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Gravesoil Microbial Community Structure During Carcass Decomposition

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GRAVESOIL MICROBIAL COMMUNITY STRUCTURE
DURING CARCASS DECOMPOSITION

by

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A THESIS

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Dead bodies placed on soil represent unique challenges for investigators. Although processes in soils can be used to estimate postmortem interval, we know very little about how carcasses and insects affect gravesoil microbial communities.

To address this, the current project was composed of two experiments. Experiment one was conducted to investigate the effect of surface type on carcass decomposition and evaluate soil ecology methods. Experiment two was conducted to investigate the presence of an insect population (*Lucilia sericata* Meigen) on gravesoil microbial communities. Both experiments were conducted in a laboratory setting using freshly killed mouse carcasses. Mouse carcasses were placed on either a plastic petri dish without soil or on a sand/soil mix (90/10 or 50/50) and calibrated to 55% water holding capacity. Carcass decomposition was measured as mass loss and total body score. Gravesoil microbial communities were analyzed as lipid-phosphorous and fatty acid methyl esters. Carcass decomposition was measured over 35 days at 20°C (experiment 1) and 22°C (experiment 2).

Major findings were that surface type affects the rate of carcass decomposition; carcasses decompose faster on soil. Also, the presence of a carcass on soil resulted in a significant increase in soil microbial biomass. Interestingly, the presence of insects did not affect soil microbial biomass, but it did affect the soil microbial community. Changes in microbial community structure were observed after seven days of decomposition. These gravesoils were associated with significantly different concentrations of bacteria, eukaryotes, and arbuscular mycorrhizal fungi. These findings are insightful, because they show that soil based methods in forensic science must account for the presence of insects to be accurate in estimating postmortem interval.

Keywords: forensic taphonomy, extended post mortem interval, ecology, *Lucilia sericata*, fatty acid methyl esters analysis, lipid-phosphorus analysis
To my family, whose never ending support lead me here.

To Dave, who’s encouragement made all the difference during this enlightening journey called graduate school.
Table of Contents

Chapter 1: Introduction 1
Chapter 2: Materials and Methods 7
Chapter 3: Results 18
Chapter 4: Discussion 43
References 48

Multimedia Objects

Figure 1  Experimental unit for experiment 1 9
Table 1  Decomposition scoring system 11
Figure 2  Growth chamber for experiment 2 13
Figure 3  Petri dishes in growth chamber 13
Figure 4  Mice carcasses in growth chamber 15
Figure 5  Soil homogenized during harvest 16
Figure 6  Mass loss graph for experiment 1 19
Figure 7  Mouse carcass on soil on day 14 20
Figure 8  Mouse carcass without soil on day 7 20
Figure 9  Mouse carcass without soil on day 14 21
Figure 10  Total body score for experiment 1 22
Figure 11  Mouse carcass on soil on day 28 23
Figure 12  Mouse carcass on soil on day 28 23
Figure 13  Microbial biomass graph for experiment 1 24
Figure 14  Soil moisture content graph for experiment 1 25
Figure 15  Mass loss graph for experiment 2 27
Figure 16  Mouse carcass on soil on day 21 with mites 27
Figure 17  Acarid mites under microscope  28
Figure 18  Mouse carcass on soil on day 35  28
Figure 19  Mouse carcass on soil on day 35  29
Figure 20  Total body score graph for experiment 2  30
Figure 21  Mouse carcass on soil with insects on day 14  31
Figure 22  Mouse carcass on soil with insects on day 15  31
Figure 23  Microbial biomass graph for experiment 2  32
Figure 24  Discriminant canonical correlation graph  34
Figure 25  Total FAMEs graph  35
Figure 26  Graphs for fatty acid taxonomic classes  36
Figure 27  Bacterial fatty acid graph  37
Figure 28  Eukaryote fatty acid graph  38
Figure 29  Fungal fatty acid graph  39
Figure 30  Arbuscular mycorrhizal fungi graph  40
Figure 31  Actinomycete fatty acid graph  41
Figure 32  Soil moisture content for experiment 2  42
Table 2  GC-MS identification of fatty acids  54
Table 3  Taxonomic classes for fatty acids  56

Appendices

Appendix A  Soil ecology method protocols and fatty acid identifications  51
Appendix B  Experiment 1 photographs  57
Appendix C  Experiment 2 photographs  913
Gravesoil Microbial Community Structure during Carcass Decomposition

Chapter 1: Introduction

In homicide investigations, decomposing bodies on or in soil present unique challenges. While bodies themselves are obviously of forensic importance, the gravesoil, any soil associated with a dead animal (Carter et al. 2007), is evidence itself. The study of gravesoil is of primary interest to the field of forensic taphonomy, the discipline concerned with death investigation and the decomposition of dead animals. Forensic taphonomists use decomposition and human remains to identify the deceased, estimate cause and manner of death, locate clandestine graves, and estimate postmortem interval (Haglund and Sorg 1997). These components of an investigation can be crucial, because they allow an investigator to assess the veracity of an alibi, exclude suspects, or focus an investigation toward a particular geographical area.

Decomposition of organic material, an essential part of ecosystem function, is a process that contributes to the cycling of energy and nutrients (Carter et al. 2009). Decomposition is a complex process that takes place under a range of abiotic and biotic conditions, with the specific progression dependent upon the quality of the resource that is being decomposed, the decomposer community, and the physicochemical environment in which the resource is located (Swift et al. 1979). For example, the decomposer community associated with carcasses includes scavengers, insects, and microbes. The activity of these decomposers will vary according to their environment (e.g. temperature, burial, etc.) and the quality of the carcass as it breaks down.
We currently recognize (e.g. Dent et al. 2004; Carter et al. 2007; Benninger et al. 2008; Carter et al. 2010) that a carcass is a significant ephemeral source of energy, nutrients, and water. A carcass acts as a hub through which biota are dispersed throughout a landscape. This dispersal is the result of several internal and external processes. Two major internal processes of carcass decomposition are autolysis and putrefaction. Autolysis is the self-destruction of cells and organic compounds caused by cellular enzymes (Carter et al. 2007; Van Belle et al. 2009) after acidity increases with elevated carbon dioxide levels within the body cavity (Gill-King 1997). This process can begin immediately following the cessation of the heart when a lack of internal oxygen inhibits metabolism by aerobic microorganisms (Carter et al. 2007). Putrefaction, the breakdown of soft tissue, occurs later when proteins, lipids, and carbohydrates are converted into gases and organic acids by anaerobic microorganisms which dominate the body cavity after oxygen is depleted (Carter et al. 2007). When hemoglobin reacts with hydrogen sulfide, hemolysis of blood vessels occur and results in green marbling, which also marks the start of the putrefaction process (Schoenly et al. 2006). An intense odor from the carcass can be detected at this time (Carter and Tibbett 2008 (B)). Physical changes follow quickly, usually in a sequential, predictable pattern. Decomposition stages (fresh, bloat, active decay, advanced decay, and skeleton) can be determined from the observation of these physical changes (Schoenly and Reid 1987; Carter and Tibbett 2008 (B)). These stages do not necessarily occur as individual stages, but are a steady progression of transformations (Schoenly and Reid 1987).

Externally, carcasses are broken down primarily by insects and scavengers (i.e., mammals, birds, reptiles). In warm weather insects will often outcompete scavengers
(DeVault et al. 2004), because they can respond rapidly to volatile compounds, such as carbon dioxide, hydrogen sulfide, ammonia, methane, hydrogen, and sulfur dioxide, released from the body after death (Vass 2001). Scavenging tends to occur either before insects dominate a carcass or after blow fly larvae have migrated. Quite simply, scavengers tend not to eat carcasses covered in maggots. Several carcass decomposition studies have been conducted to understand the structure of insect (Reed 1958; Payne 1965; Payne et al. 1968; Rodriguez and Bass 1983) and scavenger (Hill 1977; Hagland et al. 1988) communities. Insects and scavengers represent major pathways through which carcass materials are cycled particularly in outdoor death scenes. The soil also represents a major pathway for carcass materials in outdoor death scenes. Yet, processes in gravesoils, particularly the structure of gravesoil microbial communities, remains poorly understood (Parkinson et al. 2009; Howard et al. 2010).

Understanding the structure of gravesoil microbial communities is important because soil frequently excludes macrobiotic agents making microbes primarily responsible for organic decomposition. Their ability to breakdown organic material contributes to nutrient cycling and can result in an increase in microbial biomass (Carter et al. 2009). The microbial contribution to decomposition is evident in several studies (e.g. Carter et al. 2009). Howard et al. (2010) reported that microbial metabolism can produce chemical changes within the ecosystem, including an inverse relationship among proteolytic and lipolytic bacteria throughout the decomposition process. Microbial activity accounts for approximately 90% of the mobilization of carbon and nitrogen in soil (Burges et al. 1967; Swift et al. 1979; Paul 2007). In fact, decomposition is second
only to photosynthesis for the cycling of carbon in an ecosystem (McGuire and Treseder 2010).

Although most human deaths occur indoors, the turn of the 21st century has witnessed a growing interest in outdoor carcass decomposition. Much of what we know about gravesoil associated with dead human bodies is the result of work conducted at the University of Tennessee Anthropology Research Facility (UTARF). Rodriguez and Bass (1985) observed that the rate of decomposition decreases as the depth of burial increases (see also Mann et al. 1990). The pH of gravesoil also tends to increase during the early stages of cadaver breakdown when ammonium levels are elevated (Mann et al. 1990).

Changes in gravesoil chemistry coincide with putative shifts in gravesoil microbial communities which are made up of bacteria, fungi, algae, yeast, and protozoa (Carter and Tibbett 2006; Carter et al. 2009). These separate communities measure biomass as a single collective group when carbon dioxide is measured (Carter and Tibbett 2006; Carter et al. 2009). Bacterial and fungal community structures will shift and soil microbial activity will increase in less than 24 hours of carcass placement (Carter and Tibbett 2008 (B)). The shift in bacterial communities, although beginning in the fresh stage, will continue throughout all five stages (Carter et al. 2009). This shift most likely reflects the microbes need for nutrients (Moreno et al. 2011). Changes in gravesoil pH and microbial community structure, along with the concentration of short-chain fatty acids (butyric, propionic, acetic, formic, valeric, caproic, heptanoic) and other nutrients (ammonium, calcium, chloride, magnesium, potassium, sodium, sulfate), can be used to estimate the postmortem interval (Vass et al. 1992).
Unfortunately, access to human bodies for decomposition studies is rare. Oftentimes, forensic taphonomy uses analogs such as swine (Payne 1965; Payne et al. 1968; Schoenly et al. 2006; Voss et al. 2009; Van Belle et al. 2009; Howard et al. 2010), rats (Tomberlin and Adler 1998; De Jong and Hoback 2006; Carter and Tibbett 2008 (A); Carter et al. 2010), and mice (Putman 1978; Blackith and Blackith 1989; Stokes et al. 2009). Although these analogs differ greatly in size to a human body, depending on the question being asked, working with small animals can give insights into the decomposition of large animals. For instance, we know that differences in decomposition rate are harder to see in small animals than large animals, because small animals decompose faster. Therefore, if a difference in decomposition can be measured with small animals, a difference in large animals would almost certainly be detected.

Ecologically and forensically, the related question that needs to be addressed includes do microbial communities qualitatively differ for different animals? Relative to decomposition, what is the most significant microbial source that contributes to the decomposition process? Is it internal, air, surface, or transport (e.g., by carrion insects)? Of these, we expect internal microbes to be the most important, because bodies bloat, decompose in the absence of insects, and even in the absence of air. However, is this argument true for internal microbes being more influential in the decomposition process than soil microbes? There has not been any previous work done to address this question directly with soil. Therefore, to examine this question, I conducted two experiments to address three specific points.

1. Decomposition of a mouse carcass on soil versus not on soil
2. Decomposition of a mouse carcass with and without insects
3. Examination of soil ecology methods with gravesoil for measuring microbial communities and decomposition

Since one of our goals was to determine if soil ecology methods work with gravesoil, mice carcasses were chosen, because small carcasses contribute small amounts of fluid. This would limit the possibility of flooding the analyses, which would make it too difficult to determine if the microbes present were from the soil or the carcass. Additionally, there are too many microbes present where it is too hard to quantify differences of microbial communities with some methods.
Chapter 2: Materials and Methods

I conducted two experiments to explore gravesoil microbial communities. The first experiment explored the differences between carcass (mouse) decomposition on sand inoculated with soil and on a plastic surface, to distinguish between microbial communities from soil vs. microbial communities solely from the body. Controls (inoculated sand with no carcass) were used, and the experiment was replicated four times. The experiment was conducted for 35 days at approximately 20°C. The second experiment explored differences in carcass decomposition between mouse carcass on inoculated sand, mouse carcass on inoculated sand with insects, and mouse carcass on a plastic surface. Controls (inoculated sand with no carcass) were used, and the experiment was replicated four times. The experiment was conducted for 35 days at 22°C and was carried out in thermally controlled chambers.

Experiment One

Experimental Units and Treatments

Experimental units were in plastic containers (20 cm x 34 cm x 11 cm) containing ~965 g methanol-washed pea gravel, distilled water (100 ml) to regulate humidity, and a plastic petri dish (150 mm x 25 mm). Experimental treatments were imposed on petri dishes placed on the washed pea gravel. One of three treatments was applied to each unit. Treatments included inoculated sand with no carcass (control), a mouse carcass on inoculated sand, and a mouse carcass without inoculated sand. Treatments were arranged in a completely random design with four replications. Treatments were maintained for 35 days at approximately 20°C.
For treatments containing soil. Pawnee clay loam soil was collected from Nine Mile Prairie, a natural, uncultivated tall grass prairie ecosystem located approximately nine miles northwest of Lincoln, Nebraska. Collected soil was stored, frozen (-20°C), and passed through a 2 mm sieve before use in experiments. Plastic petri dishes that were designated as controls or mouse carcass on sand were filled with 360 grams (g) of washed sea sand inoculated with 40 g soil. Inoculated sand was calibrated to a water holding capacity of 55% and left to equilibrate for seven days at approximately 20°C.

Carcasses. Male mice (*Mus musculus*, ~36.8 g) were euthanized with carbon dioxide by Rhonda Griess and all treatments were established with mice dead no more than one hour postmortem. This project was approved as part of IACUC protocol 08-01-006, which approves the killing of mice from the UNL Animal Science Small Animal Laboratory. Carcasses were placed on their left side on the petri dishes designated as treatment with carcass on inoculated sand or as treatment with carcass without inoculated sand. After treatments were established, nylon mesh (0.1 mm x 0.1 mm) was used to cover each container to prevent insect colonization (Figure 1). Temperature was maintained at approximately 20°C during the experimental trial, and the water content of the inoculated sand was maintained at 55% every 3-4 days by adding distilled water.
Figure 1 Experimental units were constructed from plastic containers (20 cm x 34 cm x 11 cm) containing ~965 g methanol washed pea gravel and distilled water (100 ml) to regulate humidity. Controls (inoculated sand with no carcass), carcasses on inoculated sand, or carcasses without inoculated sand were placed in the center of a unit, and nylon mesh (0.1mm x 0.1mm) was used to cover the container to prevent insect colonization.

Experimental Procedures

After treatments were established, the experiment was conducted over 35 days. Decomposition was scored daily using a procedure described below. Every seven days a destructive harvest was conducted on previously randomized units. Mice were scored, photographed, and then removed from the unit and weighed to obtain a final mass. Carcasses were then sealed in plastic and frozen (-20°C). Soil was homogenized within its entirety then weighed for moisture content and lipid-phosphate analysis. The remaining soil was divided with half being immediately frozen and the other half left to air dry for two weeks. Lipid-phosphate analysis was conducted after the completion of the experiment.
**Decomposition Measures.** A decomposition scoring system was developed for mice based on the method of Megyesi *et al.* (2005) for human cadavers, in which physical characteristics of decomposition for different body parts or regions are assigned specific numbers. The sum of these numbers provides a Total Body Score (TBS) indicating overall decomposition. For my system, I characterized mouse carcasses based on three body regions: the head, trunk, and limbs. The head accounted for 20% of the entire body, the trunk 70%, and the limbs 10%. To obtain scores that reflected these percentages in comparison to the body as a whole, I multiplied the score of each region by their appropriate percentage. The sum of these sections provides a TBS (Table 1).

**Microbial Biomass.** Lipid-phosphate method was used to estimate total microbial biomass, because it is a precise, quantitative method used for measuring the compounds in soil extracts of living microorganisms. Protocols for this method are described in Appendix A.
Table 1 A decomposition scoring system developed using mouse carcasses to provide a Total Body Score based on physical characteristics of decomposition. TBS comprises the sum of scores from the Head, Trunk and Limbs.

<table>
<thead>
<tr>
<th>Decomposition Stage</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>*<em>Head (<em>0.2)</em></em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh Stage</td>
<td>Fresh, no discoloration</td>
<td>1</td>
</tr>
<tr>
<td>Early decomposition</td>
<td>Discoloration</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Purging of decomposition fluids out of eyes, nose, or mouth</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Bloating of neck and/or face</td>
<td>4</td>
</tr>
<tr>
<td>Advanced Decomposition</td>
<td>Sagging of flesh</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Sinking of flesh</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Caving in of flesh</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Mummification or desiccation</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Partially gone</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Gone</td>
<td>10</td>
</tr>
<tr>
<td>*<em>Trunk (<em>0.7)</em></em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh Stage</td>
<td>Fresh, no discoloration</td>
<td>1</td>
</tr>
<tr>
<td>Early decomposition</td>
<td>Discoloration</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bloating of abdominal cavity</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Rupture and/or purging of fluids</td>
<td>4</td>
</tr>
<tr>
<td>Advanced decomposition</td>
<td>Sagging of flesh</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Sinking of flesh</td>
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<td></td>
<td>Partially gone</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Gone</td>
<td>10</td>
</tr>
<tr>
<td>*<em>Limbs (<em>0.1)</em></em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>Fresh, no discoloration</td>
<td>1</td>
</tr>
<tr>
<td>Early decomposition</td>
<td>Discoloration</td>
<td>2</td>
</tr>
<tr>
<td>Advanced decomposition</td>
<td>Mummification and/or desiccation</td>
<td>3</td>
</tr>
</tbody>
</table>
Statistical Analyses

Regression analyses were generated for mass loss and TBS statistics using GraphPad Prism version 5.02 (Graph Pad Software, Inc., La Jolla, CA). Lipid-phosphate statistics were generated using the Mann-Whitney U test using SPSS version 19 (Chicago, IL). Mixed model analysis of variance was generated for moisture content using SAS 9.1 (Windows 2002-2003 SAS Institute, Cary, NC).

Experiment Two

Experimental Units and Treatments

Experimental units were plastic petri dishes housed in four thermally controlled chambers (Percival, I-30LL) (Figure 2). One of four treatments were imposed on these experimental units: controls with no carcass, carcass on inoculated sand, carcass on inoculated sand with insects, carcass on smooth, plastic surface without sand. Treatments were arranged in a completely random design with treatments and replications nested within date. Treatments were replicated four times. Within each chamber, there were twelve petri dishes on the top shelf and eight on the bottom shelf (see Figure 3). Chambers were ethanol washed prior to carcass placement and set at 22°C with diurnal settings at twelve hours light and twelve hours dark. Chambers were used during this experiment to avoid temperature variations noted in experiment one.
Figure 2 Growth chamber used for experiment two.

Figure 3 Petri dishes after random placement in chamber.
For treatments including soil. Soils were collected from the same location and sieved in the same manner as the first experiment with a few exceptions. Once collected, soils were refrigerated until sieved and inoculated. Petri dishes (150 mm x 25 mm) that were designated as controls, treatments with carcasses on inoculated sand, and treatments with carcasses on inoculated sand with insects were filled with 150 g of washed sea sand inoculated with 150 g soil. Inoculated sand was then tapped to approximately 5 mm below the top of the petri dish. Soil was calibrated to water holding capacity of 55%, placed inside temperature controlled chambers and left to equilibrate for seven days at 22°C.

Carcasses. Dead mice (~38.1 g) were obtained in the same manner as the first experiment. Same sex and species were used for easier comparison between the two experiments. Placement of carcasses began within 30 minutes of death on the petri dishes designated as carcass on inoculated soil, carcass on inoculated soil with insects, and carcass with no soil. All treatments were established with dead mice no more than three hours postmortem (Figure 4).

For treatments including insects. Insect eggs (*Lucilia sericata* Meigen) were obtained from fly colonies maintained by A. Fujikawa (Department of Entomology, University of Nebraska-Lincoln). Insect eggs were removed from liver and counted the morning of carcass placement. Ten eggs were placed on the right eye of the mouse carcass and then the petri dish was placed back into the appropriate chamber. Eggs were carefully monitored daily to be sure desiccation did not occur.
Experimental Procedures

After treatments were established, the experiment was conducted over 35 days with decomposition scored daily. Every seven days a destructive harvest was conducted on previously randomized dishes. Mice were scored, photographed, and then removed from the petri dish and weighed to obtain a final mass. Carcasses were then sealed in plastic bags and frozen (-20°C). Soil was homogenized within its entirety then weighed for moisture content, lipid-phosphate and fatty acid methyl ester (FAMEs) analysis. The remaining soil was divided with half being immediately frozen (-20°C) and the other half left to air dry for a period of two weeks (Figure 5). Lipid-phosphate and FAME analysis, on previously frozen soil, was conducted after the completion of the experiment.

Figure 4 A growth chamber that contained 12 petri dishes on the top shelf and 8 on the bottom shelf for an incubation period of 35 days at 22°C. Every row contained one treatment (control, carcass with no soil, carcass on soil, and carcass on soil with insects) and all treatments were randomized among rows and shelves.
Figure 5 Soil before and after being homogenized during harvest.

Decomposition Measures. Decomposition was scored following the same procedures as experiment 1.

Microbial Biomass. Microbial biomass was analyzed following the same procedures as experiment 1.

Microbial Community Structure. The FAMEs method, adopted from White et al. (1979), was used to analyze soil microbial community structure for this study, because it is simpler, less time consuming, and found to be the most acceptable method applied to the extraction of whole soils (Graber and Tsechansky 2010). FAMEs are also a well recognized method used to “fingerprint” soil microbial communities (Drijber et al. 2000). Protocols for this method are described in Appendix A.
Statistical Analyses

Regression analyses were generated for mass loss and TBS statistics using Graph Pad Prism version 5.02 (Graph Pad Software, Inc., La Jolla, CA). Lipid-phosphate statistics were generated using the Mann-Whitney U test using SPSS version 19 (Chicago, IL). Mixed model analysis of variance was generated for moisture content using SAS 9.1 (Windows 2002-2003 SAS Institute, Cary, NC). FAMEs were analyzed using discriminant and canonical correlation analysis with SAS 9.1 (Windows 2002-2003 SAS Institute, Cary, NC).
Chapter 3: Results

Experiment One

Gross Decomposition

Overall, carcass treatment influenced mass loss. During a 35 day incubation period, carcasses without soil lost more mass than carcasses on soil (Figure 6). Carcasses on soil showed a 7% increase in mass during the first week, indicating that they remained in early decomposition stages for a longer period of time than carcasses without soil. After a 7 day delay in mass loss, carcasses on soil lost mass 50% faster than carcasses without soil. Overall mass loss of carcasses on petri dishes reached approximately 29% ± 5% of total body weight after 35 days and carcasses on soil reached approximately 23% ± 3%. Carcasses without soil decomposed steadily throughout the experiment. Fungal growth continued throughout the experimental study on all carcasses on soil (Figure 7). Carcasses without soil decomposed through all five stages at a much faster rate than carcasses on soil, because carcasses without soil bloated rapidly then caved rapidly, leading to rapid desiccation (Figures 8 and 9). It is important to note that desiccation was most evident on Saturdays and Sundays during experiment 1 when the air conditioner was turned off within the building where the experimental units were kept. During this time, temperature increased 3° - 4°C higher than on other days. Changes in temperature most likely influenced mass loss. Fluctuations with temperature during experiment 1 were out of our control. Abdominal rupture was observed in several of the carcasses without soil after extensive bloating occurred. No mummification was observed for carcasses without soil.
Figure 6 Mass loss of mice carcasses (~38.2 g) at 20°C. Bars represent standard errors where n = 4. Linear model fit with linear regression.
Figure 7 A mouse carcass on 90/10 inoculated sand without insects on day 14.

Figure 8 A mouse carcass without sand on day 7.
Figure 9 A mouse carcass without soil on day 14.

**Total Body Score**

Total Body Score for carcasses on soil was significantly ($F_{2,64}=130.1$) different than carcasses without soil (Figure 10). The difference in slopes for the two treatments indicated that carcasses on soil decomposed at a faster rate than carcasses without soil, which was shown as a higher TBS (Figures 11 and 12). The highest TBS reached for carcasses without soil was 4.85. This score was reached on day 11, but never reached again. After day 11, the TBS plateaued at 4.3. Carcasses on soil reached a TBS of 6.75 on day 17, but never reached climax. The highest TBS possible was 9.3. Fluctuations with temperature on certain days most likely influenced physical appearance and affected TBS for carcasses. Refer to Appendix B for more photographs.
**Figure 10** Scoring system used to determine decomposition based on physical characteristics of mice carcasses without soil (empty circles) and with soil (filled circles) at 20°C. Bars represent standard errors where n=4. One phase association with plateau model: $y = \text{If}(x < 2, 1, 1 + (\text{Plateau} - 1) \cdot (1 - e^{-k(x-2)}))$, fit by non-linear regression.
Figure 11 A mouse carcass on 90/10 inoculated sand without insects on day 28.

Figure 12 A mouse carcass on 90/10 inoculated sand without insects on day 28.
Microbial Biomass

Gravesoil resulted in a significant increase in lipid-P (Figure 13). This effect was observed from day 14 onward. A decrease in lipid-P concentration was observed between 21-28 days. A similar pattern of lipid-P concentration in gravesoils was shown in previous decomposition studies (Benninger et al. 2008). Concentrations of lipid-P in inoculated sand controls remained constant throughout the study.

Figure 13 Lipid-phosphorous concentrations from controls (empty circles) and gravesoils (filled circles) after carcass decomposition over an incubation period of 35 days at 20°C on 90/10 inoculated sand with 55% water holding capacity. Bars represent standard errors where n = 4 and “*” represents a significant (p < 0.05) difference between treatments.
Moisture Content

Gravesoil moisture was influenced by the treatment and week, but there was no significant difference between the interaction of treatment and week (Figure 14). Moisture was significantly different (p < 0.01) between control soils and gravesoils. Control soils and gravesoils had the highest moisture during week 4. The lowest moisture was during week 3 for controls and week 1 for gravesoils.

Figure 14 Soil moisture content (%) for controls (empty circles) and gravesoils (filled circles) after carcass decomposition over an incubation period of 35 days at 20°C on 90/10 inoculated sand with 55% water holding capacity. Bars represent standard errors where n = 4.
Experiment Two

Gross Decomposition

Carcass mass loss was greatly affected by treatment. Overall, all carcasses on soil, regardless of the presence of insects, lost more mass than carcasses without soil (Figure 15). There was no significant difference between carcasses on soil and carcasses on soil with insects. All carcasses on soil remained in early decomposition stages for a longer period of time than carcasses without soil, which was observed as a gain in mass during the first week of the experiment. This observation was also seen with carcasses on soil during the first experiment. It was observed that the rapid decline in mass loss during the second week allowed all carcasses on soil to greatly surpass the mass loss of carcasses without soil which was not observed in experiment one. Mass loss of carcasses without soil reached approximately 35% ± 3.4% of total body weight after 35 days, carcass on soil reached approximately 62% ± 5.8%, and carcasses on soil with insects reached approximately 60% ± 5.4%.

Fungal growth was minimal, but still observed throughout the experimental study on all carcasses on soil. Acarid mites (*Caloglyphus*), identified by J. Kalisch (Department of Entomology, University of Nebraska-Lincoln), were abundant on almost all carcasses associated with soil (Figures 16 and 17). Their contribution to mass loss and decomposition is unknown. It was observed that several carcasses from all treatments showed minimal bloating while extensive bloating was observed in only a select few. Rupture was not commonly seen in any of the treatments. However, some carcasses associated with soil became completely flattened due to the enormous amount of water lost during the experimental trial (Figures 18 and 19).
Figure 15 Mass loss of mice carcasses (~38.1 g) at 22°C. Bars represent standard errors where n = 4. Carcasses without soil show a quadratic model and carcasses on gravesoil show an exponential decay model. Both models were fit by non-linear regression.

Figure 16 A mouse carcass with mites on day 21 on 50/50 inoculated sand associated with insects.
Figure 17 Acarid mites (Caloglyphus) on day 35 after removal from mice carcasses on 50/50 inoculated sand.

Figure 18 A mouse carcass on 50/50 inoculated sand with insects on day 35.
Figure 19 A mouse carcass during harvest after removal from 50/50 inoculated sand with no insects on day 35.

Total Body Score

A higher TBS was observed for carcasses on soil with insects than carcasses on soil without insects, which both were higher in TBS than carcasses without soil (Figure 20). The highest TBS reached for carcasses without soil was 5.1. This score was reached on day 35. Carcasses on soil reached a TBS of 6.25 which was also observed on day 35. Carcasses on soil with insects reached a TBS of 5.69 on day 34. The highest possible TBS for all treatments was 9.3. Carcasses associated with insects had only their face consumed and therefore it was observed that the remainder of the body decomposed in a similar fashion to carcasses on soil not associated with insects (Figures 21 and 22). All carcasses had minimal hair discoloration to where the majority of the mice remained white in color throughout the entire experiment which made it difficult to determine if desiccation occurred. This observation varied greatly from observations during the first experiment. See Appendix C for more photographs.
Figure 20 Scoring system used to determine decomposition based on physical characteristics of mice carcasses incubated at 22°C over a 35 day period. Bars represent standard errors where n = 4. One phase association with plateau model fit by non-linear regression.
**Figure 21** A mouse carcass on day 14 after a destructive harvest from 50/50 inoculated sand with insects.

**Figure 22** A mouse carcass on 50/50 inoculated sand with insects on day 15.
Microbial Biomass

There was a significant increase in lipid phosphorous in gravesoils observed after day 7 (Figure 23). Concentration decreased between 21-28 days for gravesoil and between days 28-35 for gravesoils associated with insects. Overall, there was no significant difference of lipid phosphorous concentration in gravesoil associated with insects. Lipid-phosphorous concentrations in control soils remained consistent for all five weeks of the study.

Figure 23 Lipid-phosphorous concentrations from controls (empty circles), gravesoils (filled circles), and gravesoils associated with insects (filled triangles) after carcass decomposition over an incubation period of 35 days at 22°C on 50/50 inoculated sand with 55% water holding capacity. Bars represent standard errors where n = 4.
Microbial Community Structure

There was a significant shift observed in microbial community structure after day seven due to treatment (Figure 24). Discriminant canonical correlation analysis indicated no significant difference between any of the treatments on day 7. The similarity in microbial community structure is shown in Figure 24, where all treatments are clustered near each other for week 1. On day 14, the controls remain in the same relative location as day 7, but all gravesoils shift upward. This shift indicates a change in microbial community structure during week 2. All treatments are significantly different from day 14 onward. All treatments remain significantly different during week 5. These results indicate a shift in gravesoil microbial communities after week one.

Total FAME concentrations in controls were significantly different than gravesoils after day 7 (Figure 25). There was no significant difference in total FAME concentration between gravesoils. The fatty acid biomarkers that were detected during the experiment represented bacteria, fungi, eukaryotes, actinomycetes, and arbuscular mycorrhizal fungi (AMF) (see Figures 26-32, Appendix A Tables 2 and 3).
Figure 24 Discriminate canonical correlation analysis graph. Controls are represented as white symbols, gravesoil with no insects are represented as grey symbols, and gravesoil with insects are represented as black symbols. Week 1 is represented by circles, week 2 by squares, week 3 by upside down triangles, week 4 by diamonds, and week 5 is represented by right side up triangles.
Figure 25 Total fatty acid methyl ester concentrations for controls (empty circles), gravesoil (grey circles), and gravesoil associated with insects (black circles) for 50/50 inoculated sand kept at 55% water holding capacity for a 35 day incubation period in growth chambers kept at 22°C.
Figure 26 Fatty acid taxonomic classes shown cumulatively as total FAMEs and individually by the taxonomic group.
**Bacterial Fatty Acids**

Bacterial fatty acid concentrations in controls were significantly different than concentrations in gravesoils for every week except week one (Figure 27). This pattern illustrated a week delay in fatty acid change is consistent with a time lag necessary before microbes react to their surrounding environment (see Spicka *et al.* 2011). Gravesoils were not significantly different from each other regardless of the presence of insects.

**Figure 27** Bacterial fatty acid concentrations for controls (empty circles), gravesoils (grey circles), and gravesoils associated with insects (black circles) for 50/50 inoculated sand at 55% water holding capacity incubated in growth chambers at 22°C over a 35 day period.
Eukaryote Fatty Acids

Eukaryote fatty acid concentrations significantly differed between controls and gravesoils for all weeks except week one, which is also consistent with microbial activity lag time (see Spicka et al. 2011). There was no significant difference between gravesoils with or without insects (Figure 28).

Figure 28 Eukaryote fatty acid concentrations for controls (empty circles), gravesoils (grey circles), and gravesoils associated with insects (black circles) for 50/50 inoculated sand at 55% water holding capacity incubated in growth chambers at 22°C over a 35 day period.
Fungal Fatty Acids

There was no significant difference observed in fungal fatty acids between any of the treatments for the entire experiment (Figure 29).

**Figure 29** Fungal fatty acid concentrations for controls (empty circles), gravesoils (grey circles), and gravesoils associated with insects (black circles) for 50/50 inoculated sand at 55% water holding capacity incubated in growth chambers at 22°C over a 35 day period.
Arbuscular mycorrhizal fungi

On day 21, there was a significant difference between controls and gravesoil with insects, but gravesoil without insects did not differ between controls or gravesoil with insects (Figure 30). There was no significant difference between any of the treatments for any other days other than day 21.

Figure 30 Arbuscular mycorrhizal fungi fatty acid concentrations for controls (empty circles), gravesoils (grey circles), and gravesoils associated with insects (black circles) for 50/50 inoculated sand at 55% water holding capacity incubated in growth chambers at 22°C over a 35 day period.
**Actinomycete Fatty Acids**

There was no significant difference in actinomycete fatty acid concentrations between any of the treatments for the entire experiment (Figure 31).

**Figure 31** Actinomycete fatty acid concentrations for controls (empty circles), gravesoils (grey circles), and gravesoils associated with insects (black circles) for 50/50 inoculated sand at 55% water holding capacity incubated in growth chambers at 22°C over a 35 day period.
Moisture Content

Gravesoil moisture was influenced by the treatment and week, but there was no significant difference between the interaction of treatment and week (Figure 32). Moisture was significantly different ($p < 0.01$) between all treatments. The highest moisture content was during week 2 for gravesoils associated with insects and during week 3 for control soils and gravesoils with no insects. All treatments were driest during week 4.

Figure 32 Soil moisture content (%) for controls (empty circles), gravesoils (grey circles), and gravesoils with insects (black circles) after carcass decomposition over an incubation period of 35 days at 22°C on 50/50 inoculated sand with 55% water holding capacity. Bars represent standard errors where $n = 4$. 
Chapter 4: Discussion

A carcass is a high quality resource that has a significant effect on the chemistry and biology of associated soils (Vass et al. 1992; Carter et al. 2009). Once released, decompositional fluids fill soil pores, increasing available organic compounds and prompting a reaction from localized microbial communities. Changes in resources stimulate changes in the soil community, allowing for biomass to increase and microbial groups to shift (Carter and Tibbett 2008 (B); Parkinson et al. 2009; Howard et al. 2010). Knowledge beyond this point, however, is currently restricted. The experiments that I have conducted add to this limited understanding by showing that soil contributes dramatically to the decomposition process independent of microbes on the body and that the presence of insects can significantly alter decomposition if it takes place on soil. Based on our results, we conclude that the surface on which a carcass is placed significantly affects carcass decomposition. Although the presence of insects does not alter the dynamics of total microbial biomass below the carcass, insects do alter the gravesoil microbial community structure.

The surface on which a carcass decomposes can significantly affect physical appearance of the carcass and the rate at which it breaks down. When carcasses were placed on a dry, smooth surface, mass loss was unable to persist past the point of desiccation, which occurred rapidly (see also Mann et al. 1990). Desiccation also allowed carcasses to only reach a certain TBS which was observed when physical characteristics remained at a similar state past a certain point. We refer to this as the plateau effect. Obviously, microbes from the air could have contributed to all treatments, however, only microbes present on or within the carcass at the time of death seem likely to be the most
important in breaking down and altering the appearance of the carcasses without soil. In contrast, for carcasses on soil, there was an additional significant microbial source contributed from the soil itself that affected the physical appearance of the carcass. Thus, it was not surprising to see a significant difference between carcasses without soil compared to those on gravesoil (also see Mann et al. 1990). Carcasses placed on soil underwent a different decomposition process, probably because gravesoil was able to take up carcass moisture and carcasses were exposed to a larger decomposer community. Also, with the addition of water every 3-4 days, the possibility of desiccation occurring was limited, which allowed for the TBS to continue rising until the experiment was complete.

Decomposition was associated with an increase in soil microbial biomass. Other studies show similar results where lipid-phosphorous concentrations increase with carcass placement (Benninger et al. 2008; Van Belle et al. 2009). The presence of insects had no affect on the microbial biomass; the soil biomass might only be able to increase up to a certain point until the resource is unable to supply the demand of needed nutrients brought on by the decomposer community. This effect is analogous to a zero order Michaelis-Menten reaction (see Spicka et al. 2011).

The structure of the microbial community, however, was affected by the presence of insects. These results were not surprising, because an increase in total fatty acid methyl esters has been observed in studies with human cadaver decomposition (Parkinson et al. 2009). The presence of a carcass introduces different compounds and alters the physicochemical environment of the soil (Vass et al. 1992). Not all groups of microbes are able to survive the change in their surroundings; however, some microbes thrive with
the changing environment and dominate that area while the resource is still available. In our experiment we saw this primarily as an increase in bacterial and eukaryote biomass.

Bacteria concentrations have been shown to dominate over fungi in most soils (Wardle et al. 2004). Leached fluids from carcass placement create a fertile environment that accounts for the increased concentrations of bacterial fatty acids in our gravesoil. Increasing concentrations of bacterial fatty acids were observed during the first twenty-one days of the experiment when environmental conditions were optimal in moisture and nutrients. During this time, concentrations of fungal fatty acids and, to a lesser extent, actinomycete fatty acids remained relatively constant for all treatments. The biomarker fatty acid for arbuscular mycorrhizal fungi (AMF), an obligate plant symbiont, declined during this time period, as expected, since there were no live plants from which to obtain carbon. The eukaryote fatty acid concentrations also increased during the first three weeks, but at a much smaller magnitude than bacteria.

The steady declines in bacterial biomarkers after day 21 corresponded to carcasses that were caved in, desiccated, and lacking in leachable fluids. With this decline in resources, we observed an increase in fungi, AMF, actinomycetes, and eukaryotes. Increased concentrations of these fatty acids may be attributed to a decline in bacteria that, up until this point, were the primary competitors for resources. This shift in microbial community structure signifies a shift in environmental conditions which is characterized by the high moisture content and desiccated resource. Changes in moisture conditions began on day 17 (experiment 1) and day 10 (experiment 2) and were prevalent in most gravesoils for the remainder of the experiment as evident by standing water and high saturation when water additions occurred. Since decomposition fluids filled soil
pores directly beneath and around the carcass, space for water was limited causing most gravesoils to become saturated to the point where addition of water to 55% water holding capacity was no longer possible and therefore addition ceased. Soils remained saturated for less than 24 hours and soils were dried and starting to crack by the time the next water addition was carried out (3-4 days later). This phenomenon was most likely attributed to continuous air flow when the air conditioner was on (experiment 1) or the fans within the growth chambers (experiment 2) where standing water evaporated prior to sinking into available pore space.

The shift from aerobic to anaerobic environment after water additions may have contributed to the microbial community shift. High moisture conditions are not suitable for bacteria and fungi and actinomycetes are well known to dominate areas where low nutrients are available, conditions are drier, and recalcitrant materials such as chitin, cellulose, and lignin are more abundant (Killham 1994). These resources tend to be prevalent during advanced decomposition stages and many studies have observed increased concentrations of fungi during this time (see Carter and Tibbett 2008 (B)). It is at this time when fungi and actinomycetes can be described as obligatory. The increase in concentration of AMFs during this time period may also be contributed to the sudden decline in bacteria where competition for resources is decreasing. There was a decline in all fatty acid biomarkers in gravesoils after day 28, which is most likely explained by the depletion and low quality of the available resource.

The current results complement the relevant literature. It is well known that fresh, higher quality resources are initially dominated by bacteria (Killham 1994). Also, many studies have reported observations in increasing soil pH when carcasses are used as study
materials (Hopkins et al. 2000; Stokes et al. 2009; Carter et al. 2010). This has been attributed to an increase in ammonium and nitrate ions that leach into soil from decompositional fluids, causing soil to become more basic. High levels of bacteria have been correlated in areas where ammonia is abundant, because they begin the process of nitrification by converting ammonium to nitrite (Killham 1994). Once ammonia is depleted and resources become poor in quality, fungi proliferate (Carter and Tibbett 2008 (B)).
References


Megyesi MS, Nawrocki SP, Haskell NH (2005) Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. J Forensic Sci 50(3): 618-626


Appendix A: Soil Ecology Method Protocols and Fatty Acid Identification

Lipid-phosphate

Lipid-phosphate extraction was conducted using the protocol determined by Bartlett (1959) as described by Kates (1986). Briefly, 2 g of moist soil plus 0.5 ml distilled water was placed into 10 ml teflon centrifuge tubes (in duplicate), then vortexed for 30 seconds. Next, 2 ml of solvent I (methanol:chloroform 2:1) was added, followed by 30 seconds of vortexing, 2 ml more of solvent I were added and vortexed for 30 seconds again. The sample was left at room temperature for 2 hours while being vortexed for 5 seconds every half hour. After these 2 hours, the sample was centrifuged at 6000 g for 10 minutes. The supernatant was then transferred into a 15 ml glass tube with a Pasteur pipette. The soil pellet was re-suspended in 4 ml of solvent II (methanol:chloroform:water 2:1:0.8), vortexed for 30 seconds, centrifuged as previously described, and the supernatant from this step was combined with that previously obtained. Samples were stored in a freezer at -22°C until ready for phase separation.

For phase separation, 2.5 ml chloroform and 2.5 ml ammonium sulfate was added to the supernatant. The tubes were then capped, vortexed for 30 seconds, and centrifuged at 2500 rpm for 5 minutes. The lower chloroform layer was transferred into a HNO₃-washed 15 ml glass tube with a Pasteur pipette. The transfer of water was avoided. Next, 2.5 ml chloroform was added to the supernatant, vortexed for 30 seconds, and centrifuged for 5 minutes again. Chloroform extracts were combined then evaporated under N₂ to dryness. An HNO₃-washed glass bead was added to the sample. Next, 400 µl perchloric acid was added, the tube was capped with an HNO₃-washed 10 ml beaker and the sample was placed in a digestion block until the extract was clear. Two digestion blanks were included.
After digestion was complete, phospholipid-P colorimetric assay was conducted using the protocol described by Kates. Amidol was prepared fresh by adding 0.25 g of 2,4-diaminophenol dihydrochloride to 5.0 g sodium bisulfate (20%) in 25 ml distilled deionized water. Solution was made to volume in 25 ml volumetric flask, filtered through a Whatman #1, and transferred to a 30 ml amber bottle.

Ammonium molybdate was prepared by dissolving 1.25 g in 25 ml distilled, deionized water. Solution was stored at room temperature in a 30 ml amber bottle. A phosphorous standard was prepared by dissolving 0.439 g KH₂PO₄ in 100 ml distilled deionized water and made to volume (1000 µg/ml). A working standard was prepared by diluting 1 ml to 100 ml to give 10 µg P/ml. A standard curve was prepared by adding 0, 250, 500, 750, and 1000 µL to separate HNO₃-washed tubes then diluted to 1000 µl using deionized water.

Next, 400 µl perchloric acid and 3.2 ml distilled deionized water was added to the standards. To the samples, 4.2 ml distilled deionized water was added. Ammonium molybdate, 200 µl, was then added to all tubes. Samples were vortexed for 30 seconds. All tubes were vortexed for 30 seconds again after 200 µl amidol was added. Tubes were capped with HNO₃-washed 10 ml beakers and placed in a boiling water bath for 10 minutes. Once tubes were removed from the boiling water bath, they were rapidly cooled in an ice bath for 15 minutes, then removed and left to cool to room temperature. All samples were vortexed for 30 seconds and absorbance was then read at 830 nm in the hood.
**Fatty acid methyl esters**

Once soil was collected, it was analyzed using FAMEs analysis as explained by Grigera et al. (2007) where 5 g soil in 50 ml teflon tubes were hydrolyzed with 10 ml freshly prepared 0.2 M potassium hydroxide in methanol at 37°C for 1 h with shaking of the tubes every 10 minutes. Alkaline extracts were neutralized with 1 ml 1 M acetic acid, and FAMEs were partitioned into 5 ml hexane followed by centrifuging at 3470 x g for 10 minutes. The hexane layer was transferred into a 15 ml Pyrex tube and the aqueous soil mixture was re-extracted with another 5 ml hexane. Hexane extracts were combined and filtered through Acrodisc CR 10 mm syringe filtered with 0.2 µm PTFE membrane into a clean 15 ml Pyrex tube.

Solvent was evaporated under N₂ to a small volume then 3-4 drops of benzene was added and evaporated to dryness. Residue was re-dissolved in a small volume of hexane, vortexed for 30 seconds, and transferred to a 2 ml amber vial. Next, the vial was rinsed with several small volumes of hexane and samples were stored in the freezer until ready to be prepared for the gas-chromatograph (GC).

To prepare samples for the GC, samples were removed from the freezer and the solvent was evaporated under N₂ until completely dry. Sample was re-dissolved in 250 µl to 500 µl hexane containing C19:0 (0.05 mg/ml) as an internal standard. Sample was then transferred from a 50 µl aliquot to a conical GC vial and capped.
Table 2 Identification of fatty acids in soils by gas chromatography-mass spectroscopy.

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<td>methyl tetradecanoate</td>
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<td>C14:1</td>
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</tr>
<tr>
<td>C14:1</td>
<td>methyl Z-11-tetradecenoate</td>
</tr>
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<td>C14:1</td>
<td>methyl E-11-tetradecenoate</td>
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Table 2 continued  Identification of fatty acids in soils by gas chromatography-mass spectroscopy.

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**Table 3** The taxonomic classes for fatty acids used in discriminant statistical analysis.

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Appendix B: Experiment 1

Mouse carcass without soil on day 1.

Mouse carcass on soil on day 1.
Mouse carcass on soil on day 1.

Mouse carcass on soil on day 1.
Control soil on day 1.

Mouse carcass on soil on day 1.
Mouse carcass without soil on day 1.

Mouse carcass on soil on day 1.
Mouse carcass on soil on day 1.

Mouse carcass on soil on day 1.
Mouse carcass without soil on day 1.

Mouse carcass on soil on day 1.
Mouse carcass on soil on day 1.
Control soil on day 1.
Mouse carcass without soil on day 1.

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Control soil on day 2.
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Mouse carcass without soil on day 2.
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Mouse carcass without soil on day 2.
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Mouse carcass without soil on day 2.
Mouse carcass without soil on day 2.

Control soil on day 2.
Control soil on day 2 after water additions.

Mouse carcass on soil on day 2.
Mouse carcass without soil on day 2.

Mouse carcass on soil on day 2.
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Mouse carcass without soil on day 3.
Control soil on day 3.

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Control soil on day 4.
Control soil on day 4 after water additions.

Mouse carcass without soil on day 4.
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Control soil on day 4.

Control soil on day 4 after water additions.
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Control soil on day 8.
Mouse carcass on soil on day 8.
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Mouse carcass on soil on day 8.
Mouse carcass on soil on day 8.

Mouse carcass after removal from treatment without sand during harvest on day 8.
Mouse carcass after removal from treatment without sand during harvest on day 8.

Mouse carcass after removal from treatment with sand during harvest on day 8.
Gravesoil during harvest after carcass removal on day 8.
Mouse carcass without soil on day 9.

Mouse carcass on soil on day 9.
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Mouse carcass on soil on day 9.
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Control soil on day 9 after water additions.
Mouse carcass without soil on day 9.

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Control soil on day 9.

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Mouse carcass on soil on day 9.
Control soil on day 9 after water additions.

Mouse carcass without soil on day 9.
Mouse carcass on soil on day 9.
Control soil on day 9.

Mouse carcass on soil on day 10.
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Control soil on day 10 after water additions.
Mouse carcass without soil on day 10.
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Mouse carcass without soil on day 10.
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Control soil on day 10.
Mouse carcass without soil on day 11.

Mouse carcass without soil on day 11.
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Mouse carcass on soil on day 11.

Control soil on day 11.
Control soil on day 11 after water additions.

Control soil on day 11.
Control soil on day 11.

Control soil on day 11 after water additions.
Control soil on day 11.

Control soil on day 11.
Mouse carcass without soil on day 12.

Mouse carcass without soil on day 12.
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Mouse carcass on soil on day 12.
Control soil on day 12.

Control soil on day 12 after water additions.
Control soil on day 12.

Control soil on day 12.
Control soil on day 12 after water additions.

Control soil on day 12.
Control soil on day 12.

Control soil on day 12 after water additions.
Mouse carcass on soil on day 13.
Control soil on day 13.

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Control soil on day 13.
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Control soil on day 13.
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Mouse carcass without soil on day 13.
Control soil on day 13.

Control soil on day 13 after water additions.
Control soil on day 13 after water additions.

Control soil on day 13.
Control soil on day 13.

Control soil on day 13 after water additions.
Mouse carcass on soil on day 13.
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Mouse carcass without soil on day 14.
Mouse carcass on soil on day 14.
Control soil on day 14.

Control soil on day 14 after water additions.
Mouse carcass on day 14 after removal from soil during harvest.

Gravesoil on day 14 after carcass removal during harvest.
Gravesoil on day 14 after carcass removal during harvest.

Mouse carcass on day 14 after removal from soil during harvest.
Gravesoil on day 14 after carcass removal during harvest.

Mouse carcass on day 14 after removal from soil during harvest.
Mouse carcass on day 14 after removal from petri dish without soil during harvest.
Mouse carcass without soil on day 15.

Mouse carcass without soil on day 15.
Mouse carcass on soil on day 15.

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Control soil on day 15.
Control soil on day 15.

Control soil on day 15.
Control soil on day 15 after water additions.

Control soil on day 15 after water additions.
Mouse carcass without soil on day 16.

Mouse carcass without soil on day 16.
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Mouse carcass without soil on day 17.
Control soil on day 17.

Control soil on day 17 after water additions.
Control soil on day 17.

Control soil on day 17.
Control soil on day 17 after water additions.

Control soil on day 17.
Mouse carcass on soil on day 18.
Mouse carcass on soil on day 18.
Mouse carcass on soil on day 18.
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Mouse carcass on soil on day 18.
Mouse carcass without soil on day 18.

Mouse carcass without soil on day 18.
Control soil on day 18.

Control soil on day 18 after water additions.
Control soil on day 18.

Control soil on day 18 after water additions.
Control soil on day 18 after water additions.

Control soil on day 18.
Control soil on day 18.

Mouse carcass on soil on day 18.
Mouse carcass on soil on day 18.

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Mouse carcass without soil on day 21 during harvest.
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Mouse carcass after removal from soil on day 21 during harvest.
Gravesoil after mouse carcass removal on day 21.

Mouse carcass after removal from soil on day 21 during harvest.
Gravesoil after mouse carcass removal on day 21.

Mouse carcass after removal from soil on day 21 during harvest.
Gravesoil after mouse carcass removal on day 21.

Mouse carcass after removal from soil on day 21 during harvest.
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Mouse carcass removed from treatment without soil on day 28 during harvest.
Mouse carcass removed from treatment without soil on day 28 during harvest.
Mouse carcass removed from treatment without soil on day 28 during harvest.

Mouse carcass after removal from soil on day 28 during harvest.
Gravesoil on day 28 after carcass removal during harvest.

Mouse carcass after removal from soil on day 28 during harvest.
Gravesoil on day 28 after carcass removal during harvest.

Mouse carcass after removal from soil on day 28 during harvest.
Gravesoil on day 28 after carcass removal during harvest.

Mouse carcass after removal from soil on day 28 during harvest.
Gravesoil on day 28 after carcass removal during harvest.

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Mouse carcass on soil on day 29.
Mouse carcass on soil on day 29.

Control soil on day 29.
Control soil on day 29 after water additions.

Control soil on day 29.
Control soil on day 29 after water additions.

Control soil on day 29.
Control soil on day 29.

Control soil on day 29 after water additions.
Mouse carcass without soil on day 29.

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Mouse carcass without soil on day 33.
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Mouse carcass on soil on day 33.

Control soil on day 34.
Control soil on day 34 after water additions.

Control soil on day 34.
Control soil on day 34 after water additions.

Control soil on day 34.
Control soil on day 34 after water additions.

Control soil on day 34.
Mouse carcass on soil on day 34.

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Mouse carcass on soil on day 35.
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Mouse carcass on soil on day 35.
Mouse carcass on soil on day 35.

Bottom of gravesoil petri dish on day 35 during harvest.
Mouse carcass on soil on day 35.

Mouse carcass on soil on day 35.
Mouse carcass on soil on day 35.

Bottom of gravesoil petri dish on day 35 during harvest.
Mouse carcass on soil on day 35.

Mouse carcass on soil on day 35.
Mouse carcass on soil on day 35.

Bottom of gravesoil petri dish on day 35 during harvest.
Mouse carcass on soil on day 35.
Mouse carcass on soil on day 35.
Control soil on day 35.

Control soil on day 35.
Control soil on day 35.
Mouse carcass without soil on day 35.

Mouse carcass on day 35 after removal from petri dish without soil during harvest.
Mouse carcass without soil on day 35.

Mouse carcass without soil on day 35.
Mouse carcass on day 35 after removal from petri dish without soil during harvest.

Mouse carcass without soil on day 35.
Mouse carcass without soil on day 35.

Mouse carcass on day 35 after removal from petri dish without soil during harvest.
Mouse carcass without soil on day 35.

Mouse carcass on day 35 after removal from petri dish without soil during harvest.
Mouse carcass on soil on day 35.
Mouse carcass on day 35 after removal from soil during harvest.

Gravesoil on day 35 after carcass removal during harvest.
Mouse carcass on soil on day 35.

Mouse carcass on soil on day 35.
Mouse carcass on day 35 after removal from soil during harvest.

Gravesoil on day 35 after carcass removal during harvest.
Mouse carcass on soil on day 35.

Mouse carcass on soil on day 35.
Mouse carcass on day 35 after removal from soil during harvest.

Mouse carcass on soil on day 35.
Mouse carcass on soil on day 35.

Mouse carcass on day 35 after removal from soil during harvest.
Gravesoil on day 35 after carcass removal during harvest.
Appendix C: Experiment 2

Mouse carcass on soil with insects on day 4.

Mouse carcass on soil with insects on day 4.
Mouse carcass on soil with insects on day 4.
Mouse carcass on soil with insects on day 4.

Mouse carcass on soil with insects on day 4.
Mouse carcass on soil with insects on day 5.

Mouse carcass on soil with no insects on day 5.
Mouse carcass without soil on day 5.

Mouse carcass on soil with insects on day 5.
Mouse carcass on soil with insects on day 5.
Mouse carcass without soil on day 5.

Mouse carcass on soil with insects on day 5.
Mouse carcass on soil with insects on day 5.
Mouse carcass on soil with no insects on day 5.

Mouse carcass on soil with insects on day 5.
Mouse carcass on soil with insects on day 5.

Mouse carcass on soil with insects on day 5.
Mouse carcass on soil with insects on day 5.

Maggots on day 6 after migration from mouse carcass.
Maggot on day 6 after removal from mouse carcass.

Mouse carcass on soil with insects on day 6.
Mouse carcass on soil with insects on day 6.

Mouse carcass on soil with insects on day 6.
Mouse carcass tail on soil on day 6.

Mouse carcass on soil with insects on day 6.
Mouse carcass on soil with insects on day 6.

Mouse carcass on soil with insects on day 6.
Mouse carcass on soil with insects on day 6.
Mouse carcass without soil on day 7.

Mouse carcass on soil with insects on day 7.
Mouse carcass on soil with no insects on day 7.

Mouse carcass on soil with no insects on day 7.
Mouse carcass on soil with insects on day 7.

Mouse carcass on soil with insects on day 7.
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Mouse carcass on soil with insects on day 7.
Mouse carcass on soil with insects on day 7.

Mouse carcass on soil with insects on day 7.
Mouse carcass on soil with insects on day 8.
Mouse carcass tail on soil on day 8.

Mouse carcass without soil on day 8.
Mouse carcass without soil on day 8.

Mouse carcass on soil with no insects on day 19.
Mouse carcass on soil with no insects on day 19.

Mouse carcass on soil with insects on day 20.
Mouse carcass on soil with insects on day 20.
Mouse carcass on soil with insects on day 20.
Mouse carcass on soil with insects on day 20.

Mouse carcass on soil with no insects on day 20.
Mouse carcass on soil with no insects on day 20.
Mites on mouse carcass on soil with no insects on day 20.

Mouse carcass with no soil on day 20.
Mouse carcass on soil with insects on day 21.

Mites on mouse carcass on soil with insects on day 21.
Mouse carcass on soil with insects on day 21.

Mites on mouse carcass on soil with insects on day 21.
Mites on mouse carcass on soil with insects on day 21.

Mouse carcass on soil with insects on day 21.
Mouse carcass on soil with no insects on day 21.

Mouse carcass on soil with insects on day 21.
Mouse carcass on soil with insects on day 21.

Mouse carcass on soil with no insects on day 21.
Mites on mouse carcass on soil with no insects on day 21.
Mouse carcass on soil with no insects on day 21.

Mites on mouse carcass on soil with no insects on day 21.
Mouse carcass on soil with no insects on day 21.

Mouse carcass without soil on day 21.
Control soil without carcass on day 21.

Mouse carcass on soil with insects on day 21.
Mouse carcass on soil with insects on day 21.
Mouse carcass on soil with insects on day 21.

Mouse carcass after removal from soil with insects on day 21 after harvest.
Mouse carcass after removal from soil with insects on day 21 after harvest.

Mouse carcass on soil with no insects on day 21.
Mouse carcass on soil with no insects on day 21.
Mouse carcass on soil with no insects on day 21.

Mouse carcass on soil with no insects on day 21.
Grave soil on day 21 after carcass removal during harvest.

Mouse carcass on soil with no insects on day 21.
Mouse carcass on soil with no insects on day 21.
Mouse carcass on soil with no insects on day 21.

Mouse carcass on soil with insects on day 21.
Mouse carcass on soil with insects on day 21.
Mouse carcass on soil with insects on day 21.

Mouse carcass on soil with insects on day 21.
Mouse carcass on soil with insects on day 21 after harvest.

Mouse carcass without soil on day 21.
Mouse carcass without soil on day 21.

Mouse carcass on soil with insects on day 21.
Mouse carcass on soil with insects on day 21.
Mouse carcass on soil with insects on day 21.

Mouse carcass after removal from soil with insects on day 21 after harvest.
Gravesoil on day 21 after carcass removal during harvest.
Mouse carcass on soil with no insects on day 21.
Mouse carcass on soil with no insects on day 21.

Mouse carcass without soil on day 21.
Mouse carcass without soil on day 21.

Control soil without carcass on day 21.
Mouse carcass on soil with insects on day 21.

Mouse carcass on soil with insects on day 21.
Mouse carcass on soil with insects on day 21.

Mouse carcass on soil with insects on day 21.
Gravesoil after carcass removal on day 21 during harvest.

Mouse carcass without soil on day 21.
Mouse carcass without soil on day 21.

Mouse carcass on soil with no insects on day 21.
Mouse carcass on soil with insects on day 21.

Mouse carcass on soil with insects on day 21.
Gravesoil after carcass removal on day 21 during harvest.

Mouse carcass without soil on day 22.
Mouse carcass on soil with no insects on day 22.
Mouse carcass on soil with no insects on day 22.

Mouse carcass on soil with insects on day 24.
Mouse carcass on soil with insects on day 24.

Control soil with no carcass on day 24.
Control soil with no carcass on day 24 after water additions.

Mouse carcass on soil with no insects on day 24.
Mouse carcass on soil with no insects on day 24 after water additions.

Mouse carcass on soil with no insects on day 24.
Mouse carcass on soil with no insects on day 24 after water additions.

Control soil with no carcass on day 24.
Mouse carcass on soil with insects on day 24.

Mouse carcass on soil with insects on day 28.
Mouse carcass on soil with insects on day 28.
Mouse carcass on soil with insects on day 28.

Mouse carcass on soil with insects on day 28.
Mouse carcass after removal from soil with insects after harvest on day 28.

Mouse carcass without soil on day 28.
Mouse carcass without soil on day 28.

Control soil with no carcass on day 28.
Mouse carcass on soil with no insects on day 28.
Mouse carcass on soil with no insects on day 28.

Mouse carcass on soil with no insects on day 28.
Mouse carcass on soil with no insects on day 28.
Mouse carcass after removal from soil with no insects after harvest on day 28.

Mouse carcass without soil on day 28.
Mouse carcass without soil on day 28.

Mouse carcass without soil on day 28.
Mouse carcass without soil on day 28.

Mouse carcass on soil with insects on day 28.
Mouse carcass on soil with insects on day 28.

Mouse carcass on soil with insects on day 28.
Mouse carcass on soil with insects on day 28.

Mouse carcass on soil with insects on day 28.
Mouse carcass on soil with insects on day 28.

Control soil with no carcass on day 28.
Mouse carcass on soil with no insects on day 28.
Mouse carcass on soil with no insects on day 28.
Mouse carcass on soil with no insects on day 28.

Mouse carcass on soil with insects on day 28.
Mouse carcass on soil with insects on day 28.

Mouse carcass on soil with insects on day 28.
Mouse carcass on soil with insects on day 28.
Mouse carcass on soil with insects on day 28.

Mouse carcass on soil with insects on day 28.
Mouse carcass after removal from soil with insects after harvest on day 28.
Mouse carcass without soil on day 28.
Mouse carcass without soil on day 28.

Mouse carcass without soil on day 28.
Mouse carcass without soil on day 28.

Control soil with no carcass on day 28.
Control soil with no carcass on day 28.

Mouse carcass on soil with no insects on day 28.
Mouse carcass on soil with no insects on day 28.
Mouse carcass on soil with no insects on day 28.

Mouse carcass on soil with no insects on day 28.
Mouse carcass on soil with no insects on day 28.
Mouse carcass on soil with no insects on day 28.
Mouse carcass on soil with no insects on day 28.

Mouse carcass on soil with no insects on day 28.
Mouse carcass after removal from soil with no insects after harvest on day 28.

Mouse carcass on soil with insects on day 28.
Mouse carcass on soil with insects on day 28.

Mouse carcass on soil with insects on day 28.
Mouse carcass on soil with insects on day 28.
Mouse carcass on soil with insects on day 28.

Mouse carcass on soil with insects on day 28.
Mouse carcass after removal from soil with insects after harvest on day 28.

Mouse carcass without soil on day 28.
Mouse carcass without soil on day 28.
Mouse carcass without soil on day 28.

Mouse carcass without soil on day 28.
Mouse carcass without soil on day 30.
Mouse carcass on soil with no insects on day 35.
Mouse carcass on soil with no insects on day 35.
Mouse carcass on soil with no insects on day 35.

Mouse carcass after removal from soil on day 35 during harvest.
Gravesoil after carcass removal on day 35.
Mouse carcass after removal from soil with no insects after harvest on day 35.

Mouse carcass without soil on day 35.
Mouse carcass without soil on day 35.
Mouse carcass without soil on day 35.
Control soil with no carcass on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass after removal from soil with insects after harvest on day 35.

Mouse carcass after removal from soil with insects on day 35 after harvest.
Control soil with no carcass on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass after removal from soil with insects on day 35 after harvest.
Mouse carcass after removal from soil with insects on day 35 after harvest.

Mouse carcass without soil on day 35.
Mouse carcass without soil on day 35.
Mouse carcass without soil on day 35.
Mouse carcass without soil on day 35.

Mouse carcass on soil with no insects on day 35.
Mouse carcass on soil with no insects on day 35.
Mouse carcass on soil with no insects on day 35.
Mouse carcass on soil with no insects on day 35.

Mouse carcass on soil with no insects on day 35.
Mouse carcass after removal from soil with no insects on day 35 after harvest.

Mouse carcass after removal from soil with no insects on day 28 after harvest.
Control soil with no carcass on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.

Mouse carcass on soil with insects on day 35.
Mouse carcass after removal from soil with insects on day 35 after harvest.

Gravesoil after carcass removal on day 35.
Gravesoil after carcass removal on day 35.
Mouse carcass without soil on day 35.
Mouse carcass without soil on day 35.
Mouse carcass without soil on day 35.

Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.

Mouse carcass on soil with insects on day 35.
Mouse carcass on soil on day 35 after harvest.
Gravesoil after carcass removal on day 35.
Control soil with no carcass on day 35.

Control soil with no carcass on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.

Mouse carcass after removal from soil with insects on day 35 after harvest.
Mouse carcass after removal from soil with insects on day 35 after harvest.

Mouse carcass without soil on day 35.
Mouse carcass without soil on day 35.
Mouse carcass without soil on day 35.

Mouse carcass without soil on day 35.
Mouse carcass without soil on day 35.

Mouse carcass after removal from treatment without soil on day 35 after harvest.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.
Mouse carcass on soil with insects on day 35.

Acarid mite under microscope after removal from carcass on day 35.
Acarid mites under microscope after removal from carcass on day 35.

Acarid mite under microscope after removal from carcass on day 35.
Acarid mite under microscope after removal from carcass on day 35.

Acarid mites under microscope after removal from carcass on day 35.
Acarid mites under microscope after removal from carcass on day 35.