Privacy Act request, dated September 06, 2016, and received in the Office of Justice Programs (OJP), Office of the General Counsel, on September 7, 2016. A copy of your request is attached for your convenience.

Please be advised that a search has been conducted in the OJP, and one grant document, totaling 86 pages, were located that are responsive to your request. After carefully reviewing the document responsive to your request, I have determined that 75 pages are appropriate for release with excisions made pursuant to 5 U.S.C. § 552(b)(4) and 5 U.S.C. § 552(b)(6), and 11 pages are exempt from disclosure pursuant to 5 U.S.C. § 552(b)(6). Exemption (b)(4) concerns trade secrets and commercial or financial information obtained from a person that is privileged or confidential. Exemption (b)(6) concerns material the release of which would constitute a clearly unwarranted invasion of the personal privacy of third parties. This completes the processing of your request by OJP.

For your information, Congress excluded three discrete categories of law enforcement and national security records from the requirements of the FOIA. *See* 5 U.S.C. 552(c). This response is limited to those records that are subject to the requirements of the FOIA. This is a standard notification that is given to all our requesters and should not be taken as an indication that excluded records do, or do not, exist.

If you require further assistance or wish to discuss any aspect of your request, you may contact Tyra Wiseman, Contract Paralegal Specialist III, at (202) 616-3267 or OJP's FOIA Public Liaison at (202) 307-6235. Additionally, you may contact the Office of Government Information Services (OGIS) at the National Archives and Records Administration to inquire about the FOIA mediation services they offer. The contact information for OGIS is as follows: Office of Government Information Services, National Archives and Records Administration, 8601 Adelphi Road-OGIS, College Park, Maryland 20740-6001, e-mail at ogis@nara.gov; telephone at 202-741-5770; toll free at 1-877-684-6448; or facsimile at 202-741-5769.

If you are not satisfied with my response to this request, you may administratively appeal by writing to the Director, Office of Information Policy (OIP), United States Department of Justice, Suite 11050, 1425 New York Avenue, NW, Washington, DC 20530-0001, or you may submit an appeal through OIP's FOIAonline portal by creating an account on the following web site: https://foiaonline.regulations.gov/foia/action/public/home. Your appeal must be postmarked or electronically transmitted within 90 days of the date of my response to your request. If you submit your appeal by mail, both the letter and the envelope should be clearly marked "Freedom of Information Act Appeal."

Sincerely,

Rafael A. Madan
General Counsel

Attachment(s)

Parson, Russell

16-00353

Subject: FW: FOIA REQUEST

From: John Greenewald [mailto:john@greenewald.com]
Sent: Tuesday, September 06, 2016 2:04 AM
To: FOIAOJP <Service.Accountc62d1@ojp.usdoj.gov>
Subject: FOIA REQUEST

To whom it may concern,

This is a non-commercial request made under the provisions of the Freedom of Information Act 5 U.S.C. S 552. My FOIA requester status as a "representative of the news media." I am a freelance television producer often working on documentaries related to my FOIA requests, my work is commonly featured throughout major news organizations, and I freelance writer for news sites as well. Examples can be given, if needed.

I prefer electronic delivery of the requested material either via email to john@greenewald.com or via CD-ROM or DVD via postal mail. Please contact me should this FOIA request should incur a charge.

I respectfully request a copy of records, electronic or otherwise, of the grant Statement of Work and the report from the DOJ OJP grant to the University of Tennessee: A Multidisciplinary Validation Study of Non-human Animal Models for Forensic Decomposition Research, from award number 2013DNBXK037.

Thank you so much for your time, and I am very much looking forward to your response.

Sincerely,

John Greenewald, Jr.
[redacted]

Sincerely,

John Greenewald, Jr.

The Black Vault
http://www.theblackvault.com

Phone: (805) 32-VAULT

Program Narrative

Basic Scientific Research to Support Forensic Science

for Criminal Justice Purposes

NIJ SL# 001058, Announcement NIJ-2013-3362

A MULTIDISCIPLINARY VALIDATION STUDY OF NONHUMAN ANIMAL MODELS FOR FORENSIC DECOMPOSITION RESEARCH

Prepared and Submitted by

Co-PI Jennifer M. DeBruyn, PhD
Department of Biosystems Engineering & Soil Science, The University of Tennessee
2506 EJ Chapman Dr. Knoxville, TN 37996;
865-974-7277; jdebruyn@utk.edu

Lead PI Dawnie W. Steadman, PhD,
Director, Forensic Anthropology Center
Department of Anthropology
The University of Tennessee,
250 S. Stadium Hall Knoxville, TN 37996
865-974-0909; dsteadma@utk.edu

Co-PI Lee M. Jantz, PhD
Department of Anthropology,
Associate Director, Forensic Anthropology Center, The University of Tennessee
250 S. Stadium Hall Knoxville TN 37996
865-974-4408; ljantz@utk.edu

Co-PI Giovanna Vidoli, PhD
Department of Anthropology,
Assistant Director, Forensic Anthropology Center, The University of Tennessee
250 S. Stadium Hall Knoxville TN 37996
865-974-4408; gvidoli@utk.edu

Co-PI Sean M. Schaeffer, PhD
Department of Biosystems Engineering & Soil Science, The University of Tennessee
2506 EJ Chapman Dr. Knoxville, TN 37996;
865-974-7366; sschaef5@utk.edu

Consultant - (b)(6) **PhD**
Professor Emeritus of Entomology
Purdue University
West Lafayette, IN 47907-2089
(b)(6) **@purdue.edu**

March 28, 2013

Key words

Decomposition, Postmortem Interval, Animal Models, Soil, Microbiology, Isotopes

ABSTRACT

The past three decades of human decomposition have demonstrated that certain factors promote decomposition, such as high temperatures and insect access, yet the rate of decomposition varies considerably in macro- and microenvironments. The Anthropology Research Facility (ARF) at the University of Tennessee was the first to utilize human cadavers to document decomposition in a variety of controlled settings, laying the foundation for human decomposition research. Not all researchers have access to human cadavers, however, and therefore have employed nonhuman animals as surrogates for humans in decomposition studies. The results from some of these animal model studies have been used in the courts to support postmortem interval estimations of human decedents. Nonetheless, the scientific validity of substituting data from nonhuman carcasses for human cadavers has enjoyed limited evaluation. The proposed project will directly compare the decomposition dynamics of humans and two animal models often used in decomposition research – pigs and rabbits.

Human, pig and rabbit subjects will be placed simultaneously in the same ecological niche within the Anthropology Research Facility (ARF) to ensure soil and environmental conditions are identical for all subjects. Four subjects of each species will be studied in three separate trials in which season and microenvironment will vary. Our multidisciplinary data will include not only systematic visual observations of the primary decomposition stages, but also arthropod diversity on and around the subjects, microbial assortment, and chemical variation in the underlying soils. We will utilize a quantitative scoring method of gross decomposition, DNA sequencing, and mass spectrometry platforms tied to a temperature-dependent time scale, accumulated degree hours (ADH) to provide a comprehensive picture of how body size, chemistry and composition, as well as species-specific enteric bacteria, influence decomposition. Ultimately we will determine whether, in the same settings, pig and rabbits are appropriate analogs for humans in terms of decomposition research and estimates of postmortem intervals.

Well-designed validation studies are at the heart of basic scientific research and are specifically requested in the recent National Academy of Science (NAS) report as a means to elevate the scientific merit of forensic practice. Our multidisciplinary validation study of the performance of nonhuman models for decomposition research will provide the first scientific verification or refutation that pigs or rabbits can serve as proxies for human decomposition in forensic contexts. The outcomes of the study will impact how future research is conducted with regard to animal proxies as they relate to entomology, soil science, anthropology and chemistry for postmortem interval estimation. Our multidisciplinary approach has a high probability of stimulating future applied work in forensic chemistry, biology, and soil analysis in postmortem interval estimation. Our deliverables include journal articles, conference papers and lectures in the Forensic Anthropology Center's annual short courses provided to law enforcement and medicolegal personnel.

RESUBMISSION RESPONSE
A Multidisciplinary Validation Study of Nonhuman Animal Models for Forensic Decomposition Research
NIJ SL# 000994
GMS Number: 2012-90298-TN-DN
Previously Submitted April 20, 2012

The current proposal is a resubmission of GMS Number: 2012-90298-TN-DN originally submitted to the **Basic Research Solicitation** in 2012. The thoughtful critiques provided by the reviewers have resulted in an improved research design. Specifically, we have made the following changes based on the reviews:

1) Reviewers were concerned about too much variation leading to reduced statistical power. The research design now includes more replicates to control for intra- and inter-specific variation. In particular, four cadavers per species will be placed at each of three trials (compared to one subject of each species in the first submission). This will result in a total sample of twelve (12) subjects per species at the end of the study period (total N=36). This provides greater statistical robusticity to the data, including entomology, morphology, soil microbiology, and soil chemistry, and will allow us to calculate statistical error rates.
2) We were not explicit about the environment and security of the Anthropology Research Facility (ARF) so the local ecology of the ARF is described as well as the protective fencing that not only provides security to the Facility but also serves as a deterrent to terrestrial scavengers. In addition, we describe the precautions taken to prevent scavenging of the subjects, including the wrapping of hands and feet in mesh and providing a mesh cage for the rabbits.
3) The entomology methods were seen as too broad in the earlier submission. We have enlisted a new co-PI (Williams) who specializes in forensic entomology and refined the entomological data collection and analyses to maximize our understanding of species-specific cadaver attraction and succession.
4) We have better defined the roles of the principle investigators. Further, several PIs are contributing their time at no cost to the project while the research benefits from their expertise. This reflects their commitment to the project yet minimizes project costs.

In addition to these reviewer-based changes, we have conducted a pilot study over the past year and provide preliminary data on soil changes during cadaver decomposition that demonstrates the feasibility of our proposed methods.

TABLE OF CONTENTS

STATEMENT OF THE PROBLEM

Decomposition studies have been utilized in the criminal justice system to aid in human identification and provide information on time since death. Because access to human cadavers and outdoor facilities is limited, many researchers in the U.S. and throughout the world have utilized nonhuman animal carcasses as models for human cadaver decomposition. However, systematic and simultaneous validation studies comparing human and nonhuman models are lacking. This study provides a direct comparison of human and nonhuman decomposition utilizing two animal models commonly found in the literature, pigs and rabbits.

PROJECT DESIGN AND IMPLEMENTATION

Purpose, Goals, and Objectives

The National Academy of Sciences (NAS) released a report in 2009 that scrutinized whether expert testimony and forensic evidence meet the *Daubert* criteria. A major critique of forensic science that emerged in the NAS report was the lack of standards and procedures that demonstrate the validity of forensic data, especially subjective methods. A gap currently exists concerning the scientific integrity of nonhuman animal proxies for human decay rates. Therefore, the *purpose* of the proposed project is to compare decomposition processes between humans and nonhumans. The *goal* of this study is to apply both quantitative and qualitative techniques to assess the validity of two common proxies used in human decomposition studies, pig (*Sus scrofa)* and rabbit (*Oryctolagus cuniculus*) carcasses. The *forensic implication* of the proposed research project is a better understanding of the fundamental limitations of time since death estimations utilizing animal proxies. We will disseminate the research results at national meetings, peer-reviewed publications and short courses.

This study has four primary research objectives:

1. Determine if the stages and rates of decomposition vary among species – pig, rabbit and human - using the Total Body Scoring system to compare specific decomposition observations.
2. Assess differences in diversity and density of arthropod taxa around each species.
3. Quantify differences in soil chemistry and biogeochemistry in the decomposition islands around each species.
4. Determine differences between the populations of decomposing bacteria in the decomposition islands around each species.

The research objectives will be accomplished by simultaneously placing four subjects of each species – pig, rabbit and human – in the same environment at the Anthropological Research Facility (ARF). We will determine if the resulting cadaver islands of two animal species are similar to that of humans. We will conduct three trials to obtain data in different seasons and among different microenvironments of the ARF. We will assess morphological, entomological, chemical, and microbial data for each species over three study periods.

Review of Relevant Literature

The Process of Decomposition

Most published decomposition studies recognize four or five stages of decomposition for terrestrial surfaces (Reed 1958, Payne 1965, Galloway 1989, Anderson and VanLaerhoven 1996, Bass 1997, Marks et al. 2009). Marks et al. (2009), using data from human cadavers at the ARF, define these stages as: fresh, discoloration (putrefaction), bloat (active decomposition), skeletonization (advanced decomposition), and skeletal decay. The rate at which a cadaver moves from one stage to another depends upon the local environment, particularly temperature and insect access, as well the condition of the cadaver and scavenger access (Mann et al. 1990, Haglund 1997, Sorg et al. 1998, Synstelien 2007, Reeves 2009, Parmenter and MacMahon 2009, Rippley et al. 2012).

Arthropods play a critical ecological role in the decomposition of organic material. Initial colonizers will utilize the corpses as a medium for oviposition or a source of food (Kreitlow 2009). Insects typically arrive at a body within minutes postmortem (Anderson and VanLaerhoven 1996, Catts 1990, Catts and Goff 1992), and do so in a predictable sequence, known as heterotrophic succession (Smith 1986, Kreitlow 2009). Heterotrophic succession is highly linked to environmental variables as well as processes occurring within or on the corpse, such as soft tissue putrefaction and skeletal disarticulation. When the sequence of insects colonizing carrion is known for a given area under a specific set of environmental conditions, analysis of the heterotrophic succession on a carcass can be used to estimate an elapsed time since death (e.g., Early and Goff 1986, Kreitlow 2009). While some insects do not readily discriminate among corpse species, the insect succession may differ for humans and nonhuman animals based on body composition and size (Haskell et al. 2002; Schoenly et al. 2007).

Both cadaver-associated bacteria (especially enteric bacteria) and indigenous soil bacteria play important roles in decomposition (Carter and Tibbett 2006, 2008). Early in the process, fermentative activities of the enteric microflora lead to an influx of decomposition products and enteric bacteria into the local, external environment. This introduction changes soil chemistry and soil microbial activities. Hence, any estimation of the postmortem interval must account for environment, ecological factors, and intrinsic (bodily) variables that likely vary by species (Catts and Goff 1992).

Nonhuman Models for Human Decomposition

Published literature on the postmortem interval for use in forensic contexts has been hampered by the availability of human subjects. Studies on decomposition utilizing nonhuman models far outnumber those of human studies, but relatively little work has been done to validate the use of nonhuman models. Decomposition studies throughout the world have used a plethora of different carcass types and sizes, including dogs (Reed 1958), cats (Early and Goff 1986), rats (Micozzi 1986, Keiper et al. 1997, Tomberlin and Adler 1998, Carter et al. 2010), elephants (Coe 1978), monkeys (Lee and Marzuki), guinea pigs (Bornemissza 1957), sheep (Carter and Tibbett 2006), rabbits (Al-Mesbeh et al. 2012, Denno and Cothran 1976, Tessmer and Meeks 1996, Tantawai et al. 1996, Bourel et al. 1999, Adlam and Simmons 2007, Bachmann and Simmons 2010, Cross and Simmons 2010, Simmons et al. 2010a, Simmons et al. 2010b, Widya et al 2012), and pigs (Payne 1968, Goff et al. 1988, Shean et al 1993, Anderson and VanLaerhoven, Richards and Goff 1997, Carvalho et al 2000, Shalaby et al. 2000, Centeno et al 2002, Grassberger and Frank 2004, Schoenly et al. 2007, Granrud and Dabbs 2012, Gruenthal et al. 2012; Yang et al. 2012). The most recent literature has emphasized pigs and rabbits as human proxies. Simmons et al. (2010a) argue that the knowledge gained from nonhuman models can outweigh the benefits of retrospective taphonomic studies of human cadavers (e.g. Galloway et

al. 1985). While retrospective studies have limitations, the more proximate question is whether currently utilized animal models are illustrative of any aspect of human decomposition.

Despite the vast number of nonhuman animal decomposition studies, there are warnings that human and nonhuman species vary in significant ways that can strongly affect decomposition studies. For instance, Vass et al. (2008) detected significant biochemical differences between the bones of decomposed human and other mammalian species, and Carter and Tibbett (2008) outlined differences in the chemical composition of living humans, pigs, rabbits, and rats. Notter et al. (2009) found differences in adipocere formation and composition between pig and human tissues in a laboratory setting that they argue is due to the differential amount and distribution of fat between species. Arthropod predilection for certain carrion species has also been noted (Watson and Carlton 2003, 2005), as has differences in arthropod diversity among nonhuman carrion (Kuusela and Hanski 1982). Thus, arthropod variation may be significant among humans, pigs and rabbits. Recent investigations of mammalian enteric bacteria have revealed significant differences between animals based on diet (Ley et al. 2008), suggesting bacterial-mediated decomposition of omnivore and herbivore carcasses may be different. Further, carcass size plays a significant role in the decomposition process in that smaller carcasses decompose more rapidly than larger animals (Parmenter and MacMahon 2009; Simmons et al 2010b) and smaller carcasses support fewer arthropod taxa (Denno and Cothran 1976).

Catts and Goff (1992:261) argue that any animal model must closely approximate the pattern of human decomposition and cited that the "validity of extrapolating from these studies to human corpses has been questioned in court trial cases." Despite Catts and Goff's early call for validation studies, formal validation of nonhuman models has received little attention. While a few entomological studies have directly compared pig carcasses to human cadavers to validate the former's use for training and research (Haskell et al. 2002, Schoenly et al. 2007), few other human versus nonhuman species comparisons have been reported (though see Notter et al. 2009 for an *in vitro* study of adipose tissue). Further, decomposition studies integrating data from soil chemistry, microbiology, entomology, and anthropology to validate the use of nonhuman carcasses for medico-legal applications are non-existent in the current published literature. Systematic comparative studies of animals of different weight, body composition, and integument system must be conducted to evaluate whether animal models are appropriate proxies for human decomposition.

Microbiology and Biogeochemistry of Decomposition

Microorganisms play a dominant role in organic matter decomposition in general and cadaver decomposition in particular. However, they remain a "black box" in that their identity, functional processes, and successional ecology have not been elucidated. In soils below a decaying cadaver, researchers have observed increases in total carbon, nitrogen, and phosphorous (Benninger et al. 2008) as specific compounds of the body, primarily proteins, lipids, and carbohydrates (Vass et al. 1992, Dent et al. 2004, Swann et al. 2010) leach into the soil. These polymeric compounds serve as growth substrates for microbes that use extracellular enzymes to break them down into smaller molecules so they can be absorbed by the cells (Sinsabaugh et al. 2008). Indeed, there are studies that show indications of microbial activity such as elevated respiration and microbial biomass (Hopkins et al. 2000, Carter & Tibbett 2006, Haslam & Tibbett 2009) and increase in cultivatable lipolytic bacteria (Howard et al. 2010). Additionally, elevated ammonification rates and decreases in nitrogen fixation potential indicate shifts in nitrogen cycling (Hopkins et al. 2000, Moreno et al. 2011) (Appendix II Figure 1). These shifts in microbial activity are highly sensitive to the changes in temperature, soil pH, and

the availability of oxygen that occur in soils under cadavers (Vass et al. 1992, Dent et al. 2004). However, these studies offer only a limited picture; with the complexity of compounds entering the soil, the microbial response is expected to be equally (or more) complex, with many taxa directly or indirectly involved.

It is known that two main assemblages of microbes are involved in cadaver decomposition. One is the cadaver-associated microbiome, the trillions of commensal microbes associated with the body, the majority of which reside in the gastrointestinal tract. The microbial communities in mammals have similar types of bacteria at a phylum level: the vertebrate gut microbial community is dominated by Firmicutes (ca. 66%) and Bacteroides (ca. 16%) (Ley et al. 2008). The second assemblage is the soil microbes, the indigenous microorganisms that are presumed to be involved during the active decay stages. Soil microbial communities are comprised of a wide diversity of phyla, typically dominated by Proteobacteria (ca. 40%), Acidobacteria (ca. 20%), Actinobacteria (ca.13%), and Verrucomicrobia (ca. 8%) (Janssen 2006).

During the early stages of decomposition, cadaver-associated microbes (particularly bacteria from the gut and lungs) putrefy soft tissues. As decay progresses, decomposition fluids and associated microflora leach into the soil. There is evidence that the anaerobic enteric bacteria found in the soil during decomposition may be contributing to decomposition during the active decay stage and beyond (Moreno et al. 2011). Mammalian species differ in the composition of their gut microbial communities; therefore it is reasonable that these population differences may result in differential decomposition processes, especially during the early stages. A comparison of the gut microbiome of 60 mammal species by Ley et al. (2008) has provided much insight into the types of bacteria associated with different mammals. This study revealed that the microbial communities associated with a single mammal species are statistically more similar to each other than to those associated with other species. Diet is one of the strongest drivers of these differences: omnivore humans (Order: Primates) and pigs (Order: Artiodactyla) have more similar gut microbes compared to herbivore rabbits (Order: Lagomorpha) (Ley et al. 2008). Therefore, *we expect the microbial communities in the soils below humans and pigs to have more similar communities than rabbits due to the introduction of common cadaver-associated microbes*.

RESEARCH DESIGN AND METHODOLOGY

Research Question

Our central research question is whether pig or rabbit carcasses are valid proxies for human cadavers in forensic decomposition research. By monitoring decomposition of three species simultaneously, we can address whether these two commonly used species adequately represent human decomposition events from anthropological, entomological, chemical, and microbial perspectives to justify their use in forensic science research and casework.

Hypotheses and Expectations

Our project directly assesses the validity of nonhuman models, specifically pig and rabbit, as proxies for human decomposition and postmortem intervals. Therefore, our null hypothesis is quite straightforward:

H_0: Pigs and rabbits exhibit the same decomposition processes as humans.

Our research design incorporates a multidisciplinary approach to test this hypothesis. Specifically, we will use decomposition scoring, entomology, soil chemistry, and soil microbial

analysis to quantify the similarities and differences in decomposition by species. If there are no differences in the variables that contribute to decomposition then the hypothesis will be supported. If the null hypothesis is violated, we will then test a second hypothesis:

H_1: Human decomposition processes are more similar to that of pigs than rabbits.

Our project is designed to not only detect differences in decomposition processes and rates but quantify those differences to ascertain which, if either, animal model is an appropriate substitute for humans in decomposition research. Based on body mass and composition, we expect to see variation in arthropod diversity and population size among the three species. We will also examine whether certain arthropods favor one cadaver species over others. Further, we expect decomposition to occur more rapidly in the rabbits than the larger-bodied species. The same logic applies to soil chemical composition and quantity whereby the input of decomposition fluids into the soil will be similar for humans and pigs and significantly lower in the rabbit. Finally, we expect the influx of decomposition fluid and cadaver-associated microflora to result in microbial population structures and decomposition functions that are more similar between pigs and human than for the rabbit.

Research Design and Methodology

To examine the differences between human and nonhuman models for decomposition research we will conduct three controlled trial studies using pig, rabbit, and human cadavers. The ARF at the University of Tennessee, Knoxville will be the primary data collection site for this project. Entomological evidence will be studied with the assistance of project consultant (b)(6) (b)(6) Soil chemistry and microbiology data will be analyzed by specialists in the Department of Biosystems Engineering and Soil Science by co-PIs DeBruyn and Schaeffer.

Description of Sample and Placement in the ARF

This project compares four human cadavers, four domestic pig (*Sus scrofa*) carcasses, and four domestic rabbit (*Oryctolagus cuniculus*) carcasses in each of three seasonal trials. The subjects will be placed at specified locations within the ARF following a randomized block design. Surveys of the soil, fauna, and flora in the four placement areas will be conducted to document environmental conditions prior to placement of the research subjects. The first trial will begin in early February 2014, the second in June 2014, and the final trial will begin in late November 2014. Each trial will last 60-90 days in order to capture potential differences resulting from seasonal weather patterns.

The twelve cadavers from the Forensic Anthropology Center (FAC) body donation program will be selected based on: 1) known time of death within 48 hours of the target placement period; 2) natural cause of death, 3) cadaver weight between 150 to 250 lbs., and 4) not autopsied. Upon receipt of the human cadavers, recently euthanized rabbit and pig carcasses will be transported from the University of Tennessee College of Veterinary Medicine on the morning of each placement. Animals will be selected based on weight; rabbits over 6 lbs. and pigs between 100-150 lbs. The pigs will need to be transported from a farm in Henry, Tennessee. A veterinarian will provide sedation prior to euthanasi (pentobarbital 1cc/10 lbs. for each species). A University of Tennessee Institutional Animal Care and Use Committee (IACUC) Protocol Form has been submitted and is pending approval (Appendix VII).

All subjects will be photographed and weighed upon arrival at the FAC. All subjects will be refrigerated in a 38ºF morgue cooler for at least 24 hours before placement to equalize body temperature. Date of death and date of placement will be recorded for each subject.

The four human, pig and rabbit subjects within each trial will be independently placed at the ARF on the ground surface atop an open weave mesh, which allows each cadaver to remain in contact with the soil and to facilitate rolling them onto the side to sample soils below (Parkinson 2009). This mesh was used in our pilot study (described below) and was extremely effective. Subjects will be placed in the early morning hours within a newly fenced area of the ARF that has not been previously exposed to decomposing human bodies. The soil is characterized as Coghill-Corryton Complex, well-drained clay (Damann 2012). The area is naturally forested with oak, maple and hickory trees. The land in the new area of the ARF is sloped downward toward a bluff along the Tennessee River (Appendix II Figure 2). The subjects will be placed a minimum of three meters apart to maintain similar microenvironments, topography, and exposure conditions, while avoiding differences in insect diversity and succession and cross-contamination of soil among subjects (Haskell et al. 2002; Shoenly et al. 2007). In general, the cadaver decomposition island - the zone of soils where decomposition significantly changes soil biogeochemistry - only extends 20-50 cm around a cadaver (Carter 2007, 2012 pilot study [described below]). The dimensions of this new area of the ARF will allow for the 36m required to place 12 subjects three meters apart. The subjects in the first trial will be placed along a transect near the base of the slope. The second trial will take place at least five meters up-slope from the subjects in the first trial, while subjects in the third trial will be placed near the top of the slope nearest the fence (again a minimum of five meters upslope from the second trial). This placement ensures a consistent microenvironment among the subjects in each trial while maximizing the number of subjects that can be utilized. It also reduces any potential soil contamination between trials.

Temperature and Humidity Data

Climatological information is necessary to determine correlations between ambient temperatures, carrion temperatures, maggot masses, and stages of decomposition. All temperature data used in the analyses will be converted to Accumulated Degree Days (ADD) and Accumulated Degree Hours (ADH), calculated average daily and hourly temperatures, respectively, to follow standard practice in entomology (Byrd and Castner 2010) and anthropology (Vass et al. 1992, Megyesi et al. 2005, Simmons et al. 2010b). Only temperatures above 0ºC will be included in ADD calculations (Vass 2011, Megyesi et al. 2005).

Three sources of temperature and humidity data will be collected including: core body temperature, ambient temperature and humidity adjacent to the subjects, and daily mean temperatures from a nearby weather station. Core body temperatures will be collected hourly via a permanently placed rectal probe and data logger. Site-specific ambient temperature and humidity will be collected one meter above ground level adjacent to the subjects using a temperature/humidity data logger. Daily temperature, rainfall and humidity data will be collected from the University of Tennessee Agricultural Campus Weather Station located approximately 0.5 miles from the ARF.

Photographic and Observational Data

The progression of decomposition will be observed and recorded twice daily (AM and PM). Data collected will include specific decomposition characteristics of the entire body following Marks et al.'s (2009) stage approach. The Total Body System (TBS) scoring method, which evaluates tissue coloration, bloating and loss on each body segment independently, will be recorded following Megyesi et al. (2005). Megyesi et al.'s method will allow our test subjects to be compared to earlier and later published studies.

Each subject will be photographed at the time of deposition and then twice daily (am and pm) concurrent with TBS scoring using a Nikon D7000 digital camera, with a north arrow and photo scale. Photographic methods will follow established FAC protocols to ensure that total body, body regions, and unique features of interest are well documented. Photographic images will be downloaded daily to a dedicated hard drive and then uploaded to a UTK server to ensure data maintenance.

To determine whether scavenger activity differs among the subject species, six infared, motion sensing Moultrie GAMESPY i60 digital game cameras will be strategically placed to detect motion by a scavenger until the late stages of decomposition.

Entomological Sampling and Analysis

Entomological evidence will be collected from each of the subjects to compare the insect species attracted to the subjects and insect population density between human, pig and rabbit subjects. Entomological sampling and observations will be done twice daily at the times of subject photographs and TBS observations. Documentation of the following insect life events will be recorded at each observation time: adult insect arrival (flies, beetles, and other arthropods), presence of fly eggs, presence of immature insects (fly larvae, beetle larvae, and other arthropod larvae), fly larval migration, and end of fly larval migration. Adult flies will be monitored from a distance of approximately 1.5 m so as to limit disturbance to their activities.

Following observation, samples of adult flies and immature arthropods will be collected. Adult flies will be collected using aerial sweep nets. They will be transferred to 70% ethanol for preservation. Using forceps, three vials of fly larvae will be collected per subject at each collection time. Fly larvae will be killed and preserved in KAA (77% ethanol, 15% glacial acetic acid, and 8% kerosene). Samples of any other arthropods found will be preserved in 70% ethanol. All arthropods collected will be identified using taxonomic keys (Whitworth 2006; Smith 1986, Byrd and Castner 2010, Well 1999).

Soil Sampling and Analysis

Soil sampling will be concurrent with entomological data collection to minimize the effects of movement on the subjects (Adlam and Simmons 2007). Time zero soil samples will be taken immediate prior to cadaver placement. During the trial, soil samples will be collected from under each subject by rolling the cadaver to its side using the underlying mesh. Since decomposition rates are highly temperature dependent, we will use ADH to determine the sampling times. Soils will be sampled at the same time of day each time to reduce diurnal variation effects, and we will choose the sampling days closest to the following ADHs: 125, 250, 500, 1000, and 2000. These times were chosen based on our previous observations of human cadaver decomposition, and will capture each major stage of decomposition (note: 2000 ADH is estimated to be approximately 45 days in summer; 80 days in spring and fall). Five small cores will be taken from the top 5cm using 1/2" soil probes and composited. In addition, nearby control soils will be sampled in the same manner. Subsamples will be used to assess soil chemistry. Subsamples for rates (i.e., respiration, enzyme assays) will be pre-incubated at *in situ* temperatures for 48 hours to reduce the influence of the added oxygen on rate processes. Subsamples for DNA extraction will be stored at -20°C.

Soil Chemistry

Soil chemistry beneath each subject will be tracked throughout decomposition to determine how the soil chemistry environment is changing. To estimate the relative influx of decomposition fluids, we will quantify total free amino acids, typically present in low concentrations in soils, but high concentrations in decomposition fluid (Vass et al. 2002). Total free amino acids in the

extracts will be quantified using the OPAME fluorometric method (Jones et al. 2002). Gravimetric soil moisture and pH will be determined using standard measures. Soils will be shaken with 2M KCl (5:1 extractant:soil) to extract dissolved components: total dissolved carbon (DOC), total dissolved nitrogen (TON), NO_3, NH_4^+, and PO_4^{3-} concentrations will be determined on a Shimadzu TOC/TN analyzer and Dionex ICS 5000, respectively.

Soil Microbiology

The functional changes in soil microbes will be assessed using standard methods to analyze samples for microbial biomass and respiration (CO_2 production). To measure microbial biomass, we will use the chloroform fumigation-extraction slurry method (Fierer & Schimel 2003) on soils extracted in 0.5 M K_2SO_4 (4:1 extractant:soil). We will determine bacterial: fungi ratios using quantitative PCR on DNA extracts (Fierer et al. 2005) (DNA extraction described below). Microbial respiration will be measured by CO_2 production in a sealed volume and repeated sampling of headspace CO_2 using an infrared gas analyzer (LiCor Gashound). Overall C use efficiency can be calculated as biomass C produced per unit C respired.

To characterize decomposition processes with respect to utilization of decomposition substrates and byproducts, we will measure potential extracellular enzyme activities: leucine aminopeptidase and urease (degradation of proteins), phosphodiesterase and phosphatases (degradation of nucleic acids), and lipases and fatty acid esterase (degradation of lipids). Assays will be carried out using substrates with methylumbelliferyl fluor, and assayed fluorometrically as described in Sinsabaugh et al. (2008). Phosphodiesterase activities will be performed as in Carter (2010), and lipase activities will be assayed according to (Farnet et al. 2010).

Bacterial community structures will be determined for soils collected. Two previous studies have used genetic fingerprinting techniques to track soil microbial communities during decomposition (Parkinson et al. 2009, Moreno et al. 2011). However, genetic fingerprinting is limited in that it only provides information on the 100-150 most abundant taxa in a sample, and lacks the power to identify the taxa. This project will use cutting edge next generation sequencing technology to yield > 5,000 sequences per sample. This will provide in-depth information on both identity and abundance of organisms, revealing a much more complete picture of the microbes present, including the low-abundance, more rare organisms. In addition it will allow us to clearly identify the cadaver-associated microbes that enter the soils.

Bacterial 16S rRNA genes will be sequenced using 454 pyrosequencing of barcoded 16S rRNA gene amplicon libraries, as we have previously done (Wilhelm et al. 2011). This next generation sequencing method is a high throughput, massively parallel approach to sequencing, and has been successfully employed for microbial community characterization in a variety of soils (Roesch et al. 2007, Acosta-Martinez et al. 2008, Lauber et al. 2009, Acosta-Martinez et al. 2010, Campbell et al. 2010, Teixeira et al. 2010, Yergeau et al. 2012). DNA will be extracted from each soil sample using the PowerSoil DNA isolation kit (MoBio). DNA from replicate cadavers within each trial (N=4) will be pooled. The V3 to V5 region of 16S rRNA genes will be amplified using universal bacterial primers 338F and 926R (Wang & Qian 2009) with forward primers modified with 8bp barcodes unique to each sample for later sorting. Several libraries will be duplicated to assess the technical reproducibility of the methods. In addition, a "mock community" of known sequences (40 pooled cloned 16S rRNA gene sequences) will be included to assess sequence error rates. Triplicate PCR amplification runs for each sample will be pooled. Thirty cycles (maximum) will be used for amplification so that reaction remains sub-plateau and therefore quantitative. Libraries will be pooled and submitted to the University of Tennessee/Oak Ridge National Lab Joint Institute for Biological Sciences (JIBS) to be sequenced. Sequencing

will be done unidirectionally from the forward primer end on a Roche 454 FLX Genome Sequencer Instrument using titanium chemistry (400-500 nucleotide read lengths). The ca. 50 libraries (duplicate libraries plus the mock community) will be sequenced on a full 454 plate, which should yield >10,000 reads per library. Sequences will be initially processed on Roche proprietary software, available for use on a cluster housed in the Science and Engineering Research Facility at the University of Tennessee. Quality reads (>149 bp) will be sorted by barcode (i.e., station) into a multi-FASTA file. Bioinformatic tools within Mothur (Schloss et al. 2009) will be used to remove erroneous and low quality reads, according to the Schloss lab SOP (Schloss et al. 2011): this includes de-noising (i.e., re-calling bases from flowgrams), filtering based on quality scores, and preclustering sequences. Statistical tools within Mothur will then be used to identify unique sequence clusters (i.e., OTUs), calculate richness, evenness, diversity indices, phylogenetic distances, and group abundances. These tools will also allow us to do comparisons between cadavers to reveal microbial community composition differences (e.g., Ley et al. 2008).

The above measures will monitor changes in decomposition in terms of chemistry and microbiology and will be used to compare these processes between species – namely human and nonhuman animals. This will allow us to determine the validity of these nonhuman animal models in decomposition research.

PILOT STUDY – PROOF OF CONCEPTS

We conducted a pilot study in 2012 to determine soil microbial and chemical changes during human cadaver decomposition. Four cadavers were placed at the ARF on open weave mesh so that they could be rolled aside for accessing soils underneath (Appendix II Figure 3). Soils below were collected every other day during the first few weeks to capture the dynamic changes during putrefaction and active decay. Soil chemistry and microbiological parameters were measured, revealing distinct successional changes over the course of decay. In particular we observed that ammonium concentrations increase significantly during active decay, and then decrease slowly during the advanced decay and skeletal decay periods (Appendix II Figure 4). In addition, control soils taken from 1 m away had no change in chemistry, indicating that they are unaffected by the nearby decomposition island. Thus our proposed inter-subject placement distance (3 m) will ensure no cross contamination between cadaver decomposition islands. We also observed distinct successional changes in the microbial activity in the decomposition islands (Appendix II Figure 5). These data demonstrate a clear succession in soil chemistry and microbiology during human cadaver decay. What we do not know is if other species follow the same successional patterns. Despite distinct changes in microbial activity and abundances, we have not determined composition of the microbial community (which includes both soil and cadaver associated microbes). Thus the study proposed here will bring critical insight into the role of these microbial populations in decomposition. It will reveal if the differences between species' commensal microbiomes ultimately translate to differences in decomposition dynamics and provide a critical evaluation of use of animal models for human decomposition research.

MANAGEMENT PLAN AND ORGANIZATION

The proposed research project will be conducted through the FAC at the University of Tennessee, Department of Anthropology. Analyses will also be conducted in the Department of Biosystems Engineering and Soil Science on the University of Tennessee's agriculture campus.

The project is headed by the Director of the Forensic Anthropology Center (Steadman), but each co-PI has equal responsibility fulfilling project tasks such as data collection, data analysis, data interpretation and synthesizing, as well as contributing to reports and data dissemination. Each co-PI has independent experience and expertise in different aspects of forensic science which will contribute to the multidisciplinary focus of this project. The FAC Director is responsible for overseeing the timely submissions of progress reports and the dissemination of results. The Department of Anthropology has successfully completed NIJ grant 2010-DN-BX-K229 as well as six other NIJ grants that have produced multiple publications in high quality peer-reviewed journals.

IMPLICATIONS FOR CRIMINAL JUSTICE POLICY AND PRACTICE IN THE UNITED STATES

Estimating time since death and decomposition events is central to forensic science and the criminal justice system in the United States. Currently, nonhuman animal cadavers, such as pig and rabbit, are common proxies for human cadavers in decomposition research. Methods and results from nonhuman studies are extrapolated for use in forensic human identifications and criminal investigations without adequate validation to date. Determining the validity of using nonhuman models for decomposition research and applied forensic casework will provide the basis for statistically validating court testimony relating to time since death estimates. The results of our study will directly inform the medicolegal community as to the scientific value of nonhuman models for human PMI estimation. If either or both of these nonhuman animal models can function as proxies for human cadavers then it is reasonable for the courts to continue to accept the PMI estimated derived from such studies. On the other hand, if pigs and rabbits decomposition processes significantly vary from that of humans then the courts may reject the application of nonhuman animal-derived PMI estimates to human forensic cases.

The legal value of a postmortem interval estimate increases when associated with a quantifiable error rate (Christensen and Crowder 2009, Christensen 2004). According to the *Daubert v Merrell Dow (1993)* ruling, a quantifiable estimate is often required in criminal cases pending prosecution. Even though rough, observation-based estimates for the postmortem interval have been accepted in court under the "Frye Rule" (1923), only estimates that are based on scientifically derived data and can be empirically evaluated are acceptable under the *Daubert* ruling (1993). Hence, this project could legitimize anthropological, entomological, and soil chemistry time since death estimations often utilized in the criminal justice system.

Additionally, this project will provide standard data collection protocols for decomposition research, which is often seen as subjective and unscientific compared to other forensic sub-disciplines. Observed changes in decomposing cadavers are highly qualitative and subjective because they are based on the scientist's background and experience. This research will help alleviate the subjectivity of current methods. A standard for analysis will be created and longitudinal data will be provided to the scientific community for further testing. Systematic, longitudinal studies using human cadavers are necessary for the advancement of forensic science based decomposition research.

CAPABILITIES

The Forensic Anthropology Center was established with the mission to conduct innovative research in forensic anthropology, train students and law enforcement in forensic techniques, and

demonstrate the science of forensic anthropology in the general public. The FAC is managed by a Director, an Associate Director, two Assistant Directors and between 8-10 graduate student staff. The FAC oversees the body donation program, the ARF, and the Bass Donated Skeletal Collection and other collections.

Located on approximately 2.5 acres of land along a bluff overlooking the Tennessee River, the ARF is the first outdoor natural laboratory dedicated to the study of human decomposition and related subjects. Scores of researchers have conducted scientific studies at the ARF and it serves as a training site of more than 200 students and law enforcement personnel a year. Over 3000 individuals have pre-registered to donate their body to the program, and the William M. Bass Donated Skeletal Collection now contains more than 1,100 donated skeletons.

The FAC PIs are well qualified to accomplish the objectives of this project. Dr. Steadman is the Director of the Forensic Anthropology Center and will facilitate and oversee the research at the ARF. She will be responsible for interdisciplinary data management, data analysis, and writing and submitting semi-annual reports to OJP. Dr. Steadman has been the PI for National Science Foundation (NSF) and Wenner-Gren Foundation grants. Dr. Jantz is the Associate Director of the Forensic Anthropology Center and coordinates the FAC Body Donation Program. She has worked with the FAC for over two decades, has an established publication record for human decomposition research and has been successful in acquiring funding through NIJ. She will be responsible for acquisition and placement of the human research subjects and assist with data analysis. Dr. Vidoli will liaison with the Veterinary College to procure the nonhuman animal subjects, serve as the project PI for the Institutional Animal Care and Use Committee (IACUC), analyze the meteorological data and assist Dr. Steadman with the correlation analyses of the metadata.

(b)(6) will serve as the senior entomologist, supervise the graduate research assistant conducting data collection, and assist the graduate student with taxonomic identifications at the University of Tennessee. (b)(6) and the graduate student will provide the insect biodiversity analysis associated with the decomposition environment for the twelve subject species in each trial. (b)(6) has 35 years of experience in forensic entomology and training students.

Dr. Schaeffer is an expert in soil biogeochemistry and has conducted research on soil elemental transformations in a variety of ecosystems and under range of environmental conditions. He will lead the soil chemical and enzyme analyses. Dr. DeBruyn is an expert in molecular microbial ecology, researching microbial communities in both soils and aquatic systems. She will lead the molecular biology and microbial community analysis aspects of the project. Both DeBruyn and Schaeffer are early in their career, but both have an established record of publishing in high impact journals in their fields (soil science, environmental science, ecology and microbiology), and have been successful in securing outside funding from the National Science Foundation. Along with the PIs personal labs, the Biosystems Engineering & Soil Science Department has a complete wet chemistry lab available for chemical and physical analysis of soil, with a full complement of research instrumentation required for this proposed work, including analytical equipment (HPLCs, GC-MS, IC, C/N/S analyzer, spectrophotometers, etc.). In addition, the PIs have access to the University of Tennessee Institute of Agriculture Genomic Hub lab, a molecular biology facility containing all molecular biology instruments required (next gen sequencers, fluorometer and spectrophotometer, gel electrophoresis, microplate reader etc.).

OUTCOMES, EVALUATION, AND DISSEMINATION STREATEGY

Outcomes and Evaluation

This study will have a significant impact on the field in that it will provide the first comprehensive study of simultaneous decomposition of multiple species in a controlled setting. The result will be an integrated validation study of whether pigs and rabbits are adequate models of human decomposition and postmortem interval (PMI) estimation for criminal justice purposes. Further, this study will include additional, quantitative measures of decomposition to provide more objective approaches to PMI research. The broader impact of our project contributes to the efforts to quantify forensic data that have historically been subjective in nature. By incorporating soil chemistry and microbial population structure we can not only assess whether decomposition processes vary between species, but provide specific mechanisms for how they differ. Our principal outcome will be a quantitative assessment of the forensic relevance of nonhuman models.

Dissemination Strategy

The target audience for the proposed project includes forensic science researchers including anthropologists, entomologists, and soil chemists conducting time since death research and testifying in court. Forensic pathologists and local, state, and federal law enforcement agencies depending on accurate time since death estimates for identification or investigative purposes will also benefit significantly from this research. Alternatively, a potential gain is the solid refutation that studies involving animal models should be accepted in court as proxies for human decomposition.

As the significance of this research is far reaching, the results will be disseminated in journals representing forensic science, chemistry, microbiology and soil chemistry. Specifically, abstracts will be submitted to the 2015 annual American Academy of Forensic Sciences meeting and disciplinary specific conferences such as the International Society of Microbial Ecology, and Soil Science Society of America. Manuscripts will be submitted for publication to the *Journal of Forensic Sciences* and discipline-specific results will be submitted for publication to appropriate journals such as *Applied and Environmental Microbiology, Soil Biology and Biochemistry, Multidisciplinary Journal of Microbial Ecology,* and the *Journal of Medical Entomology*.

Forensic professionals, including federal, state, and local law enforcement agents and medicolegal investigators nationwide frequently attend the forensic short courses offered by the FAC. Each summer we offer a minimum of five courses that serve over 100 professionals and pre-professionals in the medicolegal community. Course participants come from agencies and universities throughout the United States and internationally. Three co-PIs are anthropology faculty members able to disseminate these results through the short course curriculum. We will bring the multidisciplinary nature of this proposed research to these courses by having co-PI DeBruyn teach about the soil chemistry and soil microbiological aspects of decomposition and co-PI Williams to instruct about the entomology component as part of the curriculum. In addition to adult education, the PIs are committed to youth outreach. Co-PI DeBruyn is a science curriculum developer for Tennessee 4-H, a youth organization of >300,000. She often uses forensic science as a vehicle for teaching STEM to youth.

Raw data stemming from the project (anthropological/morphological, microbiological, entomological and isotopic) will be accessible through the National Archive of Criminal Justice Data (NACJD) after publication of results or after (at most) two years from the completion of the project.

APPENDIX I. REFERENCES

Acosta-Martinez V, Dowd S, Sun Y, Allen V. 2008. Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. *Soil Biology and Biochemistry* 40:2762-2770.

Acosta-Martinez V, Dowd SE, Sun Y, Wester D, Allen V. 2010. Pyrosequencing analysis for characterization of soil bacterial populations as affected by an integrated livestock-cotton production system. *Applied Soil Ecology* 45:13-25.

Adlam RE, Simmons T. 2007. The effect of repeated physical disturbance on soft tissue decomposition- Are taphonomic studies an accurate reflection of decomposition. *Journal of Forensic Sciences* 52:1007-1014.

Allison SD, Vitousek PM. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry* 37:937-944.

Anderson GS, VanLaerhoven SL. 1996. Initial studies on insect succession on carrion in southwestern British Columbia. *Journal of Forensic Sciences* 41:617-625.

Bass WM. 1984. Time interval since death: A difficult decision. In: Rathbun TA and Buikstra JE, eds. *Human Identification: Case Studies in Forensic Anthropology*. Springfield, IL: CC Thomas 136-147.

Bachmann J, Simmons T. 2010. The influence of preburial insect access on the decomposition rate. *Journal of Forensic Sciences* 55(4):893-900.

Bass WM. 1997. Outdoor decomposition rates in Tennessee. In: Haglund WD and Sorg MH, eds. *Forensic Taphonomy: The Postmortem Fate of Human Remains*. Boca Raton, FL: CRC Press. 181-186.

Benninger LA, Carter DO, Forbes SL. 2008. The biochemical alteration of soil beneath a decomposing carcass. *Forensic Science International* 180:70-75.

Bornemissza GF. 1957. An analysis of arthropod succession in carrion and the effect of its decomposition of the soil fauna. *Australian Journal of Zoology* 5:1-12.

Bourel B, Martin-Bouyer L, Hedouin V, Cailliez JC, Derout D, Gosset D. 1999. Necrophilous insect succession on rabbit carrion in sand dune habitats in northern France. *Journal of Medical Entomology* 36:420- 425.

Byrd, J, and J. Castner. 2010. *Forensic Entomology: The Utility of Arthropods in Legal Investigations*, 2nd Edition. CRC Press, Inc., Boca Raton, FL.

Campbell BJ, Polson SW, Hanson TE, Mack MC, Schuur EAG. 2010. The effect of nutrient deposition on bacterial communities in Arctic tundra soil. *Environmental Microbiology* 12:1842-1854.

Carter DO, Tibbett M. 2006. Microbial decomposition of skeletal muscle tissue (*Ovis aries)* in sandy loam soil at different temperatures. *Soil Biology and Biochemistry* 38:1139-1145.

Carter, DO and M Tibbett. 2008 Cadaver decomposition and soil: processes. In*:* Tibbett M and Carter O, eds. *Soil Analysis in Forensic Taphonomy: chemical and Biological Effects of Buried Human Remains*. CRC Press. New York. 29- 52.

Carter DO, Yellowlees D, Tibbett M. 2007. Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften* 94:12-24.

Carter DO, Yellowlees D, Tibbett M. 2008. Temperature affects microbial decomposition of cadavers (*Rattus rattus*) in contrasting soils. *Applied and Environmental Microbiology* 40:129-137.

Carter DO, Yellowlees D, Tibbett M. 2010. Moisture can be the dominant environmental parameter governing cadaver decomposition in soil. *Forensic Science International* 15(1-3):60-66.

Catts EP. 1990. Analyzing entomological data. In: Catts EP and Haskell NH eds, *Entomology and Death: A Procedural Guide*. Clemson, SC: Joy's Print Shop. pp124-137.

Catts EP, Goff ML. 1992. Forensic entomology in criminal investigations. *Annual Review of Entomology* 37:253-273.

Christensen A. 2004. The impact of Daubert: implications for testimony and research in forensic anthropology (and the use of frontal sinuses in personal identification). *Journal of Forensic Sciences* 49(3):427-430.

Christensen A, Crowder CM. 2009. Evidentiary standards for forensic anthropology. *Journal of Forensic Sciences* 54(6):1211-1216.

Clarke KR, Warwick RM. 1997. *PRIMER User Manual* (Plymouth routines in multivariate ecological research). Plymouth Marine Laboratory, Plymouth, United Kingdom.

Coe M. 1978. The decomposition of elephant carcasses in the Tsavo (East) National Park, Kenya. *Journal of Arid Environments* 1:71-86.

Cross P, Simmons T. 2010. The influence of penetrative trauma on the rate of decomposition. *Journal of Forensic Sciences* 55(2):295-301.

Damann FE, Tanittaisong A, Carter DO. 2012. Potential carcass enrichment of the University of Tennessee Anthropology Research Facility: A baseline survey of edaphic features. *Forensic Science International* 222:4-10.

Daubert v. Merrell Dow Pharmaceuticals, Inc. 509 U.S. 579 (1993).

Denno RF, Cothran WR. 1976. Competitive interactions and ecological strategies of Sacrophagid and Calliphorid flies inhabiting rabbit carrion. *Annals of Entomological Society of America* 69(1):109-113.

Dent BB, Forbes SL, Stuart BH. 2004. Review of human decomposition processes in soil. *Environmental Geology* 45:576-585.

Early M, Goff ML. 1986. Arthropod succession patterns in exposed carrion on the island of O'ahu, Hawaiian Islands, USA. *Journal of Medical Entomology* 23:520-531.

Farnet AM, Qasemian L, Goujard L, Gil G, Guiral D, Ruaudel F, Ferre E. 2010. A modified method based on p-nitrophenol assay to quantify hydrolysis activities of lipases in litters. *Soil Biology and Biochemistry* 42:386-389.

Fierer N, Jackson JA, Vilgalys R, Jackson RB. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR Assays. *Applied and Environmental Microbiology* 71:4117-4120.

Fierer NS, Schimel JP. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Science Society of America Journal* 67:798.

Frye v. United States, 293 F. 1012 (C.A.D.C. 1923).

Galloway A, Birkby WH, Jones AM, Henry TE, Barks BO. 1989. Decay rates of human remains in an arid environment. *Journal of Forensic Sciences* 34(3):607-616.

Goff ML, Omori AL, Gunatilake K. 1988. Estimation of postmortem interval by arthropod succession. Three case studies from the Hawaiian Islands. *American Journal of Forensic Medicine and Pathology* 9:220-225.

Grassberger M, Frank C. 2004. Initial study of arthropod succession on pig carrion in a central European urban habitat. *Journal of Medical Entomology* 41:1210-1214.

Granrud MA, Dabbs GR. 2012. A preliminary study of incisor exfoliation as an estimator of the postmortem interval using accumulated degree days. *Forensic Science International* DOI:10.1016/j.forsciint.2012.02.020.

Gruenthal A, Moffat C, Simmons T. 2012. Differential decomposition patterns in charred versus un-charred remains. *Journal of Forensic Sciences* 57(1):12-18.

Haglund WD. 1997. Dogs and coyotes: postmortem involvement with human remains In: Haglund WD and Sorg MH, eds. *Forensic Taphonomy: The Postmortem Fate of Human Remains*. Boca Raton, FL: CRC Press. 367-379.

Haskell N, Schoenly KG, Hall RD. 2002. Testing the reliability of animal models in research and training program in forensic entomology, Part II, Final Report. NIJ192281. January 31, 2002, Award 97-IJ-CX-0046.

Haslam TCF, Tibbett M. 2009. Soils of contrasting pH affect the decomposition of buried mammalian (*Ovis aries*) skeletal muscle tissue. *Journal of Forensic Sciences* 54:900-904.

Hopkins DW, Wiltshire PEJ, Turner BD. 2000. Microbial characteristics of soils from graves: arm investigation at the interface of soil microbiology and forensic science. *Applied Soil Ecology* 14:283-288.

Howard GT, Duos B, Watson-Horzelski EJ. 2010. Characterization of the soil microbial community associated with the decomposition of a swine carcass. *International Biodeterioration and Biodegredation* 64:300-304.

Janssen PH. 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Applied and Environmental Microbiology* 72:1719-1728.

Jones DL, Owen AG, Farrar JF. 2002. Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. *Soil Biology and Biochemistry* 34:1893-1902.

Keiper JB, Chapman EG, Foote BA. 1997. Midge larvae (Diptera: Chironomidae) as indicators of postmortem submersion interval of carcasses in a woodland stream: a preliminary report. *Journal of Forensic Sciences* 42:1074-1079.

Kreitlow KL. 2009. Insect succession in a natural environment. In: Byrd JH and Castner JL, eds. *Forensic Entomology*. CRC Press Boca Raton FL. 251-267.

Kuusela S, Hanski I. 1982. The structure of carrion fly communities: the size and type of carrion. *Holarctic Ecology* 5:337-348.

Lambdin PL, Grant JF. 1999. Rare, Threatened, and Endangered (RTE) Terrestrial Invertebrate Survey. CN F40650-95-D-0006, CH2M Hill, Atlanta, GA, No. (March). 16:168

Lambdin PL, Grant JF, Wiggins GJ, Saxton A. 2003. Diversity of the true bugs (Hemiptera: Heteroptera) on Arnold Air Force Base, Tullahoma, Tennessee. *Tennessee Academy of Science* 78(3):76-84.

Lauber CL, Hamady M, Knight R, Fierer N. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75:5111-5120

Lee HL, Marzuki T. 1993. Preliminary observation of arthropods in carrion and its application to forensic entomology in Malaysia. *Tropical Biomedicine* 10:5-8.

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JI. 2008. Evolution of mammals and their gut microbes. *Science* 320:1647-1651.

Mann RW, Bass WM, Meadows L. 1990. Time since death and decomposition of the human body: variables and observations in cause and experimental field studies. *Journal of Forensic Sciences* 35(1):103–11.

Marks MK, Love JC, Dadour IR. 2009. Taphonomy and time: estimating the postmortem interval. In: Steadman DW, ed. *Hard Evidence: Case Studies in Forensic Anthropology*, 2nd Edition. Upper Saddle River, NJ: Prentice Hall. 165-178.

Megyesi MS, Nawrocki SP, Haskell NH. 2005. Using accumulated degree days to estimate the postmortem interval from decomposed human remains. *Journal of Forensic Sciences* 50(3):618–26.

Micozzi MS. 1986. Experimental study of postmortem change under field conditions: effects of freezing, thawing, and mechanical injury. *Journal of Forensic Sciences* 31:953-961.

Moreno LI, Mills D, Fetscher J, John-Williams K, Meadows-Jantz L, McCord B. 2011. The application of amplicon length heterogeneity PCR (LH-PCR) for monitoring the dynamics of soil microbial communities associated with cadaver decomposition. *Journal of Microbiological Methods* 84:388-393.

National Academy of Science. 2009. *Strengthening Forensic Science in the United States: A Path Forward*. National Academy Press, Washington, DC.

Notter SJ, Stuart BH, Rowe R, Langlois N. 2009. The initial changes of fat deposits during the decomposition of human and pig remains. *Journal of Forensic Sciences* 54(1):195-201.

O'Brien TG, Kuehner AC. 2007. Waxing grave about adipocere; soft tissue change in an aquatic context. *Journal of Forensic Sciences* 52(2):294-301.

Parkinson RA, Dias KA, Horswell J, Greenwood P, Banning N, Tibbett M, Vass AA. 2009. Microbial Community Analysis of Human Decomposition in Soil. In: Ritz K, Dawson L, Miller D, eds. *Criminal and Environmental Soil Forensics*. Springer. 379-394.

Parmenter RR, MacMahon JA. 2009. Carrion decomposition and nutrient cycling in a semiarid shrub–steppe ecosystem. *Ecological Monographs* 79(4):637-661.

Payne JA. 1965. A summer carrion study of the baby pig *Sus scrofa*. *Ecology* 46:592-602.

Reed J. 1958. A study of dog carcass communities in Tennessee, with special reference to the insects. *American Midland Nature* 59:213-245.

Reeves N. 2009. Taphonomic effects of vulture scavenging. *Journal of Forensic Sciences* 54(3):523-528.

Richards EN, Goff ML. 1997. Arthropod succession on exposed carrion in three contrasting tropical habitats on Hawaii Island, Hawaii. *Journal of Medical Entomology* 34:328-339.

Rippley A, Larison NC, Moss KE, Kelly JD, Bytheway JA. 2012. Scavenging Behavior of *Lynx rufus* on human remains during the winter months in southeast Texas. *Journal of Forensic Sciences* (available online, onlinelibrary.wiley.com).

Rodriguez WC, Bass WM. 1983. Insect activity and its relationship to decay rates of human cadavers in east Tennessee. *Journal of Forensic Sciences* 28(2):423–32.

Rodriguez WC, Bass WM. 1985. Decomposition of buried bodies and methods that may aid in their location. *Journal of Forensic Sciences* 30:836-852.

Roesch LFW, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Kent AD, Daroub SH, Camargo FAO, Farmerie WG, Triplett EW. 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. *Multidisciplinary Journal of Microbial Ecology* 1:283-290.

SAS Institute. 2005. *SAS User's Guide: Statistics*, version 9.1. SAS Institute, Cary, NC.

Schoenly KG, Haskell NH, Hall RD, Gbur JR. 2007. Comparative performance and complementarity of four sampling methods and arthropod preference tests from human and porcine remains at the Forensic Anthropology Center in Knoxville, Tennessee. *Journal of Medical Entomology* 44:881-894.

Schloss PD, Gevers D, Westcott SL. 2011. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PLoS One* 6:e27310.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing Mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology* 75:7537-7541.

Shalaby O, DeCarvalho LK, Goff ML. 2000. Comparison of patterns of decomposition in a hanging carcass and a carcass in contact with soil in xeraphytic habitat on the Island of Oahu, Hawaii. *Journal of Forensic Sciences* 45:1267-1273.

Shean BS, Messinger L, Papworht M. 1993. Observations of differential decomposition on sun exposed v. shaded pig carrion in coastal Washington State. *Journal of Forensic Sciences* 38:938-949.

Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR, Cusack D, Frey S, Gallo ME, Gartner TB, Hobbie SE, Holland K, Keeler BL, Powers JS, Stursova M, Takacs-Vesbach C, Waldrop MP, Wallenstein MD, Zak DR, Zeglin LH. 2008. Stoichiometry of soil enzyme activity at global scale. *Ecology Letters* 11:1252-1264.

Simmons T, Adlam RE, Moffatt C. 2010a. Debugging decomposition data-comparative taphonomy studies and the influence of insects and carcass size on decomposition rate. *Journal of Forensic Sciences* 55(1):8-13.

Simmons T, Cross T, Adlam RE, Moffat C. 2010b. The influence of insects on decomposition rates in buried and surface remains. *Journal of Forensic Sciences* 55(4):889-892.

Smith KGV. 1986. *A Manual of Forensic Entomology*. Ithaca, NY: Cornell University Press.

Sorg MH, David E, Rebmann AJ. 1998. Cadaver dogs, taphonomy, and postmortem interval in the Northeast. In: Reichs KJ, ed. *Forensic Osteology: Advances in the Identification of Human Remains*, 2nd Edition. Springfield, IL: Charles C. Thomas. pp120-144.

Swann LM, Forbes SL, Lewis SW. 2010. Analytical separations of mammalian decomposition products for forensic science: a review. *Analytica Chimica Acta* 682:9-22.

Synstelien J. 2009. Raccoon (*Prodyon lotor)* soft tissue modification of human remains. *Proceedings from the American Academy of Forensic Sciences* 15:359.

Tantawi TI, El-Kady EM, Greenberg B, El-Ghaffar HA. 1996. Arthropod succession on exposed rabbit carrion in Alexandria, Egypt. *Journal of Medical Entomology* 33:566-580

Teixeira L, Peixoto RS, Cury JC, Sul WJ, Pellizari VH, Tiedje J, Rosado AS. 2010. Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. *Multidisciplinary Journal of Microbial Ecology* 4:989-1001.

Tessmer JW, Meeks CL. 1996. Dispersal and distribution of Calliphordae (Diptera) immature from animal carcasses in southern Louisiana. *Entomology Society of America* 33(4):665-669.

Tomberlin JK, Adler PH. 1998. Seasonal colonization and decomposition of rat carrion in water and on land in an open field in South Carolina. *Journal of Medical Entomology* 35:704-709.

Vass AA, Bass WM, Wolt JD, Foss JE, Ammons JT. 1992. Time since death determinations of human cadavers using soil solution. *Journal of Forensic Sciences* 37(5):1236-1253.

Vass AA, Barshick SA, Sega G, Caton J, Skeen JT, Love JC, Synstelien JA. 2002. Decomposition chemistry of human remains: A new methodology for determining the postmortem interval. *Journal of Forensic Sciences* 47:542-553.

Vass AA, Smith RR, Thompson CV, Burnett MN, Dulgerian N, Eckenrode BA. 2008. Odor Analysis of Decomposing Buried Human Remains. *Journal of Forensic Sciences* 53(2):384-391.

Vass AA. 2011. The elusive universal postmortem interval formula. *Forensic Science International* 204(1-3):34-40.

Vlach J, Lambdin P, Dilling C, Grant J, Paulsen D, Wiggins G. 2010. Biodiversity of the insect fauna within the unique Sinking Pond habitat in middle Tennessee. *Tennessee Academy of Science* 85(3-4):62-86.

Wang Y, Qian PY. 2009. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS One* 4:e7401.

Widya M, Moffatt C, Simmons T. 2012. The formation of early stage adipocere in submerged remains: a preliminary experimental study. *Journal of Forensic Sciences* 57(2): 328-333.

Watson EJ, Carlton CE. 2003. Spring succession of necrophilous insects on wildlife carcasses in Louisiana. *Journal of Medical Entomology* 40:338-347.

Watson EJ, Carlton CE. 2005. Insect succession and decomposition of wildlife carcasses during fall and winter in Louisiana. *Journal of Medical Entomology* 42:193-203.

Wells, J.D. 1999. Key to third-instar Chrysomyinae (Diptera: Calliphoridae) from carrion in the continental United States. *J. Med. Entomol*. 36:638-641.

Whitworth, T. L. 2006. Keys to the3 genera and species of blow flies (Diptera: Calliphoridae) of America north of Mexico. *Proc. Entomol. Soc*. Washington 108:689-725.

Wilhelm SW, Farnsley SE, LeCleir GR, Layton AC, Satchwell MF, DeBruyn JM, Boyer GL, Zhu G, Paerl HW (2011) The relationships between nutrients, cyanobacterial toxins and the microbial community in Taihu (Lake Tai), China. *Harmful Algae* 10:207-215.

Yergeau E, Bokhorst S, Kang S, Zhou JZ, Greer CW, Aerts R, Kowalchuk GA. 2012. Shifts in soil microorganisms in response to warming are consistent across a range of Antartic environments. *Multidisciplinary Journal of Microbial Ecology* 6:692-702.

APPENDIX II. TABLES, CHARTS, AND GRAPHS

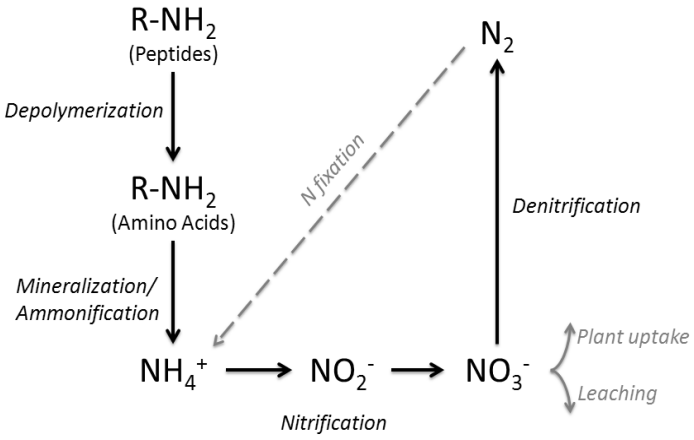


Fig. 1. Nitrogen cycling in soils. During decomposition, heavy influx of organic nitrogen (peptides, amino acids) results in increased ammonification rates and decreased nitrogen fixation rates.

Figure 2. Topographic map of the new area of the ARF where the project will take place.

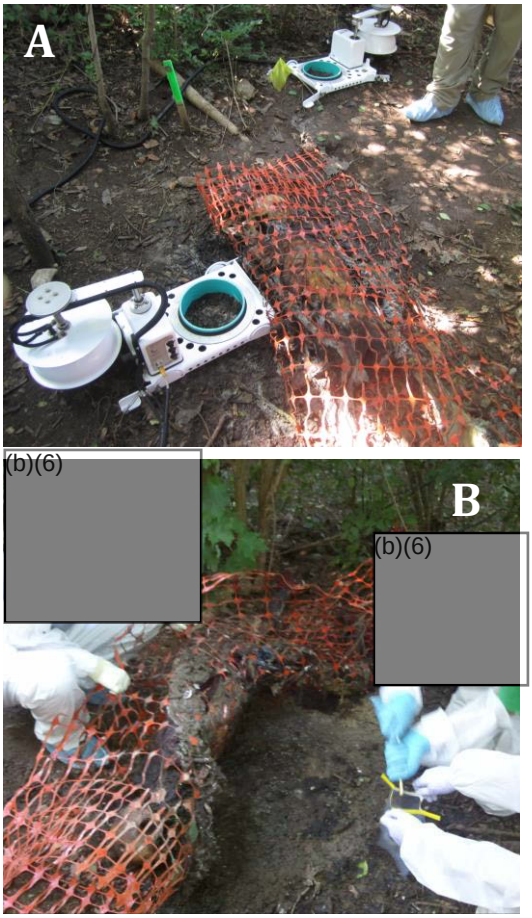


Figure 3. (A) Analytical equipment in CDI and control soils at the Anthropology Research Facility. (B) Plastic mesh is used to move the decaying cadaver to access the cadaver decomposition island underneath.

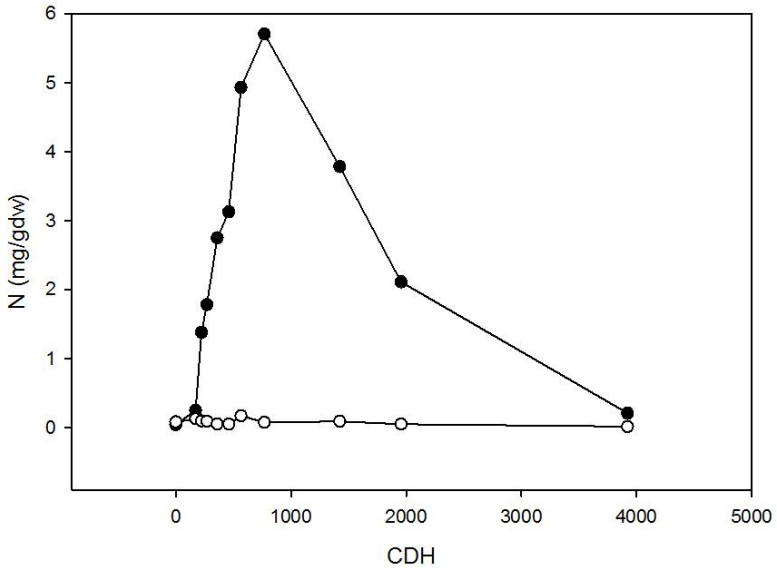


Figure 4. Total extractable nitrogen in soils below a decaying cadaver (filled symbols) increases during active decay. Soils collected one meter away remain unaffected (open symbols). CDH = cumulative degree hours.

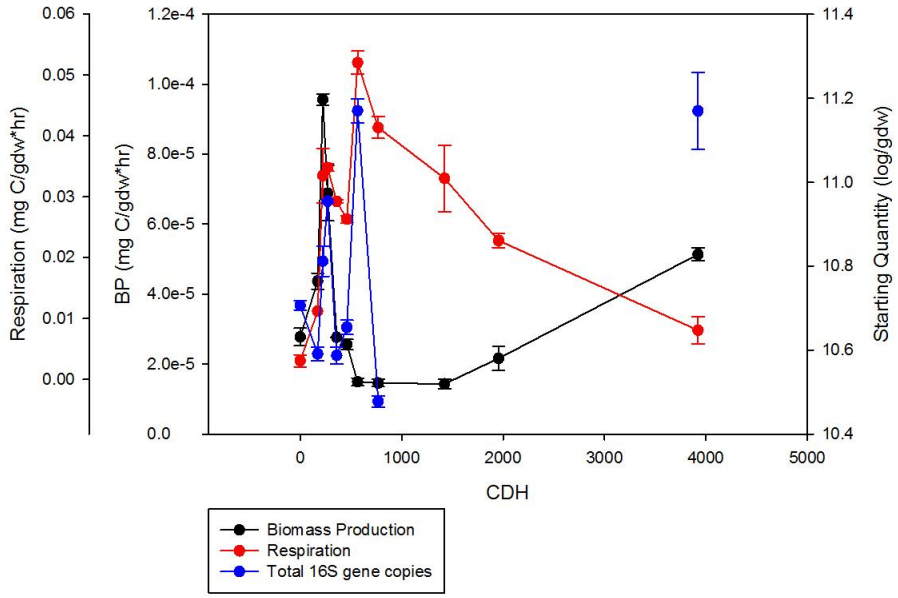


Figure 5. Succession in soil microbial activity during cadaver decay. Bacterial biomass production rates (black) peak at the start of active decay. Respiration (red) peaks at the start of advanced decay. 16S rRNA gene copies (a proxy for bacterial abundance (blue)) were closely aligned with respiration rates. These patterns were observed in all four cadavers measured (not shown). CDH = cumulative degree hours.

APPENDIX III. SUPPORTING DATA

NONE

Pages 31 through 42 redacted for the following reasons:
- -
Resumes Withheld in full pursuant to (b)(6)

APPENDIX V. CHART FOR TIMELINE, RESEARCH CALENDER, OR MILESTONES

Year 1 (January 1 - December 31, 2014)

Month	Grant Activity
January 1 to June 30	• Establish data collection guidelines and train GRA in TBS and entomological collection protocols • Build cages for rabbits and secure data loggers • Place pig, rabbit and human subjects for first trial – February 1 • Collect TBS, entomological and soil data and continue at designated ADHs • Take daily photographs and written notes of subjects • Place pig, rabbit and human subjects for second trial – June 15 and repeat data collection procedures • Begin soil chemistry and microbial analyses • Submit quarterly financial report to NIJ OJP – March 31 & June 30 • Submit semi-annual progress report to NIJ OJP – June 30
July 1 to Dec. 31	• End first trial and pick up remains • Continue soil chemistry and microbial analyses • Meet at FAC with PIs to discuss results and interpretations thus far • Submit abstract to AAFS to present preliminary results at 2015 Annual Meetings – August 1 • Submit quarterly financial report to NIJ OJP - September 30 & December 31 • Place pigs, rabbits and human subjects for final trial – November 30

Year 2 (January 1 - December 31, 2015)

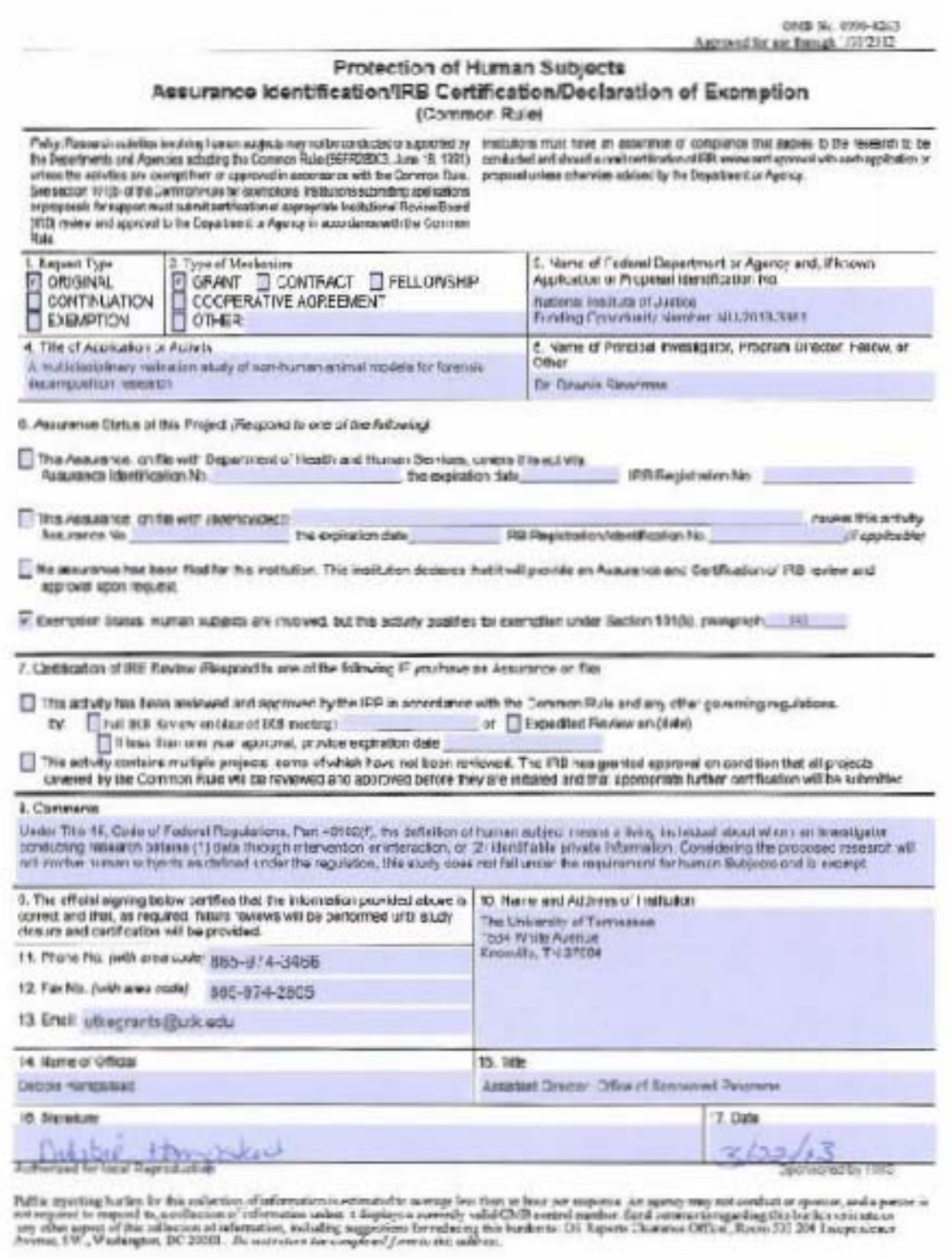
Month	Grant Activity
January 1 to June 30	• End second trial at 2000 ADH and pick up remains • Continue data collection on third trial until 2000 ADH then pick up remains • February: presentation for AAFS 2015 Annual Meetings • Continue soil chemistry and microbial analysis • Complete ADH data collection and begin time series analysis • Submit quarterly financial report to NIJ OJP – March 30 and June 30 • Submit semi-annual progress report to NIJ OJP – June 30
July 1 to September 30	• Complete all analyses • Submit abstract to AAFS to present preliminary results at 2015 Annual Meetings – August 1 • Submit quarterly financial report to NIJ OJP - September 30 • Submit final technical report - September 30
October 1 to Dec. 31	• Compile all data and complete final analyses • Prepare manuscript for submission to JFS • Submit quarterly financial report, semi-annual progress report, and final draft of Final Technical Report to NIJ OJP

APPENDIX VI. RESEARCH INDEPENDENCE AND INTEGRITY

The University of Tennessee's Conflicts of Interests policy (FI0125) is in place to monitor the outside interests of researchers and to ensure research independence and integrity. This policy requires the annual submission of an outside interests disclosure form by all faculty and exempt staff, whether or not they have interests or activities to disclose. A committee reviews disclosures to determine whether a conflict exists and individuals are notified of the results of the review. If a conflict or potential conflict is identified, the chief business officer works with the individual(s) to prevent or resolve the conflict. Resolution can include eliminating the conflict or managing conflicts that cannot be eliminated. Conflicts can be managed through independent reviewers, reassignment of responsibilities, modification of the research plan, or other methods to reduce or minimize the effects of a conflict. In addition, as part of the ongoing efforts to promote research integrity, the University offers to staff, faculty, and students two training options relative to conducting research ethically and responsibly.

The authors believe there are no potential organizational conflicts of interest with this research proposal. We do not stand to gain any monetary benefits from this proposed research.

APPENDIX VII. HUMAN SUBJECTS ASSURANCES, IRB LETTER AND PRIVACY CERTIFICATE



OMB No. 0990-0263
Approved for use through 7/31/2012

Protection of Human Subjects
Assurance Identification/IRB Certification/Declaration of Exemption
(Common Rule)

Public Reporting burden for this collection of information is estimated to average less than one hour per response. An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: HHS Reports Clearance Officer, Room 531 200 Independence Avenue, SW., Washington, DC 20201. Do not return the completed form to this address.

Page 45

THE UNIVERSITY of TENNESSEE KNOXVILLE

Institutional Review Board
Office of Research
1534 White Ave
Knoxville, TN 37996-1529
Phone: 865-974-7697
FAX: 865-974-7400

March 14, 2013

Dawnie Wolfe Steadman, Ph.D.
Anthropology Department
The University of Tennessee
250 S. Stadium Hall
Knoxville, TN 37996

Dr. Steadman:

This letter is provided in response to the request by NIJ regarding human subjects involvement in your proposed research entitled "A Multidisciplinary Validation Study of Nonhuman Animal Models for Forensic Decomposition Research".

Under Title 45, Code of Federal Regulations, Part 46102(f), the definition of human subjects is defined as a living individual about whom an investigator (whether professional or student) conducting research obtains (1) data through intervention or interaction with the individual, or (2) identifiable private information.

Considering your proposed research will not involve living human subjects as defined under the above regulation, your study does not fall under the requirement for human subjects, and is therefore exempt from Institutional Review Board (IRB) review.

If I need to provide further information, please let me know.

Sincerely,

Brenda Lawson

Brenda Lawson
Compliance Officer and IRB Administrator
FWA #00006629 Expiration date 04-11-2014
IRB #103

THE UNIVERSITY of TENNESSEE KNOXVILLE
Institutional Animal Care & Use Committee
2431 Joe Johnson Drive
336 Ellington Plant Sciences Bldg.
Knoxville, TN 37996-4564
Phone: (865) 974-5637 / 974-2631
Fax: (865) 974-3587
utiacuc@utk.edu

March 26, 2013

Dr. Giovanna Vidoli

Department of Anthropology

University of Tennessee, Knoxville

Dear Dr. Vicoli,

The University of Tennessee Institutional Animal Care and Use Committee (IACUC) has received your animal use protocol titled" A Multidisciplinary Validation Study of Nonhuman Animal Models for Forensic Decomposition Research". The protocol has completed an initial administrative review and veterinarian review during which it was assigned a pain and distress category. The protocol was distributed for Designated Member Review (DMR) on March 25, 2012. Any modifications identified during DMR will require resolution to secure approval of the protocol.

The University of Tennessee, Knoxville area holds USDA certificate 63-R-0105

The University of Tennessee, Knoxville PHS Assurance number is A3668-01 effective 12/19/2012 to 12/30/2016

The University of Tennessee, Knoxville is AAALAC file 000178.

Should you need any additional information, do not hesitate to contact me.

Elizabeth M. Bailey LAT MS CPIA

IACUC Coordinator

336 Ellington Plant Science

2431 Joe Johnson Drive

Knoxville, TN 37996 4564

ebailey@utk.edu

THE UNIVERSITY of TENNESSEE

Office of Sponsored Programs
Office of Research & Engagement
1534 White Avenue
Knoxville, TN 37996-1529
(865) 974-3466
fax (865) 974-2805

Privacy Certificate

Grantee[1], Dawnie Steadman, Debbie Hampstead, certifies that data *identifiable to a private person*[2] will not be used or revealed, except as authorized in 28 CFR Part 22, Sections 22.21 & 22.22.

Brief Description of Project (required by 28 CFR §22.23(b):

This project is a validation study to determine whether commonly used nonhuman animals, pigs and rabbits, are appropriate proxies for humans in decomposition studies. Most researchers do not have access to human subjects for natural decomposition experiments so use pigs and other animals as human models. Further, the results of these studies are often cited in forensic case reports and court testimony despite the fact that no formal validation has taken place. We will create four trials of four replicates of each species (human, pig and rabbit) at the Anthropology Research Facility (ARF) at UT. We will collect a wealth of data, including soil chemistry, soil microbes, flora, insect and observational data to assess any differences across species during decomposition.
No data identifiable to a private person will be collected here.

Grantee certifies that any private person from whom identifiable information is collected or obtained shall be notified, in accordance with 28 CFR §22.27, that such data will only be used or revealed for research or statistical purposes and that compliance with the request for information is not mandatory and participation in the project may be terminated at any time. In addition, grantee certifies that where findings in a project cannot, by virtue of sample size or uniqueness of subject, be expected to totally conceal the identity of an individual, such individual shall be so advised.

Procedures to notify subjects that such data will only be used or revealed for research or statistical purposes and that compliance with the request for information is not mandatory and participation in the project may be terminated at any time as required by 28 CFR §22.23(b)(4):

Not applicable since this stucy is not colecting identifiable data.

If notification of subjects is to be waived, pursuant to 28 CFR §22.27(c), please provide a justification:

Not applicable since this study is not collecting identifiable data.

Grantee certifies that project plans will be designed to preserve the confidentiality of private persons to whom information relates, including where appropriate, name-stripping, coding of data, or other similar procedures.

Procedures developed to preserve the confidentiality of personally identifiable information, as required by 28 CFR §22.23(b)(7):

Not applicable since this study is not collecting identifiable data.

Grantee certifies that, if applicable, a log will be maintained indicating that (1) identifiable data have been transferred to persons other than employees of NIJ, BJA, BJS, OJJDP, OVC, OJP, or grantee/contractor/subcontractor staff, and (2) such data have been returned or that alternative arrangements have been agreed upon for future maintenance of such data, in accordance with 28 CFR §22.23(b)(6).

Justification for the collection and/or maintenance of any data in identifiable form, if applicable:

Not applicable since this study is not collecting identifiable data.

Procedures for data storage, as required by 28 CFR §22.23(b)(5):

Not applicable since this study is not collecting identifiable data.

Grantee certifies that all contractors, subcontractors, and consultants requiring access to identifiable data will agree, through conditions in their subcontract or consultant agreement, to comply with the requirements of 28 CFR §22.24, regarding information transfer agreements. Grantee also certifies that NIJ will be provided with copies of any and all transfer agreements before they are executed as well as the name and title of the individual(s) with the authority to transfer data.

Description of any institutional limitations or restrictions on the transfer of data in identifiable form, if applicable:

Not applicable since this study is not collecting identifiable data

Name and title of individual with the authority to transfer data:

Not applicable since this study is not collecting identifiable data.

Grantee certifies that access to the data will be limited to those employees having a need for such data and that such employees shall be advised of and agree in writing to comply with the regulations in 28 CFR Part 22.

Grantee certifies that all project personnel, including subcontractors, have been advised of and have agreed, in writing, to comply with all procedures to protect privacy and the confidentiality of personally identifiable information.

Access to data is restricted to the following individuals, as required by 28 CFR §22.23(b)(2):

Principal Investigator(s)

Not applicable since this study is not collecting identifiable data

Project Staff

Not applicable since this study is not collecting identifiable data

Contractors, Subcontractors, and/or consultants

Not applicable since this study is not collecting identifiable data.

Grantee certifies that adequate precautions will be taken to ensure administrative and physical

security of identifiable data and to preserve the confidentiality of the personally identifiable information.

Procedures to insure the physical and administrative security of data, as required by 28 CFR §22.25(b), including, if applicable, a description of those procedures used to secure a name index :

Not applicable since this study is not collecting identifiable data.

Procedures for the final disposition of data, as required by 28 CFR §22.25:

Not applicable since this study is not collecting identifiable data.

Name and title of individual authorized to determine the final disposition of data:

Not applicable since this study is not collecting identifiable data.

Grantee certifies that copies of all questionnaires, informed consent forms and informed consent procedures designed for use in the project are attached to this Privacy Certificate.

Grantee certifies that project findings and reports prepared for dissemination will not contain information which can reasonably be expected to be identifiable to a private person, except as authorized by 28 CFR §22.22.

Grantee certifies that the procedures described above are correct and shall be carried out.

Grantee certifies that the project will be conducted in accordance with all the requirements of the Omnibus Crime Control and Safe Streets Act of 1968 as amended and the regulations contained in 28 CFR Part 22.

Grantee certifies that NIJ shall be notified of any material change in any of the information provided in this Privacy Certificate.

Signature(s):

Daniel Wolfe Stoudmire (Principal Investigator)

_____ (Principal Investigator)

Debbie Humphreys (Institutional Representative)

Date: March 21, 2013

Notes:

APPENDIX VIII. PREVIOUS AND CURRENT NIJ AWARDS

Award	Lead Unit	Sponsor	PI	Amount
2011-DN-BX-K537	Mech Aero & Biomed Eng.	NIJ	M. Mahfouz	514,495
Computerized Reconstruction of Fragmentary Skeletal Remains for Purposes of Extracting Osteometric Measurements and Estimating MNI				End Date 31-Dec-2013
2010-DN-BX-K229	Anthropology	NIJ	A. Mundorff	200,316
Developing an Empirically Based Ranking Order for Bone Sampling				End Date 31-June-2012
2010-DN-BX-K202	Chemistry	NIJ	M. Dadmun	258,816
Improving the Efficiency and Speed of Developing Latent Prints with Superglue Fumes				End Date 01-Mar-2012
2010-DN-BX-K228	Anthropology	NIJ	A. Vass	167,414
Evaluating the Use of DNA and RNA Degradation for Estimating the Post-Mortem Interval				End Date 31-Dec-2011
2008-DN-BX-K182	Anthropology	NIJ	R. Jantz	338,493
Improving Sex Estimation Using 3-Dimensional CT Scans				End Date 31-Dec-2010
2008-DN-BX-K193	Anthropology	NIJ	R. Jantz	478,500
Isotopic Analysis of the William Bass Donated Skeletal Collection and Other Modern Donated Collections				End Date 31-Dec-2010
2008-IJ-CX-K406	Geography	NIJ	B. Ralston	165,509
Development of a Web-Based GIS Application for the National Missing & Unidentified Persons System				End Date 30-June-2010
2008-DN-BX-K183	Nuclear Eng.	NIJ	J.W. Hines	107,099
Improving Facial Reproduction Using Empirical Modeling				End Date 31-May-2010

Award	Lead Unit	Sponsor	PI	Amount
2006-DN-BX-K031	Chemistry	NIJ	M. Dadmum	126,505
Cultivating Methods to Enhance the Quality of Fingerprints Developed by Cyanoacrylate Fuming				End Date 31-Aug-2009
DJJ-07W-ENR01-0492	Geography	DOJ	H. Grissino-Mayer	18,931
Arkansas Game and Fish Commission V. U.S.				End Date 06-Aug-2009
2007-DN-BX-0004	Anthropology	DOJ	R. Jantz	20,000
Determination of Age and Sex from the Human Clavicle: A Geometric Morphometric Approach				End Date 30-Apr-2009
2007-DN-BX-0013	Anthropology	NIJ	L. Konigsberg	20,000
Body Mass Estimation from the Human Skeleton				End Date 31-Aug-2008
2003-IJ-CX-K106	Anthropology	DOJ	N. Herrmann	32,229.79
Evaluation of Stature Estimation from the Database of Forensic Anthropology in the United States				End Date 28-Feb-2006

CURRENT AND PENDING NON-NIJ FUNDING

Bridget F.B. Algee-Hewitt, **Dawnie W. Steadman** & Graciela S. Cabana. PENDING
A Structured Approach to Human Variation: Interrogating the Genotype and Phenotype for Questions of Ancestry and Race in the Modern American Context. NSF: BCS-Biological Anthropology PD 98-1392. Requested $477,129.

Johnson A, **JM DeBruyn**. $75,000. Downregulation of lignin content in switchgrass plants and the impact on soil microbial communities and nutrient cycling. SunGrant Initiative 8/1/2011 to 7/31/2013.

Wilhelm SW and **JM DeBruyn**. $179,999. Collaborative Research: CREATIV: An Ecologically-Driven Strategy for Ensuring Sustainability of Anthropogenically and Climatically Impacted Lakes. National Science Foundation 9/1/2012 – 8/31/2015.

DeBruyn JM, T Sparer, L Hauser, L Jantz. $9,990. Post-Mortem Fate of the Human Gut Microbiome. UT Microbiology Across Campuses Ed. & Res. Venture 9/1/2012 – 6/30/2013.

OTHER FUNDING SOURCES TO WHICH THIS PROPOSAL HAS BEEN SUBMITTED:

None

APPENDIX IX. LETTERS OF COOPERATION FROM COLLABORATORS

(b)(6)

8448 E 100 N
Lafayette, IN 47907

March 20, 2013

Dawnie Wolfe Steadman, PhD
Director, Forensic Anthropology Center
University of Tennessee
250 South Stadium Hall
Knoxville, TN 37996

Dear Dr. Steadman:

My role on this proposed project is to consult on entomological collections as described in the plan of work, and to train a student to collect, preserve, and identify insect specimens collected. I will also assist in analysis of insect samples collected pertinent to the project plan of work.

I plan to take two trips to Knoxville at the beginning of the project to train the student collecting insect samples and a trip at the end of the project to help in insect identification and data analysis. I will also be available from Lafayette, Indiana for consultation during the course of the project. I estimate a total of 50 hr of my time to be spent on this project.

Sincerely,

(b)(6)

(b)(6) @purdue.edu

APPENDIX X. ADDITIONAL MATERIALS

ATTACHED SEPARATELY

APPENDIX XI. DATA ARCHIVING AND SHARING PLANS

Type of data generated

This study will generate a collection of data on decomposition processes, entomology, and soil chemical and biological parameters from three species (human, pig, rabbit) at the Anthropological Research Facility (ARF), University of Tennessee:

- Video: Motion sensor video at each trial site.
- Photographic: Daily photographs of each subject.
- Field notes: Daily for each subject.
- Total Body Score: Daily for each subject.
- Temperature data: Ambient temperature and relative humidity recorded hourly.
- Entomology: Arthropod collection and identification.
- Discrete field measures: Soil moisture, pH, carbon and nitrogen species, bacterial biomass, bacterial abundances, respiration rates, and various enzyme rates.
- Bacterial 16S rRNA gene sequences generated by 454 pyrosequencing.

Data Preservation

Raw photographic and video data will be saved in a designated "view only" file on the University of Tennessee's secure server. Immediate copies will be saved in a separate file allowing for manipulation and analysis. Field notes will be compiled into a relational access database and archived with the FAC completed research directory. The Access database will allow for easy comparison of research subjects and will include links to relevant temperature data and photographs. The database will be immediately available to all PIs on the project through a secure server on the University of Tennessee network. Temperature data for each research subject will be stored in an excel spreadsheets on the University of Tennessee network. Entomological samples will be incorporated into the current entomological collection at the University of Tennessee.

Samples preserved in the field will be barcoded and entered into a LIMS-type database along with all associated ancillary and metadata. For discrete field measures, technical replicates and standards will be used to ensure quality of raw data. Calculations will then be made on raw data to convert to concentrations (e.g. x g $soil^{-1}$) or a rate (e.g. x g $soil^{-1}h^{-1}$). Ultimately, all data on counts and rates collected during field work will be compiled into one spreadsheet for easy access for all PIs on the project, and archived as ASCII files on the University of Tennessee's Research and Creative Exchange (TRACE), an open access digital archive. All data acquired during this project will be stored and access preserved for a minimum of five years after completion of the project. Data will be organized into matrices for input to statistical software for analysis. All data will be stored as excel spreadsheets and statistical (e.g., NCSS, Primer6, Canoco, SigmaPlot) analysis files.

Read data from the pyrosequencing instruments will be initial processed using Roche and Illumina's software on a cluster housed at the Joint Institute for Biological Scicences in Oak Ridge, Tennessee (adjacent to the 454 instrument). The Mothur open source software package (Schloss et al. 2009) will be used to trim low quality reads and annotate and analyze the 16S rRNA gene library reads. Sequences will be stored as bioinformatic (FASTA, Mothur, UniFrac) analysis files. All nucleic acid sequences from the barcoded libraries will be deposited into MG-

RAST (http://metagenomics.anl.gov/), along with associated metadata as outlined by the TerraGenome initiative (http://www.terragenome.org/metadata-for-soil-metagenomics/).

Data sharing policy

Data collected under this project will be made available to the public with as few restrictions as possible. We will share requested data with any other researcher working on similar decomposition studies if needed as ancillary data to support their research. To the degree possible, all primary experimental data will be provided as appendices to manuscripts. We plan for public access to the data (with metadata) after the publication of results, or after (at most) two years from the completion of the study. We do not currently envision that any datasets will warrant proprietary restrictions. We will apply the following conditions for use of these data and results:

1. The data user agrees to contact the FAC prior to publishing.
2. The user agrees to cite our project in all publications by including the following statement in the Acknowledgments: "Data were provided by [data owner's name], and data collection was funded by the National Institute of Justice ([award number])".
3. The user agrees to send the full citation of any publication using our data to the data owner.
4. Users are prohibited from selling or redistributing any data provided by our project.
5. Extensive efforts are made to ensure that online data (from website) are accurate and up to date, but we will not take responsibility for any errors that may exist.
6. Any violation of the terms of this agreement will result in immediate forfeiture of the data and loss of access privileges to other project data sets.

Datasets will be accessible with the National Archive of Criminal Justice Data (NACJD) after publication of results or after (at most) two years from the completion of the study.

Budget Detail Spreadsheet

Basic Scientific Research to Support Forensic Science

for Criminal Justice Purposes

NIJ-2013-3362

A MULTIDISCIPLINARY VALIDATION STUDY OF NONHUMAN ANIMAL MODELS FOR FORENSIC DECOMPOSITION RESEARCH

Prepared and Submitted by

Co-PI Jennifer M. DeBruyn, PhD
Department of Biosystems Engineering & Soil Science, The University of Tennessee
2506 EJ Chapman Dr. Knoxville, TN 37996;
865-974-7277; jdebruyn@utk.edu

Lead PI Dawnie L.W. Steadman, PhD,
Director, Forensic Anthropology Center
Department of Anthropology
The University of Tennessee,
250 S. Stadium Hall Knoxville, TN 37996
865-974-0909; dsteadma@utk.edu

Co-PI Lee M. Jantz, PhD
Department of Anthropology,
Associate Director, Forensic Anthropology Center, The University of Tennessee
250 S. Stadium Hall Knoxville TN 37996
865-974-4408; ljantz@utk.edu

Co-PI Giovanna Vidoli, PhD
Department of Anthropology,
Assistant Director, Forensic Anthropology Center, The University of Tennessee
250 S. Stadium Hall Knoxville TN 37996
865-974-4408; gvidoli@utk.edu

Co-PI Sean M. Schaeffer, PhD
Department of Biosystems Engineering & Soil Science, The University of Tennessee
2506 EJ Chapman Dr. Knoxville, TN 37996;
865-974-7366; sschaef5@utk.edu

Consultant - (b)(6) **PhD**
Professor Emeritus of Entomology
Purdue University
West Lafayette, IN 47907-2089
(b)(6) **@purdue.edu**

March 28, 2013

Key words

Decomposition, Postmortem Interval, Animal Models, Soil, Microbiology, Isotopes

TABLE OF CONTENTS

Budget Detail Worksheet
Cumulative Budget (Years 1 and 2)

A. Personnel

Name/Position	Computation	Cost
Dawnie Steadman, Ph.D. Co-Principal Investigator	Donating time	$0
Lee Meadows Jantz, Ph.D. Co-Principal Investigator	summer pay @ $6387 (2014) per month Y1	$6,387
Jennifer DeBruyn, Ph.D. Co-Principal Investigator	(b)(4), (b)(6) yr – (b)(4), (b)(6) month summer pay @ $3,564 Y1 (b)(4), (b)(6) @ $3,671 Y2	$7,235
Sean Schaeffer, Ph.D Co-Principal Investigator	(b)(4), (b)(6) / yr – (b)(4), (b)(6) month summer pay @ $3,474Y1 (b)(4), (b)(6) @ $3,578Y2	$7,052
Giovanna Vidoli, Ph.D. Co-Principal Investigator	(b)(4), (b)(6) / yr – (b)(4), (b)(6) time @ $13,648 Y1 (b)(4), (b)(6) @ $14,057 Y2	$27,705
Graduate Research Assistant (Anthropology)	One year appointment 50% $16,500	$16,500
Graduate Research Assistant (ESS)	Two year appointment $18,000 Y1; $18,540 Y2	$36,540
Tech/Professional (EPP)	$32,004/ yr – 25% of annual salary @ $8,001 Y1 $32,964 @ $8,241 Y2	$16,242
SUB-TOTAL $117,661		

B. Fringe Benefits

Name/Position	Computation	Cost
Dawnie Steadman, Co-PI	$0	
Lee Meadows Jantz, Co-PI	(b)(4), (b)(6) x (b)(4), (b)(6) (Retirement, Social Security, Worker's Comp.)	$2,044

Giovanna Vidoli, Co-PI	(b)(4), (b)(6) x (b)(4), (b)(6) Health Insurance and Benefits	$11,914
Jennifer DeBruyn, Co-PI	(b)(4), (b)(6) x (b)(4), (b)(6) Health Insurance and Benefits	$1,874
Sean Schaeffer, Co-PI	(b)(4), (b)(6) x (b)(4), (b)(6) Health Insurance and Benefits	$2,666
GRA – Anthropology	$112/month x 8 months, $119/ea X 4 months Student Health Insurance, 1 year	$1,372
GRA – ESS	Student Health Insurance for 2 years	$5,467
Tech/Professional – EPP	$16,242 x 30%	$4,872
SUB-TOTAL $30,209 **Total Personnel & Fringe Benefits $147,972**		

C. Travel

Presentation at AAFS meeting	Orland, FL	Airfare Hotel Meals	$300 x 2 people $111/night x 3 x 2 people $56/day x 4 days x 2 people	$600 $666 $448
Consultant travel to Knoxville, TN	Knoxville	Mileage Hotel Meals	2000@ $0.47 miles $90/night x 4 nights $56/day x 5 days	$940 $344 $280
Total Travel $3,278				

D. Equipment
None Budgeted

E. Supplies

Supply Items	Computation	Cost

TGP-4520 Dual-Channel External Temperature Recorder and Data Logger	12 loggers @ $250/each	$3000
TGP-4500 Temperature and Humidity Data Logger	2 loggers @ $250/each	$500
PB-5001 Standard Thermistor Probe	24 probes @ $60/each	$1,440
Nikon D7000 Camera	1 camera @ $1,300/each	$1,300
Game Spy I-45S Game Camera	6 cameras @ $260/each	$1,560
32 gig Flash Memory Card	12 cards @ $25/each	$300
D-cell Batteries	36 packs @ $15/pack	$540
Shoe Covers	3 cases @ $60/each	$180
Sleeves	3 cases @ $110/each	$330
Gowns	3 cases @ $90/each	$270
Latex Gloves	3 cases @ $75/each	$225
Insect aerial Nets	3 @ $27.75/each	$84
1000 20 ml scintillation vials with caps		$300
Glacial acetate	1 liter @ $110	$110
Ethyl acetate	1 liter @ $85	$85
Kerosene	0.5 gal @ 20 /gal	$10
Ethyl alcohol 190 proof	10 gal @ $20/gal	$200
Pig Cadavers	12 pigs @ $200/each	$2,400
Rabbit Cadavers	12 rabbits @ $25/each	$300
Transportation of Pigs from Henry Co. to Knoxville, TN	3 trips to bring pigs to Knoxville @ $400 / trip	$1,200
Veterinarian euthanasia	12 pigs@ $58, 12 rabbits @ $1, vet tech fee @ $25 X 24 animals	$1,308
Human Cadavers	12 human cadavers – FAC	$0
Molecular Biology kits	3 kits @ $440/ea; 1 kit @ $600/ea; 1 kit @$580/ea; 1 kit @ $500/ea	$3,000
Enzymes, primers, and reagents for molecular biology	200 primers @$16/ea; Taq polymerase @ $220 X 2; Lysozyme @$45 X 2; Nuclease Free Water@ $120 X 2; Agarose @ $160 X 2; Gel loading dye @ $50 X 2; DNA ladder @ $130 X 2; Agar @ $65 X 2; Ampicilin @ $80 X 2; X-Gal/IPTG @ $170 X 2; LB media @ $160 X 2; Sybr green dye @ $200 X 2	$6,000
Chemicals for Enzyme Rate Assays and Chemical Measurements ($5000)	Enzyme analysis substrates @ $1600; Reagents for enzyme analyses @ $525; Thermo-Scientific 8-channel pipetter @ $725; Potassium sulfate extractant	$5,000

	@ $700; C-free chloroform @ $600; Sodium bicarbonate extractant @ $150; 2 Millipore Q-gard cartridges @ $350/ea $700	
Consumable laboratory supplies ($6000)	Pipette tips 9 cases @ $120/case; Gloves 7 cases @ $180/case; Petri plates @ $60; Serological pipettes 2 cases @ $280 ; Vials cases 2 @ $300/case; Bottles 96 bottles @ $4/bottle; Microcentrifuge tubes 2 cases@ $120/case; Cryovials @ $430/case; PCR plates 2 cases @ $240/case ; PCR plate seals 2 cases @ $90/case; Centrifuge tubes 2 cases @$80/case; 96 well plates @$140/case; Whirlpak bags 3 cases @ $50/case; Autoclavable biosafety disposal bags 2 cases @ $46/case; Kimwipes 2 cases @ $60/case	$6,000
		TOTAL $35,642

F. Construction
None Budgeted

G. Consultants/Contracts

Name of Consultant	Service Provided	Computation	Cost
UT/ORNL Joint Institute for Biological Sciences	One plate of 454 Pyrosequencing	$12,000/plate	$12,000
(b)(6) Ph.D.	Entomology analysis	(b)(4), (b)(6) ours @ (b)(4), (b)(6) hour	(b)(4), (b)(6)
TOTAL $15,750			

H. Other Costs

Description	Computation	Cost
GRA Tuition Waiver (Anthropology)	$4,770 / semester x 2 semesters, $5,056/semester x 1 semester	$14,596
GRA Tuition Waiver (ESS)	Graduate student fees $13,999 Y1 and $15,259 Y2	$29,258
		TOTAL $43,854

I. Indirect Costs

Description	Computation	Cost
Facilities and Administrative Costs – Anthropology	$88,592 (MTDC) x 49%	$43,410
Facilities and Administrative Costs – ESS and Entomology	$113,948 (MTDC) x 40%	$45,579
	TOTAL	**$88,989**

Cumulative Budget Summary

Budget Category	**Amount**
A. Personnel	$117,661
B. Fringe Benefits	$30,209
C. Travel	$3,278
D. Equipment	$0
E. Supplies	$35,642
F. Construction	$0
G. Consultants/Contracts	$15,750
H. Other	$43,854
Total Direct Costs	$246,394
I. Indirect Costs	$88,989
TOTAL PROJECT COSTS	**$335,383**
Federal Request	**$335,383**

Budget Detail Worksheet

Year 1

A. Personnel

Name/Position	Computation	Cost
Dawnie Steadman, Ph.D. Co-Principal Investigator	Volunteer	$0
Lee Jantz, Ph.D. Co-Principal Investigator	summer pay @ $6387 (2014) per month Y1	$6,387
Jennifer DeBruyn, Ph.D. Co-Principal Investigator	(b)(4), (b)(6) yr (b)(4), (b)(6) month summer pay @ $3,564	$3,564
Sean Schaeffer, Ph.D. Co-Principal Investigator	(b)(4), (b)(6) yr – (b)(4), (b)(6) onth summer pay @ $3,474	$3,474
Giovanna Vidoli, Ph.D. Co-Principal Investigator	(b)(4), (b)(6) yr (b)(4), (b)(6) time @ $13,648	$13,648
Graduate Research Assistant (Anthropology)	One year appointment $16,500 per year	$16,500
Graduate Research Assistant (ESS)	$18,000	$18,000
Tech/Professional	$32,004/ yr – 25% of annual salary @ $8,001	$8,001
SUB-TOTAL $69,574		

B. Fringe Benefits

Name/Position	Computation	Cost
Dawnie Steadman, Co-PI	Volunteer	$0
Lee Meadows Jantz, Co-PI	$6,387 x (b)(4), (b)(6) (Retirement, Social Security, Worker's Comp.)	$2,044
Jennifer DeBruyn, Co-PI	$3,564 x (b)(4), (b)(6) Health Insurance and Benefits	$923
Sean Schaeffer, Co-PI	$3,474 x (b)(4), (b)(6) Health Insurance and Benefits	$1,313
Giovanna Vidoli, Co-PI	$13,648 x (b)(4), (b)(6) Health Insurance and Benefits	$5,869
Tech/Professional	$8001 x 30%	$2,400

GRA - Anthropology	$112/month x 8 months, $119/ea X 4 months Student Health Insurance, 1 year	$1,372
GRA - ESS	health insurance	$2,712
SUB-TOTAL $16,633 **Total Personnel & Fringe Benefits $86,207**		

C. Travel

Consultant travel to Knoxville, TN	Knoxville	Mileage Hotel Meals	2000@ $0.47 miles $86/night x 4 nights $56/day x 5 days	$940 $344 $280
Total Travel $1,564				

D. Equipment
None Budgeted

E. Supplies

Supply Items	Computation	Cost
TGP-4520 Dual-Channel External Temperature Recorder and Data Logger	12 loggers @ $250/each	$3,000
TGP-4500 Temperature and Humidity Data Logger	2 loggers @ $250/each	$500
PB-5001 Standard Thermistor Probe	24 probes @ $60/each	$1,440
Nikon D7000 Camera	1 camera @ $1,300/each	$1,300
Game Spy I-45S Game Camera	6 cameras @ $260/each	$1,560
32 gig Flash Memory Card	12 cards @ $25/each	$300
D-cell Batteries	36 packs @ $15/pack	$540
Shoe Covers	3 cases @ $60/each	$180
Sleeves	3 cases @ $110/each	$330
Gowns	3 cases @ $90/each	$270
Latex Gloves	3 cases @ $75/each	$225

Insect aerial Nets	3 @ $28/each	$84
1000 20 ml scintillation vials with caps		$300
Glacial acetate	1 liter @ $110	$110
Ethyl acetate	1 liter @ $85	$85
Kerosene	0.5 gal @ $20/gal	$10
Ethyl alcohol 190 proof	10 gal @ $10/gal	$200
Pig Cadavers	12 pigs @ $200/ea	2400
Rabbit Cadavers	12 rabbits @ $25/each	$300
Transportation of Pigs	3 trips @ $400/trip	$1200
Veterinarian euthanasia	12 pigs@ $58, 12 rabbits @ $1, vet tech fee @ $25 X 24 animals	$1,308
Human Cadavers	12 human cadavers – FAC	$0
Molecular Biology kits ($3000)	3 kits @ $440/ea; 1 kit @ $600/ea; 1 kit @$580/ea; 1 kit @ $500/ea	$3,000
Enzymes, primers, and reagents for molecular biology ($3000)	100 primers @$16/ea; Taq polymerase @ $220; Lysozyme @$45; Nuclease Free Water@ $120; Agarose @ $160; Gel loading dye @ $50; DNA ladder @ $130; Agar @ $65; Ampicilin @ $80; X-Gal/IPTG @ $170; LB media @ $160; Sybr green dye @ $200	$3,000
Chemicals for Enzyme Rate Assays and Chemical Measurements ($5000)	Enzyme analysis substrates @ $1600; Reagents for enzyme analyses @ $525; Thermo-Scientific 8-channel pipetter @ $725; Potassium sulfate extractant @ $700; C-free chloroform @ $600; Sodium bicarbonate extractant @ $150; 2 Millipore Q-gard cartridges @ $350/ea $700	$5,000
Consumable laboratory supplies ($4000)	Pipette tips 5 cases @ $120/case = $600; Gloves 4 cases @ $180/case; Petri plates @ $60; Serological pipettes @ $280; Vials cases 2 @ $300/case; Bottles 96 bottles @ $4/bottle; Microcentrifuge tubes @ $120/case; Cryovials @ $430/case; PCR plates @ $240/case ; PCR plate seals @ $90/case; Centrifuge tubes @$80/case; 96 well plates @$140/case; Whirlpak bags 3 cases @ $50/case; Autoclavable biosafety disposal bags @ $46/case; Kimwipes @ $60/case	$4,000
		TOTAL $30,642

F. Construction
None Budgeted

G. Consultants/Contracts

Name of Consultant	Service Provided	Computation
UT/ORNL Joint Institute for Biological Sciences (ESS) (b)(6) Ph.D.	One plate of 454 Pyrosequencing Entomology Analysis	$12,000/plate (b)(4), (b)(6) hours @ (b)(4), (b)(6) hour (b)(4), (b)(6)
		Subtotal $14,250
		TOTAL $14,250

H. Other Costs

Description	Computation	Cost
GRA Tuition Waiver (Anthropology)	$4,770 / semester x 2 semesters, $5,056/semester x 1 semester	$14,596
GRA Tuition Waiver (ESS)	Graduate student fees $13,999	$13,999
		TOTAL $28,595

I. Indirect Costs

Description	Computation	Cost
Facilities and Administrative Costs – Anthropology	$65,276 (MTDC) x 49%	$31,985
Facilities and Administrative Costs – ESS and Entomology	$67,387 (MTDC) x 40%	$26,955
		TOTAL $58,940

Year 1 Budget Summary

Budget Category	COST
A. Personnel	$69,574
B. Fringe Benefits	$16,633
C. Travel	$1,564
D. Equipment	$0
E. Supplies	$30,642
F. Construction	$0
G. Consultants/Contracts	$14,250
H. Other	$28,595
Total Direct Costs	$161,258
I. Indirect Costs	$58,940
TOTAL YEAR 1	$220,198

Budget Detail Worksheet
Year 2

A. Personnel

Name/Position	Computation	Cost
Dawnie Steadman, Ph.D. Co-Principal Investigator	Volunteer	$0
Lee Meadows Jantz, Ph.D. Co-Principal Investigator	Volunteer	$0
Jennifer DeBruyn, Ph.D. Co-Principal Investigator	(b)(4), (b)(6) onth summer pay (b)(4), (b)(6) @ $3,671	$3,671
Sean Schaeffer, Ph.D Co-Principal Investigator	(b)(4), (b)(6) month summer pay (b)(4), (b)(6) @ $3,578	$3,578
Giovanna Vidoli, Ph.D. Co-Principal Investigator	(b)(4), (b)(6) ime @ (b)(4), (b)(6) $14,057	$14,057
Graduate Research Assistant (ESS)	One year appointment $18,540 per year	$18,540
Tech/Professional	25% of annual salary $32,964 @ $8,241	$8,241
SUB-TOTAL $48,087		

B. Fringe Benefits

Name/Position	Computation	Cost
Dawnie Steadman, Co-PI	Volunteer	$0
Lee Meadows Jantz, Co-PI	Volunteer	$0
Jennifer DeBruyn, Co-PI	(b)(4), (b)(6) (b)(4), (b)(6) Health Insurance and Benefits	$951
Sean Schaeffer, Co-PI	$3,578 x 38% Health Insurance and Benefits	$1,353
Giovanna Vidoli, Co-PI	$14,057 x 43% Health Insurance and Benefits	$6,045
GRA - ESS	Student Health insurance	$2,755
Tech/Professional	$8,241 x 30%	$2,472
SUB-TOTAL $13,576 **Total Personnel & Fringe Benefits $61,663**		

C. Travel

Presentation at AAFS	Orland, FL	Airfare	$300 x 2 people	$600

meeting		Hotel Meals	$111/night x 3 x 2 people $56/day x 4days x 2 people	$666 $448
Total Travel $1,714				

D. Equipment
None Budgeted

E. Supplies

Supply Items	Computation	Cost
Enzymes, primers, and reagents for molecular biology	100 primers @$16/ea; Taq polymerase @ $220; Lysozyme @$45; Nuclease Free Water@ $120; Agarose @ $160; Gel loading dye @ $50; DNA ladder @ $130; Agar @ $65; Ampicilin @ $80; X-Gal/IPTG @ $170; LB media @ $160; Sybr green dye @ $200	$3000
Consumable laboratory supplies	Pipette tips 4 cases @ $120/case = $720; Gloves 3 cases @ $180/case; Serological pipettes @ $280; Microcentrifuge tubes @ $120/case; PCR plates @ $240/case; PCR plate seals @ $90/case; Centrifuge tubes @$90/case; Autoclavable biosafety disposal bags 2 cases @ $50/case; Kimwipes @ $60/case	$2,000
TOTAL $5,000		

F. Construction
None Budgeted

G. Consultants/Contracts

(b)(6) Ph.D.	Entomology Analysis	(b)(4), (b)(6) ours @ (b)(4), (b)(6) hour	(b)(4), (b)(6)
			TOTAL $1,500

H. Other Costs

Description	Computation	Cost
GRA Tuition Waiver (ESS)	$15,259	$15,259
		TOTAL $ $15,259

I. Indirect Costs

Description	Computation	Cost
Facilities and Administrative	$23,316 (MTDC) x 49%	$11,425

Costs – Anthropology		
Facilities and Administrative Costs – ESS and Entomology	$46,561 (MTDC) x 40%	$18,624
		TOTAL $30,049

Year 2 Budget Summary

Budget Category	**COST**
A. Personnel	$48,087
B. Fringe Benefits	$13,576
C. Travel	$1714
D. Equipment	$0
E. Supplies	$5,000
F. Construction	$0
G. Consultants/Contracts	$1,500
H. Other	$15,259
Total Direct Costs	$85,136
I. Indirect Costs	$30,049
TOTAL YEAR 2	**$115,185**

Budget Narrative

Basic Scientific Research to Support Forensic Science

for Criminal Justice Purposes

NIJ-2013-3362

A MULTIDISCIPLINARY VALIDATION STUDY OF NONHUMAN ANIMAL MODELS

FOR FORENSIC DECOMPOSITION RESEARCH

Prepared and Submitted by

Co-PI Jennifer M. DeBruyn, PhD
Department of Biosystems Engineering & Soil Science, The University of Tennessee
2506 EJ Chapman Dr. Knoxville, TN 37996;
865-974-7277; jdebruyn@utk.edu

Lead PI Dawnie L.W. Steadman, PhD,
Director, Forensic Anthropology Center
Department of Anthropology
The University of Tennessee,
250 S. Stadium Hall Knoxville, TN 37996
865-974-0909; dsteadma@utk.edu

Co-PI Lee M. Jantz, PhD
Department of Anthropology,
Associate Director, Forensic Anthropology Center, The University of Tennessee
250 S. Stadium Hall Knoxville TN 37996
865-974-4408; ljantz@utk.edu

Co-PI Giovanna Vidoli, PhD
Department of Anthropology,
Assistant Director, Forensic Anthropology Center, The University of Tennessee
250 S. Stadium Hall Knoxville TN 37996
865-974-4408; gvidoli@utk.edu

Co-PI Sean M. Schaeffer, PhD
Department of Biosystems Engineering & Soil Science, The University of Tennessee
2506 EJ Chapman Dr. Knoxville, TN 37996;
865-974-7366; sschaef5@utk.edu

Consultant - (b)(6) **PhD**
Professor Emeritus of Entomology
Purdue University
West Lafayette, IN 47907-2089
(b)(6) **@purdue.edu**

March 28, 2013

Key words

Decomposition, Postmortem Interval, Animal Models, Soil, Microbiology, Isotopes

TABLE OF CONTENTS

BUDGET JUSTIFICATION – YEAR 1

A MULTIDISCIPLINARY VALIDATION STUDY OF NONHUMAN ANIMAL MODELS FOR FORENSIC DECOMPOSITION RESEARCH

Year 1 Budget: $220,198

A. Personnel: $69,574

1. **Dawnie Steadman, PhD** $0
   As the Director of the Forensic Anthropology Center (FAC), Dr. Steadman will maintain the project's overall managerial responsibility, assisted by Drs. Jantz and Vidoli. Steadman will select and supervise the graduate research assistant, and will review all reports and presentations prior to dissemination. Dr. Steadman's statistical fluency will be integral during data analysis. Salary request: None, she will donate her time to the project.
2. **Lee Meadows Jantz, PhD** $6,387
   As the Associate Director of the FAC, Dr. Jantz manages the Body Donation Program, Dr. Jantz will oversee the selection, intake, and preparation of the human cadavers. During data analysis Jantz will provide the necessary background in decomposition research to compare morphoscopic changes between each subject and trial. Salary request: 1summer month the first year, and will volunteer her time the second year. Based on (b)(4), (b)(6) annual salary.
3. **Giovanna Vidoli, Ph.D.** $13,648

   As Assistant Director of the FAC, Dr. Vidoli will liaison with the Veterinary College to procure the nonhuman animal subjects, serve as the project PI for the Institutional Animal Care and Use Committee (IACUC), analyze the meteorological data, and assist Dr. Steadman with the correlation analyses of the metadata. . Salary request: (b)(4), (b)(6) f annual salary of (b)(4), (b)(6)
4. **Graduate research assistant** $16,500
   A graduate student in the Department of Anthropology will be responsible for daily systematic photographs of the research subjects, taking detailed notes on stages of decomposition and scores for each body region, and assisting with entomological data collection on a daily basis at the Anthropology Research Facility. The GRA will also assist with soil sampling for the microbial and chemistry portion of the project on an as needed basis. Salary requested: 1 year. ($16,500 per year).
5. **Jennifer DeBruyn, PhD** $3,564
   DeBruyn will perform the molecular microbial analyses and supervise a graduate student. Prepare abstracts, presentations and manuscripts for dissemination. Salary requested for 0.5 months per year. Based on (b)(4), (b)(6) annual salary.
6. **Sean Schaeffer, PhD** $3,474

Shaeffer will perform the soil biogeochemistry and functional assays (respiration, enzyme assay etc.). Prepare abstracts, presentations and manuscripts for dissemination. Salary requested for 0.5 months per year. Based on (b)(4), (b)(6) annual salary.

7. **Graduate research assistant** $18,000
   A Master's student in Environmental & Soil Science (ESS) will work with DeBruyn and Schaeffer to complete the microbiological and biogeochemical analyses. Funds requested for full stipend ($18,000 per year.)

8. **Tech/Professional** $8,001
   Three months of salary is requested for a laboratory technician to help with molecular biology and sequence analysis ($2,667 per month).

B. Fringe Benefits: **$16,633**

1. **Dawnie Steadman (32%)** $0
   Volunteer
2. **Lee Meadows Jantz (32%)** $2,044
   Health insurance and benefits.
3. **Giovanna Vidoli (43%)** $5,869
   Health insurance and benefits.
4. **Graduate Research Assistant** $1,372
   Student health insurance, 1 year.
5. **Jennifer DeBruyn (26%)** $923
   Health insurance and benefits
6. **Sean Schaeffer (38%)** $1,313
   Health insurance and benefits
7. **Graduate research assistant (ESS)** $2,712
   Health insurance and benefits
8. **Tech/Professional (30%)** $2,400
   Health insurance and benefits

C. Travel: **$1,564**
Travel for (b)(6) to come to Knoxville and spend a week. Mileage for 2000 miles at $0.47 per mile, hotel for 4 nights at $86 per night, meals for five days at $56 per day.

D. Equipment: No equipment is requested **$0**

E. Supplies: **$30,642**

Supplies for Anthropology

1. **TGP-4520 Dual Channel External Temperature (rabbit/pig)** $3,000
   12 @ $250/ea. Tinytag dual-channel temperature recorder and data loggers can be used to record data from 2 cadavers simultaneously, using the dual probe attachments. These 12 data loggers will record core temperatures data from human, pig and rabbit cadavers at each trial site. The purchase of 12 data loggers allows 2 trials to run

concurrently. FAC also owns software associated with Tinytag data loggers and probes.

2. TGP-4500 Temperature and Humidity Data Logger $500

2 @ $250/ea. One data logger placed at 3 trial site locations to collect ambient temperature and relative humidity. Three temp/humidity loggers allow 2 trials to run concurrently.

3. PB-5001 Standard Thermistor Probe $1,440

24 @ $60/ea. Standard thermistor probe for use with Tinytag data loggers. One per cadaver, both human and nonhuman for two trials. Twenty-four probes allow 2 trials to run concurrently.

4. Nikon D7000 $1,300

The Nikon D7000 is the only Nikon camera with solo duel slots. This allows data to be backed-up automatically on a separate card when photographing, preventing data loss. The 7000 is the only series compatible with every Nikon lens. The FAC currently owns several Nikon lens, including a macro lens, which will be used during this project. A full kit is incorporated into the camera package including an additional lens and shockproof case for storage, eliminating the need to purchase a separate Pelican Case or needed accessories. Finally, the FAC currently owns the software for this camera.

5. Game Spy I-45S Game Camera $1,560

6 @ $260/ea. Cameras are dual purpose, time-lapse photography and motion sensing, however they do not capture both at the same time. Two cameras will be placed at each of the three trial sites. One camera will be set for time-lapse photography to monitor decay activity hourly and the second camera will be set to the motion sensor, monitoring scavenger activity. Upon completion of the first trial, the 2 camera set-up at the first trial site will be moved to the last trial site. This leapfrog method will prevent interference of the time-lapse photography and motion sensor scavenger monitoring during the first two trials. All three sites do not need cameras running concurrently, scavengers are typically attracted to bodies in their early stages of decomposition. Changes in decay at the later stages are minimal and won't require hourly photos. The GRA will continue to take daily photographs after the game cameras are removed.

6. 32 gig Flash Memory Card $300

12 @ $25/ea, 2 per game camera

7. D-Cell Batteries $540

6 batteries per game camera (n=36), per month (36x12) total = 432 batteries. 12 batteries per pack = 36 packs. 36 packs @ $15/ea.

8. Shoe Covers $180

3 cases @ $60/ea. Standard Personal Protective Equipment required while conducting research at the ARF.

9. Sleeves $330

3 cases @ $110/ea. Standard Personal Protective Equipment required while conducting research at the ARF.

10. Gowns $270

3 cases @ $90/ea. Standard Personal Protective Equipment required while conducting research at the ARF.

11. Latex Gloves $225

3 cases @ $75/ea. Standard Personal Protective Equipment required while conducting research at the ARF.

12. Insect Aerial Nets $84

3 (@$28) nets will be used to collect flying insects on a daily basis. One net will be used per trial.

13. 1000 20 ml Scintillation vials with caps $300

Insects collected will be placed in the vials upon collection.

14. Chemicals glacial acetate, ethyl acetate, kerosene, and ethyl alcohol 190 proof $405

These chemicals will be used to kill and preserve the insects upon collection.

15. Pig Cadavers $2,400

12 ~150 lb pigs @ $200/pig. Four pig cadavers will be used during each of the three trials.

16. Rabbit Cadavers $300

12 ~6lb rabbits @ $25/ea. Four rabbit cadavers will be used during each of the three trials.

17. Transportation of Pigs $1,200

The pigs will be transported from the vendor's location in Henry, TN to UTK. Three trips @ $400 per trip.

18. Veterinarian Euthanasia $1,308

The pigs and rabbits will be taken to the University of Tennessee Veterinarian School for euthanasia on the day each trial will begin. The drugs for the 12 pigs are $58 each, and drugs for the 12 rabbits are $1 per animal. In addition, a vet tech fee for 24 animals is $25.

19. Human Cadavers $0

5 Human cadavers provided through the Forensic Anthropology Center's Body Donation Program. One human cadaver will be used during each of the five trials.

Supplies for molecular biology and soil chemistry **$15,000**

Funds are requested to purchase consumables supplies and reagents for molecular biology, enzyme assays and chemistry:

1. Molecular biology kits (soil DNA extraction kits, DNA purification kits etc.) $3,000
2. Enzymes, primers, and reagents for molecular biology $3,000
3. Chemicals for enzyme rate assays and chemical measurements $5,000
4. Consumable supplies include filters, pipette tips, gloves, centrifuge tubes, plates, PPE, and miscellaneous laboratory supplies. $4,000

F. Construction: None budgeted **$0**

G. Consultants/Contracts: $12,000

1. 454 Pyrosequencing

Funds requested for one plate of 454 pyrosequencing for microbial community characterization. This will be done in house at the next generation sequencing facility at UT/Oak Ridge National Labs Joint Institute for Biological Sciences. The $12,000 flat rate includes library preparation supplies, technician time, and server time for initial post-run processing of flow-grams.

2. (b)(6) **Ph.D.** (b)(4), (b)(6)
(b)(6) will be training a GRA in the collection and analysis of insects that are ongoing daily with each trial. His rate is (b)(4), (b)(6) our fo (b)(4), (b)(6) ours.

H. Other Costs: **$28,595**

Tuition Waiver (Anthropology) $14,596
$4,770 per semester for two semesters, and $5,056 for one semester for one academic year.

Tuition Waiver (ESS) $13,999
Graduate student fees

I. Indirect Costs: **$58,940**

$65,276 (MTDC) x 49% (Anthropology) – on-campus research $31,985

$67,387 (MTDC) x 40% (ESS and Entomology) $26,955

TOTAL COST: $220,198

BUDGET JUSTIFICATION – YEAR 2

A MULTIDISCIPLINARY VALIDATION STUDY OF NON-HUMAN ANIMAL MODELS FOR FORENSIC DECOMPOSITION RESEARCH

YEAR 2 Budget: $115,185

A. Personnel: **$48,087**

1. Dawnie Steadman, PhD $0

As the director of the Forensic Anthropology Center, Dr. Steadman will maintain the project's overall managerial responsibility, and will review all reports and presentations prior to dissemination. Dr. Steadman's statistical fluency will be integral during data analysis. Salary request: None, will donate time to the project.

2. Lee Meadows Jantz, PhD $0

During data analysis Jantz will provide the necessary background in decomposition research to compare morphoscopic changes between each subject and trial. Salary request: None, will donate time to the project.

3. Jennifer DeBruyn, PhD $3,671

DeBruyn will perform the molecular microbial analyses and supervise a graduate student. Prepare abstracts, presentations and manuscripts for dissemination. Salary requested for 0.5 months per year. Based on (b)(4), (b)(6) annual salary.

4. Sean Schaeffer, PhD $3,578

Schaeffer will perform the soil biogeochemistry and functional assays (respiration, enzyme assay etc. Prepare abstracts, presentations and manuscripts for dissemination. Salary requested for 0.5 months per year. Based on (b)(4), (b)(6) annual salary.

5. Giovanna Vidoli, PhD $14,057

As Assistant Director of the FAC, Dr. Vidoli will liaison with the Veterinary College to procure the nonhuman animal subjects, serve as the project PI for the Institutional Animal Care and Use Committee (IACUC), analyze the meteorological data, and assist Dr. Steadman with the correlation analyses of the metadata. . Salary request: (b)(4), (b)(6) of annual salary of (b)(4), (b)(6)

6. Graduate research assistant $18,540

A masters student in Environmental & Soil Science (ESS) will work with DeBruyn and Schaeffer to complete the microbiological and biogeochemical analyses. Funds requested for full stipend ($18,540 per year).

7. Laboratory Technician $8,241

Three months of salary is requested for a laboratory technician to help with molecular biology and sequence analysis ($2,747 per month).

B. Fringe Benefits: **$13,576**

1. Dawnie Steadman, Principal Investigator (32%) $0

2. Lee Meadows Jantz, Principal Investigator (32%) $0

3. Giovanna Vidoli, Principal Investigator (43%) $6,045
Health insurance and benefits

4. Jennifer DeBruyn (25.9%) $951
Health insurance and benefits

5. Schaeffer (37.8%) $1,353
Health insurance and benefits

6. ESS GRA $2,755
8% plus Health Insurance ($1,272/year)

7. Technician (30%) $2,472
Health insurance and benefits

C. Travel: $1,714

Travel for two Co-PI's to the American Academy of Forensic Sciences annual meeting in Orlando, FL to present the results of the research. This is will include airfare ($300 x 2), hotel for two ($111/night) and meals for two ($56/day for 4 days).

D. Equipment: No equipment is requested $0

E. Supplies:

Supplies for molecular biology and soil chemistry $5,000

Funds are requested to purchase consumables supplies and reagents for molecular biology, enzyme assays and chemistry:

a. Enzymes, primers, and reagents for molecular biology ($3,000)
b. Consumable supplies include filters, pipette tips, gloves, centrifuge tubes, plates, PPE, and miscellaneous laboratory supplies. ($2,000)

F. Construction: No construction needed $0

G. Contracts: $1,500

Ralph Williams, PhD. will continue to conduct the entomological analysis for approximately 20 hours at $75/hour.

H. Other Costs:

1. Tuition and fees (ESS) $15,259
ESS student tuition waiver at $15,259

I. Indirect costs: **$30,049**

$23,316 (MTDC) x 49% (Anthropology) $11,425

$46,561 (MTDC) x 40% (ESS and Entomology) $18,624

TOTAL COST: $115,185

BUDGET JUSTIFICATION – CUMULATIVE (2-YEAR) BUDGET

A MULTIDISCIPLINARY VALIDATION STUDY OF NON-HUMAN ANIMAL MODELS FOR FORENSIC DECOMPOSITION RESEARCH

Overall 2-year combined Budget $335,383

A. Personnel: **$117,661**

1. Dawnie Steadman, PhD $0

As the director of the Forensic Anthropology Center, Dr. Steadman will maintain the project's overall managerial responsibility, assisted by Drs. Jantz and Vidoli. Steadman will select and supervise the graduate research assistant, and will review all reports and presentations prior to dissemination. Dr. Steadman's statistical fluency will be integral during data analysis. Salary request: None. She has donated her time to save costs to the project.

2. Lee Meadows Jantz, PhD $6,387

As the FAC coordinator for the Body Donation Program, Dr. Jantz will oversee the selection, intake, and preparation of the human cadavers, and will be responsible for the acquisition of the rabbit and pig carcasses. During data analysis Jantz will provide the necessary background in decomposition research to compare morphoscopic changes between each subject and trial. Salary request: 1 summer month. Based on (b)(4), (b)(6) annual salary.

3. Giovanna Vidoli, Ph.D. $27,705

As Assistant Director of the FAC, Dr. Vidoli will liaison with the Veterinary College to procure the nonhuman animal subjects, serve as the project PI for the Institutional Animal Care and Use Committee (IACUC), analyze the meteorological data, and assist Dr. Steadman with the correlation analyses of the metadata. . Salary request (b)(4), (b)(6) f annual salary of (b)(4), (b)(6) 2014) and (b)(4), (b)(6) 2015).

4. Jennifer DeBruyn, PhD $7,235

DeBruyn will perform the molecular microbial analyses and supervise a graduate student. Prepare abstracts, presentations and manuscripts for dissemination. Salary requested for 0.5 months per year. Based on (b)(4), (b)(6) annual salary (2014) and (b)(4), (b)(6) (2015).

5. Sean Schaeffer, PhD $7,052

Schaeffer will perform the soil biogeochemistry and functional assays (respiration, enzyme assay etc. Prepare abstracts, presentations and manuscripts for dissemination. Salary requested for 0.5 months per year. Based on (b)(4), (b)(6) nnual salary (2014) and (b)(4), (b)(6) (2015).

6. Graduate Research Assistant (Anthropology) $16,500

A graduate student in the Department of Anthropology will be responsible for daily systematic photographs of the research subjects, taking detailed notes on stages of decomposition and scores for each body region, and assisting with entomological data

collection on a daily basis at the Anthropology Research Facility. The GRA will also assist with soil sampling for the microbial and chemistry portion of the project on an as needed basis. Salary requested: $16,500..

7. Graduate research assistant (ESS) $36,540

A masters student in Environmental & Soil Science (ESS) will work with DeBruyn and Schaeffer to complete the microbiological and biogeochemical analyses. Funds requested for full stipend. Salary requested: $18,000 Y1; $18,540 Y2.

8. Tech/Professional $16,242

A laboratory technician will help with molecular biology and sequence analysis DeBruyn and Schaeffer. Salary request is 25% salary for each year ($32,004 for 2014 annual salary and $32,964 for 2015 annual salary).

B. Fringe Benefits: **$30,209**

1. **Dawnie Steadman (32%)** $0
   Benefits (Retirement, Social Security, Worker's Comp).
2. **Lee Meadows Jantz (32%)** $2,044
   Benefits (Retirement, Social Security, Worker's Comp).
3. **Giovann Vidoli, (43%)** $11,914
4. **Jennifer DeBruyn (26%)** $1,874
   Health insurance and benefits
5. **Sean Schaeffer (38.1%)** $2,666
   Health insurance and benefits
6. **Graduate Research Assistant (Anthropology)** $1,372
   Student health insurance, 1 year .
7. **ESS GRA** $5,467
   Student Health benefits
8. **Tech/Professional (30%)** $4,872

C. Travel: **$3,278**

1. Travel to Knoxville by (b)(6) in year 1 including mileage of 2000 miles@ $0.47/mile, hotel for 4 nights at $86 per night, and five days of meals at $56 per day in Y1. ($1,796)

2. Travel to Orland to attend American Academy of Forensic Sciences in 2015 by two CO-PI's. Airfare of $300 for two people, hotel for two people at a rate of $111 per night for three nights, and meals for two people at $56 per day for four days. ($1,714)

D. Equipment: None budgeted $0

E. Supplies: **$35,642**

Supplies for Anthropology

1. **TGP-4520 Dual Channel External Temperature (rabbit/pig)** $3,000

12 @ $250/ea. Tinytag dual-channel temperature recorder and data loggers can be used to record data from 2 cadavers simultaneously, using the dual probe attachments. These 12 data loggers will record core temperatures data from human, pig and rabbit cadavers at each trial site. The purchase of 12 data loggers allows 2 trials to run concurrently. FAC also owns software associated with Tinytag data loggers and probes.

2. TGP-4500 Temperature and Humidity Data Logger $500

2 @ $250/ea. One data logger placed at 3 trial site locations to collect ambient temperature and relative humidity. Three temp/humidity loggers allow 2 trials to run concurrently.

3. PB-5001 Standard Thermistor Probe $1,440

24 @ $60/ea. Standard thermistor probe for use with Tinytag data loggers. One per cadaver, both human and nonhuman for two trials. Twenty-four probes allow 2 trials to run concurrently.

4. Nikon D7000 $1,300

The Nikon D7000 is the only Nikon camera with solo duel slots. This allows data to be backed-up automatically on a separate card when photographing, preventing data loss. The 7000 is the only series compatible with every Nikon lens. The FAC currently owns several Nikon lens, including a macro lens, which will be used during this project. A full kit is incorporated into the camera package including an additional lens and shockproof case for storage, eliminating the need to purchase a separate Pelican Case or needed accessories. Finally, the FAC currently owns the software for this camera.

5. Game Spy I-45S Game Camera $1,560

6 @ $260/ea. Cameras are dual purpose, time-lapse photography and motion sensing, however they do not capture both at the same time. Two cameras will be placed at each of the three trial sites. One camera will be set for time-lapse photography to monitor decay activity hourly and the second camera will be set to the motion sensor, monitoring scavenger activity. Upon completion of the first trial, the 2 camera set-up at the first trial site will be moved to the last trial site. This leapfrog method will prevent interference of the time-lapse photography and motion sensor scavenger monitoring during the first two trials. All three sites do not need cameras running concurrently, scavengers are typically attracted to bodies in their early stages of decomposition. Changes in decay at the later stages are minimal and won't require hourly photos. The GRA will continue to take daily photographs after the game cameras are removed.

6. 32 gig Flash Memory Card $300

12 @ $25/ea, 2 per game camera

7. D-Cell Batteries $540

6 batteries per game camera (n=36), per month (36x12) total = 432 batteries.
12 batteries per pack = 36 packs. 36 packs @ $15/ea.

8. Shoe Covers $180

3 cases @ $60/ea. Standard Personal Protective Equipment required while conducting research at the ARF.

9. Sleeves $330

3 cases @ $110/ea. Standard Personal Protective Equipment required while conducting research at the ARF.

10. Gowns $270

3 cases @ $90/ea. Standard Personal Protective Equipment required while conducting research at the ARF.

11. Latex Gloves $225

3 cases @ $75/ea. Standard Personal Protective Equipment required while conducting research at the ARF.

12. Insect Aerial Nets

3 nets will be used to collect flying insects on a daily basis. One net will be used per trial.

13. 1000 20 ml Scintillation vials with caps $300

Insects collected will be placed in the vials upon collection.

14. Chemicals glacial acetate, ethyl acetate, kerosene, and ethyl alcohol 190 proof

$705

These chemicals will be used to kill and preserve the insects upon collection.

15. Pig Cadavers $2,400

12 ~150 lb pigs @ $200/pig. Four pig cadavers will be used during each of the three trials.

16. Rabbit Cadavers $300

12 ~6lb rabbits @ $25/ea. Four rabbit cadaver swill be used during each of the three trials.

17. Transportation of Pigs $1,200

The pigs will be transported from the vendor's location in Henry, TN to UTK. 3 trips @ $400/trip

18. Veterinarian Euthanasia $1,308

The pigs and rabbits will be taken to the University of Tennessee Veterinarian School for euthanasia on the day each trial will begin. The drugs for the 12 pigs are $58 each, and drugs for the 12 rabbits are $1 per animal. In addition, a vet tech fee for 24 animals is $25.

19. Human Cadavers $0

5 Human cadavers provided through the Forensic Anthropology Center's Body Donation Program. One human cadaver will be used during each of the five trials.

Supplies for molecular biology and soil chemistry

1.Supplies for molecular biology and soil chemistry $20,000

Funds are requested to purchase consumables supplies and reagents for molecular biology, enzyme assays and chemistry:

Molecular biology kits (soil DNA extraction kits, DNA purification kits etc.) $3,000
Enzymes, primers, and reagents for molecular biology $6,000
Chemicals for enzyme rate assays and chemical measurements $5,000
Consumable supplies include filters, pipette tips, gloves, centrifuge tubes, plates, PPE, and miscellaneous laboratory supplies. $6,000

F. Construction: None budgeted $0

G. Consultants/Contracts **$15,750**

1. 454 Pyrosequencing

Funds requested for one plate of 454 pyrosequencing for microbial community characterization. This will be done in house at the next generation sequencing facility at UT/Oak Ridge National Labs Joint Institute for Biological Sciences. The $11,000 charge includes library preparation supplies, technician time, and server time for initial post-run processing of flow-grams. $12,000

2 (b)(6) **Ph.D.**

(b)(6) will be training a GRA in the collection and analysis of insects that are ongoing daily with each trial. Hi rate i(b)(4), (b)(6) our fo(b)(4), (b)(6) ours. (b)(4), (b)(6)

H. Other Costs **$43,854**

1. Tuition waiver (Anthropology) $14,596

$4770/semester x 2 semesters; 1 semester @ $5056

2. Tuition and fees (ESS) $36,540

ESS student: $12,873 for tuition for 2012-2013; $14,032 for 2013-2014

I. Indirect Costs: **$88,989**

$88,592 (MTDC) x 49% (Anthropology) $43,410
$113,948 (MTDC) x 40% (ESS and Entomology) $45,579

TOTAL COST: $335,383