THE IMPORTANCE OF MICROBIAL AND PRIMARY COLONIZER INTERACTIONS ON AN EPHEMERAL RESOURCE

A Dissertation

by

JENNIFER LYNNE PECHAL

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

DOCTOR OF PHILOSOPHY

May 2012

Major Subject: Entomology

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ABSTRACT

The Importance of Microbial and Primary Colonizer Interactions on an Ephemeral Resource. (May 2012)

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Carrion decomposition is an essential ecosystem function as it is an important component of nutrient cycling. Carrion decomposition has primarily been attributed to insect consumption, with little attention given to microbial communities or their potential interactions with insects. The first objective was to use passive insect-trapping methods to assess primary colonizer communities on swine carcasses between two treatments: 1) carrion with access to insects and 2) carrion excluded from insect access for five days using exclusion cages. Despite similarities between succession patterns within each treatment, carcasses initially exposed to insects had significantly fewer insect taxa. Therefore, collections of adult insect communities associated with carrion are promising as an indication of whether or not there has been a delay in insect colonization of a resource.

There has yet to be a study documenting bacterial communities during carrion decomposition. The second objective was to describe bacterial community succession and composition during decomposition in the presence and absence of naturally occurring insects. Total genomic DNA was used to identify bacterial community composition via a modified bacterial tagged encoded FLX amplicon pyrosequencing. I obtained 378,904 sequences and documented distinct bacterial community successional trajectories associated with insect access and exclusion carcasses. By the fifth day of decomposition, *Proteus* was the dominant (72%) bacterial genus on exclusion carcasses

while *Psychrobacillus* (58%) and *Ignatzschineria* (18%) were dominant bacterial genera on insect carcasses. These data are the first to document bacterial community composition and succession on carrion.

My final objective was to assess microbial community function in response to carrion insect colonization using metabolic profiling. I characterized microbial community metabolic function in the presence and absence of the primary necrophagous insects. I documented significant microbial community metabolic profile changes during active decomposition of carcasses. Mean carcass microbial community metabolic function with insect access continuously decreased over decomposition during both field seasons. Thus demonstrating microbial metabolic activity may have discriminatory power to differentiate early and late stages of decomposition.

Overall, my data contributes to an understudied area of microbial research important to organic matter decomposition, forensic entomology, and microbial-insect ecological interactions.

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NOMENCLATURE

EXC	Insect exclusion carcasses
ACC	Insect access carcasses
ADH	Accumulated degree hours
RM-ANOVA	Repeated measures analysis of variance
NMDS	Nonmetric multidimensional scaling
MRPP	Multiple-response permutation procedure
ISA	Indicator species analysis
MMCPs	Microbial metabolic community profiles

TABLE OF CONTENTS

CHAPTER Page

CHAPTER Page

LIST OF FIGURES

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

LIST OF TABLES

TABLE Page

TABLE Page

TABLE Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Decomposition ecology

The decomposition of organic matter is an essential ecosystem function (Hooper et al. 2005). Decomposition is vital for nutrient cycling (Putman 1978a), food web dynamics (Polis and Strong 1996), and can impact the biodiversity of ecosystems (Hines et al. 2006). Detrital decomposition is considered a fundamental ecosystem process (Srivastava et al. 2009). Organic matter in the form of leaf litter (Srivastava et al. 2009, Gessner et al. 2010) and rotting fruit (Janzen 1977) are well-documented models for studying decomposition ecology. However, there are limited data on the impact of high-quality resources such as decomposing vertebrate carcasses, or carrion, to ecosystem functions (Putman 1978a, Hocking and Reimchen 2006, Wilson and Wolkovich 2011).

Carrion represents an ecological unit within a larger ecosystem (Odum 1969) resulting in a nutrient surge to the immediate soil, insect and plant communities (Towne 2000, Yang 2006). Carrion is part of the decaying organic matter of most ecosystems, and is considered to be an primary level of energy flow (DeVault et al. 2003). The introduction of carrion into an ecosystem can be considered a disturbance, or food-fall for the soil microbial community immediately underneath the carcass with adjacent soil responses occurring throughout decomposition (Hopkins 2008, Stokes et al. 2009). Because these resources are unpredictable and result in short bursts of intense responsive biological activity (i.e., microbial and insect community assembly and succession), they are referred to as resource pulses (Yang et al. 2008). For example, the study of ungulate carcasses post deposition indicated vegetation growing from the carcass site was significantly different in species richness and density when compared to zones radiating

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This dissertation follows the style of Ecology.

from the carcass site (Towne 2000). Introducing carrion into an ecosystem can impact the associated soil microbial community (Hopkins 2008, Stokes et al. 2009) by influencing soil chemistry and composition, which may lead to diverging soil communities (Post and Kwon 2000).

Decomposing remains such as deep-sea whale carcasses or anadromous salmon, *Oncorhynchus* spp.) (Salmoniformes: Salmonidae), in Pacific watersheds (Hocking and Reimchen 2006, Janetski et al. 2009) can be primary resource subsidies for ecosystems (Klages et al. 2001, Burkepile et al. 2006). Caloric values and mass lost estimated from decomposing brown laboratory mice ranged from 3,146-6,064 calories g^{-1} (640-858 mg organic matter) over a temporal gradient (Putman 1978a), providing a first assessment of the amount of energy made available by this small, but abundant species. Other ephemeral resources, such as dead vegetation sampled from forests has up to 5,187 calories g^{-1}, have been found to supply an ecosystem with nutrients (Ovington and Heitkamp 1960). Carrion decomposition introduces nitrogen, potassium, calcium and magnesium, back into the ecosystem (Carter et al. 2007). The processes of nutrient transfer back into the ecosystem from decomposing carcasses is variable (Gessner et al. 2010). In some systems, such as salmon carcasses in Alaskan streams, nutrients derived from the carrion results in a positive feedback as seen in increased growth of riparian vegetation by 25% (Helfield and Naiman 2002). Conversely, decomposition of red alder (*Alnus rubra* Bongard) leaf litter with salmon carcasses was significantly lower than without carcasses (Zhang et al. 2003). For example, nitrogen concentration in soil collected one year after the decomposition of a bison, *Bos bison* L. (Artiodactyla: Bovinae), carcass are approximately six fold higher than control soil samples (Towne 2000).

Carrion decomposition rates

The rate of carrion decomposition has been examined in terrestrial habitats (Reed 1958, Payne 1965), tropical (Jiron and Cartin 1981, Tullis and Goff 1987), marine (Anderson and Hobischak 2004, Burkepile et al. 2006), and freshwater (Anderson and

Hobischak 2004, Hocking and Reimchen 2006). Payne and Reed performed some of the classic studies in decomposition ecology of carrion. Both studies tracked insect succession patterns while monitoring physiological progression of the carrion though the decomposition process (Reed 1958, Payne 1965). Fresh stage began at the time of death and continued until bloat was evident, with no odor emitted or evidence of decomposition (1965). Bloat stage was characterized by swelling of the body (the abdomen was the first, and most prominent area, swollen on each carcass) and color changes sometimes resulting in marbling of the tissue as the result of gas build up during decomposition. Odors were prominent during bloat, with fluid drainage from the head and anal areas, along with any other area where the skin had become disrupted. Active decay stage was determined by the removal of soft tissue from the head and neck by Calliphoridae larvae with the remainder of the carcass beginning to deflate. The odors of decomposition were very strong during this stage. Advanced decay stage was similar to the active decay stage; however, most soft tissue had been removed by large maggot masses. The putrification odors were not as strong at the end of advanced decay. The dry stage represented the end of decomposition when all soft tissue was gone leaving only bone, cartilage and skin. Transition characteristics between stages were also noted (Kelly et al. 2009). It was during these transitional stages that multiple characteristics from various stages of decomposition were present (i.e., bloated abdomen with an active maggot mass on the head).

Swine carcasses on land were reduced to the dry stage of decomposition in approximately five days during summer months in southern parts of the US (Payne 1965). In tropical habitats, decomposition of canine carcasses followed similar patterns of decomposition in temperate areas (Jiron and Cartin 1981). Swine, *Sus scrofa* L. (Artidodactyla: Suidae), carcasses submerged in marine locations and the rate of decomposition was dependent on whether or not the carcass was floating or submerged with the latter associated with increasing decomposition rates due to scavenging (Anderson and Hobischak 2004). Swine carcasses placed in freshwater habitats, which

have different insect fauna, can still be detected 336 days post-submersion (Anderson and Hobischak 2004).

Physical structure or barriers to insect colonization can also influence decomposition rates. Swine carcasses placed inside a house took an additional 18 days to reach dry stage in comparison to carcasses placed outside (Anderson 2011). When insects are excluded from a carcass, the body will mummify before soft tissue has been consumed by microbes (Payne 1965). Insect colonization can also be delayed by two or more weeks if a body is buried (VanLaerhoven and Anderson 1999). Additionally, a delay of colonization may be seen if a body is wrapped; a swine carcass wrapped in heavy blankets was used to mimic a homicide scene and resulted in a delay in colonization by approximately 2.5 days (Goff 1992). However, other studies have demonstrated swine carcasses either with clothing, wrapped in a sheet, or a combination of clothing and being wrapped in a sheet does not delay colonization (Kelly et al. 2009).

Vertebrate scavengers (e.g., raccoons, vultures, and coyotes) also influence rates of decomposition of carrion. There is competition for carrion between invertebrate detritivores and vertebrate scavengers. In one instance, vertebrates were found to scavenge rodent carcasses 35% of the time (DeVault et al. 2003). Vertebrates scavenged year round however decomposition rates increased in the summer (warmer) months due to the increased insect activity on carrion (DeVault et al. 2004).

Consumer interactions on carrion

Species commonly associated with carrion have been divided into three trophic levels: microbial decomposers (Burkepile et al. 2006), arthropod primary consumers (Norris 1965, Payne 1965, Putman 1978b), and vertebrate scavenger secondary consumers (DeVault et al. 2003, DeVault et al. 2004). Community composition including biodiversity, dominant species, keystone species and interactions amongst species influence community function (Hooper et al. 2005). For example, the introduction of ungulate carrion into a terrestrial system has been reported to facilitate a localized succession of insect colonizers, such as blow flies (Diptera: Calliphoridae)

(Čeřovský et al. 2010). However, overall community structure of the ecosystem is stable because of the total community species composition within the ecosystem (Horn 1974).

Role of microbial communities in decomposition

Microbial assemblages are important for many ecosystem processes (Hattenschwiler et al. 2005, Parmenter and MacMahon 2009, Nemergut et al. 2010). Microbial communities in terrestrial systems have been suggested to be just as important as primary producers (Tiunov and Scheu 2005). They convert decaying organic matter into low molecular organic forms, which can then be used by other organisms (Tiunov and Scheu 2005). For example, nitrogen is a limiting nutrient in terrestrial systems. The loss of nitrogen from forest canopy results from events such as insect herbivory and premature leaf fall (Lovett et al. 2002). Leaf litter is broken down by complex microbial communities and is reintroduced into the system where it can be absorbed by plants, thus facilitating new plant growth (Witkamp 1966, Lee 1999, Lovett et al. 2002). Empirical data describing microbial community dynamics on carrion are lacking even though their role in trophic level interactions and food webs is well appreciated (Zak et al. 2003, Chung et al. 2007, Rohlfs 2008, Strickland et al. 2009).

Microbial community assembly influence in other decomposition model systems, such as leaf litter, has varied considerably in previous studies with results emphasizing either the importance of species diversity or individual species and functional composition (Loreau et al. 2001). The relationship between biodiversity and ecosystem function has been well documented through experimentally altering community composition and analyzing the functional response (e.g., respiration or succession) (Venner et al. 2011). Leaf litter decomposed by complex microbial communities results in energy and nutrients being reintroduced into the ecosystem (Witkamp 1966, Lee 1999, Lovett et al. 2002). For example, microbial community biomass increased significantly when plant richness increased from 1 to 16 species, thus influencing functional processes such as nitrogen cycling (Zak et al. 2003). However, some studies have not found significant correlation between microorganisms and functional response within an

ecosystem (Longmuir et al. 2007, Andersen et al. 2010). For instance, microbes, such as fungi and bacteria, have been documented to initially decompose carrion (Jiron and Cartin 1981, Burkepile et al. 2006).

Carrion provides a discrete and ephemeral ecological unit that is also colonized by microbes, including those that directly consume the carrion and those that are fed upon by insects. Understanding carrion microbial communities and potential interactions with primary colonizers is important for understanding mechanisms driving the decomposition process (Strickland et al. 2009). Empirical data quantifying and qualifying microbial community dynamics including species composition, abundance and succession patterns on carrion are limited (Vass 2001).

Insects associated with carrion

Arthropod succession patterns on carrion have been documented for at least 115 years and follow predictable patterns (Benecke 2001). Species colonize remains in a predictable pattern (Braack 1987, Archer 2004, VanLaerhoven 2008), but several factors such as ecoregion, season, and abiotic factors influence the colonization patterns and community structure of the arthropods present on carrion (Byrd and Castner 2001). Dipteran and coleopteran species are the most common arthropods to colonize carrion.

Blow flies are spatially and temporally distributed throughout North America (Whitworth 2006). *Cochliomyia macellaria* (F.) (Diptera: Calliphoridae) and *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) are primary colonizers, such that they colonize a carcass early in decomposition. *Chrysomya rufifacies* Macquart (Diptera: Calliphoridae) on the other hand is a secondary colonizer (Tenorio et al. 2003), using the resource in more advanced stages of decomposition (Jiron and Cartin 1981, Wells and Greenberg 1994). *Phormia regina* (Meigen) (Diptera: Calliphoridae) has a wide distribution and is considered to be a cool weather species (Hall 1948, Byrd and Allen 2001, Schroeder et al. 2003). This is a prominent species found in southwest Virginia (Joy et al. 2006), eastern Tennessee (Rodriguez and Bass 1983) and Canada (Sharanowski et al. 2008) during summer months. However, it is a winter/ early spring

species in Texas (Tenorio et al. 2003). There are multiple development data sets available for *P. regina* (Kamal 1958, Byrd and Allen 2001, Nabity et al. 2006). *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) is also a commonly found species throughout the United States with populations found in Michigan (Tarone and Foran 2006), Texas (Tenorio et al. 2003), Southern British Columbia (Anderson 2000) and available development data (Kamal 1958, Anderson 2000, Grassberger and Reiter 2001). The predictability of insects colonizing remains is the foundation for determining a minimum post-mortem interval or period of insect activity in forensic investigations (Tomberlin et al. 2011b). These two predictions can be the same time but are not necessarily synonymous. After dipteran arrivals there is a wave of coleopterans species including staphylinids (Coleoptera: Staphylinidae) and histerids (Coleoptera: Histeridae), which are predaceous on blow fly larvae and heterospecific coleopteran immatures (Byrd and Castner 2001).

Studies of interactions occurring on carrion have primarily focused on describing relationships amongst blow fly species. As the resource decomposes, the number of organisms occupying that resource increases, as does the complexity of the interactions occurring amongst organisms (Jiron and Cartin 1981). The increase of interactions results from the number of species utilizing the resource, thus based on insect succession data, species interactions may be occurring on carrion in a normal distribution pattern. Few species utilize carrion during fresh decomposition, a maximum species diversity during active decomposition, and the finally a few specialist utilizing the resource during the dry stages of decomposition (Payne 1965). Previous studies have described blow fly species composition (Wells and Greenberg 1992, Faria et al. 1999), densities (Goodbrod and Goff 1990), priority effects (Hanski and Kuusela 1977; A. Brundage, personal communication), and competition (Burkepile et al. 2006) on corresponding population dynamics in controlled laboratory settings.

For instance, blow fly species composition can be influenced by interactions between non-predacious and predacious blow fly larvae. *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) demonstrated a preference (60-80% predation

rates) for *C. macellaria* over *Chrysomya putoria* (Weidemann) (Diptera: Calliphoridae) and *Chrysomya megacephala* (Fabricus) (Diptera: Calliphoridae) larvae (Faria et al. 1999).

Role of insects in decomposition

Insects facilitate decomposition of carrion (Payne 1965, Simmons et al. 2010a, Simmons et al. 2010b). The influence of decomposition by blow fly larvae has been previously studied by analyzing decomposition rates (Simmons et al. 2010a, Simmons et al. 2010b). Swine carcasses in terrestrial environments can lose 90% of its mass in less than six days by the larvae facilitating removal of soft tissue (Payne 1965). While carcasses placed in similar habitats and protected from insect colonization still had 20% remaining after 100 days (Payne 1965). Despite the influence larvae have on the removal of carrion, the quantitative role of blow flies in nutrient cycling process remains relatively unknown (Tomberlin et al. 2011b). Approximately 48% of salmon carcasses (energy) can be transferred to riparian zones by blow flies (Hocking and Reimchen 2006), and rat, *Rattus rattus* L. (Rodentia: Muridae), carcasses placed in a temperate ecosystem during summer and winter seasons introduced approximately 1.25-2.5 mg C g^{-1} (dry weight) into the soil (Carter et al. 2007). One study demonstrated that once blow fly larvae disperse from a human cadaver, the highest rates of ions (e.g., magnesium, calcium, and sodium) were found in the soil, beneath the cadaver (Carter et al. 2007). However, this study failed to restrict insect access to replicate cadavers, not providing a control, and thus the role of insects in the reintroduction of nutrients to the system is unknown.

Microbe-insect interactions

Competition for ephemeral resources between micro- and macroorganisms is well documented (Janzen 1977, Polis and Strong 1996, DeVault et al. 2004). *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) and fungi (*Aspergillus* spp.) interact on fruit, with larval mortality correlating to age and species of fungi (Trienens et al. 2010).

In the presence of *Aspergillus fumigatus* Fresenius (Eurotiales: Trichocomaceae), *Drosophila* larval mortality increased from 40 to 85% within a day of exposure, and no larvae survived when placed on two-day old fungal colonies (Trienens et al. 2010). Secondary metabolites synthesized by the fungi are toxic to the fly larvae, thus demonstrating competition between fungi and fruit fly (Trienens et al. 2010). Scavenging rates in marine systems varied from 66% when microbial communities were allowed to proliferate undisturbed to 89% in the absence of mature microbial communities (Barlocher 1979, Burkepile et al. 2006). It has yet to be determined what, and if, the microbial threshold is for outcompeting higher trophic levels (e.g., insects and vertebrate scavengers) in terrestrial systems.

Mutualistic interactions between insects and microbes have been described. Fungal species provide nutrients to southern pine beetle, *Dendroctonus frontalis* (Zimmerman) (Coleoptera: Scolytidae) larvae (Scott et al. 2008); fungus-growing ants (Hymenoptera: Formicidae) cultivate fungus gardens (e.g., *Pseudonocardia,*) for food (Sen et al. 2009); and tsetse flies (Diptera: Glossinidae) regulate their symbiont density to increase survival rates (Rio et al. 2006). Cytoplasmically inherited symbionts can be lethal or detrimental to male reproduction while remaining beneficial or neutral to females (Engelstadter and Hurst 2009).

Insects can reduce microbial populations by producing antibiotics such as those found in adult and larval excretions/secretions (ES). Several categories of peptides including cercropins and defensins are found in ES and contribute to antimicrobial activity in insects (Kerridge et al. 2005, Altincicek and Vilcinskas 2009). Low molecular-weight, cationic antimicrobial peptides were first characterized in the early 1980's from the heamolymph of cercropia moth pupae (Kerridge et al. 2005). These molecules were effective against Gram-negative organisms and have been found to be crucial during larval development (Kerridge et al. 2005). Antibiotic compounds have been isolated in specific dipteran and coleopteran species commonly associated with carrion (Greenberg and Klowden 1972, Hoback et al. 2004). Antibacterial agents were initially reported in dipteran larvae secretions approximately 80 years ago (Simmons

1935). *Lucilia sericata* larvae secrete antimicrobial compounds such as lucifensin, which is effective at the degradation of *Psuedomonas aeruginosa* biofilms (Čeřovský et al. 2010). The antibacterial activity analyzed from excretions/secretions (ES) collected from third instar *L. sericata* larvae contain a small compound (<500 Da), which has been effective against *Bacillus cereus* (Frankland and Frankland), *Staphylococcus aureus* (Rosenbach), and *Escherichia coli* (Migula) Castellani and Chalmers (Bexfield et al. 2008). The antimicrobial compound lucifensin, a defensin, is effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Schroeter) Migula bioflims (van der Plas et al. 2007, Čeřovský et al. 2010). In addition to the potential role of insect ES in mediating microbial communities, microbes can be physically removed through larval feeding of a resource (Mumcuoglu et al. 2001, Lerch et al. 2003). Microbes are destroyed in the alimentary canal of calliphorid larvae; *E. coli* prevalence in *L. sericata* larvae was reduced almost 4-fold when comparing the posterior to the anterior end of the alimentary canal (Mumcuoglu et al. 2001, Huberman et al. 2007). *Nicrophorus* beetles (Coleoptera: Silphidae) care for their brood balls by coating them with oral and anal secretions, which are thought to maintain antibiotic molecules (Hoback et al. 2004). Although not all carrion beetles use antibiotics in such a direct manner, some beetles are not attracted to carrion until a species that utilizes antibiotics has been at the same resource (Hoback et al. 2004).

Novel methodologies in decomposition ecology

Microbial community profiles based on function have been used to study ecosystem processes (Stefanowicz 2006, Bell et al. 2009). Biolog EcoPlates™ provide quantifiable functional responses of environmental microbial communities by generating microbial metabolic community profiles (MMCPs) (Garland 1997, Garcia-Villaraco Velasco et al. 2009). Biolog EcoPlates™ are inexpensive and require minimal equipment yet generate substantial data that can be used to study the microbial community dynamics. Biolog EcoPlates™ contain 33 carbon sources (carbohydrates, amino acids, carboxylic acids, polymers and controls), in triplicate, along with a

tetrazolium violet dye; a color is produced when microbes reduce the carbon source and is measured with a spectrophotometer at 590 nm (Harbell 2001, Weber and Legge 2010). By quantifying MMCPs, it is possible to trace the functional responses of a microbial community through a natural process such as decomposition.

Pyrosequencing was first introduced in 1998 and is a high-throughput sequencing technology based on the sequence-by-synthesis theory (Ronaghi et al. 1998). High-throughput sequencing methods generate large amounts of data and obtain sequences for un-culturable or newly discovered microbial species (Hudson 2008, Rothberg and Leamon 2008). For instance, 454 pyrosequencing of microbial samples collected from 27 body regions produced 4,949 species level phylotypes out of a total of 250,000 16S rRNA sequences; thus less than 2% of the sequences had been previously characterized (Turnbaugh et al. 2010). Utilizing 454-pyrosequencing technology will allow, for the first time ever, for the characterization of microbial species composition, community change and interactions with arthropods that occur throughout decomposition. The principle of this technique is based on exploiting enzymatic reactions to determine the sequence of samples. The iterative addition of four enzymes (Klenow fragment of DNA polymerase I, ATP sulfurylase, Luciferase and Apyrase) produces a pattern of light, which then is used to determine the sequence of nucleotides incorporated (Ahmadian et al. 2006). Pyrosequencing has been used for several applications including bacterial sequencing, analyzing small RNA, metagenomics, whole genome human sequencing and comparative genomics (Rothberg and Leamon 2008). Limitations of using pyrosequencing include a lack of known reference genomes for sequence comparisons, an increased error rate when sequencing insertions-deletions, and this technology is costly (Shendure and Ji 2008). Additionally, computation limitations of pyrosequencing include costs associated with running and analyzing samples, a lack of sufficient computing power, and not having algorithms capable of analyzing the output in a biologically relevant manner (Rothberg and Leamon 2008, Petrosino et al. 2009).

Objectives

Previous literature has described insect succession patterns and species interactions occurring on carrion (Fuller 1934, Reed 1958, Payne 1965). Most research has examined the ecology of carrion decomposition using descriptive approaches to solely evaluate insect community structure over time with no emphasis on the associated microbial communities. In carrion systems, necrophagous insects may alter the microbial community structure and function on the resource, but there are no data from field studies at this time.

The objectives of this research were: (i) to assess insect arrival patterns to swine carcasses that were either excluded from insect access for five days using insect exclusion cages or were exposed to insects throughout decomposition, (ii) to identify bacterial communities during carrion decomposition and assess related bacterial community composition changes in the presence or absence of naturally occurring necrophagous insects, and (iii) to evaluate the microbial metabolic profiles during succession throughout carrion decomposition under conditions that allowed natural insect colonization compared to insect exclusion treated carrion

A survey of insect and microbial communities occurring throughout decomposition of a resource is vital information important for discovering inter-kingdom interactions that can contribute to general theory in decomposition ecology. In addition to elucidating mechanisms governing insect attraction and competition for an ephemeral resource. This research also has practical applications in forensic entomology as it may provide an additional parameter to predict decomposition times.

CHAPTER II

INSECT COMMUNITY ARRIVAL PATTERNS DURING DELAYED COLONIZATION OF PATCHY, EPHEMERAL RESOURCES

Introduction

The relationship between biodiversity and ecosystem function has been well documented through experimentally altering community composition and analyzing functional response (e.g., respiration or succession) (Venner et al. 2011). Biodiversity including species richness, keystone species, relative abundance and species interactions is key to ecosystem function (Hooper et al. 2005). Studies have demonstrated a positive relationship between ecosystem function and biodiversity (Jousset et al. 2011). Variation in species arrival patterns influence community assembly and can affect ecosystem processes (Chase 2003, Fukami and Nakajima 2011). Despite the importance of carrion in an ecosystem, there have been few examinations of the mechanisms, driving forces, and impact of carrion within terrestrial ecosystems.

Carrion represents an ecological unit within a larger ecosystem (Odum 1969) resulting in an energy and nutrient surge to the immediate soil, insect and plant communities at its location (Towne 2000, Yang 2006). Decomposing remains such as deep-sea whale carcasses (Klages et al. 2001, Burkepile et al. 2006) or anadromous salmon (*Oncorhynchus* spp. (Salmoniformes: Salmonidae)) in Pacific watersheds (Hocking and Reimchen 2006, Janetski et al. 2009) can be the primary resource subsidy for certain ecosystems. Carrion decomposition reintroduces essential nutrients such as nitrogen, potassium, calcium and magnesium into an ecosystem (Carter et al. 2007). For example, nitrogen concentration in soil collected beneath a bison, *Bos bison* L. (Artiodactyla: Bovinae), decomposition site are approximately 6 fold higher than control soil (Carter et al. 2007). Also, rat (*Rattus rattus* L. (Rodentia: Muridae)) carcasses had an estimated 1.25-2.5 mg C g^{-1} (dry weight) introduced into the soil (Carter et al. 2007). This macromolecule reintroduction influences soil chemistry and composition, which

may lead to different species occupying the space beneath decomposed resource (Post and Kwon 2000).

Understanding nutrient transfer back into the ecosystems is vital to elucidating mechanisms, rates, and efficacy of ecosystem functions (Gessner et al. 2010). Insect communities are key factors in carrion decomposition (Srivastava et al. 2009). The introduction of ungulate carrion into a terrestrial system has been reported to facilitate a localized succession of insect colonizers, such as blow flies (Diptera: Calliphoridae) (Čeřovský et al. 2010). Blow fly larvae consume most of the soft tissue of carrion (Simmons et al. 2010a, Simmons et al. 2010b). Carcasses can lose 90% of their mass in less than six days when exposed to insect colonizers (Payne 1965). Swine, *Sus scrofa* L. (Artiodactyla: Suidae), carcasses were primarily consumed by insects and were in the dry stage of decomposition after approximately 5 days during summer months in South Carolina (Payne 1965). It also has been shown that up to 48% of salmon carcass biomass is translocated to riparian zones by Diptera (Hocking and Reimchen 2006). One study demonstrated that blow fly larvae transferred the highest rates of ions (e.g., magnesium, calcium, and sodium) from human cadavers when they migrated from the body (Carter et al. 2007). However, the role of blow flies in nutrient cycling remains limited.

Arthropod colonizers follow succession patterns (Reed 1958, Payne 1965, MacArthur and Wilson 1967). For example, in southwestern regions of the USA *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) initially colonizing carrion followed by *Chrysomya rufifacies (*Macquart) (Diptera: Calliphoridae) secondarily colonizing the carcasses, using the resource in more advanced stages of decomposition (Jiron and Cartin 1981, Wells and Greenberg 1994). There are then waves of coleopterans species such as Staphylinidae and Histeridae, which are predaceous on blow fly larvae and heterospecific coleopteran immatures (Reed 1958, Payne 1965). Secondary consumers remove large quantities of carrion biomass (estimated 6,250 kg/y) from an ecosystem (Wilmers et al. 2003). It is important to understand how necrophagous species diversity influences the decomposition process. Delaying or excluding insect colonization of remains results in altering the decomposition rate and

stages associated with carrion (Payne 1965). Decomposition becomes prolonged in carcasses excluded from insect access (Payne 1965, Simmons et al. 2010b). However, there has yet to be a study that excludes insect access under natural environmental conditions to test the hypothesis that delayed colonization would alter insect community assembly.

The objectives of this study were to quantify decomposition rates and insect community structure in response to delayed access to carrion over two field seasons. Here I test the null hypothesis that delayed colonization will not affect insect species arrival sequence and community composition on carrion. The alternative hypothesis is that insect species arrival due to delayed colonization will reduce community richness and diversity.

Methods

Site description and experimental design

Swine carcass decomposition was studied in a Midwestern temperate forest habitat surrounded by agricultural fields in Xenia, Ohio, USA (39°38'14.83"N, 84°1'37.82"W). Carcasses were sampled from 5 - 14 August 2010 and 26 July - 2 August 2011. The dominant tree fauna consisted of oaks (*Quercus* spp.) and maples (*Acer* spp.). The 95% canopy cover was relatively homogenous over all carcasses. In 2010, six male swine ranging from 10.4 - 30.1 kg (TABLE 1), euthanized by cranial blunt force at approximately 16:30 h, were purchased from a local farm on 5 August 2010. Carcasses were double bagged, transported for about 1 hour, and randomly placed minimally 20 m apart along three transects (FIG. 1) two hours before National Oceanic and Atmospheric Administration (NOAA) defined sunset at approximately 19:00 h on 5 August 2010.

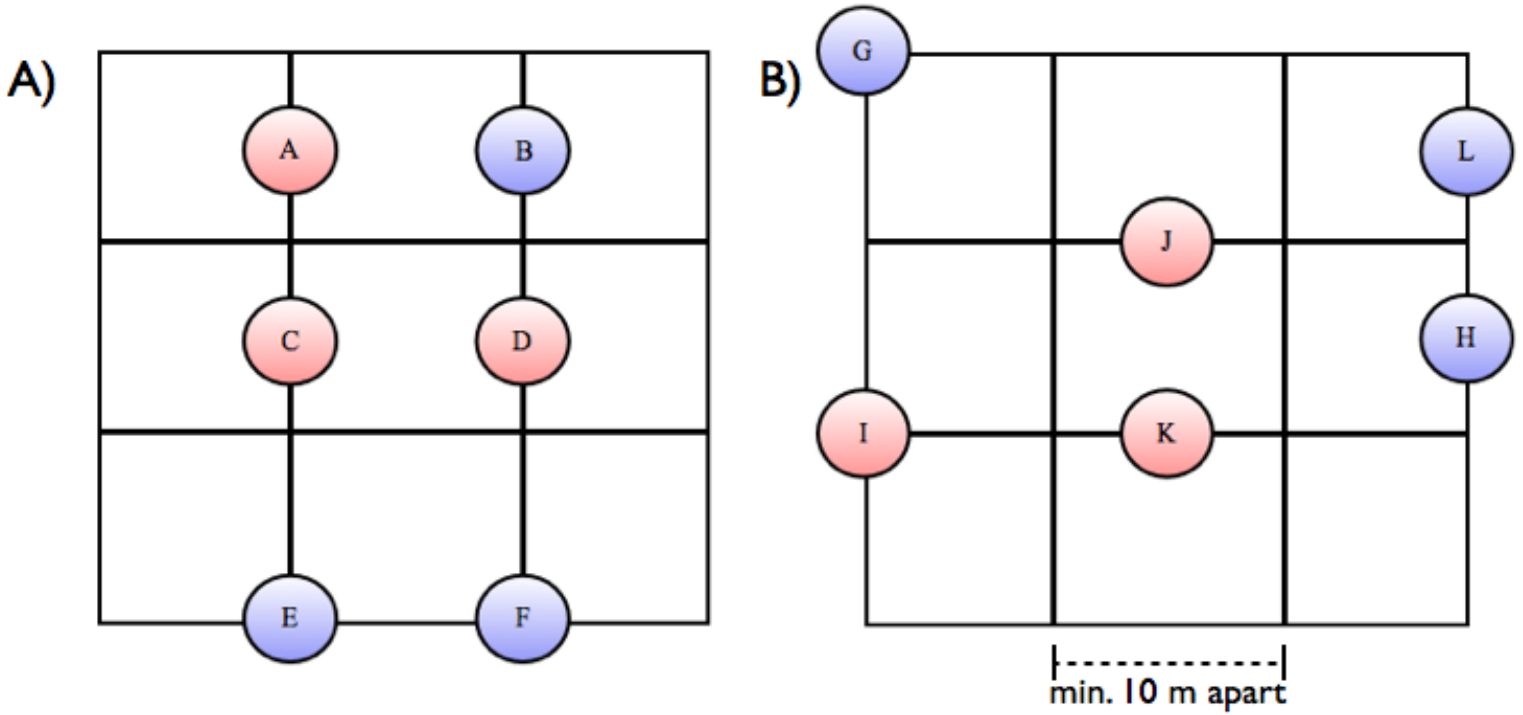

FIG. 1. (A) Schematic of the carcass deposition sites and treatments for the 2010 trial. Blue circles represent the insect access (ACC) carcasses; red circles represent insect exclusion (EXC) carcasses. (B) Schematic of the carcass deposition sites and treatments for the 2011 trial. Blue circles represent the ACC treatments; red circles represent EXC carcasses. All carcasses were a minimum of 10 m apart.

TABLE 1. The sex, weight (kg), and treatment for each carcass.

Year	Carcass	Sex	Weight (kg)	Treatment
2010	A	Male	10.4	EXC
2010	B	Male	13.7	ACC
2010	C	Male	20.6	EXC
2010	D	Male	18.1	EXC
2010	E	Male	16.5	ACC
2010	F	Male	30.1	ACC
2011	G	Male	6.8	ACC
2011	H	Female	6.4	ACC
2011	I	Female	4.1	EXC
2011	J	Female	6.8	EXC
2011	K	Male	7.3	EXC
2011	L	Male	5.0	ACC

In 2011, using the same methods, six swine (three females and three males) carcasses were purchased from the same local farm on 26 July 2011 after being euthanized at approximately 17:45 h. Carcasses ranged from 5.0 - 7.3 kg (TABLE 1) and were randomly placed along three new transects at approximately 18:30 h on 26 July 2011 (FIG. 1).

All carcasses were oriented with heads to cardinal north and dorsal side towards the east. Each was labeled alphabetically with "A" through "F" representing the 2010 field season and "G" through "L" representing carcasses in 2011. During each field season, three random carcasses were enclosed in individual 1.8 m^3 Lumite® screen (18 x 14 mesh size) portable field cages (BioQuip Products, Rancho Dominguez, CA, USA) to reduce and delay insect access. These carcasses were considered the insect exclusion

FIG. 2. (A) Example of an insect exclusion carcass (EXC) and (B) an example of an insect access carcass (ACC). All carcasses were placed within an anti-scavenging cage.

treatment (EXC), while insects were allowed access to the remaining three carcasses (ACC); all carcasses were covered with anti-scavenging cages (0.9 x 0.6 x 0.6 m) constructed of wooden frames enclosed with poultry netting (FIG. 2). Fresh, bloat, active decay, advanced decay and dry stages of decomposition, as defined by Payne (1965), were recorded throughout the study. NexSens DS1923 micro-T temperatures loggers (Fondriest Environmental, Inc., Alpha, OH, USA) were placed within 0.6 m of each carcass approximately 0.3 m above the ground to measure local ambient temperature every 15 min. Temperature data were later converted into accumulated degree hours (ADH), which accounts for temperature variation over decomposition time (Megyesi et al. 2005). Additionally, local ambient temperature was compared to air temperature collected at the nearest NOAA weather station , located approximately 34 km away.

Flying insects attracted to carcasses were sampled using two Trapper® max glue traps (16.5 x 11 cm) (Bell Laboratories, Inc., Madison WI, USA). Glue traps were attached to the anti-scavenging cage, each approximately 0.15 m from the anterior and posterior region of the carcass (FIG. 3). Glue traps were replaced every 12 h. The exclusion cages were removed from insect exclusion carcasses after five days at approximately 19:30 in 2010 and 18:30 in 2011. The same insect sampling protocol was followed in 2011; however, sampling of insect access carcasses concluded after three

days due to rapid carcass decomposition. Carcasses were considered in the advanced active decay stage when there was no soft tissue in the buccal cavity and the skin had become indistinguishable from the internal anatomy (Payne 1965). Adult insects were identified to the lowest taxonomic level possible while remaining on the glue trap (Triplehorn and Johnson 2005, Whitworth 2006).

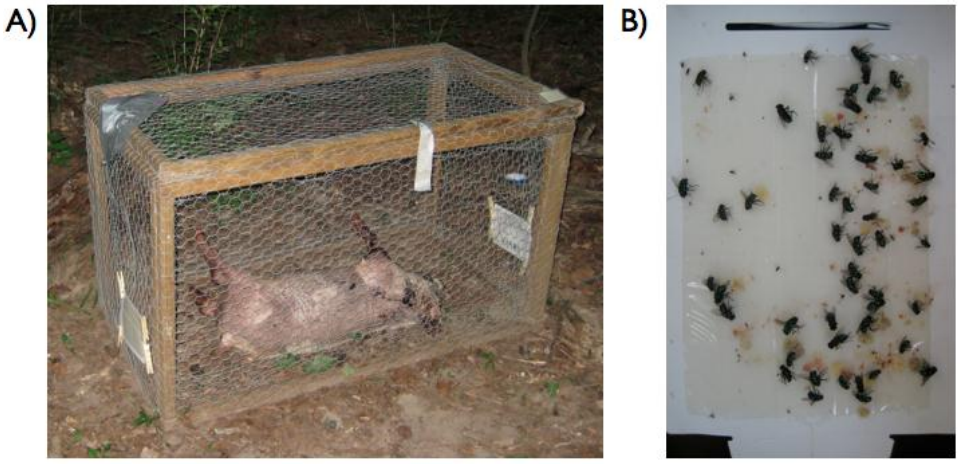

FIG. 3. (A) Example of glue traps attached to an anti-scavenging cages near the anterior and posterior ends of the carcasses. (B) Example of a single glue trap with adult insects collected after 12 h.

Statistical analyses

All insect data were arcsine-square root transformed to accommodate assumptions of normality and homogeneity of variance for parametric analyses. Simpson's diversity, Shannon-Weaver diversity, richness, and evenness were calculated according to methods of Zak et. al (1994). Simpson's diversity index (*D*) was determined as:

$$D = \Sigma\, p_i^2$$

Shannon-Weaver diversity (*H*) was determined as:

$$H = -\Sigma\, p_i \ln(p_i)$$

where p_i is the ratio of relative abundance of a taxon (Simpson 1949). Species richness (S) was the number of taxa. Evenness (E) measured by the equitability of taxa in the community was determined by:

$$E = H/H_{max} = H/\log S \text{ (Zak et al. 1994).}$$

The effects of decomposition day, insect access treatment and their interaction were tested using two-way repeated measures analysis of variance (RM-ANOVA) with multiple comparisons evaluated after Bonferroni corrections using Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) for diversity, richness and evenness.

Non-metric multidimensional scaling (NMDS) was used to evaluate insect community composition between treatments and over decomposition in PC-ORD 5 (MjM Software, Gleneden Beach, Oregon, USA) (McCune and Mefford 2006). NMDS is a nonparametric ordination technique that avoids assuming linearity among community variables (McCune and Grace 2002). First, I identified outliers using Jackknife distances in JMP 9.0.0 (SAS Institute Inc., Cary, NC, USA) as recommended by McCune and Grace (2002). Multi-response permutation procedure (MRPP) was used for testing statistical differences in insect community composition between or among covariates (i.e., treatment or days, respectively) within the ordination using methods described elsewhere (Biondini et al. 1985). Indicator species analysis (ISA) complemented MRPP by assigning significant indicator values to insect taxon that were indicative of community separation between treatments and over decomposition (McCune and Grace 2002). The indicator value represents the taxon best predicting decomposition day or treatment (EXC or ACC carcasses) with 0 representing no indication and 100 being a perfect indication for each grouping.

Results

Abiotic conditions

The mean daily ambient temperature among carcasses was 23.2 ± 2.1°C during 2010, and 25.1 ± 1.0°C in 2011. Mean ADH for each carcass in 2011 was significantly higher (8 - 18%) than 2010 (FIG. 4; TABLE 2) throughout decomposition, except at initial placement in the field when ADH for all carcasses was zero. Mean daily temperature recorded at the weather station was 25.5 ± 2.5°C during 2010 and 27.9 ± 1.4°C during 2011. Weather station air temperature was significantly related to local ambient temperature in 2010 (r^2 = 0.83, F = 23.64, P = 0.0046) and 2011 (r^2 = 0.83, F = 24.83, P = 0.0042). Total precipitation was two-orders of magnitude higher in 2011.

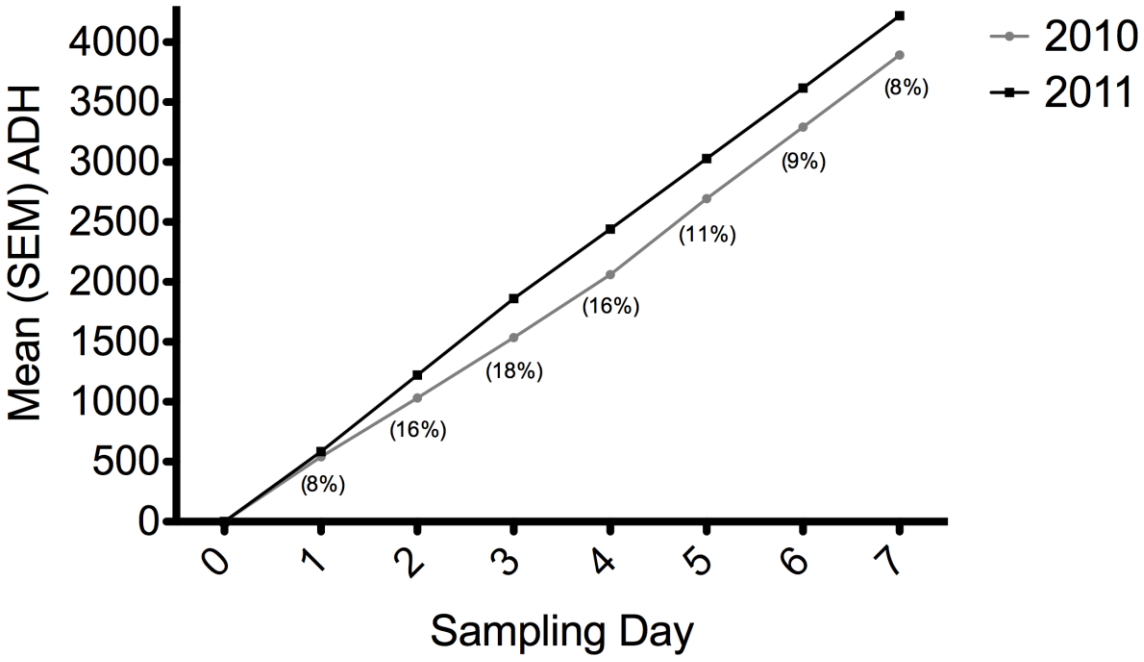

FIG. 4. Mean (SEM) accumulated degree hours (ADH) between 2010 and 2011 field seasons. The mean ADH for each day in 2011 was significantly higher (P < 0.0001) than 2010 except on day 0. Each number beneath the data points represents difference between 2010 and 2011 mean ADH.

TABLE 2. RM-ANOVA results testing mean ADH differences between 2010 and 2011 and over days of decomposition.

Factor	*F* test	df	*P* value
Day	91273	7	<0.0001
Year	423.9	1	<0.0001
Day x Year	242.0	7	<0.0001

Decomposition stages

Fresh stage began at time of death and continued until bloat was evident, with no odor emitted or evidence of decomposition (Payne 1965). Bloat stage was characterized by swelling of the body (the abdomen was the first and most prominent area swollen on each carcass) and color changes sometimes resulting from tissue marbling as a result of gas accumulation. All EXC carcasses in 2010 were in bloat stage until insect access was allowed on day 5 (FIG. 5). While EXC carcasses in 2011 had the abdomen burst during bloat stage (day 2) with internal organs protruding through the epidermal layer. Decomposition odors were prominent during bloat, with fluid drainage from the head and anal areas, along with any other area where the skin had become disrupted. Active decay stage was determined by the removal of soft tissue from the head and neck by Calliphoridae larvae with remainder of the carcass beginning to deflate. Odors of decomposition were very strong during this stage. Advanced decay stage was similar to the active decay stage; however, most soft tissue had been removed by calliphorid larval masses. The putrification odors were not as strong at the end of advanced decay. The dry stage represented the end of decomposition when all soft tissue was gone leaving only bone, cartilage and skin. Transition characteristics between stages were also noted (Kelly et al. 2009). It was during these transitional stages that multiple characteristics from various stages of decomposition were present (i.e., bloated abdomen with an active larval mass on the head).

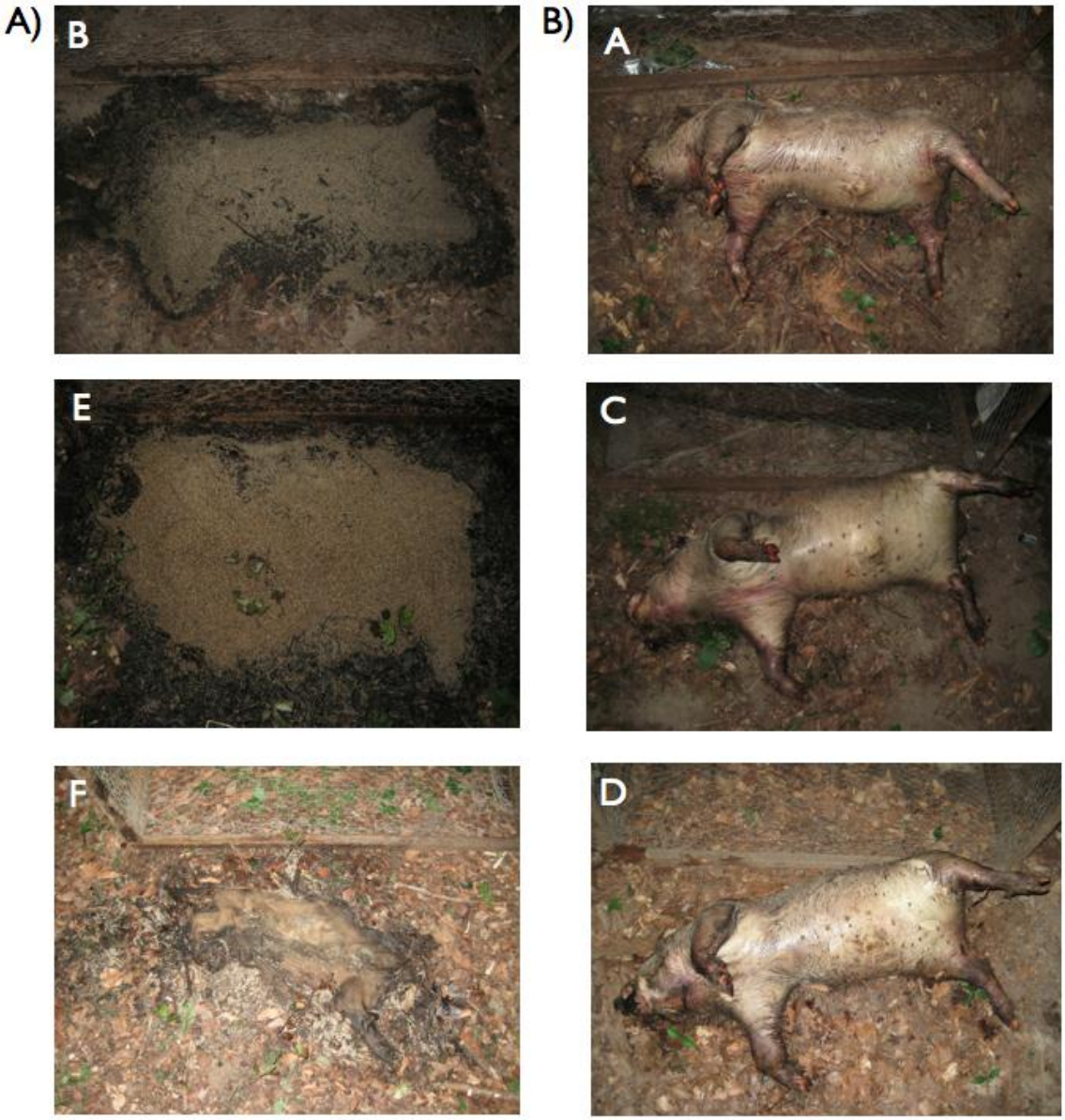

FIG. 5. Images of 2010 carcasses on the fifth day of decomposition (10 August at 19:00). Insect exclusion cages were removed from the carcasses on this day. (A) Carcasses with insect access (B, E and F) were in active or advanced decay while (B) carcasses excluded from insect access (A, C, and D) were still in bloat.

In 2010, ACC carcasses were in the dry stage between the sixth and seventh day of decomposition. While in 2011, ACC carcasses were in the dry stage within five days. During both 2010 and 2011, EXC carcasses were in dry stage by the fourth day of decomposition once insect exclusion netting was removed.

Insect arrival patterns

Sixty arthropod taxa, representing eight orders and 49 families, were collected during both field seasons (TABLE 3).

2010 field season

Necrophagous insect taxa were analyzed for arrival patterns (FIG. 6). Carcasses exposed to insect access (ACC) had blow fly oviposition within 24 h of exposure. *Phormia regina* (Meigen) (Diptera: Calliphoridae) was the dominant (71 - 98%) calliphorid taxon during fresh, bloat, and active decomposition stages. Larval masses were present on the head on the third day of decomposition as bloat stage was beginning to transition into active decay. Decomposition progressed into active and advance decay stages with beetles became the prominent taxa. On day 5, carcasses were covered with larval masses and a dispersal event occurred within 12 h. Rove beetles (Coleoptera: Staphylinidae) represented 59% of taxa present during later portions of advanced decay. Finally, black scavenger flies (Diptera: Sepsidae) were dominant (58%) during the dry stage.

Carcasses with delayed insect access (EXC) had oviposition occur within the first 24 h after exclusion net removal on day 5. The insect exclusion cages were approximately 99% efficient; however, exclusion cages did not exclude any ground dwelling insects present in the soil. Post-exclusion insect access will be used hence forth to define the period of time when insect exclusion carcasses were exposed to insect access upon exclusion cage removal on day 5. *Phormia regina* was the dominant taxon (42 - 87%) arriving to carcasses throughout initial active decomposition. On the second day of post-exclusion insect access, larval masses were present on the head. Carcasses

TABLE 3. Adult insects collected throughout decomposition from insect access (ACC) and exclusion (EXC) carcasses during 2010 and 2011. Necrophagous insects are indicated with an asterisk (*).

Order	Family	Genus and species
Diptera	Calliphoriadae	*Calliphora vicina* Robineau-Desvoidy*
Diptera	Calliphoriadae	*Calliphora vomitoria* (L.)*
Diptera	Calliphoriadae	*Cochliomyia macellaria* (Fabricius)*
Diptera	Calliphoriadae	*Cynomya cadaverina* (Robineau-Desvoidy)*
Diptera	Calliphoriadae	*Lucilia coeruliviridis* (Macquart)*
Diptera	Calliphoriadae	*Phormia regina* (Meigen)*
Diptera	Calliphoriadae	*Protophormia terraenovae* (Robineau-Desvoidy)*
Diptera	Sarcophagidae	unknown sp.*
Diptera	Tachinidae	unknown sp.
Diptera	Muscidae	unknown sp.
Diptera	Muscidae	*Ophyra* spp.*
Diptera	Anthomyiidae	unknown sp.
Diptera	Conopidae	unknown sp.
Diptera	Piophilidae	*Piophila casei* (L.)*
Diptera	Piophilidae	*Prochyliza* sp. (Walker)*
Diptera	Phoridae	unknown sp.*
Diptera	Sepsidae	*Sepsia* sp.*
Diptera	Psychodidae	*Psychoda* sp.*
Diptera	Sciaridae	unknown sp.
Diptera	Tepididae	unknown sp.
Diptera	Cecidomyiidae	unknown sp.
Diptera	Dolichopodidae	unknown sp.
Diptera	Ceratopogonidae	unknown sp.
Diptera	Drosophilidae	unknown sp.
Diptera	Tipulidae	unknown sp.
Diptera	Tabanidae	unknown sp.

TABLE 3. Continued

Order	Family	Genus and species
Coleoptera	Staphylinidae	*Creophilus maxillosus* (Gravenhorst)*
Coleoptera	Staphylinidae	*Philonthus caeruleipennis* (Mannerheim)*
Coleoptera	Staphylinidae	*Platydracus maculosus* (Gravenhorst)*
Coleoptera	Staphylinidae	unknown spp.*
Coleoptera	Trogidae	unknown sp.*
Coleoptera	Histeridae	unknown sp.*
Coleoptera	Anobiidae	unknown sp.
Coleoptera	Tenebrionidae	unknown sp.
Coleoptera	Dermestidae	unknown sp.*
Coleoptera	Mycetophagidae	unknown sp.
Coleoptera	Nitidulidae	unknown sp.
Coleoptera	Scarabidae	unknown sp.
Coleoptera	Curculionidae	unknown sp.
Coleoptera	Elateridae	unknown sp.
Coleoptera	Latriidae	unknown sp.
Hympenoptera	Vespidae	unknown sp.
Hympenoptera	Apidae	unknown sp.
Hympenoptera	Ichnuemonidae	unknown sp.
Hympenoptera	Chalcidonidae	unknown sp.
Hympenoptera	Sphecidae	unknown sp.
Hympenoptera	Halticidae	unknown sp.
Hympenoptera	Formicidae	unknown sp.
Lepidoptera	Nymphalidae	unknown sp.
Lepidoptera	Erebidae	unknown sp.
Hympenoptera	Braconidae	unknown sp.

TABLE 3. Continued

Order	Family	Genus and species
Lepidoptera	Papilionidae	unknown sp.
Lepidoptera	Noctuidae	unknown sp.
Lepidoptera	Pyralidae	unknown sp.
Mecoptera	Meropeidae	*Merope tuber* Newman
Mecoptera	Panopidae	*Panorpa* sp.
Siphonaptera		unknown sp.
Acari		unknown sp.

were covered with larvae by the third day of post-exclusion insect access, with a larval dispersal event occurring within the next 12 h. Black scavenger flies were 33% of adult taxa during the dry stage.

2011 field season

As in the previous year, necrophagous insect taxa were analyzed for arrival patterns (FIG. 7). Oviposition was documented on carcasses exposed to insects (ACC) within 12 h of field placement. *Lucilia coeruleiviridis* (Macquart) (Diptera: Calliphoridae) was dominant (55 - 83%) from fresh to early stages of active decomposition, with *P. regina* (41 - 69%) becoming the dominant from active decomposition until the early stages of dry. Larval masses were present on the head of each carcass while in active and advanced decay. Rove beetles arrived from beginning of bloat stage until dry stage. Carcasses were covered with larvae during transition from active to advanced decay with a larval dispersal event occurring in 24 h. Black scavenger flies were the most dominant adult taxon collected (43%) during dry stage.

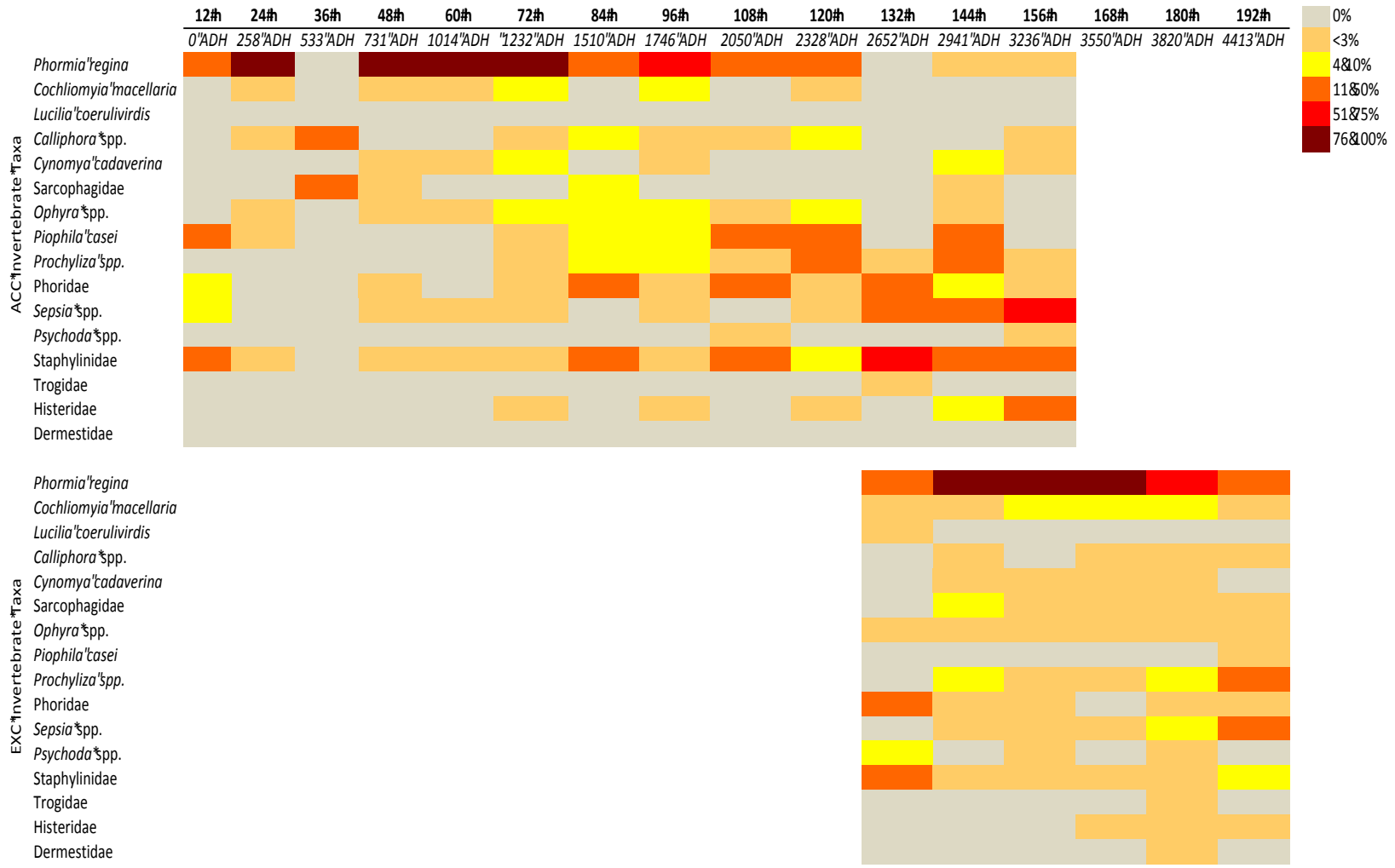

FIG. 6. Arthropod succession of carcasses excluded from insects (EXC) and carcasses allowing insect access (ACC) during 2010. The number of hours since field placement is along the top axis with the corresponding accumulated degree hours (ADH) directly below. Decomposition stages are also above each set of carcasses (EXC and ACC).

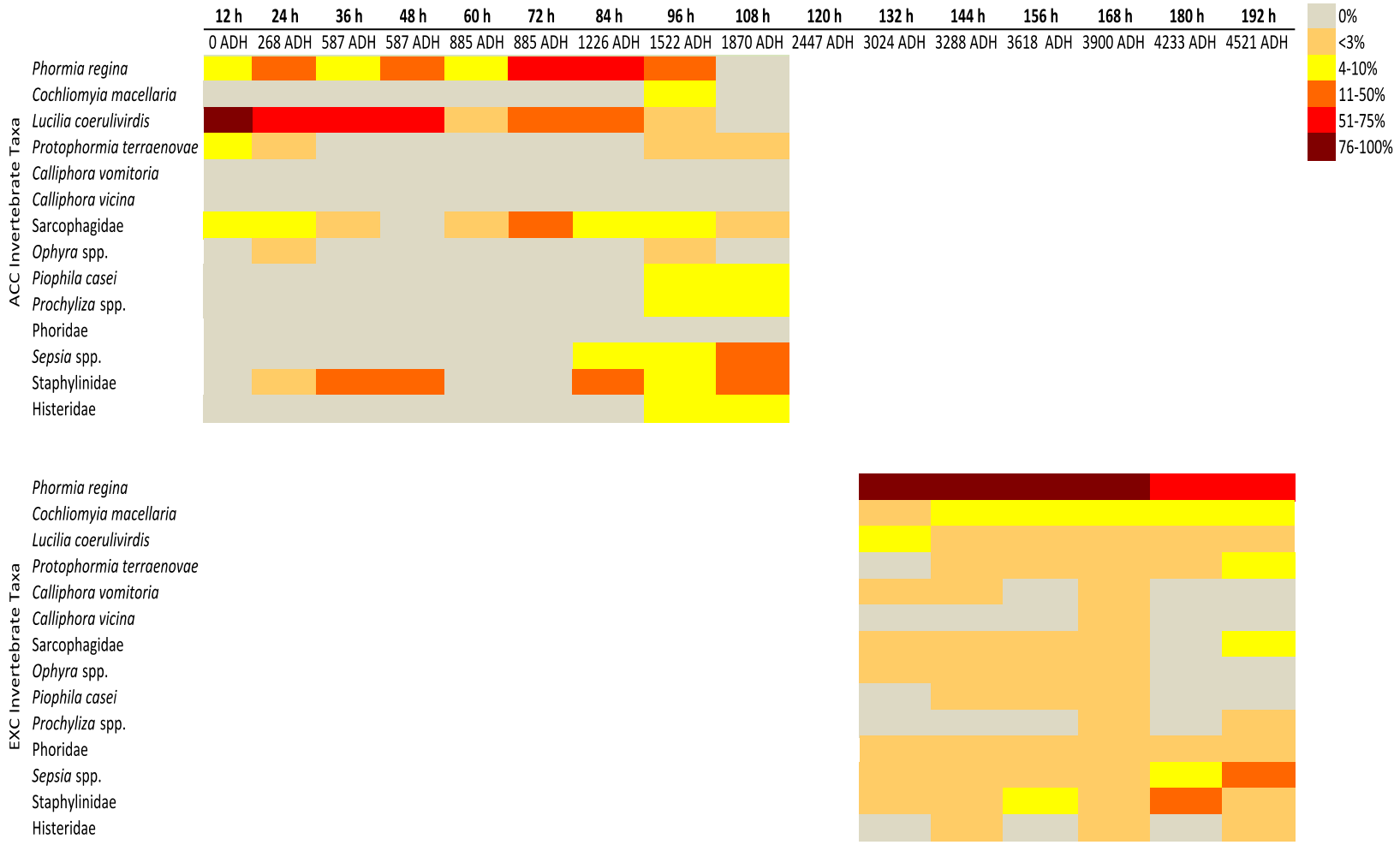

FIG. 7. Insect succession of carcasses excluded from insects (EXC) and carcasses allowing insect access (ACC) during 2011. The number of hours since field placement is along the top axis with the corresponding accumulated degree hours (ADH) directly below. Decomposition stages are also above each set of carcasses (EXC and ACC).

Insect exclusion cages were approximately 95% effective but again did not exclude any ground dwelling insects present in the soil. Carcasses excluded from insects had oviposition observed during the first 24 h of post-exclusion insect access. *Phormia regina* was dominant (58 - 85%) throughout decomposition. Larval masses were present on the head and in areas where the legs attached to the body during the second day of post-exclusion insect access. The body was completely covered with larvae by the third day of post-exclusion insect access, followed by a dispersal event within 12 h.

Insect community composition

2010 and 2011 field seasons combined

All insect taxa data from both years were combined to evaluate year effects on insect community structure. There were no significant differences between insect Shannon-Weaver diversity, Simpson's diversity, richness, and evenness between years. However, there was a significant difference over decomposition days and a significant interaction effect (TABLE 4). The interaction being significant results in data that are difficult to interpret, therefore each year was analyzed separately. It was not possible to find a stable NMDS ordination using combined insect community data from each year.

2010 field season

There were significant differences in Shannon-Weaver diversity of insect communities between insect access and insect exclusion carcasses and over time with no significant interaction (FIG. 8, TABLE 5). Carcasses excluded from insects resulted in higher amounts of diversity over time. Simpson's diversity (FIG. 9, TABLE 5) and evenness (FIG. 10, TABLE 5) both had significant differences over decomposition time and no significant differences between insect access and insect exclusion carcasses and no interaction based on insect communities. Richness was the only metric that had significant differences (FIG. 11, TABLE 5) over decomposition days, between exclusion

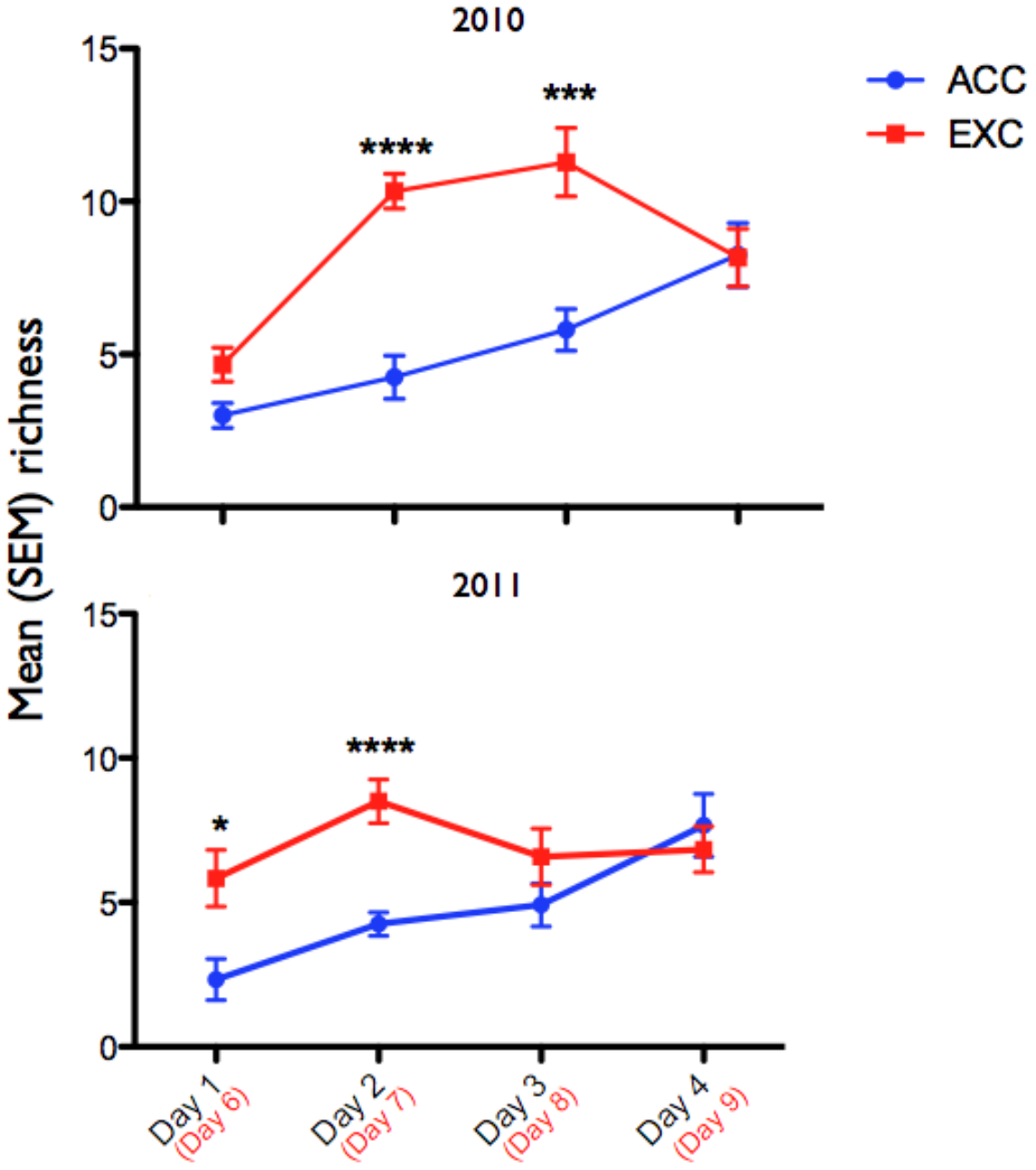

FIG. 8. Insect richness during decomposition for carcasses with insects present (ACC) and insects excluded for 5 d (EXC). Adult insects were collected using glue traps. Each sampling day is representative of insects having access to the carcass. However, due to the insect exclusion for 5 d in EXC carcass the days in parentheses represent how long the carcasses have been in the field.

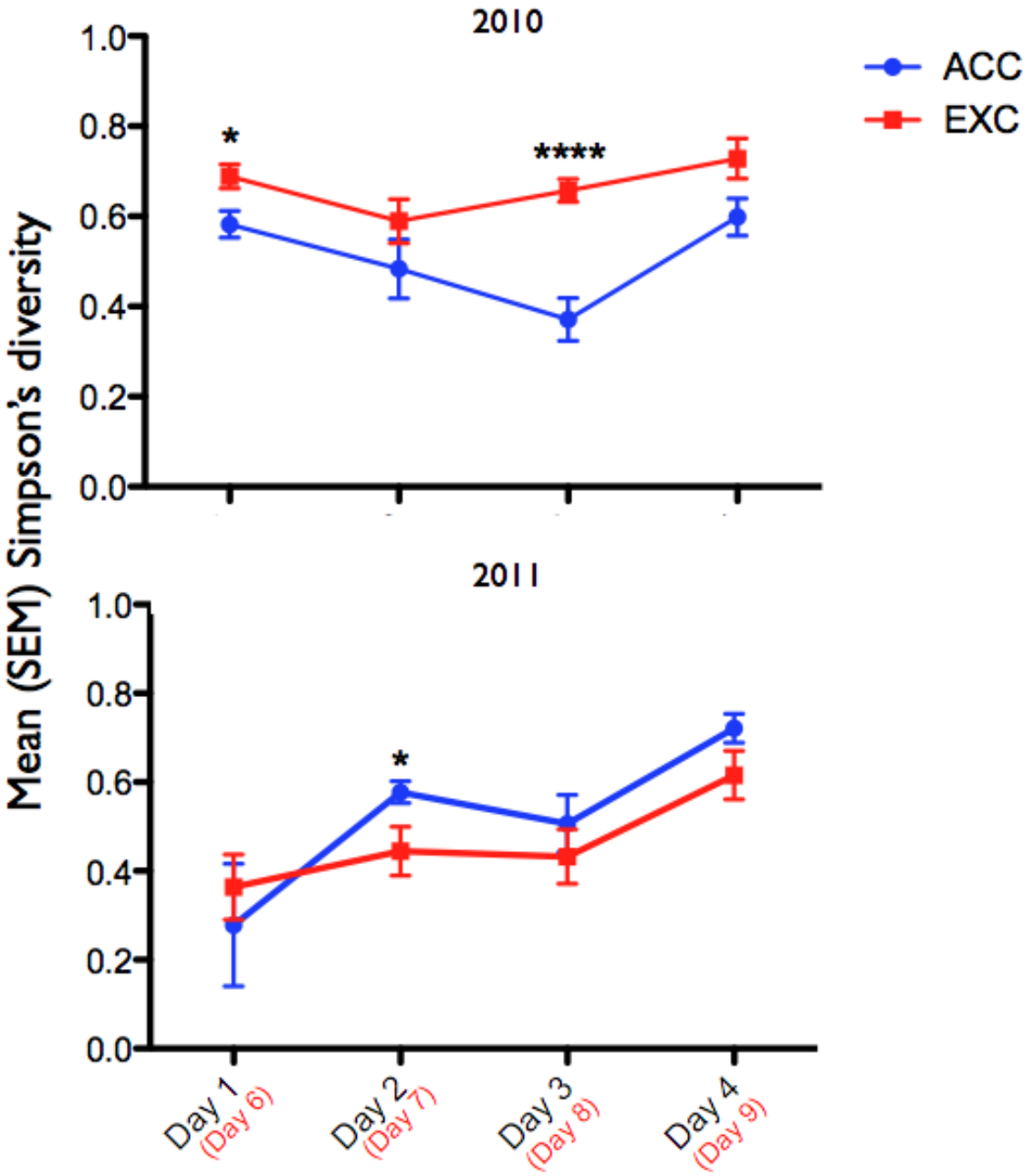

FIG. 9. Simpson's diversity of insects during decomposition for carcasses with insects present (ACC) and insects excluded for 5 d (EXC). Adult insects were collected using glue traps. Each sampling day is representative of insects having access to the carcass. However, due to the insect exclusion for 5 d in EXC carcass the days in parentheses represent how long the carcasses have been in the field.

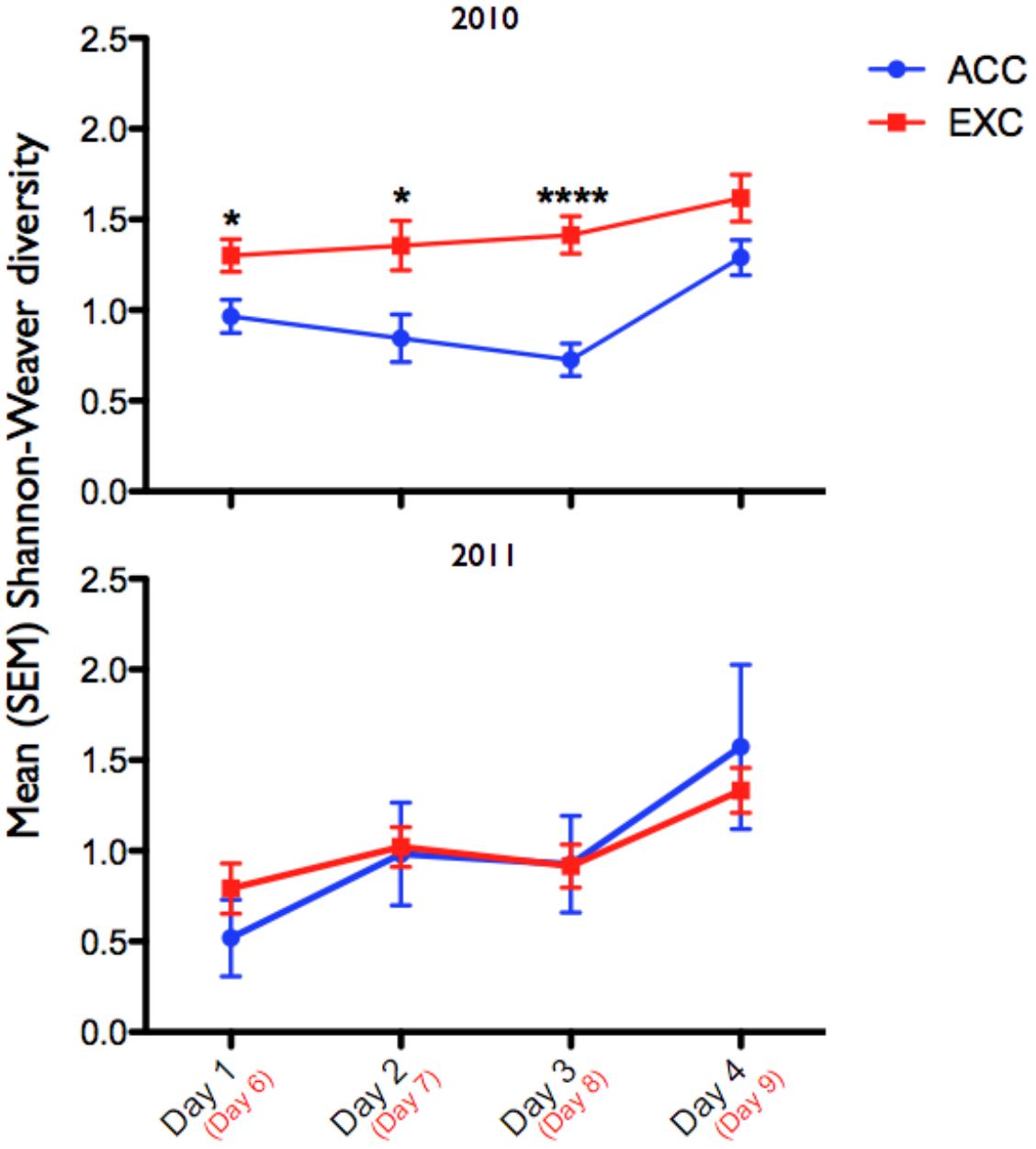

FIG. 10. Shannon-weaver diversity of insects during decomposition for carcasses with insects present (ACC) and insects excluded for 5 d (EXC). Adult insects were collected using glue traps. Each sampling day is representative of insects having access to the carcass. However, due to the insect exclusion for 5 d in EXC carcass the days in parentheses represent how long the carcasses have been in the field.

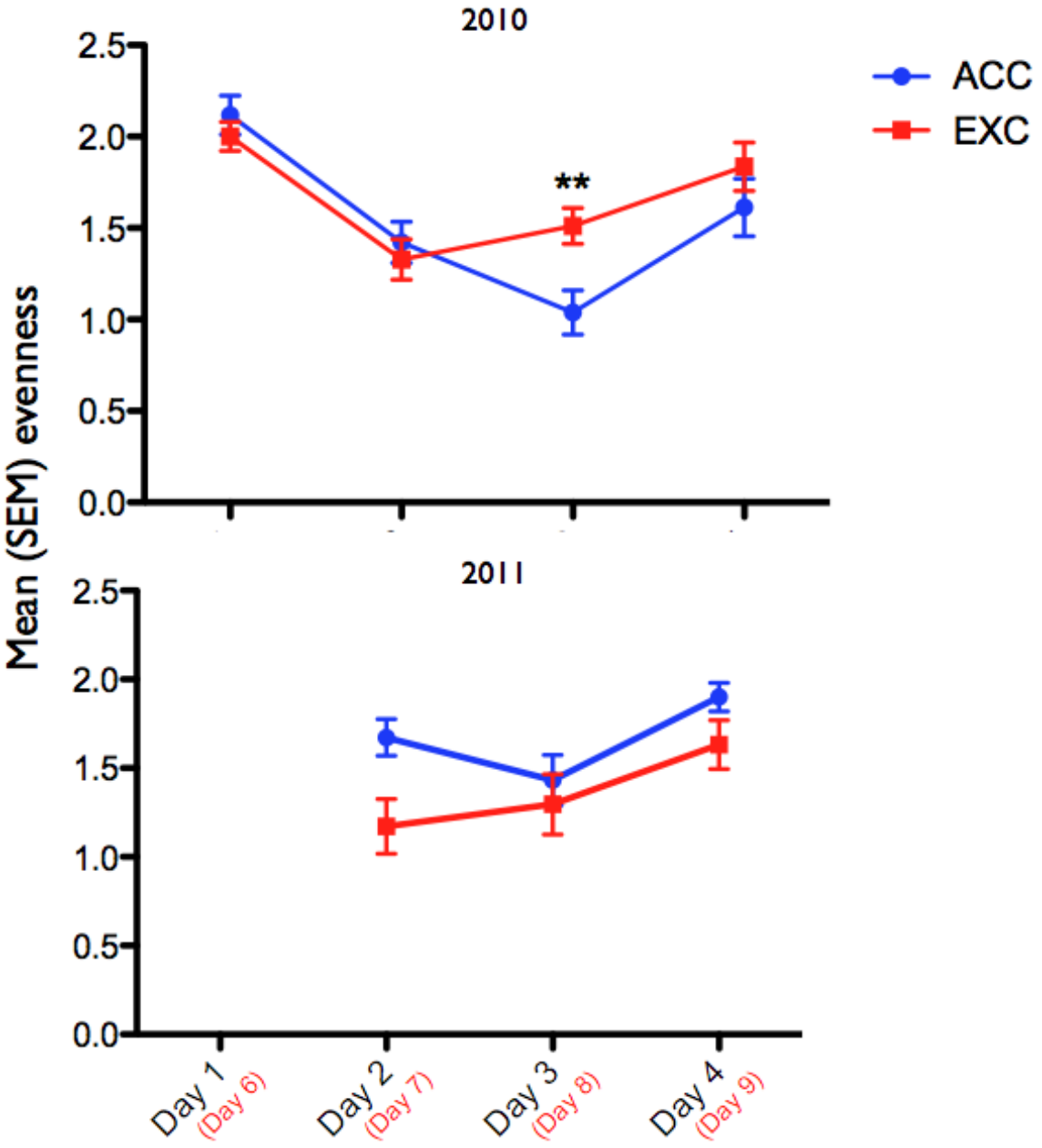

FIG. 11. Insect evenness during decomposition for carcasses with insects present (ACC) and insects excluded for 5 d (EXC). Adult insects were collected using glue traps. Each sampling day is representative of insects having access to the carcass. However, due to the insect exclusion for 5 d in EXC carcass the days in parentheses represent how long the carcasses have been in the field.

TABLE 4. RM-ANOVA results testing insect community metrics (Shannon-Weaver diversity, Simpson's diversity, richness, and evenness) between 2010 and 2011 (Year) and decomposition days (Day).

Ecological metric	Factor	*F* test	df	*P* value
Shannon-Weaver diversity	Day	6.95	7	<0.0001
	Year	0.01	1	0.9418
	Day x Year	3.56	7	0.0015
Simpson's diversity	Day	5.94	7	<0.0001
	Year	0.24	1	0.6238
	Day x Year	4.51	7	0.0001
Richness	Day	14.65	7	<0.0001
	Year	2.71	1	0.1021
	Day x Year	3.03	7	0.0053
Evenness	Day	9.63	7	<0.0001
	Year	0.47	1	0.4963
	Day x Year	5.40	7	<0.0001

TABLE 5. RM-ANOVA results testing insect community metrics (Shannon-Weaver diversity, Simpson's diversity, richness and evenness) between insect exclusion and insect access carcasses (Treatment) over decomposition days (Day).

Year	Ecological metric	Factor	*F* test	df	*P* value
2010	Shannon-Weaver diversity	Day	7.12	3	0.0003
		Treatment	11.45	1	0.0012
		Day x Treatment	0.25	3	0.8645
	Simpson's diversity	Day	7.91	3	0.0001
		Treatment	0.48	1	0.4893
		Day x Treatment	1.92	3	0.1331
	Richness	Day	14.46	3	<0.0001
		Treatment	38.09	1	<0.0001
		Day x Treatment	5.47	3	0.0020
	Evenness	Day	19.81	3	<0.0001
		Treatment	0.93	1	0.3382
		Day x Treatment	2.03	3	0.1175
2011	Shannon-Weaver diversity	Day	2.53	3	0.8573
		Treatment	0.01	1	0.9416
		Day x Treatment	0.26	3	0.8573
	Simpson's diversity	Day	10.05	3	<0.0001
		Treatment	1.58	1	0.2123
		Day x Treatment	1.18	3	0.3237
	Richness	Day	4.25	3	0.0082
		Treatment	10.93	1	0.0015
		Day x Treatment	3.04	3	0.0346
	Evenness	Day	4.70	3	0.0128
		Treatment	6.78	1	0.0116
		Day x Treatment	0.85	3	0.4322

and access carcasses and an interaction effect thus making the results difficult to interpret.

A two-axis NMDS ordination explained 89.4% of the variation in carcass insect community (FIG. 12, TABLE 6). There were significant differences between insect exclusion and insect access carcass insect community (MRPP: T = -9.38, P = <0.0001), among days (MRPP: T = -19.53 P = <0.0001) and among carcasses (MRPP: T = -3.41, P = 0.0031) (TABLE 7), thus demonstrating significant insect community change throughout decomposition. *Phormia regina, C. macellaria*, flesh flies (Diptera: Sarcophagidae), *Prochyliza* sp. (Walker) (Diptera: Piophilidae), *Sepsia* spp., and histerid beetles were significant indicator taxa for carcasses excluded from insect access. While *L. coeruleiviridis* was a significant indicator taxon for insect access carcasses. Over decomposition days, carcasses excluded from insects had *Cynomya cadaverina* (Robineau-Desvoidy) (Diptera: Calliphoridae) as a significant indicator taxon of the second day of post-exclusion insect access, which was the active decay stage. *Phormia regina*, *C. macellaria*, and flesh flies were indicator taxa of the third day of post-exclusion insect access with *Piophila casei* (L.) (Diptera: Piophilidae), *Prochyliza* sp., and *Sepsia* spp. as significant indicators of the dry stage, which occurred on the fourth day of decomposition (TABLE 9). Insect access carcasses had no significant indicator taxa throughout decomposition.

2011 field season

There were no significant differences in Shannon-Weaver diversity of insect communities between insect access and insect exclusion carcasses and over decomposition time with no significant interaction (FIG. 8, TABLE 5). Simpson's diversity had significant differences over decomposition time and no significant differences between insect access and insect exclusion carcasses and no interaction based on insect communities (FIG. 9, TABLE 5). The evenness (FIG. 10, TABLE 5) of insect communities was significantly different over decomposition day and between insect exclusion and insect access carcasses with no interaction. Richness was the

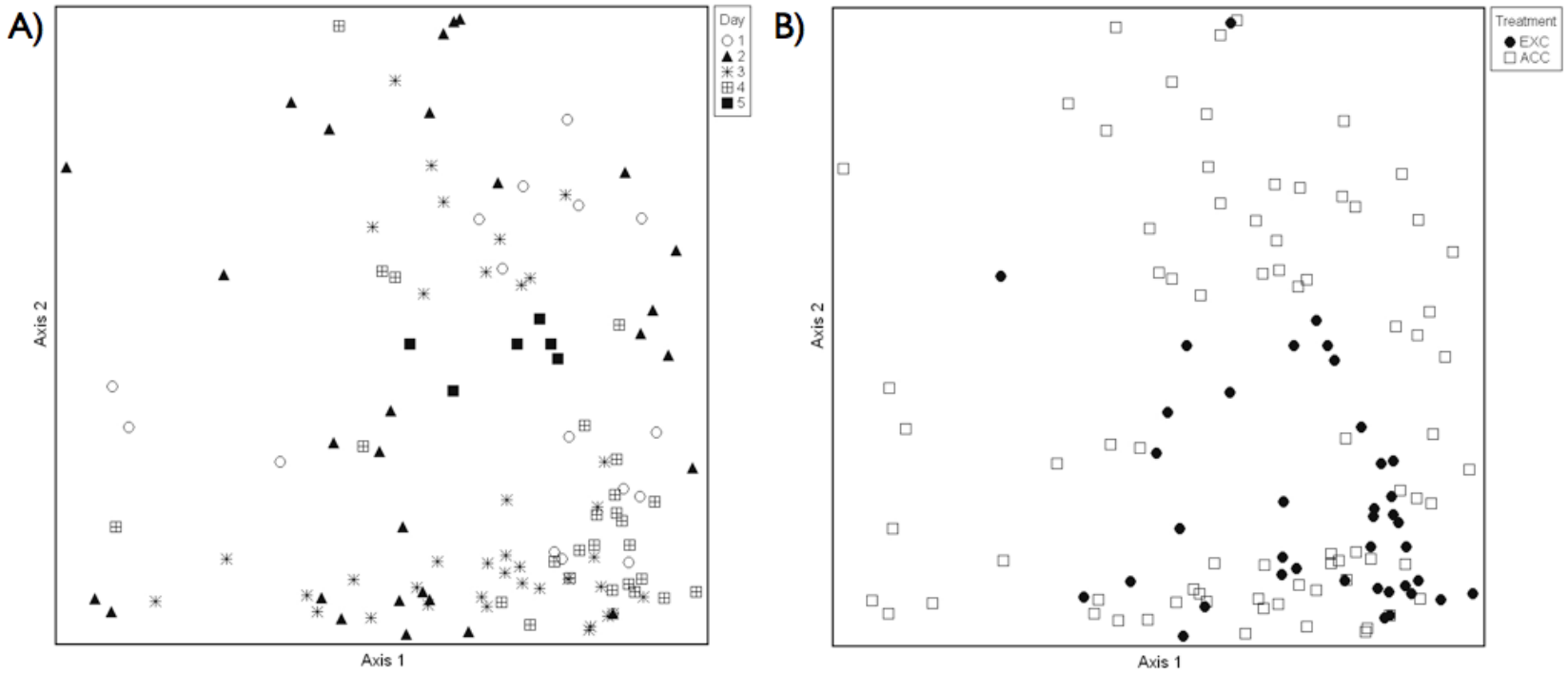

FIG. 12. NMDS ordination of A) insect communities from 2010 with sampling day overlay. Total stress was 13.39 and B) insect communities from 2010 with treatment (EXC and ACC) overlay. Axis 1 explained 22.8% of the variation among communities, while axis 2 explained 48.3% and axis 3 explained 18.3% for a total of 89.4% of the variation explained by this ordination.

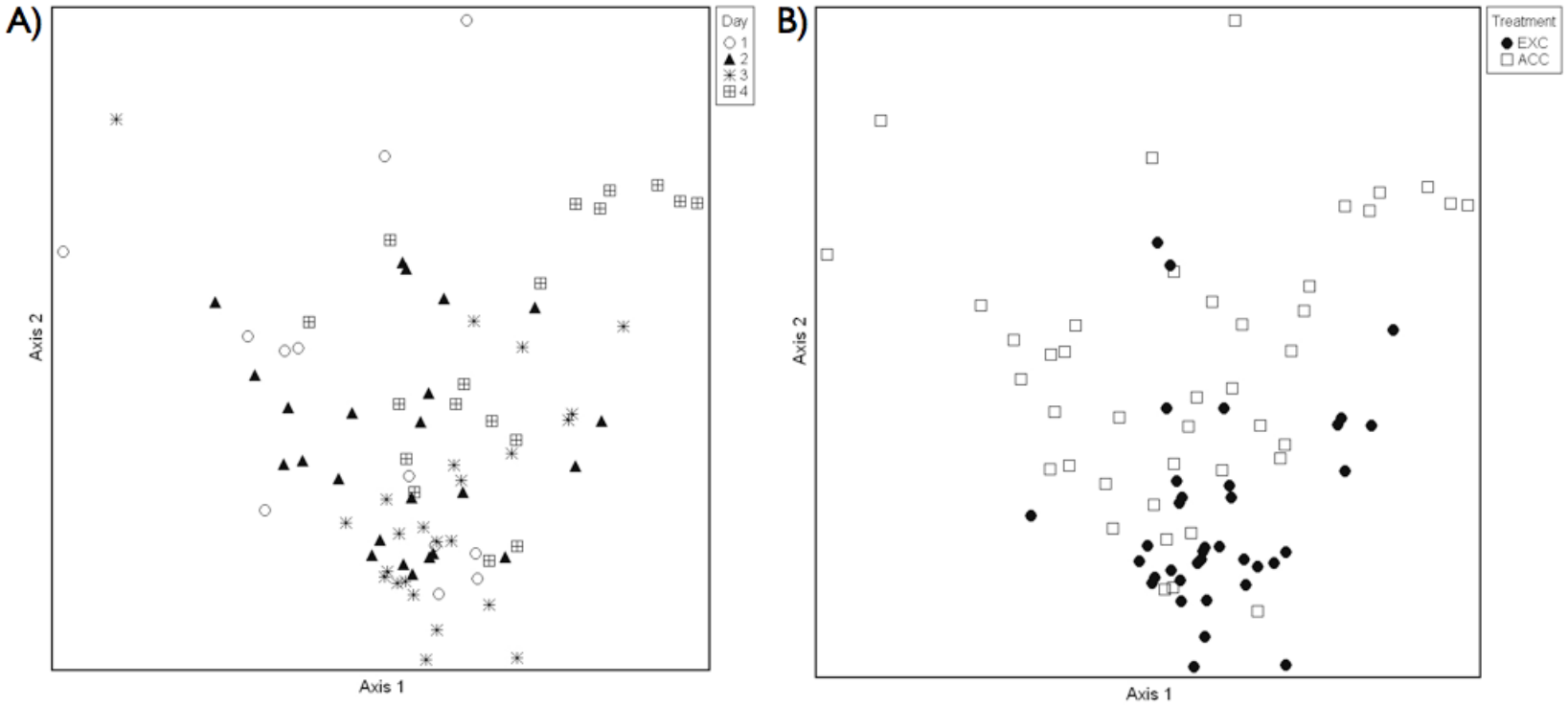

FIG. 13. NMDS ordination of A) insect communities from 2011 with sampling day overlay and B) insect communities from 2011 with treatment (EXC and ACC) overlay. Total stress was 12.38. Axis 1 explained 33.7% of the variation among communities, while axis 2 explained 33.5% and axis 3 explained 24.5% for a total of 91.7% the variation explained by this ordination.

TABLE 6. The stress and percent variation explained (total and by each axis) as determined by NMDS for insect communities during 2010 and 2011.

			Percent variation explained		
Year	Stress	Total	Axis 1	Axis 2	Axis 3
2010	13.39	89.4	22.8	48.3	18.3
2011	12.83	91.7	33.7	33.5	24.5

TABLE 7. Summary statistics for MRPP between 2010 insect communities of ACC and EXC carcasses, across decomposition day, and among carcass replicates. All pair-wise comparisons were significantly different at $\alpha = 0.0056$ (day) and $\alpha = 0.0063$ (carcass) after Bonferroni correction and are indicated with an asterisk (*).

	δ under null hypothesis						
	Observed δ	Expected	Variance	Skewness	*T*	*p*	*A*
EXC vs. ACC	0.656	0.675	0.40E-05	-1.43	-9.38	<0.0001	0.028
Day	0.631	0.675	0.17E-04	-0.70	-10.66	<0.0001	0.065
1 vs. 3					-2.45	0.0287	0.017
1 vs. 4					-2.75	0.0215	0.024
1 vs. 5					-5.71	0.0002*	0.085
2 vs. 3					-4.82	0.0020*	0.027
2 vs. 4					-10.64	<0.0001*	0.068
2 vs. 5					-6.45	0.0001*	0.069
3 vs. 4					-5.24	0.0010*	0.031
3 vs. 5					-5.12	0.0016*	0.057
4 vs. 5					-8.72	<0.0001*	0.104

TABLE 7. Continued

	δ under null hypothesis						
	Observed δ	Expected	Variance	Skewness	*T*	*p*	*A*
Carcass	0.660	0.675	0.21E-04	-0.63	-3.41	0.0031	0.023
B vs. A					-2.49	0.0242	0.024
B vs. C					-2.36	0.0299	0.024
B vs. D					-2.39	0.0282	0.024
E vs. A					-2.72	0.0190	0.024
E vs. C					-3.58	0.0067	0.034
E vs. D					-2.14	0.0392	0.021
F vs. A					-2.54	0.0240	0.021
F vs. C					-3.87	0.0044*	0.034

TABLE 8. Summary statistics for MRPP between 2011 insect communities of ACC and EXC carcasses, across decomposition day, and among carcass replicates. All pair-wise comparisons were significantly different at $\alpha = 0.01$ (day) and $\alpha = 0.0056$ (carcass) after Bonferroni correction, and are indicated with an asterisk (*).

	δ under null hypothesis						
	Observed δ	Expected	Variance	Skewness	*T*	*p*	*A*
EXC vs. ACC	0.577	0.631	0.85E-05	-1.53	-18.55	<0.0001	0.086
Day	0.573	0. 631	0.26E-04	-0.86	-11.36	<0.0001	0.092
1 vs. 3					-5.26	0.0007*	0.047
1 vs. 4					-8.30	<0.0001*	0.099
2 vs. 3					-4.14	0.0050*	0.035
2 vs. 4					-9.88	<0.0001*	0.098
3 vs. 4					-9.85	<0.0001*	0.094

TABLE 8. Continued

		δ under null hypothesis					
	Observed δ	Expected	Variance	Skewness	*T*	*p*	*A*
Carcass	0.585	0. 631	0.45E-04	-0.66	-6.94	<0.0001	0.074
G vs. I					-5.34	0.0004*	0.071
G vs. J					-5.72	0.0004*	0.079
G vs. K					-7.79	<0.0001*	0.106
H vs. I					-4.80	0.0012*	0.064
H vs. J					-5.05	0.0012*	0.070
H vs. K					-6.18	0.0002*	0.084
L vs. I					-5.20	0.0010*	0.076
L vs. J					-5.08	0.0015*	0.080
L vs. K					-6.36	0.0003*	0.099

TABLE 9. Results from ISA for 2010 insect communities. Insect taxon is given along with the indicator value and p value for the respective group. All pair-wise corrections that are significantly different using $\alpha = 0.0071$, $\alpha = 0.0063$, and $\alpha = 0.0167$ after Bonferroni correction for multiple pair-wise comparisons of treatment (EXC and ACC), day and carcass, respectively, are indicated with an asterisk (*).

Group	Insect Taxon	Indicator Value	Mean	Std. Dev.	*p*
ACC	*Piophila casei*	25.6	13.0	3.11	0.0040*
EXC	*Phormia regina*	63.9	33.8	3.48	0.0002*
EXC	Sarcophagidae	46.1	16.3	3.24	0.0002*
EXC	*Cochliomyia macellaria*	39.8	20.7	3.39	0.0004*
EXC	*Ophyra* spp.	39.6	29.2	3.55	0.0120
EXC	*Prochyliza* spp.	36.1	25.4	3.62	0.0120
EXC	*Psychoda* spp.	13.3	6.1	2.22	0.0096
Day 3	*Cynomya cadaverina*	18.3	9.2	4.99	0.0498
Day 4	*Phormia regina*	32.3	19.1	4.38	0.0176
Day 4	*Cochliomyia macellaria*	29.6	14.0	4.95	0.0156
Day 4	Sarcophagidae	23.6	12.0	4.85	0.0324
Day 5	*Sepsia* spp.	55.5	15.9	5.24	0.0004*
Day 5	*Prochyliza* spp.	45.2	16.2	5.13	0.0020*
Day 5	Drosophilidae	45.2	17.5	4.81	0.0010*
Day 5	Staphylinidae	30.4	18.1	4.81	0.0294
Carcass C	*Phormia regina*	25.0	16.1	2.93	0.0094*
Carcass D	*Psychoda* spp.	17.6	6.7	3.63	0.0172
Carcass F	*Piophila casei*	16.9	9.4	3.60	0.0420

TABLE 10. Results from ISA for 2011 insect communities. Insect taxon is given along with the indicator value and p value for the respective group. All pair-wise corrections that are significantly different using $\alpha = 0.0071$, $\alpha = 0.005$, and $\alpha = 0.0167$ after Bonferroni correction for multiple pair-wise comparisons of treatment (EXC and ACC), day and carcass, respectively, are indicated with an asterisk (*).

Group	Insect Taxon	Indicator Value	Mean	Std. Dev.	*p*
ACC	*Lucilia coeruliviridis*	56.3	35.0	4.40	0.0004*
EXC	*Phormia regina*	68.5	43.8	3.25	0.0002*
EXC	*Cochliomyia macellaria*	52.6	24.1	4.01	0.0002*
EXC	Vespidae	27.4	11.7	3.33	0.0018*
EXC	*Ophyra* spp.	25.5	16.8	3.84	0.0292
EXC	Chalcidonidae	13.6	7.8	2.80	0.0442
EXC	*Calliphora vicina*	11.1	5.2	2.23	0.0366
Day 1	Nitidulidae	20.2	9.7	4.86	0.0372
Day 1	Tachinidae	16.9	9.1	4.23	0.0538
Day 2	*Lucilia coeruliviridis*	35.5	22.7	4.32	0.0098
Day 3	*Phormia regina*	33.6	25.3	2.72	0.0070
Day 4	*Sepsia* spp.	65.9	18.2	4.39	0.0002*
Day 4	Histeridae	51.8	13.2	4.69	0.0002*
Day 4	*Prochyliza* spp.	33.8	14.2	4.29	0.0028*
Day 4	*Piophila casei*	29.3	13.6	4.36	0.0068
Day 4	Staphylinidae	27.5	19.0	4.17	0.0420
Day 4	*Creophilus maxillosus*	22.1	8.9	3.90	0.0110
Carcass I	Vespidae	19.8	9.7	4.06	0.0262
Carcass K	*Phormia regina*	24.6	18.6	2.04	0.0022*
Carcass L	*Merope tuber*	21.4	6.4	4.38	0.0226*

only metric that had significant differences over decomposition days, between exclusion and access carcasses (FIG. 11, TABLE 5) and an interaction effect thus making the results difficult to interpret.

A three-axis NMDS ordination explained 91.7% of the variation in insect communities (FIG. 13, TABLE 6). There were significant differences between insect exclusion and insect access carcass community insect communities (MRPP: T = -18.55, P = <0.0001), among days (MRPP: T = -18.69 P = <0.0001) and among carcasses (MRPP: T = -6.94, P = <0.0001) (TABLE 8). Thus demonstrating significant insect community change throughout decomposition. *Phormia regina* and *C. macellaria* were significant indicator taxa for carcasses excluded from insect access while *L. coeruleiviridis* was a significant indicator taxon for insect access carcasses. Over decomposition day, *P. regina* was the indicator taxon of the second day of decomposition, which was the active decay stage, for EXC carcasses. *Ophyra* spp. was a significant indicator of the third day of decomposition as active decay was transitioning into advanced decay, and histerid beetles were a significant indicator taxon of the last day of decomposition as the carcasses entered the dry stage. Insect access carcasses had different indicator taxa throughout decomposition. *Lucilia coeruleiviridis* was the indicator taxa of the second day of decomposition as the carcasses were beginning active decay. *Piophila casei*, *Prochyliza* sp., and *Sepsia* sp. were significant indicators of the dry stage (TABLE 10), which occurred on the fourth day of decomposition.

Discussion

My results demonstrate consistency in arrival patterns of blow flies initially arriving to the resource followed by secondary colonizers such as rove beetles and black scavenger flies; even after delayed colonization, arrival sequence of blow flies followed by beetle and lesser flies. These succession data are beneficial towards understanding interactions of organisms utilizing carrion, which will build upon the basic knowledge about behaviors and species interactions.

Variability of insect succession patterns and community composition over years in the same habitat occurred as well as between treatments. Based on the ecological metrics (i.e., Shannon-Weaver diversity, Simpson's diversity, richness and evenness) there were inconsistent patterns between years. The variation occurring between years could be attributed to differences in resource size (Braack 1987) or priority effects of initial colonizers altering subsequent community structure (Chase 2010, Fukami and Nakajima 2011). The exclusion carcasses attracted a different insect community once they were exposed to insects. Succession patterns are governed by various environmental factors and biodiversity results from spatial and temporal heterogeneity (Grinnell 1917, Elton 1927, Hutchinson 1961, Leibold 1995). Alternatively, succession can be governed by immigration and extinction rates of species within a habitat (Hubbell 2001). There has been a documentation of variation occurring during colonization of vertebrate resources in similar habitats. Asynchronous blow fly succession have been documented on alligator *Alligator mississippiensis* Daudin (Crocodilia: Alligatoridae) carcasses, with *L. coeruleivirdis* arriving during early stages of decomposition and *Chrysomya* spp., *C. macellaria*, and *P. regina* arriving at later stages of decomposition, which suggests niche theory is driving colonization patterns (Nelder et al. 2009). Clearly, there is an overlap in which ecological theory (i.e., niche vs. neutral) explaining how insect species carrion with neither fully explaining the interactions occurring on carrion.

Arthropod community structure on carrion over time has often been described as a process of competition between species for a resource (Norris 1965, Hanski and Kuusela 1977). Competition for ephemeral resources between micro- and macroorganisms is well documented. *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) and fungi (*Aspergillus* spp.) interact on fruit, with larval mortality correlating with the age and species of fungi present on the resource (Trienens et al. 2010). Microbe and insect interactions have been documented in other habitats with varying results on organic matter processing. Specifically, leaf litter decomposition studies have reported substantial variation in the influence of substrate type and microbial and insect communities on decomposition rates. For example, microbial and

insect communities have been shown to facilitate nutrient cycling and decomposition rates in leaf litter systems (Hieber and Gessner 2002, Srivastava et al. 2009). Alternatively, other studies have demonstrated that leaf litter composition drives decomposition rates with little or no influence of microbial and insect communities (Kominoski et al. 2011). However, the influence of microbes in succession patterns has been greatly overlooked, and species interactions between microbes and blow flies throughout decomposition has only yet to be thoroughly analyzed in carrion systems using field trials.

Changes in decomposition rates is more apparent under the influence of insects associated with carrion, specifically the necrophagous insect community that utilize carrion as a food source (Denno and Cothran 1975). Microbial communities associated with the carcasses could be altering the quality of the resource and thus mediating insect community assembly as demonstrated with fungi governing phytophagous insect composition in a plant-based system (Tack et al. 2012). As insects consume the resource, many substrates are made available for utilization by microbes, which then can produce volatile organic compounds (VOCs) (Boumba et al. 2008, Boumba et al. 2012). Biogenic amines (e.g., cadaverine and putrescine) are commonly associated decomposition and correspond to microbial growth on vertebrate carcasses; however, these compounds have a low volatility and have not been detected in either human or swine carrion decomposition (Statheropoulos et al. 2005, Dekeirsschieter et al. 2009, Paczkowski and Schütz 2011). VOCs have been proposed as the governing mechanisms of necrophagous insect colonization patterns on carrion with species-specific cues from the resource dictating successional patterns. (Dekeirsschieter et al. 2009). Blow fly oviposition can be induced by bacteria and their associated semiochemicals; specifically, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) females have a preference for five out of eight Enterobacteriaceae volatiles, such as *Proteus* spp. in oviposition assays (Chaudhury et al. 2010), and gravid females 10-12 d old are attracted to *Providencia* sp. (Hammack et al. 1987). Therefore, the blow fly species that secondarily colonize remains may be responding to cues released by the bacteria that subsequently

stimulate oviposition on the resource (Ashworth and Wall 1994). However, there is a upper threshold in the usability of a resource once microbes are allowed to proliferate and microbial laden carcasses actually deterred scavenging (Burkepile et al. 2006).

During my study, I documented a retarded decomposition rate of the carcasses that were not exposed to insect access with these carcasses remaining in the bloat stage of decomposition until the insects were allowed to access the remains. Thus, further considerations should be taken when remains are found excluded from insects as the stages of decomposition (e.g., bloat) may last longer over time than if there were insects present utilizing the remains. This work provides a foundation for more accurate estimations of how long a resource has been decomposing, which directly translates to improving forensics estimations of how long a body has been exposed for colonization by arthropods, or the period of insect activity that is often similar to the postmortem interval in forensic investigations.

CHAPTER III

CHANGES IN CARRION BACTERIAL COMMUNITIES THROUGHOUT DECOMPOSITION CHARACTERIZED BY 16S rRNA AMPLICON PYROSEQUENCING

Introduction

Carrion represents an ecological unit within a larger ecosystem (Odum 1969). Decomposing remains such as deep-sea whale carcasses (Klages et al. 2001, Burkepile et al. 2006) or anadromous salmon, *Oncorhynchus* spp. (Salmoniformes: Salmonidae), in Pacific watersheds (Hocking and Reimchen 2006, Janetski et al. 2009) can be a primary resource subsidy for certain ecosystems. Decomposition results in a nutrient surge to the immediate soil, insect, and plant communities associated with the site (Towne 2000, Yang 2006). Carrion decomposition introduces essential molecules such as nitrogen, potassium, calcium and magnesium back into the ecosystem (Carter et al. 2007). Recycling of carrion nutrients and energy is often facilitated by insect communities (Putman 1978b, Parmenter and Lamarra 1991, Carter et al. 2007) and scavenging vertebrates (Parmenter and MacMahon 2009, Wilson and Wolkovich 2011). However, most studies have only focused on the role of necrophagous arthropods during decomposition.

Microbial assemblages are important for many ecosystem processes (Hattenschwiler et al. 2005, Parmenter and MacMahon 2009, Nemergut et al. 2010). Approximately 90% of all decomposition in terrestrial systems is a result of microbial (i.e., bacteria and fungi) activity (Swift et al. 1979). Bacterial community composition may mediate biotic interactions throughout decomposition of carrion, yet there remains a paucity of data related to bacterial communities associated with carrion. Bacterial communities have previously been assumed to be selected by "filters" or environmental conditions, which in turn regulates bacterial composition (Baas Becking 1934). However, no two naturally occurring communities appear to be the same (Curtis

et al. 2002). Therefore other biotic interactions, such as competition with necrophagous insects and vertebrate scavengers, could be important to community assembly and succession (Hooper et al. 2005). Many studies have analyzed microbial assemblages in terrestrial (e.g., soil and leaf litter) (Bell et al. 2009, Redford and Fierer 2009) and aquatic habitats (Jones and McMahon 2009, Burke et al. 2011, Kominoski et al. 2011). A positive relationship between ecosystem function and biodiversity (e.g., species richness) has been demonstrated (Jousset et al. 2011). Despite the importance of carrion in ecosystem processes, there are few researchers examining the mechanisms, driving forces, and impact of carrion within terrestrial ecosystems.

Empirical data describing bacterial community dynamics on carrion are currently sparse even though their role in trophic level interactions and food webs is well appreciated (Vass 2001, Zak et al. 2003, Chung et al. 2007, Rohlfs 2008, Strickland et al. 2009). Microbial species richness has been suggested to predict soil decomposition (McGuire and Treseder 2010) with approximately 70% of models including microbial communities (Manzoni and Porporato 2009). Aggregation models have been proposed to describe how competitors (e.g., blow flies and bacteria) coexist on ephemeral resources (Woodcock et al. 2002). Aggregation models of coexistence state that several species can utilize the same resource simultaneously, and studies performed using fruit-breeding Diptera on decomposing organic matter indicate that a single species may never completely exclude another species (Shorrocks et al. 1979, Atkinson and Shorrocks 1984). This demonstration of coexistence may result from groupings of individual species consuming one resource patch and not other patches (Hartley and Shorrocks 2002, Abos et al. 2006).

From the limited number of studies available it is evident that bacterial communities are incredibly diverse in the environment (Pace 1997, Green and Bohannan 2006); however, methodological limitations have previously made it difficult to describe microbial succession, dispersal, and species interactions. These limitations result from limited sampling efforts and technological ability to identify non-culturable bacterial species from the environment (Schmeisser et al. 2007), which

are estimated to make up > 99% of the bacterial species (Pace 1997, Green and Bohannan 2006). Pyrosequencing is a high-throughput sequencing technology based on the sequence-by-synthesis theory (Ronaghi et al. 1998). 454-pyrosequencing had detected bacteria in habitats ranging from human gut flora (Turnbaugh et al. 2010) to deep mine microbial communities (Edwards et al. 2006). Based on the microbial fauna identified by high-throughput sequencing, humans maintain a high level of species diversity and richness (Mahowald et al. 2009, Petrosino et al. 2009, Price et al. 2009, The Human Microbiome Jumpstart Reference Strains Consortium 2010, Turnbaugh et al. 2010). For instance, 454 sequencing of microbial samples collected from 27 body regions produced 4,949 species level phylotypes out of a total of 250,000 16S rRNA sequences, thus less than 2% of the sequences had been previously characterized (Turnbaugh et al. 2010). Utilizing 454-pyrosequencing will allow, for the first time ever, the characterization of bacterial species composition, community change, and interactions that occur during decomposition.

The goal of this study was to analyze bacterial community succession during decomposition of vertebrate carrion using 454-pyrosequencing. To better understand variation and assembly patterns of bacterial communities on carrion I asked two questions 1) what bacteria are associated with carrion throughout decomposition and 2) how does the presence and absence of primary insect colonizers (i.e., blow flies and beetles) influence bacterial communities during decomposition? Here I tested the null hypothesis that bacterial community composition does not change over time or in the presence/absence of primary colonizers on decomposing ephemeral resources.

Methods

Site description and experimental design

The microbial ecology of swine carcass decomposition was studied in a Midwestern temperate forest habitat surrounded by agricultural fields in Xenia, Ohio, USA (39°38'14.83"N, 84°1'37.82"W) during August 2010. The dominant tree fauna consisted of oaks (*Quercus* spp.) and maples (*Acer* spp.). The 95% canopy cover was

relatively homogenous over all carcasses. Six male swine ranging from 10.4 - 30.1 kg (TABLE 1), euthanized by cranial blunt force at approximately 16:30 h, were purchased from a local farm on 5 August 2010. Carcasses were double bagged, transported for approximately 1 h, and randomly placed minimally 20 m apart along three transects (FIG. 1) two hours before U.S. National Oceanic and Atmospheric Administration (NOAA) defined sunset at approximately 19:00 h on 5 August.

All carcasses were oriented with heads to cardinal north and the dorsal side of the carcass towards the east. Carcasses were labeled alphabetically "A" through "F". Three random carcasses ("A", "C", and "D") were individually enclosed in 1.8 m^3 Lumite® screen (18 x 14 mesh size) portable field cages (BioQuip Products, Rancho Dominguez, CA, USA) to reduce insect access and were considered the exclusion treatment (EXC), while the remaining three carcasses ("B", "E", and "F") were allowed insect access
(ACC); all carcasses were covered with anti-scavenging cages (0.9 x 0.6 x 0.6 m) constructed of wooden frames enclosed with poultry netting (FIG. 2).

Trapper® max glue traps (16.5 x 11 cm) (Bell Laboratories, Inc., Madison WI, USA) were used as a passive trapping method to collect adult insects arriving to each carcass. A single glue trap was located approximately 0.15 m from the anterior and posterior region of each carcass (FIG. 3). Glue traps were replaced every 12 h. Insects were identified to the lowest taxonomic level while remaining on the glue trap (Triplehorn and Johnson 2005, Whitworth 2006). NexSens DS1923 micro-T temperatures loggers (Fondriest Environmental, Inc., Alpha, OH, USA) were placed within 0.6 m of each carcass approximately 0.3 m above the ground and temperature recorded every 15 min. Temperature data were later converted into accumulated degree hours (ADH), which accounts for variation in temperature over decomposition time (Megyesi et al. 2005).

Bacteria sampling protocol

Bacterial communities of all carcasses were sampled immediately, 1, 3, and 5 d after carcass placement for a total of four sampling points. Sterile cotton applicators (Fischer Healthcare, Houston, TX, USA) were used to sample bacterial communities from two regions on each carcass for 60 s: the buccal cavity (the top area of the mouth and under the tongue) and the skin, which consisted of combining three areas (approximately 2.54 cm x 15.24 cm) along a single transect of the carcasses, while talking caution not to sample the same areas throughout decomposition. Samples were stored at 4^{o}C until further processing.

DNA extraction

DNA extraction took place within 24 h of the samples being collected. I used a modified chloroform-phenol extraction method from Current Protocols in Molecular Biology and all extractions were performed under a fume hood. The following was added to each sample: 567 µl of TE Buffer pH 8.0 (Applied Biosystems/Ambion, Austin, TX, USA), 30 µl Caliber™ SDS 10% solution (Hoefer, Inc., San Franscisco, CA, USA), 10 µl lysozyme (15 mg/ml) (Lot No.57H7045) (Sigma Chemical Co., St. Louis, MO, USA), and 3 µl proteinase K from *Tritirachium album* (20 mg/ml) (Fischer Scientific, Fair Lawn, NJ, USA). Samples were homogenized thoroughly by hand and shaker-incubated at 56^{o}C for 1 h at 900 rmp on a LabNet VorTemp 56™ incubator/shaker (LabNet International, Inc., Woodbridge, NJ, USA). The aqueous component of each sample was transferred to a new, sterile 1.5 ml microcentrifuge tube. Each sample had 4 µl of Ribonuclease A (100 mg/ml) (Lot No. 086607) (Fischer Scientific, Fair Lawn, NJ, USA) and was allowed to incubate at room temperature for 2 min. 100 µl of 5 M Sigma NaCl (Sigma Chemical Co., St. Louis, MO, USA) and 80 µl of CTAB/NaCl solution consisting of Cetyl Trimethyl Ammonium Bromide Extraction Solution (Teknova, Inc., Holister, CA, USA), EDTA pH 8.0, NaCl and double distilled water was added. Each sample was thoroughly mixed by repeated hand inversions and incubated for 10 min at 65^{o}C on a Multi-block Heater (Lab-line Instruments, Inc.,

Melrose Park, IL, USA). Each sample then had 780 µl of chloroform/isoamyl alcohol (24:1) (Lot: A0264565) (Acros Organics, NJ, USA) added; each sample was thoroughly mixed by repeated hand inversions and centrifuged for 6 min at 4,000 x g at room temperature in an Fisher accuSpin Micro 17 (Fisher Scientific, Fair Lawn, NJ, USA). The supernatent was removed, with caution to leave the interface behind, and placed in a new, sterile 1.5 ml microcentrifuge tube. Equal volume (approximately 600 µl) of phenol/chloroform/isoamyl alcohol (25:24:1) (Lot: A0267233) (Acros Organics, NJ, USA) was added and samples were again mixed by repeated hand inversions and centrifuged for 6 min at 4,000 x g at room temperature. The supernatent was removed, with caution to leave the interface behind, and placed in a new, sterile 1.5 ml microcentrifuge tube. Approximately 0.6 volume (300 - 400 µl) of 100% Fisher Chemical 2-propanol (Lot No. 902218 A451-1) (Fisher Scientific, Fair Lawn, NJ, USA) was added to each tube and gently inverted by hand until a DNA precipitate began to form (~ 15 s). Samples were centrifuged for 6 min at 4,000 x g at room temperature and the supernatant carefully removed and the pellet retained. The DNA pellet was washed twice by 1 ml 70% EtOH (Aldrich® Chemical Co., Inc., Milwaukee, WI, USA) and centrifuging for 6 min at 4,000 x g at room temperature. The remaining EtOH was allowed to evaporate and the pellet was redissolved in 100 µl of RNase-free water (Qiagen Sciences, Maryland, USA) on a Roto-Shake Genie (Scientific Industries, Inc., Bohemia, NY, USA) at a speed of 4 for 16 - 18 h. Samples were stored at -20^{o}C until DNA quantification and quality assessment using a NanoDrop spectrophotometer (Nyxor Biotech, Paris, France).

Massive parallel bTEFAP

Bacterial community structure was determined by modified bacterial tagged encoded FLX amplicon pyrosequencing (bTEFAP). Each sample's extracted DNA (~100 ng) was sent to the Research and Testing Laboratory (Lubbock, TX). PCR amplification was performed using the primers for bacterial populations Gray28F (5'TTTGATCNTGGCTCAG) and Gray519r (5' GTNTTACNGCGGCKGCTG), as

previously described (Dowd et al. 2008b, Sen et al. 2009, Andreotti et al. 2011, Handl et al. 2011). Sequencing reactions utilized a Roche 454 FLX instrument with Titanium reagents and titanium procedures were employed to perform the tag-encoded FLX amplicon pyrosequencing analyses based on RTL protocols (www.researchandtesting.com). Initial generation of the sequencing library occurred with a one-step PCR, a mixture of Hot Start, HotStar high-fidelity *Taq* polymerases, and amplicons originating and extending from the 28F for bacterial diversity.

Pyrosequencing data analysis

Following sequencing, all failed sequence reads, low quality sequence ends, and tags and primers were removed and sequences' collections depleted of any non-bacterial ribosome sequences and chimeras using B2C2 (Gontcharova et al. 2010), as has been described previously (Dowd et al. 2008a, Li et al. 2009, Suchodolski et al. 2009, Wolcott et al. 2009, Ishak et al. 2011). Sequences were aligned, using the Ribosomal Database Project (RDP) under tool Aligner (accessed on January 13, 2012), based on the 16S rRNA secondary structure in Infernal aligner (Nawrocki and Eddy 2007, Nawrocki et al. 2009). Hierarchal classification of the 378,904 16S rRNA sequences were performed using Naïve Bayesian rRNA classifier version 2.2 in RDP (accessed on January 15, 2012) according to the Bergey's bacterial taxonomy (Garrity et al. 2004, Wang et al. 2007). Only sequences having ≥ 80% bootstrap support were considered classified at a given hierarchal level (phylum to genus), while sequences with either a bootstrap support of < 80% or that were not assigned were considered "unclassified." Bacterial taxa relative abundance was determined at each taxonomic hierarchal level. "Rare taxa" represented those with < 3% of total abundance. Richness was calculated based on Zak *et al*. (1994). Rarefaction curves were generated in Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) using data obtained from the tools aligner, complete linkage clustering, and rarefaction in RDP (accessed on January 17, 2012) at the level of 3% dissimilarity, approximately species level (Stackbrandt and Goebel 1994, Cole et al. 2009, Handl et al. 2011). Shannon-Weaver and Chao1

diversity indices were calculated at 3% dissimilarity from the complete linkage clustered files using the tool Shannon & Chao1 index in RDP (accessed on January 17, 2012) (Cole et al. 2009).

Bacterial community analysis

Bacterial community composition, as determined by operational taxonomic units (OTUs) richness was analyzed using linear regression over decomposition days. Changes in taxon richness was tested over decomposition time, insect presence/absence, and time-insect interaction using two-way repeated measures analysis of variance (RM-ANOVA) using Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). Nonmetric multidimensional scaling (NMDS) in PC-ORD 5 (MjM Software, Gleneden Beach, OR, USA) (McCune and Mefford 2006) was then used to analyze OTUs during decomposition when rare taxa were excluded. NMDS is a nonparametric ordination technique that avoids assuming linearity among community variables (McCune and Grace 2002). Multi-response permutation procedure (MRPP) was used for testing statistical differences between overlay groups of bacterial community composition within the ordination using methods described elsewhere (Biondini et al. 1985). Indicator species analysis (ISA) complemented MRPP by assigning significant indicator values to bacteria taxa that were indicative of community structure separation among treatments and over decomposition (McCune and Grace 2002). Indicator value determine which bacterial taxon best represents communities important to insect presence/absence and over decomposition based on OTUs, with 0 representing no indication and 100 being a perfect indication of each grouping.

To determine if decomposition time, based on accumulated degree hours (ADH), could be predicted based from bacterial community composition, I used a tiered statistical approach. Random forest models identify significant bacteria taxon predictors of ADH. The highest-ranking predictor variables from the random forest algorithm were then used to predict ADH using generalized additive models (GAMs). Random forest models were constructed using the randomForest 4.5-36 library in the R

statistical package (R Development Core Team 2010). Random forest modeling is a machine learning classification and regression tree that uses a deterministic algorithm to build trees based on splitting each node from a random subset of predictors randomly chosen and a bootstrap sample of the observations (Breiman 2001, Liaw and Wiener 2002). A random one-third of the data, the out-of-bag (OOB) data, was used to determine the accuracy of each tree based on the order and value of the predictor variables (i.e., bacteria genera). Each tree was constructed using only OOB data, and the overall prediction was calculated by averaging data from all trees. The mgcv library in the R statistical package was used to construct GAMs. This approach modeled ADH using covariates (important bacteria genera in the random forest models) and treatment by summing nonparametric covariates using a smoothing function (Hastie and Tibshirani 1990).

Results

A total of 378,904 sequences were obtained throughout carcass decomposition (TABLE 9). At immediate placement (D0) there was an average of 20,500 (± 3,480) and 17,289 (± 200) for EXC and ACC carcasses, respectively. After one day of decomposition (D1), an average of 11,268 (± 3,679) for EXC carcasses and 8,708 (± 1,657) for ACC carcasses. On the third decomposition day (D3) EXC carcasses had an average of 20,294 (± 2,242) sequences and ACC averaged 16,189 (± 6,074). Finally, on the last day of decomposition (D5) there was an average of 26,307 (± 1706) sequences for EXC while ACC carcasses had an average of 8,177 (± 2242) sequences.

Bacterial richness and diversity indices

I determined significant bacterial community composition differences based on bacterial genera richness between insect access and insect exclusion carcasses and over time with a significant interaction (TABLE 10). There was a 38 % (r^2 =0.42, F = 7.314, P = 0.0221) and 75% (r^2 =0.91, F = 106.7, P = <0.0001) reduction of bacteria taxa at the genus level associated with insect exclusion and insect access carcasses,

respectively, over decomposition (FIG. 14, TABLE 10). The Shannon diversity index at species, genus, and phylum level (3, 5, and 20% dissimilarity, respectively) was significantly different over day and decreased in diversity over time (TABLE 12). Similar trends were observed with the rarefaction index and Chao1 estimators (FIG. 15). However, there were treatment differences between insect access carcasses having less Chao1 diversity than carcasses excluded from insect access (TABLE 10).

Taxonomic distribution

Bacterial taxa relative abundance excluding rare taxa (< 3% relative abundance) at phylum level demonstrated unique trends and patterns of bacteria composition throughout decomposition (FIG. 16, TABLE 11). *Proteobacteria* was the dominant taxon for insect exclusion (62%) and access (70%) carcasses with *Firmicutes* being the next dominant taxon representing 33% and 20% of exclusion and access bacterial communities, respectively. *Proteobacteria* remained a dominant taxon on the first (35%), third (63%), and fifth (82%) sampling days for insect exclusion carcasses. While *Firmicutes* relative abundance decreased as decomposition progressed on the first (43%), third (28%), and fifth (16%) day. On the other hand, carcasses with insect access displayed an inversed pattern of bacterial taxa composition. *Proteobacteria* decreased over time and transitioned from a dominant taxon on the first (42%) and third (44%) day and then decreased to only 3% of taxa abundance on the fifth day. *Firmicutes* became the dominant taxon as decomposition of insect access carcasses progressed comprising of 36, 48, and 96% on the first, third and fifth sampling day, respectively.

TABLE 11. Number of observed sequences (mean ± SD), OTUs (mean ± SD), richness and diversity estimators (mean ± SD) that predicted number of species in each treatment over decomposition time at species, genera, and phylum level (3%, 5%, and 20% dissimilarity).

		% Dissimilarity	Number of reads	Number of OTUs	Shannon Diversity	Chao1 Diversity
Day 0	EXC	3	20,500 ± 3,480	1,383 ± 109	5.01 ± 0.62	1,892 ± 78
		5	20,500 ± 3,480	823 ± 88	4.34 ± 0.60	1,047 ± 144
		20	20,500 ± 3,480	83 ± 14	2.57 ± 0.49	88 ± 19
	ACC	3	17,289 ± 200	1,266 ± 82	4.87 ± 0.40	1,770 ± 160
		5	17,289 ± 200	778 ± 41	4.23 ± 0.36	1,005 ± 78
		20	17,289 ± 200	88 ± 19	2.48 ± 0.21	90 ± 21
Day 1	EXC	3	11,268 ± 3,679	1,085 ± 380	5.12 ± 0.42	1,606 ± 605
		5	11,268 ± 3,679	652 ± 223	4.47 ± 0.32	894 ± 290
		20	11,268 ± 3,679	67 ± 17	2.64 ± 0.29	69 ± 16
	ACC	3	8,780 ± 1,657	992 ± 278	5.19 ± 0.38	1,439 ± 451
		5	8,780 ± 1,657	606 ± 168	4.53 ± 0.41	809 ± 228
		20	8,780 ± 1,657	66 ± 6	2.83 ± 0.23	69 ± 6
Day 3	EXC	3	20,294 ± 2,242	1,156 ± 199	4.36 ± 0.76	1,644 ± 349
		5	20,294 ± 2,242	698 ± 108	3.69 ± 0.68	902 ± 137
		20	20,294 ± 2,242	71 ± 8	2.35 ± 0.48	75 ± 11
	ACC	3	16,189 ± 6,074	745 ± 208	4.48 ± 0.60	1,027 ± 356
		5	16,189 ± 6,074	414 ± 116	3.69 ± 0.68	529 ± 160
		20	16,189 ± 6,074	46 ± 10	1.87 ± 0.57	51 ± 21

TABLE 11. Continued

		% Dissimilarity	Number of reads	Number of OTUs	Shannon Diversity	Chao1 Diversity
Day 5	EXC	3	26,307 ± 1,706	680 ± 65	3.39 ± 0.34	906 ± 106
		5	26,307 ± 1,706	391 ± 56	2.87 ± 0.20	496 ± 88
		20	26,307 ± 1,706	43 ± 9	1.61 ± 0.53	46 ± 8
	ACC	3	8,177 ± 2,242	414 ± 199	4.25 ± 0.76	535 ± 349
		5	8,177 ± 2,242	221 ± 108	3.23 ± 0.68	281 ± 137
		20	8,177 ± 2,242	30 ± 3	1.74 ± 0.48	37 ± 11

TABLE 12. RM-ANOVA results testing mean bacterial genera taxa richness and diversity at species, genera, and phylum level (3%, 5%, and 20% dissimilarity) between insect exclusion and access carcasses (Treatment) over days of decomposition (Day).

Diversity Index	% Dissimilarity	Factor	*F* test	df	*P* value
Richness	-	Day	25.62	3	<0.0001
		Treatment	29.04	1	0.0057
		Day x Treatment	6.464	3	0.0075
Shannon Diversity	3	Day	7.867	3	0.0036
		Treatment	1.948	1	0.2353
		Day x Treatment	0.9954	3	0.4281
	5	Day	12.30	3	0.0006
		Treatment	0.2410	1	0.6492
		Day x Treatment	0.2286	3	0.8747
	20	Day	7.33	3	0.0047
		Treatment	0.0947	1	0.7737
		Day x Treatment	0.3606	3	0.7826
Chao1 Diversity	3	Day	10.17	3	0.0013
		Treatment	14.72	1	0.0185
		Day x Treatment	0.7023	3	0.5686
	5	Day	14.23	3	0.0003
		Treatment	11.72	1	0.0267
		Day x Treatment	1.170	3	0.3617
	20	Day	10.90	3	0.0010
		Treatment	0.5341	1	0.5054
		Day x Treatment	0.3708	3	0.7756

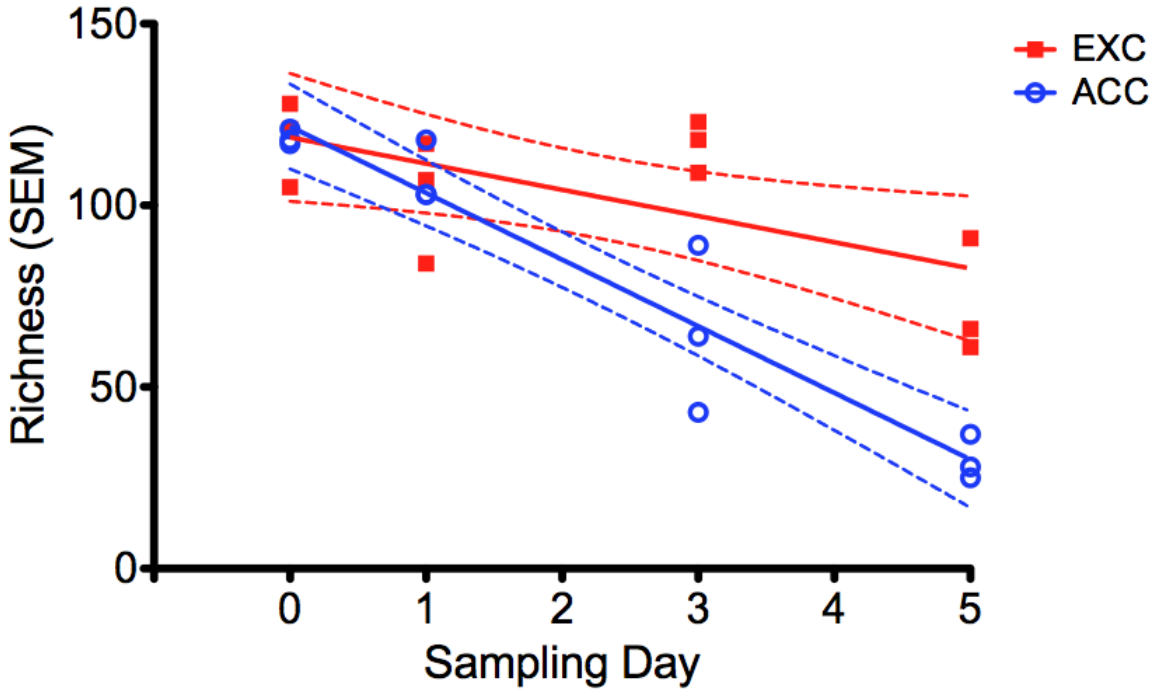

FIG. 14. Linear regression of the bacterial taxa richness at the genus level over decomposition time and between treatments (EXC and ACC) with 95% confidence intervals represented by the dotted bands. There is a 38% and 75% reduction of genera richness for EXC and ACC, respectively, over decomposition days.

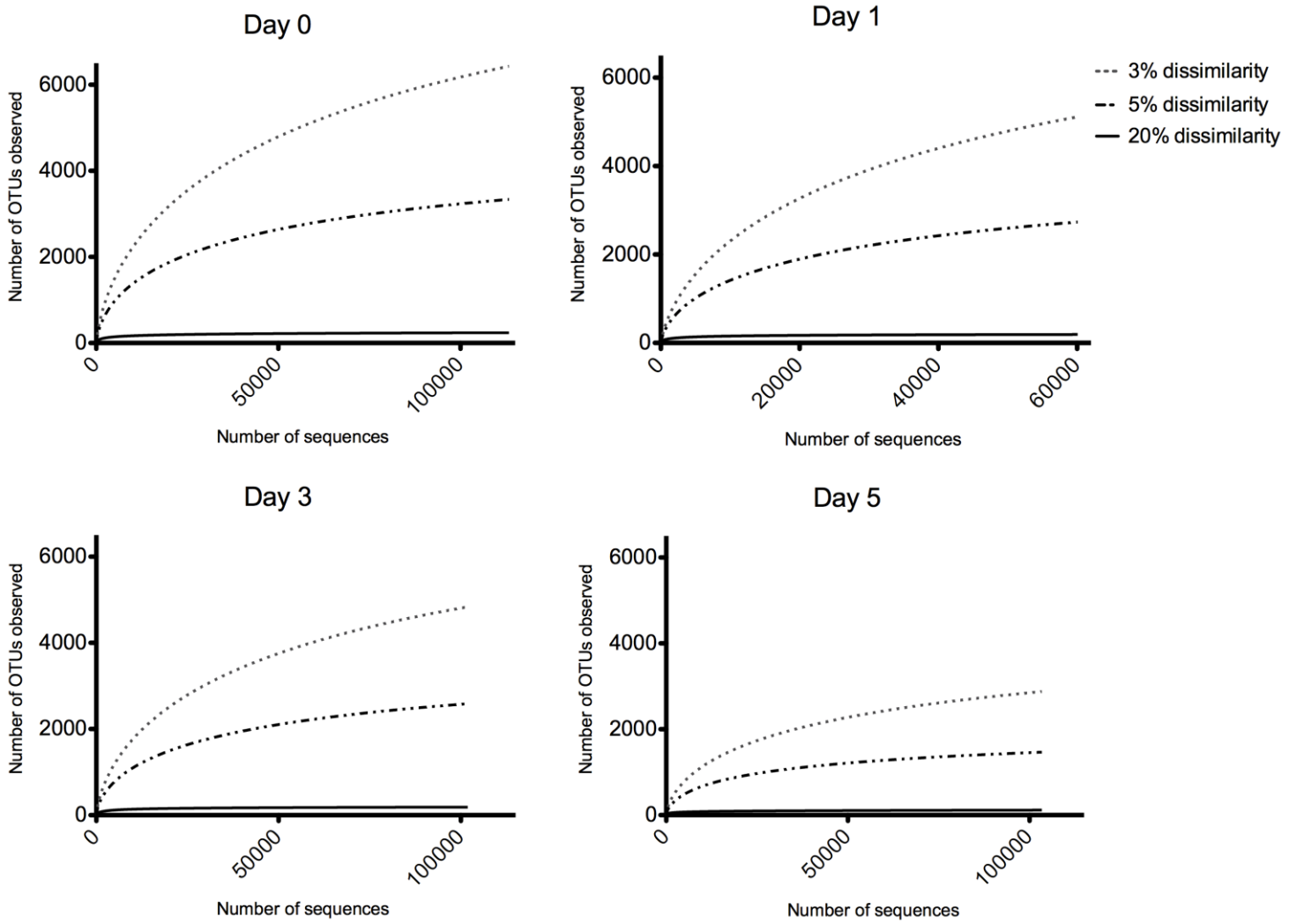

FIG. 15. Rarefaction index over decomposition at species, class, and phylum level (3, 5, and 20% dissimilarity).

The class taxonomic level had similar bacterial compositions between insect access and exclusion carcasses during early stages of decomposition followed by distinct community profiles on the fifth sampling day (FIG. 16, TABLE 14). During initial field placement, *Gammaproteobacteria* was the dominant taxon for insect exclusion (62%) and insect access (70%) carcasses with *Bacilli* being the next most dominant taxon at 27% and 17% relative abundance for insect exclusion and access, respectively. On the first sampling day, *Gamamaproteobacteria* reduced in abundance but remained a dominant taxon for insect exclusion (33%) and access (39%) carcasses. While *Bacilli* had similar abundance levels at 35% and 31% of exclusion and access carcasses, respectively. *Flavobacteria* was only detected during the first sampling day on insect exclusion (7%) and access (10%) carcasses. On the third sampling day, there were similar levels of abundances with *Gamamproteobacteria* representing 59% and 44% of the bacteria community with *Bacilli* representing 23% and 44% of insect exclusion and access carcasses, respectively. Differences between bacterial communities on carcasses became apparent on the fifth sampling day with insect exclusion carcasses having *Gammaproteobacteria* (79%), *Bacilli* (7%), and *Clostridia* (9%) as the dominant taxa. Insect access carcasses had *Gammaproteobacteria* (3%), *Bacilli* (71%), and *Clostridia* (25%). Additionally, *Betaproteobacteria* was detected on both sets of carcasses; however, at different stages during the decomposition process. Initially detected on insect access carcasses on the first sampling day (3%) and *Betaproteobacteria* was only detected on the fifth day of insect exclusion carcasses (3%).

As with previous taxonomic levels, insect access and exclusion carcasses based on genera level relative abundance excluding rare taxa (< 3% relative abundance) had independent, well-defined succession trajectories occurring during decomposition (FIG. 16, TABLE 15). *Psychrobacter* represented a dominant taxon for both insect exclusion (29%) and access (29%) carcasses at initial field placement along with *Moraxella*, which was 23% and 25% of bacteria taxa associated with exclusion and access carcasses, respectively. Bacterial succession patterns differed with insect access to the decomposing carrion. *Aeromonas* (18%) and *Shewanellaceae* (4%) were only detected

on the first day on insect exclusion carcasses, while Proteus transitioned to the dominant taxa on the third (50%) and fifth day (72%). Additionally, *Peptostretpococcus* was only detected on the fifth day (5%) of decomposition on exclusion carcasses. On ACC carcasses, *Providencia* was present on the first (16%) and third (9%) day of decomposition and was dominant on the first (22%) and third day (30%), and transitioned to *Bacillales* (51%) by the fifth day. *Proteus* was also a dominant taxon (27%) on the third day of decomposition of insect access carcasses with *Corynebacterium* (3%) only being detected on this day. By the fifth day, *Psychrobacillus* (58%), which was unique to this time of decomposition and ACC carcass, and *Ignatzschineria* (18%) were the dominant taxa. *Clostridium sensu stricto* (10%) was also only detected during this stage of decomposition for carcasses exposed to insects.

Community analysis

A three-axis NMDS ordination explained 86.8% of the variation in bacterial communities at the genera taxonomic level once rare taxa were excluded (FIG. 17). There was not a significant difference between insect exclusion and control carcass bacterial community composition (MRPP: T = -1.3851, P = 0.0940) and significant differences between sampling days (MRPP: T =-7.8131, P = < 0.0001) (TABLE 16). Pair-wise comparisons indicated significant differences in bacterial community composition between all pairs of sampling days (initial placement, 1, 3 an 5 days) except between days 3 and 5 (TABLE 16).

Thus, demonstrating significant bacterial community changes during decomposition. There were no significant differences between carcasses.

Indicator species analysis determined that *Moraxella* and *Acinetobacter* were significant indicator taxa for the initial sampling, while *Clostridium sensu stricto* was a significant indicator of sampling day 5. There were no other significant bacterial genera indicators between insect access or exclusion carcasses nor among replicate carcasses (TABLE 17).

Three genera (*Acinetobacter*, *Aerococcus* and *Clostridium*) explained 92.5% of the variation in ADH (FIG. 18). Increased abundance of *Acinetobacter* corresponded with low ADH. These data are consistent with the indicator species analysis results with *Acinetobacter* being a predictor of the early stages of decomposition and *Clostridium* an indicator of the later stages of decomposition.

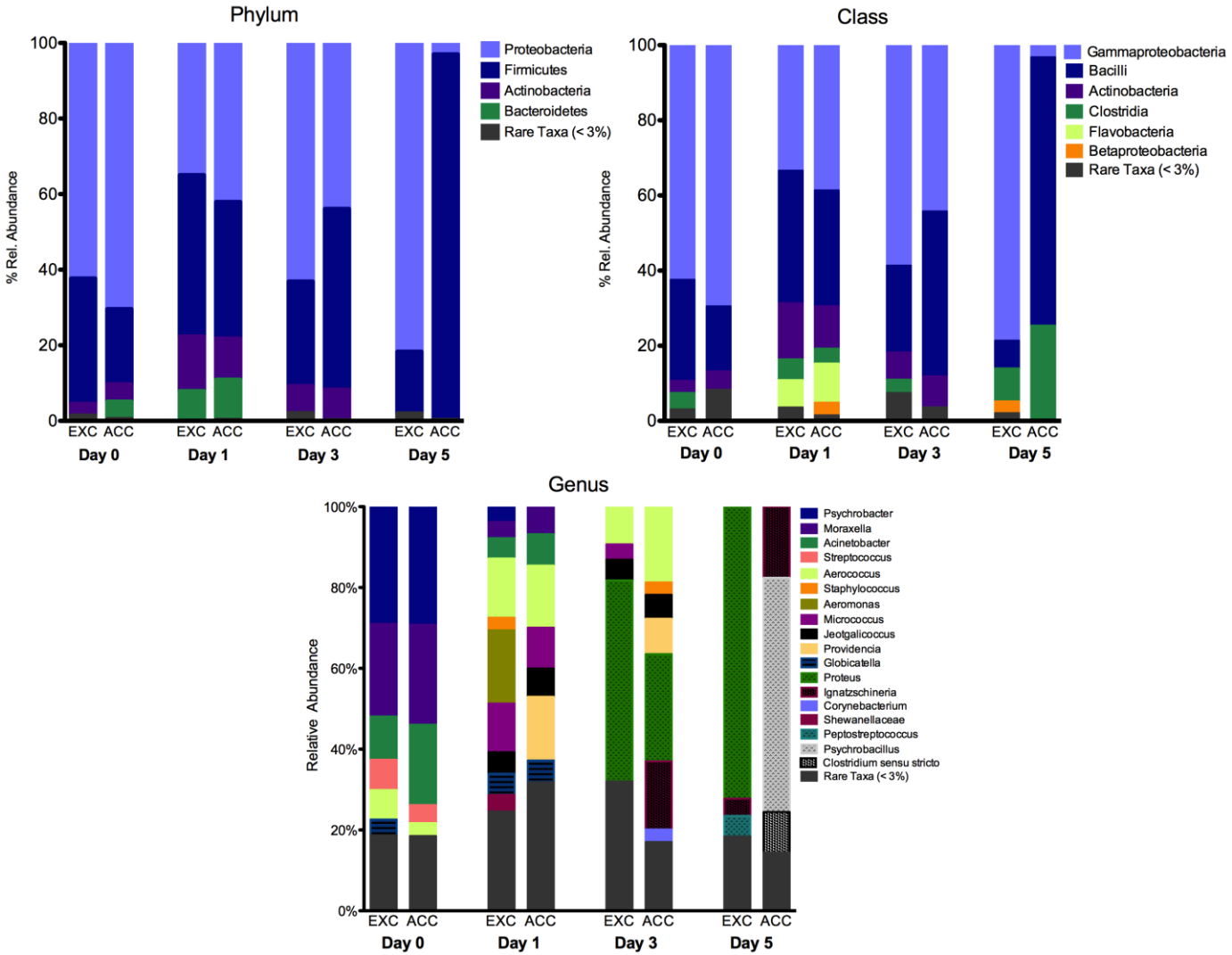

FIG. 16. Relative abundance of phylum, class, and genus level throughout decomposition between treatments. Rare taxa are < 3% of the relative abundance.

TABLE 13. Phylum level classifications and percent relative abundance with rare taxa (< 3% relative abundance) pooled over decomposition day for insect access (ACC) and insect exclusion (EXC) carcasses.

	Initial Placement		Day 1		Day 3		Day 5	
	EXC	*ACC*	*EXC*	*ACC*	*EXC*	*ACC*	*EXC*	*ACC*
Proteobacteria (%)	62.4	70.4	35.0	42.1	63.1	43.9	81.7	3.0
Firmicutes (%)	33.2	19.9	42.7	36.1	27.7	47.8	16.3	96.7
Actinobacteria (%)	3.1	4.6	14.5	10.9	7.1	8.1	0.0	0.0
Bacteroidetes (%)	0.0	4.6	7.8	10.7	0.0	0.0	0.0	0.0
Rare Taxa (%)	1.4	0.5	0.0	0.2	2.0	0.2	2.0	0.2

TABLE 14. Class level classifications and percent relative abundance with rare taxa (< 3% relative abundance) pooled over decomposition day for insect access (ACC) and insect exclusion (EXC) carcasses.

	Initial Placement		Day 1		Day 3		Day 5	
	EXC	ACC	EXC	ACC	EXC	ACC	EXC	ACC
Gammaproteobacteria (%)	62.5	69.5	33.4	38.7	58.7	44.2	78.6	3.2
Bacilli (%)	26.8	17.3	35.3	30.8	23.1	43.9	7.4	71.4
Actinobacteria (%)	3.2	4.9	14.9	11.3	7.2	8.3	0.0	0.0
Clostridia (%)	4.4	0.0	5.5	4.0	3.6	0.0	8.8	25.0
Flavobacteria (%)	0.0	0.0	7.3	10.4	0.0	0.0	0.0	0.0
Betaproteobacteria (%)	0.0	0.0	0.0	3.4	0.0	0.0	3.2	0.0
Rare Taxa (3%)	3.1	8.3	3.6	1.5	7.4	3.6	2.1	0.4

TABLE 15. Genera level classifications and percent relative abundance with rare taxa (< 3% relative abundance) pooled over decomposition day for insect access (ACC) and insect exclusion (EXC) carcasses.

	Initial Placement		Day 1		Day 3		Day 5	
	EXC	ACC	EXC	ACC	EXC	ACC	EXC	ACC
Psychrobacter (%)	29.0	29.2	3.7	0.0	0.0	0.0	0.0	0.0
Moraxella (%)	22.9	24.7	4.0	6.8	0.0	0.0	0.0	0.0
Acinetobacter (%)	10.7	19.9	5.0	7.8	0.0	0.0	0.0	0.0
Streptococcus (%)	7.5	4.5	0.0	0.0	0.0	0.0	0.0	0.0
Aerococcus (%)	7.3	3.2	14.7	15.4	9.3	18.7	0.0	0.0
Globicatella (%)	3.9	0.0	5.2	5.4	0.0	0.0	0.0	0.0
Aeromonas (%)	0.0	0.0	18.2	0.0	0.0	0.0	0.0	0.0
Micrococcus (%)	0.0	0.0	12.0	10.1	3.8	0.0	0.0	0.0
Jeotgalicoccus (%)	0.0	0.0	5.3	7.0	5.1	5.9	0.0	0.0
Shewanellaceae (%)	0.0	0.0	4.1	0.0	0.0	0.0	0.0	0.0
Staphylococcus (%)	0.0	0.0	3.1	0.0	0.0	3.0	0.0	0.0
Proteus (%)	0.0	0.0	0.0	0.0	49.8	26.6	72.2	0.0
Peptostreptococcus (%)	0.0	0.0	0.0	0.0	0.0	0.0	5.1	0.0
Ignatzschineria (%)	0.0	0.0	0.0	0.0	0.0	16.8	4.3	17.6
Providencia (%)	0.0	0.0	0.0	15.8	0.0	8.7	0.0	0.0
Corynebacterium (%)	0.0	0.0	0.0	0.0	0.0	3.1	0.0	0.0

TABLE 15. Continued

	Initial Placement		Day 1		Day 3		Day 5	
	EXC	ACC	EXC	ACC	EXC	ACC	EXC	ACC
Psychrobacillus (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	58.0
Clostridium (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0
Rare Taxa (< 3%)	18.7	18.5	24.6	31.8	32.0	17.0	18.4	14.4

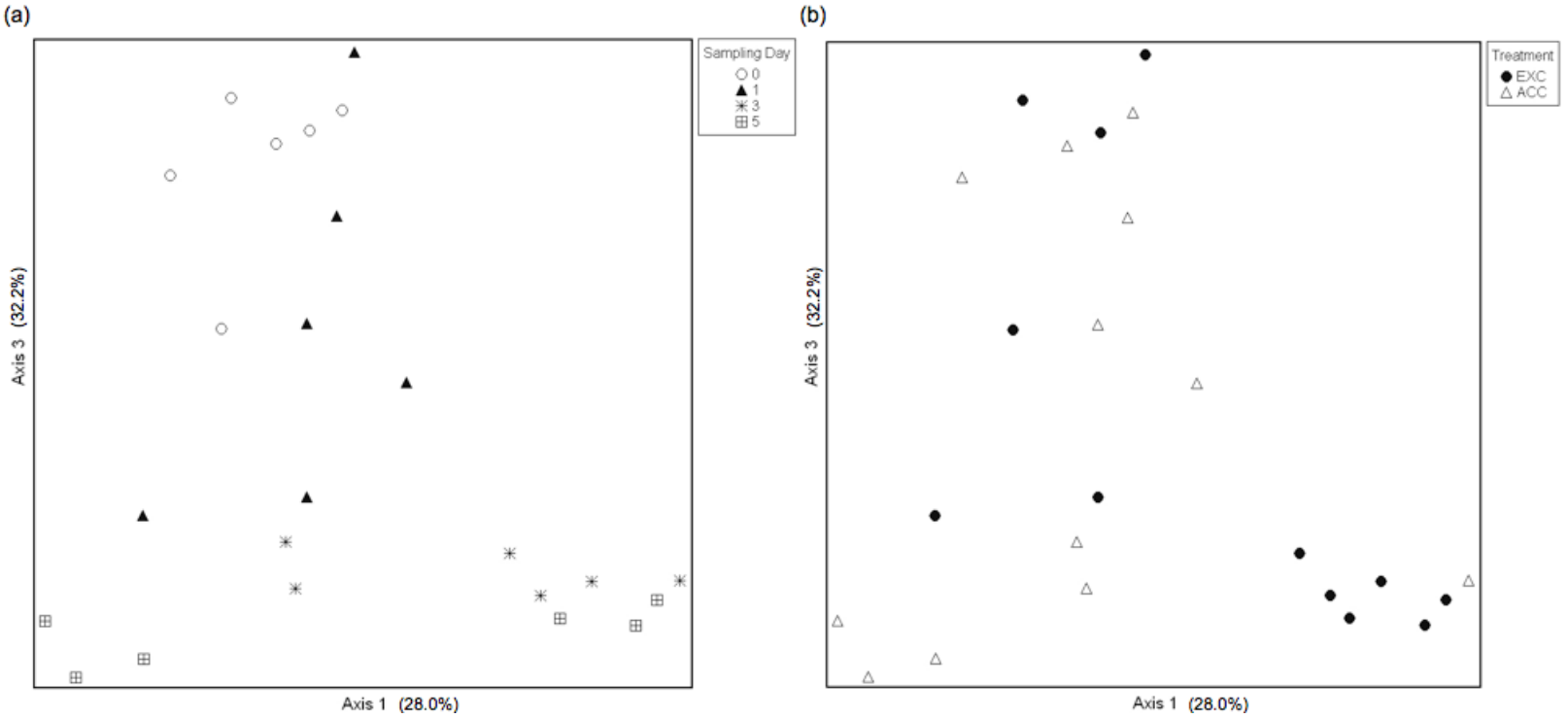

FIG. 17. NMDS of bacterial community at the genera level with rare taxa (<3%) relative abundance) removed. The bacterial community composition ordination with (A) sampling day overlay and (B) a treatment overlay of insect exclusion (EXC) and access (ACC) carcasses. Total stress was 9.17. Axis 1 explained 28.0% of the variation among communities, while axis 2 explained 26.5% and axis 3 explained 32.2% for a total of 86.8% the variation explained by this ordination.

TABLE 16. Summary statistics for MRPP of bacterial genera richness based on RDP classification between microbial communities of ACC and EXC carcasses, across decomposition day, and among carcass replicates. All pair-wise comparisons were significantly different at $\alpha = 0.01$ (day), after Bonferroni correction, and are indicated with an asterisk (*).

	δ under null hypothesis						
	Observed δ	Expected	Variance	Skewness	*T*	*p*	*A*
EXC vs. ACC	0.811	0.838	0.16E-03	-1.26	-1.39	0.0948	0.021
Day	0.657	0.838	0.54E-03	-0.74	-7.81	<0.0001	0.217
0 vs. 1					-3.85	0.0059*	0.135
0 vs. 3					-5.38	0.0010*	0.231
0 vs. 5					-5.17	0.0005*	0.211
1 vs. 3					-3.60	0.0063*	0.122
1 vs. 5					-4.85	0.0007*	0.175
Carcass	0.482	0.838	0.33E-04	-0.47	-0.17	0.4028	0.002

TABLE 17. Results from ISA for bacterial genera richness based on RDP classification. The bacteria taxon is given along with the indicator value and p value for insect access (ACC) and exclusion (EXC) carcasses and sampling day. All pair-wise corrections that are significantly different using $\alpha = 0.0056$ (day) after Bonferroni correction for multiple pair-wise are indicated with an asterisk (*).

Group	Bacteria Genera	Indicator Value	Mean	Std. Dev.	*p*
EXC	*Proteus*	44.3	24.0	7.97	0.0414
Day 0	*Acinetobacter*	84.7	25.0	9.59	0.0002*
Day 0	*Moraxella*	75.1	27.2	10.98	0.0010*
Day 0	*Psychrobacter*	64.2	23.0	10.96	0.0062
Day 0	*Streptococcus*	50.0	18.7	10.16	0.0402
Day 1	*Micrococcus*	57.0	24.0	9.68	0.0100
Day 3	*Jeotgalicoccus*	50.4	27.2	9.18	0.0212
Day 3	*Corynebacterium*	50.0	17.1	10.12	0.0388
Day 5	*Clostridium sensu stricto*	66.7	21.3	11.10	0.0050*
Day 5	*Psychrobacillus*	50.0	18.0	10.14	0.0444

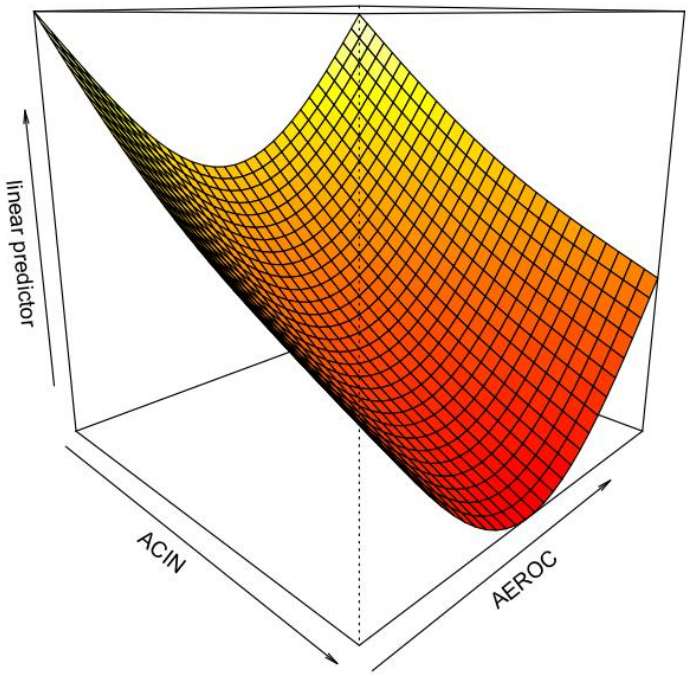

FIG. 18. Generalized additive model predicting ADH. *Acinetobacter*, *Aerococcus* and *Clostridium* were identified as important predictors in random forest and explained 92.5% of deviance in the data when predicting ADH based on bacteria community composition.

Discussion

I observed a decrease in bacterial genera taxa richness in the presence of insects over decomposition time while richness remained relatively stable across decomposition of carcasses excluded from insects. Community analysis (NMDS, MRPP, and ISA) determined significant separation of decomposition days based on the bacterial genera taxonomic level composition, but did not distinguish insect access carcasses from insect exclusion carcasses. I have clearly demonstrated a significant decrease in bacteria genera taxa richness over time (FIG. 14) therefore the lack of differentiation may result from the time (decomposition day) masking the effect of treatment in the analysis.

My data would suggest that carcasses exposed to insects decrease in richness during decomposition. Competition for ephemeral resources between micro- and macroorganisms is well documented. *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) and fungi (*Aspergillus* spp.) interact on fruit, with larval mortality correlating with the age and species of fungi present on the resource (Trienens et al. 2010). Increased microbial growth on a resource, as suggested in this study, has implications on food chains with bottom-up effects demonstrated to be driven by microbial communities (Strickland et al. 2009). The community composition of microbes can affect the community ecology of carrion decomposition. For instance, microbial community change may alter the resource in a way that attracts or repels specific consumers, influencing secondary consumer colonization and succession. *Musca domestica* L. (Diptera: Muscidae) will avoid fungi laden resources for oviposition sites as the fungal communities compete with the developing larvae for consumption of the resource (Lam et al. 2010). Microbes such *as Clostridium botulinum*, *E. coli, Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella enterica enterica* serovar Typhi produce toxins (e.g., botulism) that deter vertebrate scavengers from consuming carrion (Janzen 1977). In marine systems, carrion was scavenged 66% of the time when microbial communities were initially allowed to proliferate undisturbed, compared to 89% scavenging without mature microbial communities (Barlocher 1979, Burkepile et al. 2006).

Studies examining this process primarily focus on the "observable" data that can be collected, with little regard for what might be occurring at the microbial scale. Insect colonizers (e.g., blow flies and beetles) utilize carrion as a nutrition, mating or oviposition site. Arthropod community structure on carrion over time has often been described as a process of competition between species for a resource (Norris 1965, Hanski and Kuusela 1977). Subsequent larval development may disrupt established microbial communities through direct or indirect competitive interactions on the carcass. Insects have many strategies for combating microbial communities including initiating immune responses after pathogen detection (Gottar et al. 2002, Ferrandon et al. 2007,

Gerardo et al. 2010), pathogen avoidance as seen in honey bee colonies rejecting nest mates whom have been infected or parasitized (Wilson-Rich et al. 2009), and insects producing secretions containing antibiotic properties (Kerridge et al. 2005, Nigam et al. 2006, Rozen et al. 2008). Blow flies may directly impact microbial species through chemical secretions while consuming carrion tissue (Sherman et al. 2000, Mumcuoglu et al. 2001). Further analysis needs to be conducted to determine how changes in the bacterial community structure alter insect arrival and colonization patterns of carrion once microbial proliferation has occurred.

The relationship between biodiversity and ecosystem function has been well documented (Venner et al. 2011). For instance, leaf litter is decomposed by complex microbial communities and reintroduces nutrients into the system where it can be utilized by plants, thus facilitating new plant growth (Witkamp 1966, Lee 1999, Lovett et al. 2002). Detritus and biodiversity demonstrate a positive feedback loop in the environment but questions still remain about the processes regulating species richness in these communities (Moore et al. 2004). My results have provided the foundation for identifying bacterial communities present on carcasses throughout decomposition and the effect of insects mediating bacterial community richness.

In previous ecological field studies, it has been more common to estimate overall species diversity by sampling a small area of the ecosystem and extrapolating the diversity of the remaining components (Margalef 1963). Pyrosequencing provides a powerful tool that will better predict species richness, diversity and abundance during field studies. However, microbial taxa distribution is important to consider because sampling time at discrete intervals provides a snapshot of the microbial community at that time and may miss rare taxa events throughout decomposition. Rare species can be difficult to isolate in large ecological surveys but may influence how specific processes are occurring. Abundance and taxa distribution of organisms within microbial assemblages are correlated. More than 85% of the taxa isolated from soil, seawater, insect and sponge-associated microbial assemblages were present in a single assemblage, and no single taxon was found in more than 12% of the assemblages (Nemergut et al.

2010). There is an intrinsic error rate of overestimating rare phylotypes using pyrosequencing (Shendure and Ji 2008, Kunin et al. 2010). In my results, the bacteria communities associated with each set of carcasses (EXC or ACC) comprised of one to three dominant genera on any given sampling day. These genera could represent up to 72% relative abundance (FIG. 16; APPENDIX B) for the bacterial community on any given day. However, these six genera represented 30% (ACC) and 36% (EXC) of those encountered in the study.

It has been determined that human microbiota varies significantly less within an individual than amongst all individuals sampled on a specific day (Costello et al. 2009). However, my replicates were more similar in bacterial composition during the initial decomposition days (initial field placement and the first sampling day) and then diverged to have unique bacterial community composition as resulting from the presence or absence of insects as decomposition progressed on the third and fifth sampling days.

The pyrosequencing technique utilized for this study demonstrates the robust structure of the bacterial community associated with carcasses and how insects mediate bacterial diversity, richness and succession patterns during decomposition (FIG. 19). This demonstrates a pattern of the bacteria first being associated with the carcasses. Then as decomposition progresses, there appears to be an influence of the insects utilizing the resource and modifying the environment, and by the final day there is an influence of the environment on the carcass. This is interesting as I have demonstrated a relationship of the carcass with the environment, and the patterns of bacterial succession associated with the carcass decomposition.

Psychrobacter, *Moraxella* and *Acinetobacter* are all members of the *Moraxellaceae* family, which is in the *Proteobacteria* phylum, and dominated the initial sampling period (Rossau et al. 1991). Species within this family are commonly associated with the spoiling of meat and are found on the hides of slaughtered animals (Gill and Newton 1978). *Psychrobacter* are gram-negative rods or coccobacilli isolated from a variety of sources including the skin of fish and poultry, meat products, clinical sources but are also found in extreme conditions such as Antarctic costal marine habitats (Juni and

Heym 1986, Bozal et al. 2003). *Psychrobacter* was a dominant taxon associated with both insect exclusion and access carcasses on the initial sampling day (FIG. 16). *Moraxella* has been established as a significant human pathogen in children and adults resulting in lower and upper respiratory tract infections (Verduin et al. 2002). Predicted as an important taxon based on indicator species analysis (TABLE 17), *Moraxella* was another dominant taxon associated with exclusion and access carcasses on the initial sampling day (FIG. 16). These are gram-negative genera of bacteria rods, cocci or diplococcic. *Acinetobacter* was also a significant indicator of the initial sampling day based on indicator species analysis. These gram-negative rods are opportunistic pathogens in humans resulting in nosocomial infections (Siegman-Igra et al. 1993, Bergogne-Bérézin and Towner 1996), endocarditis (Gradon et al. 1992), and thyroid abscesses (Jacobs et al. 2003).

Bacterial community structure on carcasses excluded from insect access were dominated by *Aeromonas* and *Shewanella*, Gram-negative rods that are commonly associated with aquatic environments and fecal contamination (Araujo et al. 1989). *Aeromonas* sp. have been found to suppress stable fly (*Stomoxys calcitrans* (L.) (Diptera: Muscidae) larval development in lab studies (Romero et al. 2006). *Shewanella* is a facultative anaerobic bacteria capable of thriving in conditions without oxygen because it can utilize several final electron acceptors in the absences of oxygen (Hau and Gralnick 2007). *Aerococcus* was an abundant (15% relative abundance) (APPENDIX B) Gram-positive cocci associated with these carcasses on the first day of decomposition, (Williams et al. 1953). These bacteria have been found in cold-smoke salmon production facilities (Bagge-Ravn et al. 2003) and are pathogenic in humans causing systemic infections (Parker and Ball 1976). *Proteus* was the dominant taxon associated with insect exclusion carcasses on the third and fifth sampling day. This Gram-negative is a common inhabitant of soil, aquatic, and damp environments (Trevors et al. 1987) and has a well-known swarming behavior (O'Hara et al. 2000). Finally, *Peptostreptococcus* is a Gram-positive anaerobic cocci that was only detected on the fifth sampling day of the insect exclusion carcasses. This bacteria has been isolated in bovine rumen and

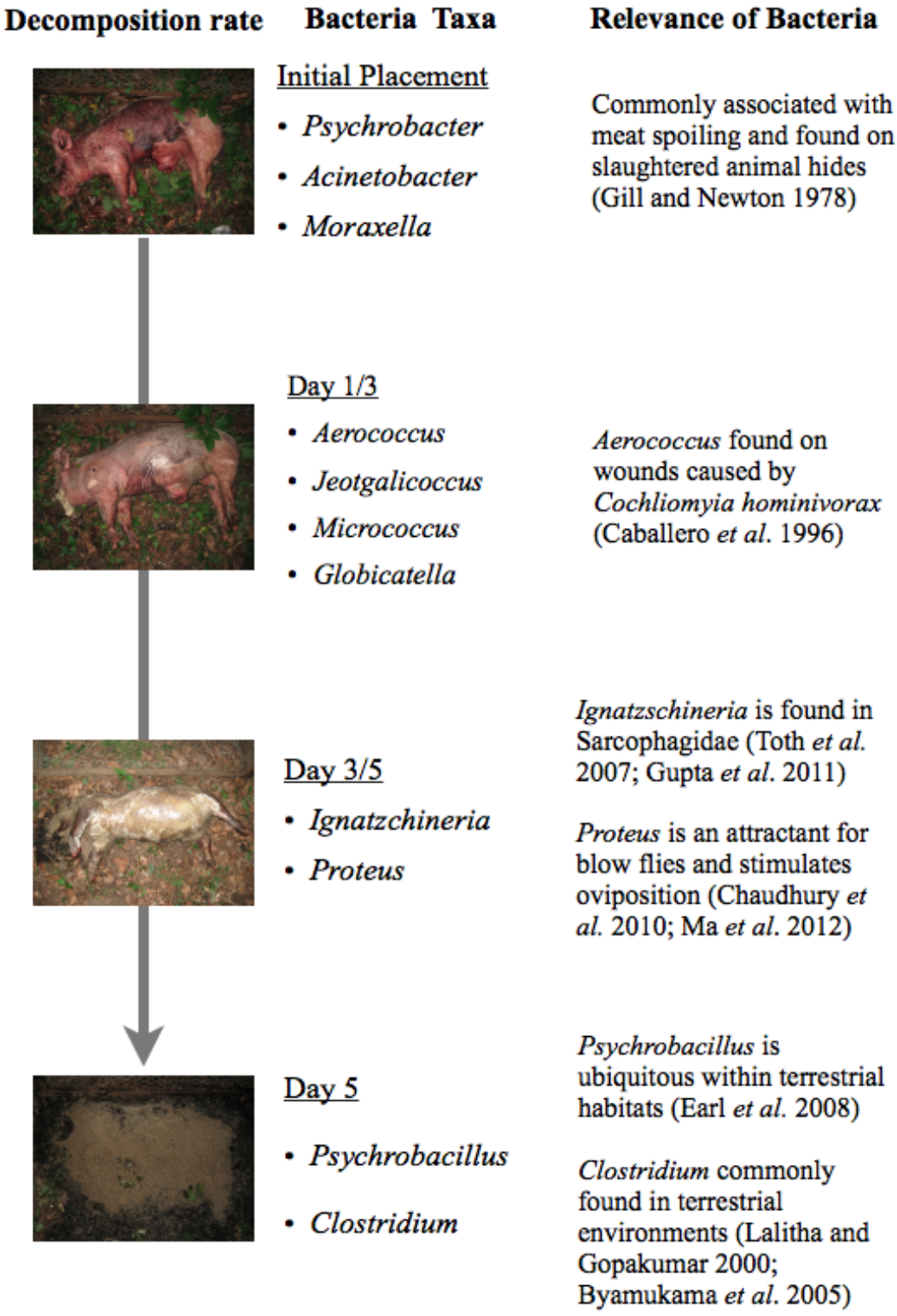

FIG. 19. Important bacteria taxa associated with carcasses throughout decomposition.

produces ammonia (Paster et al. 1993) and members of this genera are a root cause of periodontitis in humans (Rams et al. 1992).

Insect access carcasses have a distinct bacterial succession pattern, and the associated bacterial communities could alter the quality of the resource, thus mediating insect community assembly, as demonstrated with fungi governing phytophagous insect composition in a plant-based system (Tack et al. 2012). *Providencia* was a dominant taxon on the first and third sampling days. Interestingly, this Gram-negative rod was only associated with the carcasses allowed insect access. It like, *Proteus* species, can be a common pathogen of humans resulting in urinary tract infections but also has non-pathogenic roles in vertebrates (Juneja and Lazzaro 2009). *Aerococcus* were also associated with the first and third sampling days of insect access carcasses. Members of this genera have been detected in the guts of laboratory strains of Mexican fruit flies, *Anastrepha ludens* (Diptera: Tephritidae) (Kuzina et al. 2001) and on wounds caused by *Cochliomyia hominivorax* (Caballero et al. 1996). While *Corynebacterium*, another Gram-positive rod, was only detected on the third sampling day for these carcasses. Members within this genera have been detected soil (Takai et al. 1986), freshwater (Leifson 1962) habitats and can be a pathogen in humans causing infections in the human oral cavity (Gibbons and Haute 1975). Muscoid flies commonly associated with agricultural settings have been determined to vector these pathogens (Yeruham et al. 1996, Braverman et al. 1999, Spier et al. 2004). *Proteus* was a dominant taxon on the third day. *Proteus* has been detected in wild *Drosophila melanogaster* populations (Corby-Harris et al. 2007, Juneja and Lazzaro 2009). *Proteus* can produce hydrogen sulfide (Manos and Belas 2006), which is an attractant to blow flies *Lucilia cuprina* (Wiedmann) (Diptera: Calliphoridae), *Chrysomya* spp., and *Calliphora* spp. (Urech et al. 2004). Blow fly oviposition can be induced by bacteria and their associated semiochemicals; specifically, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) females have a preference for five out of eight Enterobacteriaceae volatiles, such as *Proteus* spp. in oviposition assays (Chaudhury et al. 2010), and gravid females 10 - 12 d old are attracted to *Providencia* sp. (Hammack et al. 1987); both

genera of bacteria were present on insect access carcasses during the initial stages of decomposition. Additionally, *P. mirabilis* is an attractant for *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) adults and results in higher oviposition rates on a resource (Ma et al. 2012). *Ignatzschineria* is a Gram-negative rod associated with the third and fifth day of insect access decomposition. Species within the genera have been detected in the larval (Toth et al. 2007) and adult stages (Gupta et al. 2011) of flesh flies (Diptera: Sarcophagidae). The dominant taxon of the fifth decomposition day for insect access carcasses was *Psychrobacillus* was only found during this stage of decomposition and on these carcasses. This genera is closely related to *Bacillus* (Krishnamurthi et al. 2010), which is ubiquitous within terrestrial and aquatic ecosystems (Earl et al. 2008). Finally, *Clostridium* (i.e., *Clostridium sensu stricto*), a Gram-positive rod, was only detected on insect access carcasses and was a significant indicator based on indicator species analysis and relative abundance levels. *Clostridium* has been detected in numerous environments (Lalitha and Gopakumar 2000, Byamukama et al. 2005) and is a common part of the human gut flora that can be pathogenic (van d'er Waaij 1989). Microbes such *as Clostridium botulinum*, *E. coli, Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella enterica enterica,* serovar Typhi produce toxins (e.g., botulism) that deter vertebrate scavengers from consuming carrion (Janzen 1977). However, it is unknown if they deter insect from utilizing the resource.

Due to the number of opportunistic pathogens found in humans it is important to consider the vectoring capabilities of insects associated with the carcasses. *Musca domestica* has been reported to carry many pathogens causing foodborne illnesses (Förster et al. 2007), *Escherichia coli* 0157:H7 (Alam and Zurek 2004), and over 100 known human pathogens have been reported to be transported by flies (Greenberg and Klowden 1972). Blow flies arriving to a resource may be vectoring their own endogenous fauna and may also be acting to transport microbes present on the carcass to another carcass or similar resource, thus, affecting trophic interactions occurring during decomposition (Tomberlin et al. 2011b).

Most carrion decomposition ecology research has taken a descriptive approach to examining arthropod community structure over time and often completely ignoring the microbial communities. However, the lack of microbial community analysis present on a resource is not due an oversight by the researchers, rather because of limitations of methods used to describe microbial communities. It is clear from the deep-sequencing of carcasses throughout decomposition time and in the presences or absence of necrophagous insects, there are distinct bacterial community structure and assembly patterns on carrion. Based on these findings, it is evident the bacterial community is important in the decomposition and insects can mediate bacterial structure. There should be continued studies utilizing high-throughput techniques exploring prokaryotes and their interactions with higher-level organisms in decomposition ecology.

CHAPTER IV

INSECT EFFECTS ON MICROBIAL COMMUNITY METABOLIC ACTIVITY DURING CARRION DECOMPOSITION

Introduction

The decomposition of organic matter is an essential ecosystem function (Hooper et al. 2005); it is vital for nutrient cycling (Putman 1978a), food web dynamics (Polis and Strong 1996), and can impact the biodiversity of ecosystems (Hines et al. 2006, Srivastava et al. 2009). Limited data are available on the impact of complex resources such as decomposing vertebrate carcasses to ecosystem function (Putman 1978a, Hocking and Reimchen 2006, Wilson and Wolkovich 2011). Carrion represents a part of decaying organic matter in most ecosystems, as an initial level of energy flow (DeVault et al. 2003) and can be a primary resource subsidy (e.g., whale carcasses (Klages et al. 2001, Burkepile et al. 2006) or anadromous salmon, *Oncorhynchus* spp. (Salmoniformes: Salmonidae) (Hocking and Reimchen 2006, Janetski et al. 2009). Further, carrion represents an ecological unit within a larger ecosystem (Odum 1969). Within terrestrial systems, the presence of carrion results in nutrient pulses to associated soil, plant and insect communities, which are a primary force for decomposition (Towne 2000, Yang 2006).

The relationship between biodiversity and ecosystem function has been well documented (Venner et al. 2011). For instance, leaf litter is decomposed by complex microbial communities and reintroduces nutrients into the system where it can then be utilized by plants, thus facilitating new plant growth (Witkamp 1966, Lee 1999, Lovett et al. 2002). Carrion provides nutrients to an ecosystem in a similar matter (Putman 1978a). Approximately 48% of salmon carcasses (nutrients) can be transferred to riparian zones by blow flies (Diptera: Calliphoridae) (Hocking and Reimchen 2006). Rat, *Rattus rattus* L. (Rodentia: Muridae), carcasses placed in a temperate ecosystem during summer and winter seasons introduced approximately 1.25 - 2.5 mg C g^{-1} (dry weight)

into the soil (Carter et al. 2007). However, a significant correlation between microorganism diversity and functional response within an ecosystem has not yet been demonstrated (Longmuir et al. 2007, Andersen et al. 2010). In part, this resulted of technical difficulties in identifying non-culturable bacterial species from the environment (Vass 2001, Schmeisser et al. 2007).

Insect succession patterns and corresponding interactions occurring on carrion have been well documented (Fuller 1934, Reed 1958, Payne 1965). Early studies that used sheep, *Ovis aries* L. (Artiodactyla: Bovidae), carcasses to assess insect community assembly patterns under natural decomposition determined blow flies were primary colonizers while coleopterans arrived later as secondary colonizers (Fuller 1934). Later studies analyzed arthropod succession patterns on carrion in more detail. For example, 240 arthropod taxa colonized the remains from a dog, *Canis familiaris* L. (Carnivora: Canidae) carcass in a predictable, defined succession pattern (Reed 1958). In another study, swine, *Sus scrofa* L. (Artiodactyla: Suidae), carcasses attracted 522 arthropod species throughout the decomposition process (Payne 1965). Succession patterns were consistent across all carcass types in all seasons with the arrival of Diptera first followed by Coleoptera colonizers. These studies demonstrate the tendency of carrion decomposition research to focus on insect succession; however, there have been few studies to examine the microbial communities of carrion decomposition, or their potential interactions with colonizing arthropod species (Byrd and Castner 2001).

Microbial assemblages are important for many ecosystem processes (Hattenschwiler et al. 2005, Parmenter and MacMahon 2009, Nemergut et al. 2010), such as recycling of carrion. In terrestrial systems they have been suggested to be as important as primary producers (Tiunov and Scheu 2005) since they convert decaying organic matter into low-weight, inorganic molecules, which can be utilized by other organisms. Microbes compete with other consumers for carrion (Janzen 1977, Polis and Strong 1996, DeVault et al. 2004). For instance, scavenging rates in marine systems varied from 66% when microbial communities proliferated undisturbed to 89% in the absence of microbes (Burkepile et al. 2006). It has yet to be determined if a microbial

threshold exists where microorganisms outcompete higher trophic levels (e.g., insects and vertebrate scavengers) in terrestrial systems. Even though their role in trophic level interactions and food webs is well appreciated, quantitative and qualitative empirical data describing microbial community dynamics are lacking for carrion systems (Vass 2001, Zak et al. 2003, Chung et al. 2007, Rohlfs 2008, Strickland et al. 2009). Understanding carrion microbial communities and potential interactions with primary colonizers, such as blow flies, is important for describing ecological mechanisms driving the decomposition process (Strickland et al. 2009).

My objective was to test the role of insect colonization on microbial community function throughout carrion decomposition, and to determine if this effect varied between two years. Here I tested the null hypothesis that microbial community function as determined by microbial metabolic response would not change over time or in the presence/absence of insects (i.e., blow flies and beetles) on decomposing ephemeral resources. Secondly, I wanted to assess if the duration of decomposition, as represented by accumulated degree hours (ADH), could be predicted based on microbial community metabolic profiles. Lastly, I evaluated habitat functional responses of microbial metabolic change of soil communities underneath carcass by testing the null hypothesis that microbial metabolic profiles of control soil and soil under carcasses would not be different given insect access to the resource.

Methods

Site description and experimental design

Swine carcass decomposition was described in a Midwestern temperate forest habitat surrounded by agricultural fields in Xenia, Ohio, USA (39°38'14.83"N, 84°1'37.82"W). Carcasses were sampled from 5 - 14 August 2010 and from 26 July - 2 August 2011. The dominant tree fauna consisted of oak (*Quercus* spp.) and maple trees (*Acer* spp.). During the 2010 field season, six male swine ranging from 10.4 - 30.1 kg (TABLE 1), euthanized by cranial blunt force at approximately 16:30 h, were purchased from a local farm on 5 August 2010. Carcasses were double bagged, transported for

about 1 hour, and randomly placed minimally 20 m apart along three transects (FIG. 1) two hours before U.S. National Oceanic and Atmospheric Administration (NOAA) defined sunset approximately 19:00 h on 5 August 2010. In 2011, using the same methods, six swine (three females and three males) carcasses were purchased from the same local farm on 26 July 2011 after being euthanized by cranial blunt force trauma at approximately 17:45 h. Carcasses ranged from 5.0 - 7.3 kg and were randomly placed along three new transects (FIG. 1) at approximately 18:30 h on 26 July 2011.

All carcasses were oriented with the head directed cardinal north and the dorsal side towards the east. Carcasses were labeled alphabetically with A through F representing the 2010 field season and G through L representing the 2011 field season. During each field season, three random carcasses (2010: "A", "C" and "D"; 2011: "I", "J" and "K") were enclosed in individual 1.8 m^3 Lumite® screen (18x14 mesh size) portable field cages (BioQuip Products, Rancho Dominguez, CA, USA) to reduce insect access and were considered the exclusion treatment (EXC), while the remaining three carcasses (2010: "B", "E" and "F"; 2011: "G", "H" and "L") were allowed access to insects (ACC); all carcasses were covered with anti-scavenging cages (0.9 x 0.6 x 0.6 m) constructed of wooden frames enclosed with poultry netting (FIG. 2). Trapper® max glue traps (16.5 x 11 cm) (Bell Laboratories, Inc., Madison WI, USA) were used as a passive trapping method to collect adult insects arriving to each carcass. On each anti-scavenging cage a single glue trap was attached approximately 0.15 m from the anterior and posterior region of each carcass (FIG. 3). Glue traps were replaced every 12 h. Insects were identified to the lowest taxonomic level possible while remaining on the glue trap (Triplehorn and Johnson 2005, Whitworth 2006).

NexSens DS1923 micro-T temperatures loggers (Fondriest Environmental, Inc., Alpha, OH, USA) were placed within 0.6 m of each carcass approximately 0.3 m above the ground and temperature was recorded at 0.25 h intervals. Temperature data were later converted into ADH to account for temperature variation over decomposition time (Megyesi et al. 2005).

Microbial communities of all 2010 carcasses were sampled immediately, 1, 3, and 5 d after carcass placement for a total of four decomposition time points. The exclusion cages were removed from EXC carcasses after sampling on the fifth sampling day. During 2011, microbial samples were taken immediate after carcass placement and daily throughout decomposition. Sampling of ACC was concluded after three days due to rapid decomposition of all carcasses. EXC carcasses were sampled for two additional days after removal of the insect exclusion netting, which were prior to advanced active stage. Carcasses were considered to be in the advanced active decay stage when there was no soft tissue in the buccal cavity and the skin had become indistinguishable from the internal anatomy (Payne 1965).

Microbe sampling protocol

Sterile cotton applicators (Fischer Healthcare, Houston, TX, USA) were used to sample microbial communities from two regions on each carcass for 60 s: the buccal cavity (the top area of the mouth and under the tongue) and the skin, which consisted of combining three areas (approximately 2.54 x 15.24 cm) along a single transect of a carcass. Care was taken to assure that new areas were swabbed at subsequent samplings. An additional composite sample was taken during the first two sampling points of 2011, combining both the buccal and skin regions on the same swab for direct comparison with the regions sampled individually.

Three replicate samples of soil were taken from directly beneath each carcass and amalgamated for analysis. Three replicate samples of soil were taken from a 1 m distance from each carcass and amalgamated for analysis. Each sample was taken at a depth of 5 cm (approximately 0.5 g per sample) using sterile 310 mm disposable spatulas (VWR™ International, Randor, PA, USA).

Microbial metabolic community profiles

Samples (swabs and 1 g of soil) were added individually to 50 ml Falcon tubes (VWR™ International, Randor, PA, USA) containing 40 ml of 25% Ringer solution and

15 sterilized 3 mm glass beads (Fisher Scientific, Fair Lawn, NJ, USA) within 24 h of sampling. All samples were homogenized using a Burrell Wrist-Action® shaker (Burrell Scientific, Pittsburg, PA, USA) at the power ranking 9 for 10 min. Samples were then centrifuged at 500 x g for 2 min and the supernatant was retained. Microbial metabolic community profiles (MMCPs) were determined using phenotype MicroPlate EcoPlates™ (BIOLOG Inc., Hayward, CA, USA) (Garland and Mills 1991, Garcia-Villaraco Velasco et al. 2009). Biolog EcoPlates™ provide quantifiable functional responses of environmental microbial communities as MMCPs (FIG. 20) (Garland 1997, Garcia-Villaraco Velasco et al. 2009). These profiles are described through the differential use of 31 different carbon substrates, where each carbon substrate is represented in triplicate on a plate (Weber and Legge 2010).

The microplates were inoculated with 100 µl per well of the retained supernatant for each of the 94 and 152 samples from 2010 and 2011, respectively. Plates were incubated at 25°C in darkness. Absorbance, or overall plate metabolic activity, was measured at 590 nm every 12 h up to 120 h or until the average plate absorbance reached 0.7 OD using a Wallac 1420 VICTOR^{2}™ (Perkin Elmer, Inc., Waltham, MA, USA) and Wallac 1420 Workstation software version 2.0 (Perkin Elmer, Inc., Waltham, MA, USA) during 2010 and a Tecan Sunrise™ (Tecan Group Ltd., Männedorf, Switzerland) and Magellan™ software version 7.0 (Tecan Group Ltd., Männedorf, Switzerland) during 2011. A two-tailed paired t-test was used to compare differences between the plate reader models.

Microbial community functional diversity, richness, and evenness

To evaluate microbial metabolic community profiles Simpson's diversity, Shannon-Weaver diversity, carbon substrate use richness, and evenness were calculated according to methods of Zak et. al (1994). Simpson's diversity index (*D*) was determined as:

$$D = \Sigma\, p_i^2$$

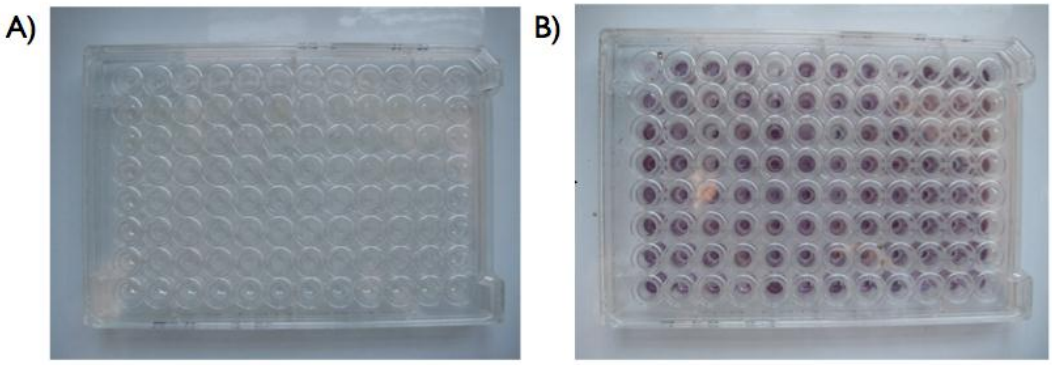

FIG. 20. Biolog EcoPlates™ (A) un-inoculated and (B) inoculated after 120 h at approximately 27°C.

Shannon-Weaver diversity (*H*) was determined as:

$$H = - \Sigma\, p_i \ln(p_i)$$

where p_i is the ratio of metabolic use of a particular carbon substrate to the sum of all carbon substrate metabolic use (Simpson 1949). Substrate richness (*S*) is the number of carbon substrate utilized by the microbial population and was based on the number of wells with positive normalized absorbance values (see below). Substrate evenness (*E*) measured the equitability of activities of all metabolized substrates and was calculated as:

$$E = H/H_{max} = H/\log S \text{ (Zak et al. 1994).}$$

Statistical analyses

The overall plate metabolic activity (mean plate absorbance) was initially used to compare functional activity over decomposition day, treatment (ACC vs. EXC), and day-treatment interaction using two-way repeated measures analysis of variance (two-way RM-ANOVA) followed by multiple comparisons tested with Bonferroni corrections using Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). Bonferroni corrections were used to test for significance of pair-wise comparisons without an increased

probability of rejecting the null when it was actually true, or a Type I error (Cabin and Mitchell 2000). Comparisons were also made using RM-ANOVA between the sampling areas (buccal and skin) and the composite sample activity over decomposition day, and the sampling area-day interaction. After this initial analysis, microbial community metabolic activity was normalized by correcting each carbon substrate absorbance (A_i) by the mean (the three replicate wells) water absorbance (A_O) and then dividing by the sum of the corrected plate absorbance as described by Weber and Legge (2010) to account for possible density differences among samples using the following equation:

$$A_k = \frac{Ai - AO}{\frac{1}{31}\sum_{i=1}^{31}(Ai - AO)}$$

Where A_k was the normalized well (individual carbon substrate) metabolic activity. Negative well responses were coded as zeros for further data analysis. Each measure of microbial community functional diversity, richness, and evenness was tested statistically for effects of sampling day, treatment and sampling day-treatment interactions using two-way RM-ANOVA corrected for multiple tests with Bonferroni corrections using Prism 5.

Non-metric multidimensional scaling (NMDS) was used to evaluate MMCPs between treatments and over decomposition in PC-ORD 5 (MjM Software, Gleneden Beach, OR, USA) (McCune and Mefford 2006). NMDS is a nonparametric ordination technique that avoids assuming linearity among community variables (McCune and Grace 2002). Before the ordinations, outliers were identified and removed using Jackknife distances in JMP 9.0.0 (SAS Institute Inc., Cary, NC, USA) as recommended by McCune and Grace (2002). Multi-response permutation procedure (MRPP) was used for testing statistical differences between overlay groups of MMCPs within the ordination using methods described elsewhere (Biondini et al. 1985). Indicator species analysis (ISA) complemented MRPP by assigning significant indicator values to carbon substrates that were indicative of community functional separation among treatments

and over decomposition (McCune and Grace 2002). The indicator value describes which carbon substrate was the best indicator of community functional response to insect access or exclusion treatments, decomposition day, sampling area (i.e., buccal or skin) and soil sampling area (i.e., under the carcass or control soil) based on normalized microbial activity, with 0 representing no indication and 100 being a perfect indication for each grouping.

To determine if decomposition time, as accumulated degree hours (ADH), could be predicted based on the normalized microbial activity, I used a tiered approach of initially using random forest models to identify significant carbon substrate predictors of ADH (i.e., normalized microbial metabolic activity of each carbon substrate). The highest-ranking predictor variables from the random forest algorithm were then used to predict ADH using generalized additive models (GAMs). Random forest models were constructed using the randomForest 4.5-36 library in the R statistical package (R Development Core Team 2010). Random forest modeling is a machine learning classification and regression tree that uses a deterministic algorithm to build trees based on splitting each node from a random subset of predictors randomly chosen and a bootstrap sample of the observations (Breiman 2001, Liaw and Wiener 2002). A random one-third of the data (out-of-bag (OOB) data) was used to determine the accuracy of each tree based on the order and value of the predictor variables (i.e., normalized activity of each carbon substrate). Each tree was constructed using only OOB data, and the overall prediction was calculated by averaging data from all trees. The mgcv library in the R statistical package was used to construct GAMs. This approach modeled ADH using covariates (important carbon substrates identified in the random forest models) and treatment by summing nonparametric covariates (i.e., MMCPs) using a smoothing function (Hastie and Tibshirani 1990).

Results

Abiotic conditions and insect communities

Neither mean ambient temperature (t=0.3333, df=3, P = 0.7608) or precipitation (t=0.8452, df=3, P = 0.4601) for the four weeks preceding each field study was significantly different between years based on climatological data collected at a NOAA weather station (TABLE 18). There were also not significant differences in mean ambient air temperature of the four weeks preceding each field study compared to temperature during each study. The mean data logger temperature recorded at each carcass during 2010 and 2011 was 23.18 ± 2.05^{o}C and 25.13 ± 1.04^{o}C, respectively. The carcass ambient temperatures were converted to ADH to account for temperature variation between study years over decomposition time. The mean carcass ADH in 2011 was significantly higher than 2010 (FIG. 4; TABLE 2) throughout decomposition except during the initial sampling period when the carcasses were placed in the field and the ADH was 0 for all carcasses. ADH in 2011 ranged from 8 - 18% higher than ADH in 2010.

Sixty arthropod taxa, representing eight orders and 49 families, were collected during both field seasons (TABLE 19). Forty-seven taxa represented insect access carcasses while forty-six were found on the insect exclusion carcasses once the insect exclusion cages were removed. *Musca* spp., Anthomyiidae, Conopidae, Sciaridae, Tipulidae, Mycetophagidae, and Fulgoridae were the only taxa collected from insect access carcasses during 2010, while Apidae and Sphecidae were collected during 2011, and Ceratopogonidae was the only taxon collected in both field seasons. Dolichopodidae, Psychodidae, Trogidae, Dermestidae, Curculionidae, Nymphalidae, Papilionidae and Noctuidae were taxa exclusive to insect exclusion carcasses once the insect exclusion cages were removed during 2010 while *Calliphora vomitoria* L. (Diptera: Calliphoridae) was the only taxon collected during 2011. The insect exclusion cages had approximately a 99% and 95% exclusion rate of insects during 2010 and 2011, respectively. However, exclusion cages did not exclude any ground dwelling insects present in the soil.

TABLE 18. Mean temperature and precipitation preceding and during each field season. Climatological data of Dayton, OH from NOAA for the four weeks preceding the start of the field studies and during the field study for each year.

		Max. Temp. (C)	Min. Temp. (C)	Mean Temp. (C)	Precipitation (cm)
2010	Week 1	30	19	25	0.86
	Week 2	31	21	26	0.05
	Week 3	31	21	26	0.16
	Week 4	29	19	24	0.32
	Field Study Mean	*31*	*19*	*25*	*0.00*
2011	Week 1	30	18	24	0.04
	Week 2	32	18	25	0.40
	Week 3	31	19	25	0.00
	Week 4	34	24	29	0.14
	Field Study Mean	*34*	*22*	*28*	*0.21*

TABLE 19. Adult insect taxa. The presence and absence of adult insects collected throughout decomposition from the insect access (ACC) and exclusion (EXC) carcasses. The ACC taxa represent specimens collected after the insect exclusion cages were removed.

Insect Taxa			2010		2011	
			ACC	*EXC*	*ACC*	*EXC*
Diptera	Calliphoridae	*Phormia regina*	○	■	○	■
		Cochliomyia macellaria	○	■	○	■
		Lucilia coeruleiviridis	○	■	○	■
		Protophormia terraenovae			○	■
		Calliphora vicina	○	■		■
		Calliphora vomitoria				■
		Cynomya cadaverina	○	■		
	Sarcophagidae	Unknown sp.	○	■	○	■
	Tachinidae	Unknown sp.	○	■	○	■
	Muscidae	*Ophyra* spp.	○	■	○	■
		Musca spp.	○			
	Anthomyiidae	Unknown sp.	○			
	Conopidae	Unknown sp.	○			
	Piophilidae	*Piophila casei*	○	■	○	■
		Prochyliza spp.	○	■	○	■
	Phoridae	Unknown sp.	○	■		■
	Drosophilidae	Unknown sp.	○	■	○	■
	Sepsidae	*Sepsia spp.*	○	■	○	■
	Psychodidae	*Psychoda spp.*		■		
	Sciaridae	Unknown sp.	○			
	Tepididae	Unknown sp.	○			■
	Dolichopodidae	Unknown sp.		■		
	Ceratopogonidae	Unknown sp.	○		○	
	Tipulidae	Unknown sp.	○			
	Tabanidae	Unknown sp.			○	■
Coleoptera	Staphylinidae	*Platydracus maculosus*	○	■	○	■
		Creophilus maxillosus		■	○	■
		Philonthus caeruleipennis	○	■		
		Unknown sp.	○	■	○	■
Coleoptera	Trogidae	Unknown sp.		■		

TABLE 19. Continued

Insect Taxa			2010		2011	
			ACC	*EXC*	*ACC*	*EXC*
Coleoptera	Histeridae	Unknown sp.	○	■	○	■
	Anobiidae	Unknown sp.	○	■		
	Tenebrionidae	Unknown sp.	○	■		■
	Dermestidae	Unknown sp.		■		
	Mycetophagidae	Unknown sp.	○			
	Nitidulidae	Unknown sp.			○	■
	Scarabidae	Unknown sp.	○		○	■
	Curculionidae	Unknown sp.		■		
	Elateridae	Unknown sp.			○	■
	Latriidae	Unknown sp.			○	■
Hymenoptera	Vespidae	Unknown sp.	○	■	○	■
	Apidae	Unknown sp.			○	
	Ichnuemonidae	Unknown sp.	○	■	○	■
	Chalcidonidae	Unknown sp.	○	■	○	■
	Sphecid	Unknown sp.			○	
	Braconidae	Unknown sp.	○		○	■
	Halticidae	Unknown sp.	○	■	○	■
	Formicidae	Unknown sp.		■	○	■
Hemiptera	Derbidae	Unknown sp.	○	■	○	■
	Fulgoridae	Unknown sp.	○			
Lepidoptera	Nymphalidae	Unknown sp.		■		
	Papilionidae	Unknown sp.		■		
	Noctuidae	Unknown sp.		■		
	Pyralidae	Unknown sp.			○	■
Mecoptera	Meropeidae	Merope tuber	○	■	○	
Siphonatera		Unknown sp.	○	■		

2010 and 2011 field seasons combined

Carcass MMCPs

I found no significant overall carcass microbial community metabolic activity differences over decomposition but significant difference between years, and no significant interaction (TABLE 20). Overall metabolic activity was significantly higher ($P = 0.0005$) in 2011. Data from both years were combined for an NMDS to evaluate year effects on microbial functional profiles. A three-axis NMDS ordination explained 86.5% of the variation in normalized carcass microbial community metabolic activity (FIG. 21). There was a significant difference in MMCPs between years (MRPP: $T = -20.64$, $P < 0.0001$). Because year was a significant factor differentiating MMCPs, each year was subsequently analyzed separately. There were no differences in year due to the change in plate readers ($t = 0.4620$, $df = 95$, $P = 0.6452$).

Soil MMCPs

I found no significant overall carcass microbial community metabolic activity differences over decomposition but a significant difference between years, and no significant interaction (TABLE 20). Overall metabolic activity was significantly higher ($P = <0.0001$) in 2011. A two-axis NMDS ordination explained 90.2% of the variation in soil microbial community metabolic profiles (FIG. 21). There was a significant difference in normalized soil MMCPs between years (MRPP: $T = -51.88$, $P < 0.0001$). Because year was a significant factor differentiating soil MMCPs, each year was analyzed separately.

2010 field season

Carcass MMCPs

I found significant overall carcass microbial community metabolic activity over decomposition and an approaching significant difference between treatments, and a significant interaction (TABLE 21). Overall, microbial metabolic activity decreased over decomposition in ACC carcasses, however increased in EXC carcasses. Additionally,

there were significant differences over decomposition, but no significant difference between sampling region (i.e., buccal and skin), and a significant interaction (TABLE 21); buccal samples initially had an increase in microbial activity followed by a decrease while skin exhibited the inverse pattern of decreased overall microbial activity followed by increased activity as decomposition time progressed. There was also a 53.3% reduction in overall microbial community activity of the insect access compared to insect exclusion carcass microbial communities (FIG. 22). Throughout decomposition there were no significant differences in Shannon-Weaver diversity, Simpson's diversity, richness, and evenness of microbial metabolic activity between insect exclusion and access carcasses.

TABLE 20. Carcass and soil microbial community function between field seasons. Two way RM-ANOVA results testing mean carcass and mean soil microbial community metabolic activity between field seasons (Year) and over days of decomposition (Day).

	Factor	*F* test	df	*P* value
Carrion	Day	0.362	3	0.7807
	Year	13.21	1	0.0005
	Day x Year	0.872	3	0.4594
Soil	Day	1.86	3	0.5981
	Year	18.14	1	<0.0001
	Day x Year	2.210	3	0.5458

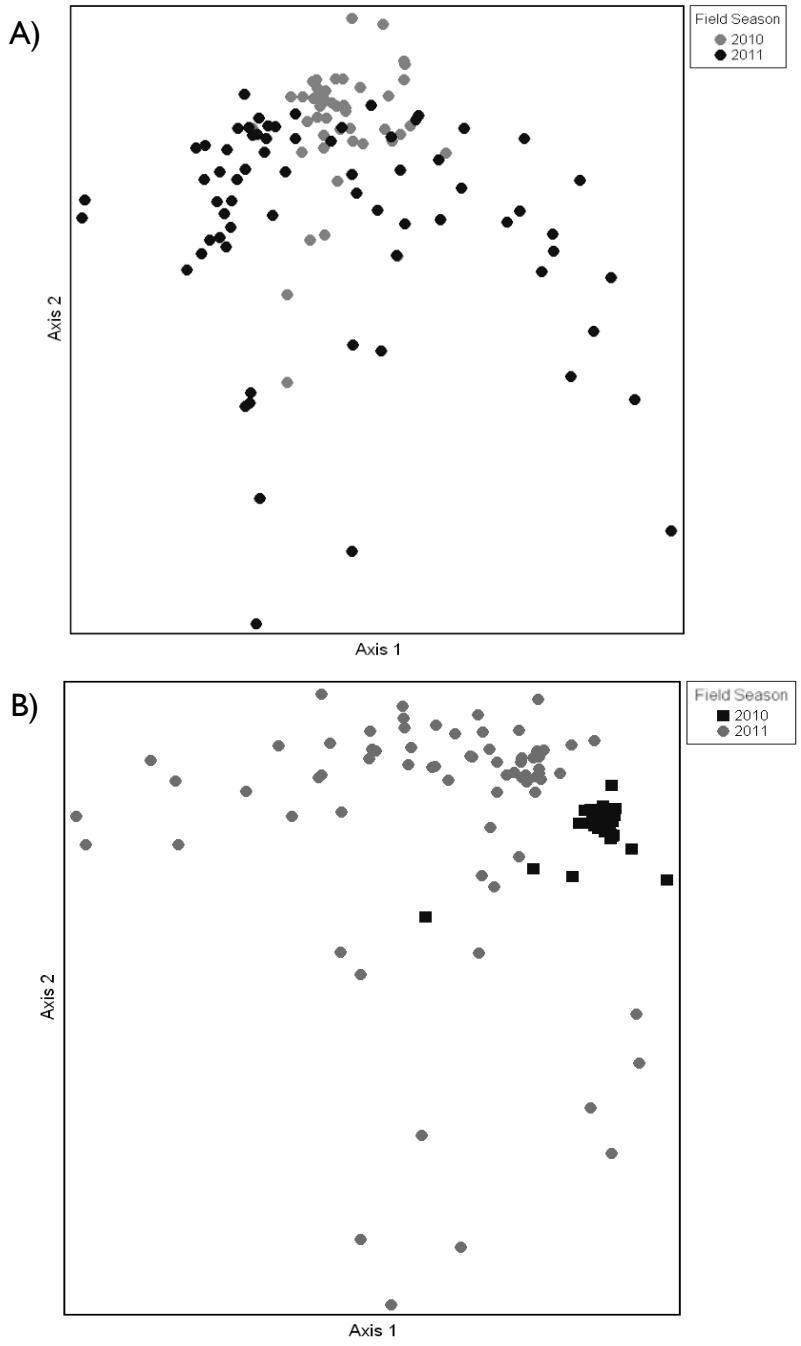

FIG. 21. NMDS ordination of (A) normalized carcass microbial community activity from both 2010 and 2011 field season with year overlay. Total stress was 13.24. Axis 1 explained 35.6% of the variation among communities, while axis 2 explained 39.0% and axis 3 explained 11.9% for a total of 86.5% the variation explained by this ordination, and (B) normalized soil microbial community activity from both 2010 and 2011 field season with year overlay. Total stress was 11.53. Axis 1 explained 51.8% of the variation among communities and axis 2 explained 38.4% for a total of 90.2% the variation explained by this ordination.

A three-axis NMDS ordination explained 87.7% of the variation in carcass MMCPs (FIG. 23; TABLE 22). There was not a significant difference between insect exclusion and control carcass microbial community metabolic activity (MRPP: T = -1.22, P = 0.1161), thus data from each treatment were pooled for further analysis. Pair-wise comparisons indicated significant differences in MMCPs between the initial day (Day 0) and each subsequent day of decomposition (Days 1, 3 and 5), thus demonstrating significant microbial community metabolic change later in decomposition (TABLE 23). The MMCPs between sampling regions (buccal vs. skin) were significantly different (MRPP: T = -6.74, $P < 0.0001$), while the metabolic profiles of two carcasses (D and E) were also significantly different (MRPP: T = -0.17, P = 0.0209) (TABLE 23).

Using indicator species analysis I identified carbon sources that differed in use between treatments. The compounds α-D-Lactose, glycogen, D,L-α-glycerol phosphate were significant indicator carbon substrates for insect exclusion carcass microbial community metabolic activity; itaconic acid, 2-hydroxy benzoic acid and α-ketobutyric acid were significant indicators of day 0; three carbon substrates (L-asparagine, D-malic acid, and L-arginine) were significant indicators of the buccal communities; and i-eryhtritol was a significant indicator of carcass E (TABLE 24). There were no other significant carbon substrate indicators among days of decomposition or replicate carcasses.

Two carbon substrates (itaconic acid and putrescine) explained 54.7% of the variation in ADH (FIG. 24) and were associated with specific time points of decomposition based on the random forest and GAM models. An increased use of both carbon substrates indicated early stages of decomposition (low ADH) while low use predicted later stages of decomposition (high ADH). These data were consistent with the indicator species analysis results with itaconic acid predicting early stages of decomposition.

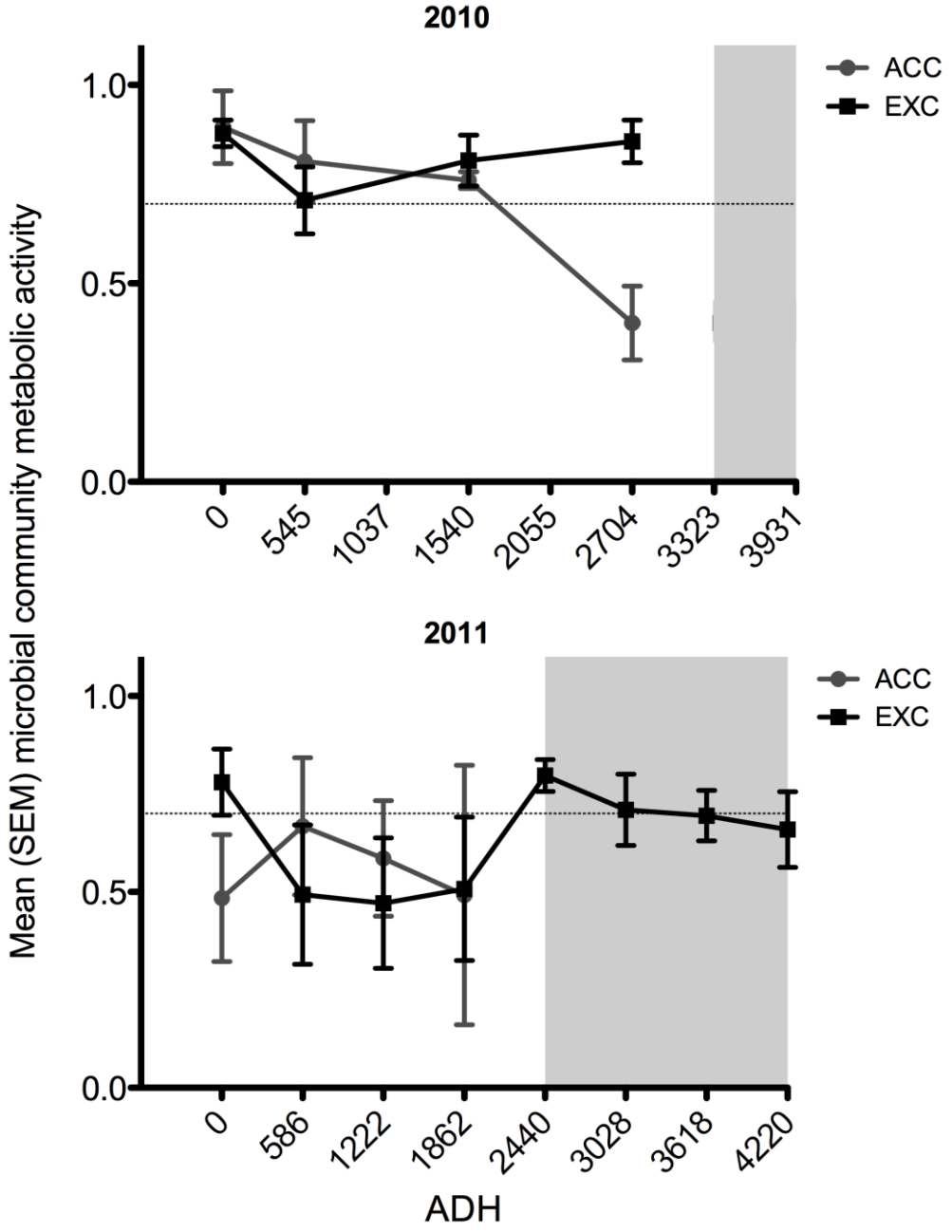

FIG. 22. Carcass microbial community activity over decomposition time (ADH) in 2010 and 2011. Mean (SEM) microbial community metabolic activity between insect exclusion (EXC) and access (ACC) carcasses over accumulated degree hours (ADH). The gray boxes indicate when carcasses were not sampled due to advanced stages of decomposition with the buccal and skin areas no longer clearly distinguishable.

TABLE 21. Carcass microbial community function. Two way RM-ANOVA results testing mean carcass microbial community metabolic activity between insect exclusion and access carcasses (Treatment) over days of decomposition (Day), and between buccal and skin sampling regions (Region) and composite samples over decomposition day in 2010 and 2011.

Year	Factor	*F* test	df	*P* value
2010	Day	4.128	3	0.0122
	Treatment	3.565	1	0.0663
	Day x Treatment	5.634	3	0.0026
2010	Day	3.535	3	0.0231
	Region	0.9301	1	0.3406
	Day x Region	3.617	3	0.0211
2011	Day	0.2118	3	0.8876
	Treatment	0.0021	1	0.9636
	Day x Treatment	0.6763	3	0.5723
2011	Day	0.4363	3	0.7284
	Region	95.72	1	<0.0001
	Day x Region	0.8699	3	0.4656
2011 Composite	Day	0.3607	1	0.5593
	Region	34.35	5	<0.0001
	Day x Region	5.537	5	0.0072

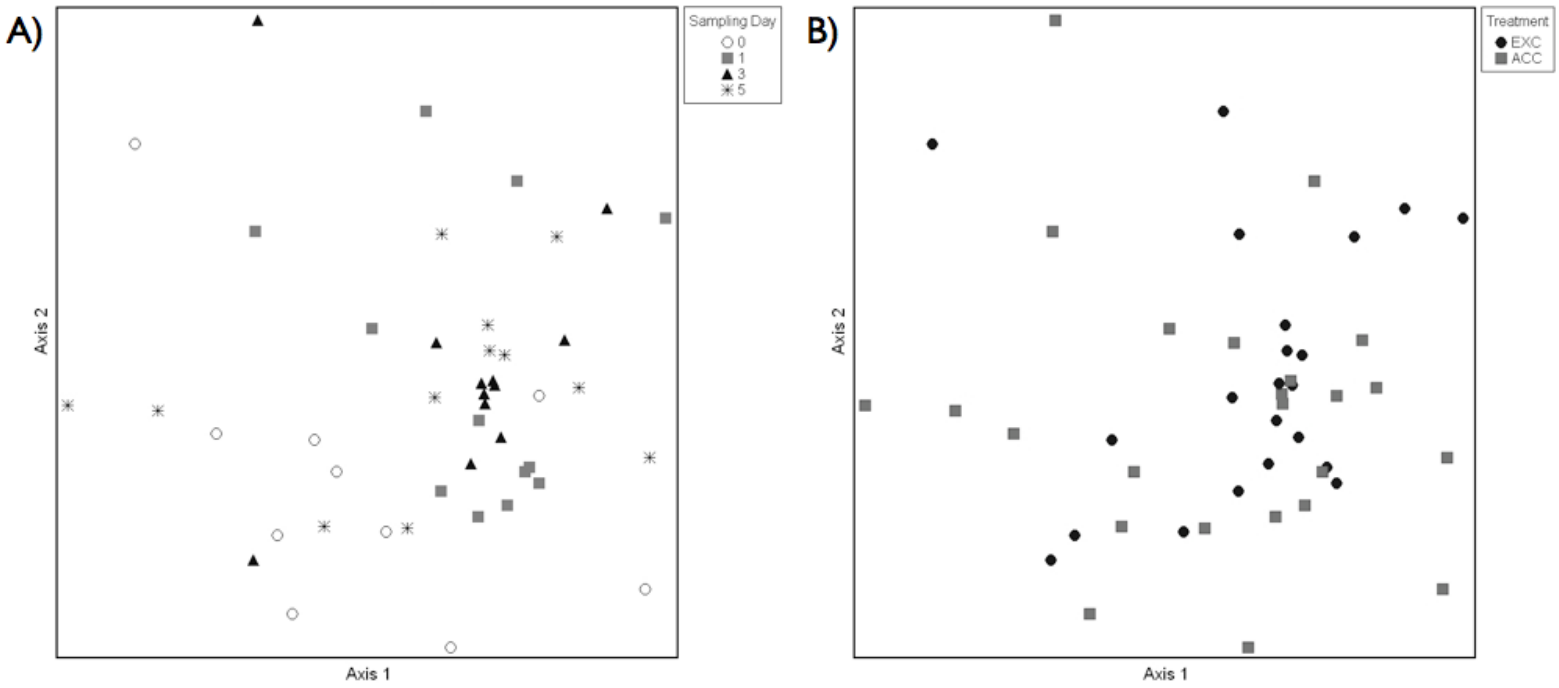

FIG. 23. NMDS ordination of normalized carcass microbial community activity from 2010 with (A) sampling day and (B) insect access (ACC) and exclusion (EXC) overlay. Total stress was 13.07. Axis 1 explained 41.9% of the variation among communities, while axis 2 explained 25.7% and axis 3 explained 20.1% for a total of 87.7% the variation explained by this ordination.

TABLE 22. NMDS ordination statistics of normalized microbial community metabolic activity. The stress and percent variation explained in community metabolic activity (total and by each axis) is given for each year and for carcass or soil microbial communities.

			Percent variation explained			
Year	Carcass/Soil	Stress	Total	Axis 1	Axis 2	Axis 3
2010	Carcass	13.07	87.7	41.9	25.7	20.1
2011	Carcass	11.03	88.6	36.7	26.4	25.4
2011	Soil	13.28	91.0	57.3	33.7	-

TABLE 23. Summary statistics for MRPP of normalized carcass microbial activity for 2010 and 2011 between microbial communities of ACC and EXC carcasses, across decomposition day, between sampling region (buccal and skin), and among carcass replicates. All pair-wise comparisons were significantly different at $\alpha = 0.0167$, after Bonferroni correction, and are indicated with an asterisk (*).

		δ under null hypothesis						
		Observed δ	Expected	Variance	Skewness	*T*	*p*	*A*
2010	EXC vs. ACC	0.480	0.483	0.60E-05	-1.11	-1.22	0.1161	0.006
	Day	0.464	0.483	0.19E-04	-0.63	-4.28	0.0006	0.038
	0 vs. 1					-3.55	0.0065*	0.044
	0 vs. 3					-4.79	0.0012*	0.061
	0 vs. 5					-3.35	0.0077*	0.037
	Buccal vs. Skin	0.466	0.483	0.59E-05	-1.11	-6.74	<0.0001	0.034
	Carcass	0.482	0.483	0.33E-04	-0.47	-0.17	0.4028	0.002
	D vs. E					-2.50	0.0209*	0.048

TABLE 23. Continued

		δ under null hypothesis						
		Observed δ	Expected	Variance	Skewness	*T*	*p*	*A*
2011	EXC vs. ACC	0.685	0.683	0.86E-05	-1.55	0.728	0.7475	-0.003
	Day	0.681	0.683	0.67E-04	-0.54	-0.30	0.3529	0.004
	0 vs. 4					-2.20	0.0381	0.052
	Buccal vs. Skin	0.680	0.683	0.82E-05	-1.57	-1.29	0.1043	0.006
	Carcass	0.674	0.683	0.46E-04	-0.65	-1.42	0.0881	0.014
	I vs. K					-2.37	0.0315	0.035
	I vs. L					-2.63	0.0217	0.065
	J vs. K					-2.95	0.0157*	0.029

TABLE 24. Results from ISA for 2010 and 2011 normalized carcass microbial activity. The carbon source is given along with the indicator value and p value for the respective group. All pair-wise corrections that are significantly different using $\alpha = 0.0167$, $\alpha = 0.0071$ and $\alpha = 0.0125$ after Bonferroni correction for multiple pair-wise comparisons of treatment, day and sampling region, respectively, during 2010 and $\alpha = 0.0056$ during 2011, are indicated with an asterisk (*).

	Group	Carbon Source	Indicator Value	Mean	Std. Dev.	*p*
2010	EXC	α-D-Lactose	69.9	45.5	5.12	0.0004*
	EXC	Glycogen	60.7	52.4	3.36	0.0158*
	EXC	D,L-α-Glycerol Phosphate	58.3	48.7	4.03	0.0252*
	Day 0	2-Hydroxy Benzoic Acid	53.6	18.8	6.08	0.0004*
	Day 0	Itaconic Acid	54.8	26.6	5.76	0.0010*
	Day 0	α-Ketobutyric Acid	53.1	26.1	7.04	0.0022*
	Day 0	4-Hydroxy Benzoic Acid	39.0	24.2	5.10	0.0110
	Day 0	L-Threonine	39.3	27.6	5.90	0.0436
	Day 1	Putrescine	41.7	29.1	4.62	0.0118
	Day 5	D-Xylose	44.1	32.5	6.04	0.0500
	Buccal	L-Asparagine	68.8	49.5	4.68	0.0006*
	Buccal	D-Malic Acid	62.5	47.2	4.61	0.0052*
	Buccal	L-Arginine	56.6	35.5	5.91	0.0038*
	Buccal	4-Hydroxy Benzoic Acid	47.2	36.1	5.50	0.0454
	Carcass E	i-Erythritol	31.0	22.9	3.74	0.0322*
2011	EXC	D-Malic Acid	52.8	38.9	7.61	0.0478
	Day 6	L-Arginine	29.3	18.2	4.63	0.0228
	Carcass H	D-Galacturonic Acid	74.1	36.1	11.19	0.0056
	Carcass H	Glycogen	66.2	41.1	11.42	0.0306
	Carcass H	D-Malic Acid	69.5	30.3	10.57	0.0030*
	Carcass H	λ-Hydroxybutyric Acid	66.7	28.7	10.29	0.0032*
	Carcass H	α-D-Lactose	62.7	29.5	9.78	0.0044*
	Carcass H	DL-α-Glycerol Phosphate	61.6	39.0	10.26	0.0286
	Carcass H	α-Ketobutyric Acid	60.8	34.4	10.23	0.0204
	Carcass H	2-Hydroxybenzoic Acid	56.9	29.5	9.24	0.0104
	Carcass H	L-Asparagine	46.0	26.0	7.19	0.0160

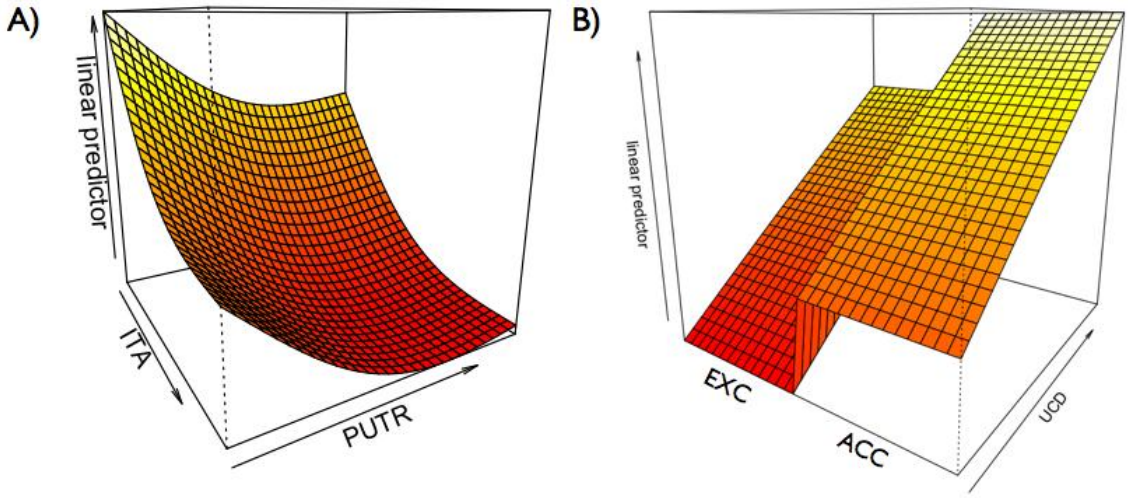

FIG. 24. Generalized additive models predicting ADH using carbon substrates identified using random forest models. (A) Random forest analysis identified itaconic acid and putrescine that explained 18.9% of deviance in the data when predicting ADH based on normalized carcass microbial community activity in 2010. Generalized additive models were then used to predict ADH based on the carbons identified in Random Forest; each carbon was identified as a linear predictor, and explained 54.7% of the variation in the data. (B) Random forest analysis identified a carboxylic acid and an amine that explained 18.9% of deviance in the data when predicting ADH from normalized carcass microbial activity in 2011. Treatment (ACC vs. EXC) was determined to have a significant effect (P = 0.0033) on this relationship in 2011. Generalized additive models were then used to predict ADH based on the carbons identified in Random Forest; each carbon was identified as a linear predictor, and explained 45.9% of the variation in the data.

TABLE 25. Soil microbial community function. Two way RM-ANOVA results testing mean soil microbial community metabolic activity between insect exclusion and access carcasses (Treatment) over days of decomposition (Day), and the mean soil microbial metabolic activity between sampling areas (under the body and the control soil) (Area) over days of decomposition.

Field Season	Factor	*F* test	df	*P* value
2010 Carcass	Day	0.0416	3	0.9885
	Treatment	0.0067	1	0.9352
	Day x treatment	0.1026	3	0.9580
2010 Soil	Day	0.6769	3	0.5716
	Area	0.0015	1	0.9689
	Day x area	0.1906	3	0.9021
2011 Carcass	Day	10.27	3	<0.0001
	Treatment	0.1006	1	0.7576
	Day x treatment	0.7387	3	0.5373
2011 Soil	Day	7.087	7	<0.0001
	Area	1.218	1	0.2745
	Day x area	1.282	7	0.2759

Soil MMCPs

I found no significant overall soil microbial community metabolic differences over decomposition time, between treatments and no interaction (TABLE 25). There were also no significant differences between soil sampling areas, over decomposition time and no interaction based on overall soil microbial community metabolic activity (TABLE 25). On each date there were no significant treatment differences in Shannon-Weaver diversity, Simpson's diversity, richness, and evenness based on soil microbial metabolic activity. It was not possible to find a stable NMDS ordination using the normalized soil microbial community activity.

2011 field season

Carcass MMCPs

I found no significant overall differences in carcass microbial metabolic activity over decomposition, between treatments nor was there a significant interaction (TABLE 21). I also found a significant difference in microbial metabolic activity between the buccal and skin communities and the composite sample but there was no significant difference over decomposition and no significant interaction (TABLE 21). Overall microbial activity was greater in buccal communities in comparison to skin communities. There were no significant differences of carcass microbial metabolic activity described by Shannon-Weaver diversity, Simpson's diversity, richness, and evenness over decomposition.

A three-axis NMDS ordination explained 88.4% of the variation in carcass microbial community metabolic profiles (FIG. 25; TABLE 24). There were no microbial metabolic profile differences between insect exclusion and access carcasses (MRPP: T = 0.74, P = 0.7475), thus data were pooled for further analysis. There was a significant pair-wise MMCP difference between Day 0 and decomposition Day 4 (MRPP: T = -0.30, P = 0.0381), while buccal and skin communities were not significantly different (MRPP: T = -1.29, P = 0.1043) (TABLE 23). The MMCPs of the J and K carcasses were significantly different (MRPP: T = -1.42, P = 0.0157) (TABLE 23).

D-malic acid was a significant indicator of EXC carcasses, which did not correspond to any of the carboxylic acid predictors from 2010. L-arginine was a significant indicator of day 6, which also different from 2010; and three carbons (D-malic acid, λ-hydroxybutyric acid, and α-D-lactose) were significant indicators of carcass H (TABLE 24). There were no other significant carbon substrates indicators among days of decomposition or replicate carcasses.

Compared to 2010 there were four different carbon substrates (α-cyclodextrin, 2-hydroxy benzoic acid, i-erythritol, and L-serine) that predicted ADH, in addition to treatment having a significant effect (P = 0.0033), which accounted for 45.9% of the variation using random forest and GAM models (FIG. 24). These data demonstrate that as ADH increases (and decomposition simultaneously progresses) there is significantly lower carbon utilization activity for EXC carcasses based on the four important carbons predicted using random forest.

Soil MMCPs

Overall soil microbial community metabolic activity was significantly different over decomposition time while there was no significant difference between insect exclusion and access treatments and there was not a significant interaction (TABLE 25). Microbial activity decreased on the second day of decomposition and then increased throughout the remainder of decomposition. I did not find significant overall soil microbial community metabolic differences between sampling areas (under the carcass vs. 1 m soil) or an interaction, however there was a significant difference over decomposition time (TABLE 25); overall microbial activity followed a similar pattern as treatment differences over decomposition with a decrease on the second day of decomposition and then increased throughout the remainder of decomposition. Throughout decomposition there were no significant differences in Shannon-Weaver diversity, Simpson's diversity or richness between treatment and control community metabolic activity; however, there was a weak significant difference in evenness.

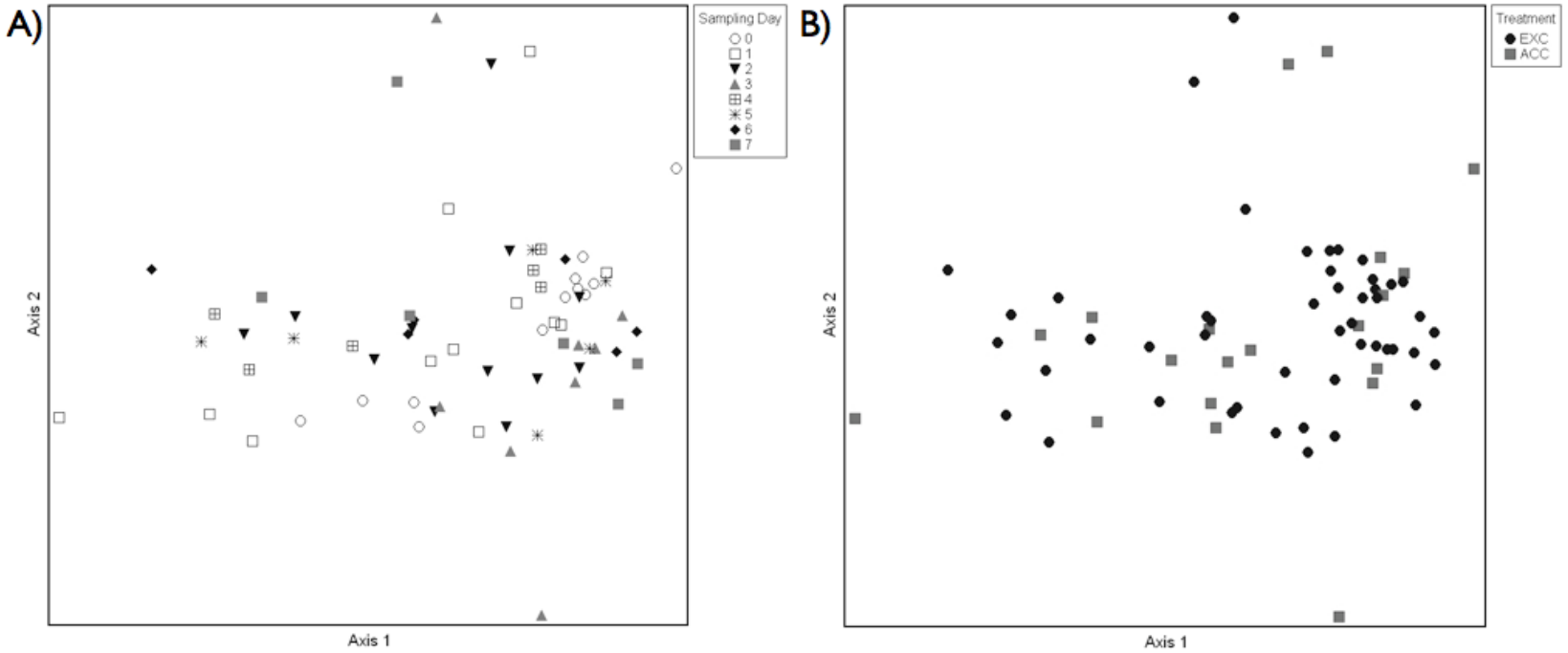

FIG. 25. NMDS ordination of normalized carcass microbial community activity from 2011 with (A) sampling day and (B) insect access (ACC) and exclusion (EXC) overlay. Total stress was 11.03. Axis 1 explained 36.7% of the variation among communities, while axis 2 explained 26.4% and axis 3 explained 25.4% for a total of 88.6% the variation explained by this ordination.

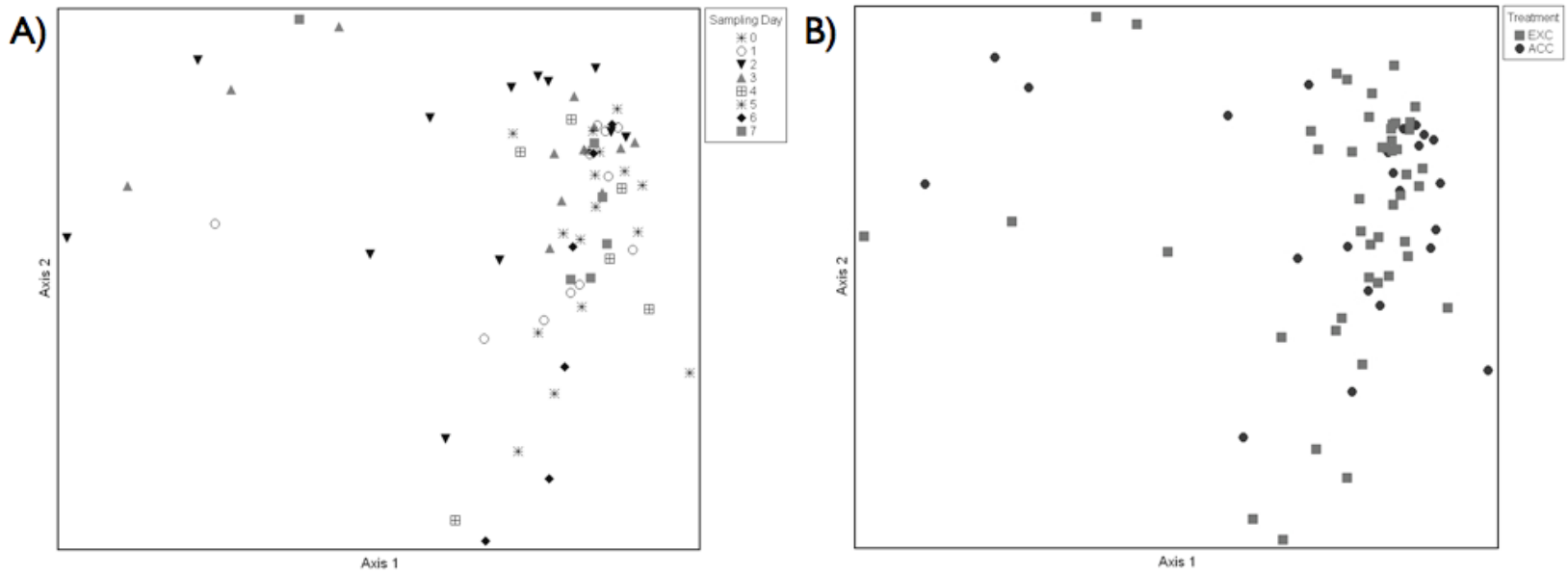

FIG. 26. NMDS ordination of normalized carcass soil community activity from 2011 with (A) sampling day and (B) insect access (ACC) and exclusion (EXC) overlay. Total stress was 13.28. Axis 1 explained 57.3% of the variation among communities, while axis 2 explained 33.7% for a total of 91.0% the variation explained by this ordination.

TABLE 26. Summary statistics for MRPP of normalized soil microbial activity for 2011 between microbial communities of ACC and EXC carcasses, across decomposition day, between sampling area (soil from under the carcass and 1 m away soil), and among carcass replicates. All pair-wise comparisons were significantly different at $\alpha = 0.0167$, after Bonferroni correction, and are indicated with an asterisk (*).

	δ under null hypothesis						
	Observed δ	**Expected**	**Variance**	**Skewness**	***T***	***p***	***A***
EXC vs. ACC	0.559	0.557	0.67E-05	-1.52	0.490	0.6193	-0.002
Day	0.545	0.557	0.52E-04	-0.57	-1.68	0.0596	0.004
0 vs. 4					-3.49	0.0057	0.040
0 vs. 3					-2.82	0.0153	0.039
2 vs. 5					-1.94	0.0467	0.035
2 vs. 6					-2.80	0.0403	0.041
Buccal vs. Skin	0.553	0.557	0.67E-05	-1.54	-1.55	0.0784	0.007
Carcass	0.549	0.557	0.36E-04	-0.68	-1.36	0.0957	0.015
I vs. K					-4.56	0.0027	0.050
J vs. K					-2.32	0.0329	0.026

TABLE 27. Results from ISA for 2011 normalized soil microbial activity. The carbon source is given along with the indicator value and p value for the respective group. All pair-wise corrections that are significantly different using $\alpha = 0.0070$ after Bonferroni correction for multiple pair-wise comparisons of carcass are indicated with an asterisk (*).

Group	Carbon Source	Indicator Value	Mean	Std. Dev.	*p*
EXC	L-Serine	64.5	52.3	5.70	0.0300
Day 6	Putrescine	48.4	24.6	5.93	0.0032*
Under body	D-Malic Acid	54.2	40.8	5.70	0.0302

A two-axis NMDS ordination explained 91.1% of the variation for normalized soil microbial community metabolic profiles (TABLE 22). There was a significant difference in MCPPs between soil underneath compared to away from the carcass (MRPP: T = -1.55, $P = 0.0072$); however, there was no significant difference between soil communities of insect exclusion and access carcasses (MRPP: T = 0.49, $P > 0.05$), among decomposition days (MRPP: T = -1.68, $P > 0.05$) or among replicate carcasses (MRPP: T = -1.36, $P > 0.05$) (TABLE 26). Lastly, putrescine indicated Day 6 and D-malic acid indicated soil communities under the carcass (TABLE 27).

Discussion

I documented variability in microbial community function, as determined by carbon utilization, during decomposition in both samples taken directly from carcasses and carrion associated soils. Decomposition could be differentiated in both carcass and soil samples and insects appear to be reducing microbial community over decomposition. However, these trends were not consistent between years.

The lack of similarities between years may result from various abiotic (e.g., temperature) and biotic factors (e.g., pre-existing microbial communities). Abiotic factors including temperature (as I have defined using ADH, which was greater for 2011) may account for microbial community function variation between years and

throughout decomposition. Ambient temperatures were up to 18% higher in 2011. Previous studies, however, have reported conflicting microbial species response to changing temperature. For instance, soil bacteria abundance increased with temperature (+3°C) in the presence of elevated carbon dioxide, but decreased under conditions of similar temperature conditions without elevated carbon dioxide (Castro et al. 2010). Also, soil microbial functional responses have been directly correlated with temperature when there was higher carbon availability in soil for respiration processes (Zogg et al. 1997). Therefore during 2011, higher average temperature may have been associated with increased available carbon and overall activity of the carcass microbial communities regardless of treatment. Rising ambient temperatures may have also contributed to an increased respiration rate of microbial species thus increasing the overall metabolic activity at the time of sampling, which has been reported in other decomposition systems (Witkamp 1966, Toljander et al. 2006, Heimann and Reichstein 2008). Finally, the variation may be associated with other parameters or interactions (e.g., pH changes occurring as part of the carrion or soil) that were not assessed during this study (Salt 1979).

Microbial assemblages are ubiquitous and important to many ecosystem processes (Baas Becking 1934, Hooper et al. 2005). Microbes are known as pathogens, mutualists, commensalists, a food source, and more commonly as decomposers within communities (Cochran-Starifa and Ende 1998, Burkepile et al. 2006). Fungi and bacteria have been documented as facilitators of the initial decomposition processes of carrion (Jiron and Cartin 1981, Burkepile et al. 2006). The inherent stochastic spatial and temporal nature of microbial communities on ephemeral resources, such as carrion, may contribute to the variation both within and across carcasses (Ramette and Tiedje 2007). I demonstrated that microbial community function was more highly variable within a carcass compared to across carcasses as indicated by the functional profile changes over decomposition days than between carcasses (insect access compared to insect exclusion carcasses). My results correspond with a study of bacterial succession patterns on humans using high-throughput sequencing (i.e., pyrosequencing), which demonstrated

significantly less variation in microbial diversity in communities sampled 24 h apart compared to 3 months apart (Costello et al. 2009). Therefore, changes in functional profiles may result from variation of microbial community structure throughout the decomposition process.

Vertebrate decomposition releases carbohydrates, fats, amino acids, minerals and water for microbial growth (Dent et al. 2004). The increased microbial growth on a resource has implications for food chains with bottom-up effects demonstrated to be driven by microbial communities (Strickland et al. 2009). The proliferation of microbes can affect the community ecology of carrion decomposition. For instance, microbial community change may alter the resource in a way that attracts or repels specific consumers, influencing secondary consumer colonization and succession. Microbes such *as Clostridium botulinum*, *E. coli, Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella enterica enterica,* serovar Typhi produce toxins (e.g., botulism) that deter vertebrate scavengers from consuming carrion (Janzen 1977). In marine systems, carrion was scavenged 66% of the time when microbial communities were initially allowed to proliferate undisturbed, compared to 89% scavenging without mature microbial communities (Barlocher 1979, Burkepile et al. 2006).

Competition for ephemeral resources between micro- and macroorganisms is well documented. *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) and fungi (*Aspergillus* spp.) interact on fruit, with larval mortality correlating with the age and species of fungi present on the resource (Trienens et al. 2010). Microbe and insect interactions have been documented in other habitats with varying results on organic matter processing. Specifically, leaf litter decomposition studies have reported substantial variation in the influence of substrate type and microbial and insect communities on decomposition rates. For example, microbial and insect communities have been shown to facilitate nutrient cycling and decomposition rates in leaf litter systems (Hieber and Gessner 2002, Srivastava et al. 2009). Alternatively, other studies have demonstrated that leaf litter composition drives decomposition rates with little or no influence of microbial and insect communities (Kominoski et al. 2011). My goal was

to test these microbe-insect interactions using a carrion system, which has never been investigated to date.

My data would suggest that carcasses exposed to insects have a decreased microbial community function. Arthropod community structure on carrion over time has often been described as a process of competition between species for a resource (Norris 1965, Hanski and Kuusela 1977). Studies examining this process primarily focus on the "observable" data that can be collected, with little regard for what might be occurring at the microbial scale. Primary colonizers utilize the resource as nutrition, mating or an oviposition site. Subsequent larval development may disrupt established microbial communities through direct or indirect competitive interactions on the carcass. It is known that insects facilitate decomposition of carrion (Payne 1965, Simmons et al. 2010a, Simmons et al. 2010b). Blow flies may directly impact microbial species through chemical secretions while consuming carrion tissue (Sherman et al. 2000, Mumcuoglu et al. 2001). Arthropods arriving to colonize carrion could introduce their own exogenous microbial community (Nayduch et al. 2002). *Musca domestica* L. (Diptera: Muscidae) carries over 100 known pathogens including *E. coli* 0157:H7 (Greenberg and Klowden 1972, Alam and Zurek 2004). Blow flies arriving at a resource can mechanically vector pathogens (Asgari et al. 1998, Conn et al. 2007). The introduction of insect associated microbial communities may influence carrion microbial community function through microbially-mediated competitive mechanism, which alters the metacommunity dynamics and biogeography of microbial communities in the landscape (Jones and McMahon 2009, Langenheder and Szekely 2011).

Primary colonizers utilizing a carcass may suppress microbial community function. Disturbances of natural communities have also been proposed as a mechanism to promote species coexistence and an influence on community assembly (Hutchinson 1961, Horn 1974, Kuusela and Hanski 1982). I define a disturbance event in this study as the utilization, whether by nutrient acquisition or oviposition with subsequent larval development site, of carrion by insect colonizers (i.e., blow flies). The undisturbed carcass microbial communities showed increased overall carbon resource utilization,

which may result from an increase in prokaryotic cell proliferation, or reduced consumption by blow fly larvae. Alternatively, specific microbes or functional groups may be outcompeting other species during later stages of undisturbed decomposition, which has been found in other systems (Setälä and McLean 2004, Hattenschwiler et al. 2005). The undisturbed carcasses displayed a similar trend during both field seasons with an initial decline in activity followed by a steady increase of microbial community function throughout the remainder of decomposition when the carcasses were excluded from insect access. To verify the role of insects on microbial function, once the insect exclusion cages were removed after the fifth day of decomposition there was a continuation of sampling the insect exclusion carcasses until they no longer had defined buccal or skin areas during the 2011 field season. There was an immediate effect on the microbial community function in the presence of insects as seen by a steady decline in microbial activity throughout the remainder of decomposition. Also, undisturbed vernal rain pools produced significantly higher species richness of protozoan and metazoan species (McGrady-Steed and Morin 1996) and higher protozoan and rotifer richness in undisturbed artificial container communities of rain (Kneitel and Chase 2004, Kneitel and Perrault 2006). It is unknown at this time whether or not there is a difference of species composition or functional groups based on taxonomic identification of bacteria, but there is an overall increase in microbial activity for the undisturbed carrion.

Carrion decomposition influences the surrounding areas and alters the soil chemistry and soil microbial communities (Carter and Tibbett 2006, Carter et al. 2007, Hopkins 2008, Stokes et al. 2009). My results only demonstrated a significant change of microbial community function over decomposition time in the second field season. The lack of variation among soil communities could relate to the influence of the microbial communities already established in the environment. As decomposition progresses, the functional responses of the microbial communities may remain similar because the environment microbial fauna may be acquired equally by all carcasses and remain stable within the soil. Additionally, Carcasses in the field season were a smaller size (based on weight) and thus decomposed 43% (ACC carcasses) and 10% (EXC carcasses) faster in

2011 when compared to 2010. The presumed increased rate of nutrient reintroduction into the soil may have facilitated an increase in soil associated microbial activity. Increased microbial activity resulting from the nutrient surge may have contributed to the differentiation of sampling days based on function. However, the lack of differences in soil activity has been previously documented. Microbial community structure of soil beneath rat carcasses were not significantly different until week 2 (< 7 days) of decomposition base on lipid-phosphate and fatty acid methyl ester analysis (Maile 2011). Similarly, increased microbial biomass in soil 28 days after the introduction of cicada carcasses was reported (Yang 2004). Group I lipase gene copy number in soils samples collected beneath swine carcasses, which are found in lipolytic bacteria such as *Acinetobacter*, increased with calliphorid larvae activity on the carcass and decreased after dispersal of the larvae (Howard et al. 2010). The introduction of mouse (*Mus musculus* L. (Rodentia: Muridae)) carrion significantly increased carbon dioxide respiration rates in soil with a peak of microbial activity occurring within the first 10 days (Stokes et al. 2009). Thus the carbons I used during this study may not provide effective discriminatory power over such a short range of decomposition time. Future studies should compare functional approaches with microbial community structure (species identification and species abundance) to better assess structure-function relationships of carrion processes.

The last objective of this study was to determine if there were any carbons that would be good predictors of decomposition time as defined by ADH. The first year had two carbons, itaconic acid and putrescine, that were able to explain 54.7% of the variation in the data. Itaconic acid can be produced by *Aspergillus terreus* (Larsen and Eimhjellen 1955). Since microbial community taxa identification was not the focus of this study, fungi may be present on the carrion and producing this compound. It should be noted that *A. terreus* has demonstrated an insecticidal effect against *Lucilia cuprina* first-instar larvae by inhibiting growth by approximately 15% (Blaney et al. 1985) Putrescine has been found during the active decay stage of swine carrion (Dekeirsschieter et al. 2009) and cadaver decomposition (Statheropoulos et al. 2005).

Dipteran species are attracted to putrescine (Leblanc et al. 2010). Thus, blow flies may be utilizing putrescine as long-distant cues for locating the carcass. My results indicate that these two carbons may be candidates to differentiate decomposition time in the presence or absence of insect colonizers (e.g., blow flies), and future work associated with these specific carbons could help elucidate the microbe-insect interactions during decomposition.

Overall, empirical data are sparse within the microbe-insect-carrion model in terrestrial ecosystems. I have demonstrated that insects may have cascading effects on decomposition by mediating microbial function. It is important to further investigate the role of microbes and their importance in determining underlying mechanisms controlling community assembly, biomass turnover and nutrient cycling of ephemeral resources. Furthermore, empirical data collected from well-designed experiments in decomposition ecology has been advocated for the enhancement of forensic sciences (Tomberlin et al. 2011b). As part of forensics, decedents represent a single entity of carrion within an ecosystem and in application, microbial communities may be another component to consider when making predications about how long a body has been decomposing. Tomberlin *et al*. proposed a new framework in decomposition ecology that divided the decomposition process of a resource into a pre-colonization interval and a post-colonization interval (Tomberlin et al. 2011a). There is much known about the post-colonization interval (e.g., necrophagous arthropod succession patterns); however, the pre-colonization interval is still in its infancy and there is sparse amount of information available about the changes of a body that make it a suitable resource for insects. Thus, understanding this process with terrestrial vertebrate carrion has application within the fields of forensics.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Discussion

Community structure on carrion over time has often been described as a process of competition between species for a resource (Janzen 1977, Polis and Strong 1996, DeVault et al. 2003). Studies examining this process primarily focus on the "observable" data that can be collected with little regard for what might be occurring at the microscopic level. Despite our understanding of decomposition rates under specific environmental conditions, the dynamics of microbe and blow fly (Diptera: Calliphoridae) communities and how they influence decomposition have not been adequately explored. Interactions between blow flies and microbial species possibly facilitate subsequent arthropod succession patterns on carrion, therefore, indirectly influencing decomposition rates and associated reintroduction of nutrients back into the environment (Hocking and Reimchen 2006).

My results document insect community assembly after a delayed colonization period and the bacterial community structure and function in the presence and absence of necrophagous insects throughout decomposition. Shifts in insect community arrival patterns and composition after delayed colonization suggests different cues are being emitted from the microbial community associated with the resource when insects have access to the remains. Thus, bacterial species may be mediating insect arrival patterns to a resource. Additionally, subsequent utilization of the remains by insects appears to be governing the resulting microbial community. This research provides a foundation for the ecology of how necrophagous insect and microbial interactions, including coexistence and community assembly, occur on decomposing carrion.

Immediately after death a succession of organisms occurs on the resource to a climax community state (Horn 1974, Yang et al. 2008), which is defined as a stable composition of species within an environment although the localized individual species

abundances may fluctuate (Horn 1974). Aggregation models have been proposed to describe how competitors (e.g., blow flies and microbes) coexist on resource pulses (Woodcock et al. 2002). This demonstration of coexistence can be explained by aggregations of individual species consuming a resource patch and not others thus providing opportunities for another species (Hartley and Shorrocks 2002, Abos et al. 2006). Coexistence of blow flies on carrion resources is common (Hanski 1987, Kouki and Hanski 1995, Woodcock et al. 2002). However, my results demonstrated the impact of delayed colonization on insect colonization, which caused a shift in species utilizing the remains. Interactions between blow flies and microbial species possibly facilitate subsequent arthropod succession patterns on carrion, therefore, indirectly influencing decomposition rates and associated nutrient cycling. My data correlates with previous research indicating the effect of arrival patterns influenced by initial insect colonization (Spivak et al. 1991). Early colonization may allow for better access to and acquisition of nutrients on fresh carrion for some consumers, while other may require some degree of resource processing by these pioneer communities before consumption as seen in marine insects (Barlocher 1979, Burkepile et al. 2006). A waste by-product of dipteran larvae is ammonia, which increases the pH of the surrounding area and inhibits the growth of many bacteria species while providing favorable conditions for proteolytic enzyme activity (Nigam et al 2006; Beasley & Hirst 2004). Thus the increased number of blow fly larvae utilizing the remains may have altered the microbial communities and lead to one insect succession pattern. While the undisturbed resource had a different microbial community present by the time the insects had access to the remains and demonstrated an alternative insect succession pattern.

Microbial organisms are ubiquitous in the environment, and their role in community structure on ephemeral resources is unknown and underestimated (Finn 2001). Microbes associated with the exposed surfaces of carcasses may play a vital role in succession patterns and inter- and intraspecific interactions of blow flies associated with decomposing remains. Necrophagous insects arriving at a resource might be vectoring their own endogenous fauna and transporting microbes present on the carcass

to other carcasses or similar resources. Conversely, I documented a reduced richness of bacterial taxa in the presence of insects, which indicates the inverts are either directly (e.g., consumption) or indirectly (e.g., larval excretions) modifying the bacterial community. Thus, affecting trophic interactions occurring during decomposition and the metacommunity dynamics and biogeography of microbial communities in the landscape.

Bacterial taxa distribution is important to consider because sampling time at discrete intervals provides a snapshot of the microbial community at that time and may miss rare taxa events throughout decomposition. Rare species can be difficult to isolate in large ecological surveys but may influence how specific processes, in this instance decomposition, are occurring. Based on the microbial fauna identified by high-throughput sequencing, humans maintain a high level of species diversity and richness (Claesson et al. 2009, Mahowald et al. 2009, Petrosino et al. 2009, Price et al. 2009, The Human Microbiome Jumpstart Reference Strains Consortium 2010, Turnbaugh et al. 2010). Detection of microbial communities on living patients implies that there is a diverse microbial fauna pre-existing on cadavers, which may be important in determining the variation of insect colonization and decomposition rates.

Ecologically, functional responses by the microbial communities have allowed a better understanding of natural groupings of microbial species present throughout the decomposition process. The utilization of the selected carbon sources by the microbial community created a profile that was used to determine microbial community function (Weber and Legge 2010). However, there were inconsistencies between the microbial responses across field seasons. The inherent variation of microbial community function was considerable in this study, and may be associated with other parameters or interactions (e.g., pH changes occurring as part of the carrion or soil) that were not assessed during this study (Salt 1979). I noted an increase in abiotic temperatures during the second field season year. This could contribute to a shift in the functional response of the microbial communities, as the increase temperatures may have selected for different microbial communities. Additionally, the changes in functional responses may result from fungi, which was not the focus of the community structural analysis. Biotic factors

could also contribute to the discrepancies between field seasons microbial function. The first field season had an increased insect population attracted to the remains. The introduction of carrion into an ecosystem facilitates a localized succession of insect colonizers that continuously alters the microbial species composition, but the overall community of the entire ecosystem is stable, as determined by undetectable changes in species function (Horn 1974). Any primary colonizers utilizing the resource as a nutrition acquisition, mating, or oviposition site and subsequent larval development may have disrupt established microbial communities, hence altering the functional response.

Practical applications that stem from this research include better understanding how microbe-insect interactions may mediate insect colonization. Better understanding these mechanism could potentially lead to better predictions of period of insect activity (PIA) (Tomberlin et al. 2011b). The PIA is defined as the time in which a cadaver can be colonized by insects (i.e., how long has the cadaver been in the environment and insects have had access for colonization); this time interval may correspond with a postmortem interval but is not necessarily synonymous with time of death estimations (Tomberlin et al. 2011b). Within an agricultural setting, understanding these inter-kingdom interactions will allow for more efficient control of diseases mechanically vectored by flies in facilities ranging form confined animal facilities to hospitals. Large numbers of carcasses accumulate at large animal production operations (Meeker 2009) and could be a hotspot for pathogen transmission to encroaching urbanization on rural habitats.

Conclusions

My work has provided a foundation for future controlled experiments where more specific questions relating to community assembly of insects and microbes on carrion can be assessed. Specifically, how do interactions of specific colonizers (e.g., *Phormia regina, Lucilia sericata, Cochliomyia macellaria, and Chrysomya* spp.) impact the bacterial community composition and microbial community function? How does microbial community titer impact insect succession rates? Conversely, how do insects respond to specific bacteria present on a resource? What is the role of fungi in the

decomposition process? and how does it interact with insects? More research is needed to develop the structural and functional relationships of microbial communities on carrion. It appears that microbial carbon utilization does not occur at the same rate as the bacterial taxa succession, which perhaps is an indication that functionally and ecologically similar bacteria are coexisting on the remains. An increased temporal sampling strategy may be necessary to assess subtle dynamic changes occurring within the bacterial community structure. Additionally, determining if key microbial members can predict insect succession patterns will further advance the mechanisms governing decomposition processes. Furthermore, research needs to be conducted to determine if and how these microbe-insect interactions occurring on swine carrion relate to human cadavers.

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APPENDIX A

TABLE A1. A list of date, sampling day, carcass, sampling regions, and treatment for microbial community structural analysis from the 2010 field trial.

Date	Sampling Day	Carcass	Sampling Region	Treatment
5 August	0	A	Carcass - Buccal	EXC
5 August	0	A	Carcass - Skin	EXC
5 August	0	B	Carcass - Buccal	ACC
5 August	0	B	Carcass - Skin	ACC
5 August	0	C	Carcass - Buccal	EXC
5 August	0	C	Carcass - Skin	EXC
5 August	0	D	Carcass - Buccal	EXC
5 August	0	D	Carcass - Skin	EXC
5 August	0	E	Carcass - Buccal	ACC
5 August	0	E	Carcass - Skin	ACC
5 August	0	F	Carcass - Buccal	ACC
6 August	1	A	Carcass - Buccal	EXC
6 August	1	A	Carcass - Skin	EXC
6 August	1	B	Carcass - Buccal	ACC
6 August	1	B	Carcass - Skin	ACC
6 August	1	C	Carcass - Buccal	EXC
6 August	1	C	Carcass - Skin	EXC
6 August	1	D	Carcass - Buccal	EXC
6 August	1	D	Carcass - Skin	EXC
6 August	1	E	Carcass - Buccal	ACC
6 August	1	E	Carcass - Skin	ACC
6 August	1	F	Carcass - Buccal	ACC
6 August	1	F	Carcass - Skin	ACC
8 August	3	A	Carcass - Buccal	EXC
8 August	3	A	Carcass - Skin	EXC
8 August	3	B	Carcass - Buccal	ACC
8 August	3	B	Carcass - Skin	ACC
8 August	3	C	Carcass - Buccal	EXC
8 August	3	C	Carcass - Skin	EXC

TABLE A1. Continued

Date	Sampling Day	Pig	Sampling Region	Treatment
8 August	3	D	Carcass - Buccal	EXC
8 August	3	D	Carcass - Skin	EXC
8 August	3	E	Carcass - Buccal	ACC
8 August	3	E	Carcass - Skin	ACC
8 August	3	F	Carcass - Buccal	ACC
8 August	3	F	Carcass - Skin	ACC
10 August	5	A	Carcass - Buccal	EXC
10 August	5	A	Carcass - Skin	EXC
10 August	5	B	Carcass - Buccal	ACC
10 August	5	B	Carcass - Skin	ACC
10 August	5	C	Carcass - Buccal	EXC
10 August	5	C	Carcass - Skin	EXC
10 August	5	D	Carcass - Buccal	EXC
10 August	5	D	Carcass - Skin	EXC
10 August	5	E	Carcass - Buccal	ACC
10 August	5	E	Carcass - Skin	ACC
10 August	5	F	Carcass - Buccal	ACC
10 August	5	F	Carcass - Skin	ACC

TABLE A2. A list of year, date, sampling day, pig, sampling regions, and treatment for microbial community functional analysis from both field trials (2010 and 2011).

Year	Date	Sampling Day	Pig	Sampling Region	Treatment
2010	5 August	0	A	Carcass - Buccal	EXC
2010	5 August	0	A	Carcass - Skin	EXC
2010	5 August	0	A	Soil - Under body	EXC
2010	5 August	0	A	Soil - 1 m away	EXC
2010	5 August	0	B	Carcass - Buccal	ACC
2010	5 August	0	B	Carcass - Skin	ACC
2010	5 August	0	B	Soil - 1 m away	ACC
2010	5 August	0	C	Carcass - Buccal	EXC
2010	5 August	0	C	Carcass - Skin	EXC
2010	5 August	0	C	Soil - Under body	EXC
2010	5 August	0	C	Soil - 1 m away	EXC
2010	5 August	0	D	Carcass - Buccal	EXC
2010	5 August	0	D	Carcass - Skin	EXC
2010	5 August	0	D	Soil - Under body	EXC
2010	5 August	0	D	Soil - 1 m away	EXC
2010	5 August	0	E	Carcass - Buccal	ACC
2010	5 August	0	E	Carcass - Skin	ACC
2010	5 August	0	E	Soil - Under body	ACC
2010	5 August	0	E	Soil - 1 m away	ACC
2010	5 August	0	F	Carcass - Buccal	ACC
2010	5 August	0	F	Soil - Under body	ACC
2010	5 August	0	F	Soil - 1 m away	ACC
2010	6 August	1	A	Carcass - Buccal	EXC
2010	6 August	1	A	Carcass - Skin	EXC
2010	6 August	1	A	Soil - Under body	EXC
2010	6 August	1	A	Soil - 1 m away	EXC
2010	6 August	1	B	Carcass - Buccal	ACC
2010	6 August	1	B	Carcass - Skin	ACC
2010	5 August	0	F	Carcass - Skin	ACC
2010	6 August	1	B	Soil - Under body	ACC

TABLE A2. Continued

Year	Date	Sampling Day	Pig	Sampling Region	Treatment
2010	6 August	1	B	Soil - 1 m away	ACC
2010	6 August	1	C	Carcass - Buccal	EXC
2010	6 August	1	C	Carcass - Skin	EXC
2010	6 August	1	C	Soil - Under body	EXC
2010	6 August	1	C	Soil - 1 m away	EXC
2010	6 August	1	D	Carcass - Buccal	EXC
2010	6 August	1	D	Carcass - Skin	EXC
2010	6 August	1	D	Soil - Under body	EXC
2010	6 August	1	D	Soil - 1 m away	EXC
2010	6 August	1	E	Carcass - Buccal	ACC
2010	6 August	1	E	Carcass - Skin	ACC
2010	6 August	1	E	Soil - Under body	ACC
2010	6 August	1	E	Soil - 1 m away	ACC
2010	6 August	1	F	Carcass - Buccal	ACC
2010	6 August	1	F	Carcass - Skin	ACC
2010	6 August	1	F	Soil - Under body	ACC
2010	6 August	1	F	Soil - 1 m away	ACC
2010	8 August	3	A	Carcass - Buccal	EXC
2010	8 August	3	A	Carcass - Skin	EXC
2010	8 August	3	A	Soil - Under body	EXC
2010	8 August	3	A	Soil - 1 m away	EXC
2010	8 August	3	B	Carcass - Buccal	ACC
2010	8 August	3	B	Carcass - Skin	ACC
2010	8 August	3	B	Soil - Under body	ACC
2010	8 August	3	B	Soil - 1 m away	ACC
2010	8 August	3	C	Carcass - Buccal	EXC
2010	8 August	3	C	Carcass - Skin	EXC
2010	8 August	3	C	Soil - Under body	EXC
2010	8 August	3	C	Soil - 1 m away	EXC
2010	8 August	3	D	Carcass - Buccal	EXC
2010	8 August	3	D	Carcass - Skin	EXC

TABLE A2. Continued

Year	Date	Sampling Day	Pig	Sampling Region	Treatment
2010	8 August	3	D	Soil - Under body	EXC
2010	8 August	3	D	Soil - 1 m away	EXC
2010	8 August	3	E	Carcass - Buccal	ACC
2010	8 August	3	E	Carcass - Skin	ACC
2010	8 August	3	E	Soil - 1 m away	ACC
2010	8 August	3	F	Carcass - Buccal	ACC
2010	8 August	3	F	Carcass - Skin	ACC
2010	8 August	3	F	Soil - Under body	ACC
2010	8 August	3	F	Soil - 1 m away	ACC
2010	10 August	5	A	Carcass - Buccal	EXC
2010	10 August	5	A	Carcass - Skin	EXC
2010	10 August	5	A	Soil - Under body	EXC
2010	10 August	5	A	Soil - 1 m away	EXC
2010	10 August	5	B	Carcass - Buccal	ACC
2010	10 August	5	B	Carcass - Skin	ACC
2010	10 August	5	B	Soil - Under body	ACC
2010	10 August	5	B	Soil - 1 m away	ACC
2010	10 August	5	C	Carcass - Buccal	EXC
2010	10 August	5	C	Carcass - Skin	EXC
2010	10 August	5	C	Soil - Under body	EXC
2010	10 August	5	C	Soil - 1 m away	EXC
2010	10 August	5	D	Carcass - Buccal	EXC
2010	10 August	5	D	Carcass - Skin	EXC
2010	10 August	5	D	Soil - Under body	EXC
2010	10 August	5	D	Soil - 1 m away	EXC
2010	10 August	5	E	Carcass - Buccal	ACC
2010	10 August	5	E	Carcass - Skin	ACC
2010	10 August	5	E	Soil - Under body	ACC
2010	10 August	5	E	Soil - 1 m away	ACC
2010	10 August	5	F	Carcass - Buccal	ACC
2010	10 August	5	F	Carcass - Skin	ACC
2010	10 August	5	F	Soil - Under body	ACC

TABLE A2. Continued

Year	Date	Sampling Day	Pig	Sampling Region	Treatment
2010	10 August	5	F	Soil - 1 m away	ACC
2011	26 July	0	G	Carcass - Composite	ACC
2011	26 July	0	G	Carcass - Buccal	ACC
2011	26 July	0	G	Carcass - Skin	ACC
2011	26 July	0	G	Soil - Under body	ACC
2011	26 July	0	G	Soil - 1 m away	ACC
2011	26 July	0	H	Carcass - Composite	ACC
2011	26 July	0	H	Carcass - Buccal	ACC
2011	26 July	0	H	Carcass - Skin	ACC
2011	26 July	0	H	Soil - Under body	ACC
2011	26 July	0	H	Soil - 1 m away	ACC
2011	26 July	0	I	Carcass - Composite	EXC
2011	26 July	0	I	Carcass - Buccal	EXC
2011	26 July	0	I	Carcass - Skin	EXC
2011	26 July	0	I	Soil - Under body	EXC
2011	26 July	0	I	Soil - 1 m away	EXC
2011	26 July	0	J	Carcass - Composite	EXC
2011	26 July	0	J	Carcass - Buccal	EXC
2011	26 July	0	J	Carcass - Skin	EXC
2011	26 July	0	J	Soil - Under body	EXC
2011	26 July	0	J	Soil - 1 m away	EXC
2011	26 July	0	K	Carcass - Composite	EXC
2011	26 July	0	K	Carcass - Buccal	EXC
2011	26 July	0	K	Carcass - Skin	EXC
2011	26 July	0	K	Soil - Under body	EXC
2011	26 July	0	K	Soil - 1 m away	EXC
2011	26 July	0	L	Carcass - Composite	ACC
2011	26 July	0	L	Carcass - Buccal	ACC
2011	26 July	0	L	Carcass - Skin	ACC
2011	26 July	0	L	Soil - Under body	ACC
2011	26 July	0	L	Soil - 1 m away	ACC

TABLE A2. Continued

Year	Date	Sampling Day	Pig	Sampling Region	Treatment
2011	27 July	1	G	Carcass - Composite	ACC
2011	27 July	1	G	Carcass - Buccal	ACC
2011	27 July	1	G	Carcass - Skin	ACC
2011	27 July	1	G	Soil - Under body	ACC
2011	27 July	1	G	Soil - 1 m away	ACC
2011	27 July	1	H	Carcass - Composite	ACC
2011	27 July	1	H	Carcass - Buccal	ACC
2011	27 July	1	H	Carcass - Skin	ACC
2011	27 July	1	H	Soil - Under body	ACC
2011	27 July	1	H	Soil - 1 m away	ACC
2011	27 July	1	I	Carcass - Composite	EXC
2011	27 July	1	I	Carcass - Buccal	EXC
2011	27 July	1	I	Carcass - Skin	EXC
2011	27 July	1	I	Soil - Under body	EXC
2011	27 July	1	I	Soil - 1 m away	EXC
2011	27 July	1	J	Carcass - Composite	EXC
2011	27 July	1	J	Carcass - Buccal	EXC
2011	27 July	1	J	Carcass - Skin	EXC
2011	27 July	1	J	Soil - Under body	EXC
2011	27 July	1	J	Soil - 1 m away	EXC
2011	27 July	1	K	Carcass - Composite	EXC
2011	27 July	1	K	Carcass - Buccal	EXC
2011	27 July	1	K	Carcass - Skin	EXC
2011	27 July	1	K	Soil - Under body	EXC
2011	27 July	1	K	Soil - 1 m away	EXC
2011	27 July	1	L	Carcass - Composite	ACC
2011	27 July	1	L	Carcass - Buccal	ACC
2011	27 July	1	L	Carcass - Skin	ACC
2011	27 July	1	L	Soil - Under body	ACC
2011	27 July	1	L	Soil - 1 m away	ACC
2011	28 July	2	G	Carcass - Buccal	ACC
2011	28 July	2	G	Carcass - Skin	ACC

TABLE A2. Continued

Year	Date	Sampling Day	Pig	Sampling Region	Treatment
2011	28 July	2	G	Soil - Under body	ACC
2011	28 July	2	G	Soil - 1 m away	ACC
2011	28 July	2	H	Carcass - Buccal	ACC
2011	28 July	2	H	Carcass - Skin	ACC
2011	28 July	2	H	Soil - Under body	ACC
2011	28 July	2	H	Soil - 1 m away	ACC
2011	28 July	2	I	Carcass - Buccal	EXC
2011	28 July	2	I	Carcass - Skin	EXC
2011	28 July	2	I	Soil - Under body	EXC
2011	28 July	2	I	Soil - 1 m away	EXC
2011	28 July	2	J	Carcass - Buccal	EXC
2011	28 July	2	J	Carcass - Skin	EXC
2011	28 July	2	J	Soil - Under body	EXC
2011	28 July	2	J	Soil - 1 m away	EXC
2011	28 July	2	K	Carcass - Buccal	EXC
2011	28 July	2	K	Carcass - Skin	EXC
2011	28 July	2	K	Soil - Under body	EXC
2011	28 July	2	K	Soil - 1 m away	EXC
2011	28 July	2	L	Carcass - Buccal	ACC
2011	28 July	2	L	Carcass - Skin	ACC
2011	28 July	2	L	Soil - Under body	ACC
2011	28 July	2	L	Soil - 1 m away	ACC
2011	29 July	3	G	Carcass - Skin	ACC
2011	29 July	3	G	Soil - Under body	ACC
2011	29 July	3	G	Soil - 1 m away	ACC
2011	29 July	3	H	Carcass - Skin	ACC
2011	29 July	3	H	Soil - Under body	ACC
2011	29 July	3	H	Soil - 1 m away	ACC
2011	29 July	3	I	Carcass - Buccal	EXC
2011	29 July	3	I	Carcass - Skin	EXC
2011	29 July	3	I	Soil - Under body	EXC
2011	29 July	3	I	Soil - 1 m away	EXC

TABLE A2. Continued

Year	Date	Sampling Day	Pig	Sampling Region	Treatment
2011	29 July	3	J	Carcass - Buccal	EXC
2011	29 July	3	J	Carcass - Skin	EXC
2011	29 July	3	J	Soil - Under body	EXC
2011	29 July	3	J	Soil - 1 m away	EXC
2011	29 July	3	K	Carcass - Buccal	EXC
2011	29 July	3	K	Carcass - Skin	EXC
2011	29 July	3	K	Soil - Under body	EXC
2011	29 July	3	K	Soil - 1 m away	EXC
2011	29 July	3	L	Soil - Under body	ACC
2011	29 July	3	L	Soil - 1 m away	ACC
2011	30 July	4	I	Carcass - Buccal	EXC
2011	30 July	4	I	Carcass - Skin	EXC
2011	30 July	4	I	Soil - Under body	EXC
2011	30 July	4	I	Soil - 1 m away	EXC
2011	30 July	4	J	Carcass - Buccal	EXC
2011	30 July	4	J	Carcass - Skin	EXC
2011	30 July	4	J	Soil - Under body	EXC
2011	30 July	4	J	Soil - 1 m away	EXC
2011	30 July	4	K	Carcass - Buccal	EXC
2011	30 July	4	K	Carcass - Skin	EXC
2011	30 July	4	K	Soil - Under body	EXC
2011	30 July	4	K	Soil - 1 m away	EXC
2011	31 July	5	I	Carcass - Buccal	EXC
2011	31 July	5	I	Carcass - Skin	EXC
2011	31 July	5	I	Soil - Under body	EXC
2011	31 July	5	I	Soil - 1 m away	EXC
2011	31 July	5	J	Carcass - Buccal	EXC
2011	31 July	5	J	Carcass - Skin	EXC
2011	31 July	5	J	Soil - Under body	EXC
2011	31 July	5	J	Soil - 1 m away	EXC
2011	31 July	5	K	Carcass - Buccal	EXC
2011	31 July	5	K	Carcass - Skin	EXC

TABLE A2. Continued

Year	Date	Sampling Day	Pig	Sampling Region	Treatment
2011	31 July	5	K	Soil - Under body	EXC
2011	31 July	5	K	Soil - 1 m away	EXC
2011	1 August	6	I	Carcass - Buccal	EXC
2011	1 August	6	I	Carcass - Skin	EXC
2011	1 August	6	I	Soil - Under body	EXC
2011	1 August	6	I	Soil - 1 m away	EXC
2011	1 August	6	J	Carcass - Buccal	EXC
2011	1 August	6	J	Carcass - Skin	EXC
2011	1 August	6	J	Soil - Under body	EXC
2011	1 August	6	J	Soil - 1 m away	EXC
2011	1 August	6	K	Carcass - Buccal	EXC
2011	1 August	6	K	Carcass - Skin	EXC
2011	1 August	6	K	Soil - Under body	EXC
2011	1 August	6	K	Soil - 1 m away	EXC
2011	2 August	7	I	Carcass - Buccal	EXC
2011	2 August	7	I	Carcass - Skin	EXC
2011	2 August	7	I	Soil - Under body	EXC
2011	2 August	7	I	Soil - 1 m away	EXC
2011	2 August	7	J	Carcass - Buccal	EXC
2011	2 August	7	J	Carcass - Skin	EXC
2011	2 August	7	J	Soil - Under body	EXC
2011	2 August	7	J	Soil - 1 m away	EXC
2011	2 August	7	K	Carcass - Buccal	EXC
2011	2 August	7	K	Carcass - Skin	EXC
2011	2 August	7	K	Soil - Under body	EXC
2011	2 August	7	K	Soil - 1 m away	EXC

APPENDIX B

Table B1. Taxa and relative abundance at the phylum level (20% dissimilarity) for carcasses across decomposition day based on RDP classifications.

Day 0					
EXC			ACC		
Phlyum	Count	Rel. Abundance (%)	Phlyum	Count	Rel. Abundance (%)
Proteobacteria	36,841	62.35	*Proteobacteria*	34,827	70.44
Firmicutes	19,605	33.18	*Firmicutes*	9,830	19.88
Actinobacteria	1,833	3.10	*Actinobacteria*	2,274	4.60
Bacteroidetes	648	1.10	*Bacteroidetes*	2,273	4.60
Fusobacteria	143	0.24	*Fusobacteria*	229	0.46
TM7	9	0.02	*TM7*	3	0.01
SR1	3	0.01	*Cyanobacteria/Chloroplast*	2	0.00
Synergistetes	2	0.00	*Acidobacteria*	2	0.00
Tenericutes	1	0.00	*Deinococcus-Thermus*	2	0.00
			Planctomycetes	1	0.00
			Tenericutes	1	0.00
N =	59,085		N =	49,444	

Table B1. Continued

Day 1					
EXC			ACC		
Phlyum	Count	Rel. Abundance (%)	Phlyum	Count	Rel. Abundance (%)
Firmicutes	14,006	42.69	*Proteobacteria*	10,712	42.09
Proteobacteria	11,477	34.98	*Firmicutes*	9,192	36.11
Actinobacteria	4,744	14.46	*Actinobacteria*	2,777	10.91
Bacteroidetes	2,564	7.82	*Bacteroidetes*	2,713	10.66
Fusobacteria	5	0.02	*Fusobacteria*	49	0.19
Cyanobacteria/Chloroplast	3	0.01	*Acidobacteria*	2	0.01
Acidobacteria	2	0.01	*Deinococcus-Thermus*	2	0.01
Planctomycetes	2	0.01	*TM7*	1	0.00
Synergistetes	1	0.00	*Spirochaetes*	1	0.00
Tenericutes	1	0.00	*Cyanobacteria/Chloroplast*	1	0.00
Lentisphaerae	1	0.00	*Synergistetes*	1	0.00
			Verrucomicrobia	1	0.00
			Chloroflexi	1	0.00
N =	32,806		N =	25,453	

Table B1. Continued

Day 3					
EXC			ACC		
Phlyum	Count	Rel. Abundance (%)	Phlyum	Count	Rel. Abundance (%)
Proteobacteria	37,906	63.14	*Firmicutes*	19,085	47.81
Firmicutes	16,615	27.67	*Proteobacteria*	17,535	43.93
Actinobacteria	4,292	7.15	*Actinobacteria*	3,235	8.10
Bacteroidetes	1,122	1.87	*Bacteroidetes*	61	0.15
Fusobacteria	91	0.15	*Cyanobacteria/Chloroplast*	2	0.01
Deinococcus-Thermus	5	0.01	*Synergistetes*	1	0.00
Cyanobacteria/Chloroplast	3	0.00			
Acidobacteria	2	0.00			
TM7	1	0.00			
Synergistetes	1	0.00			
Chloroflexi	1	0.00			
N =	60,039		N =	39,919	

Table B1. Continued

Day 5					
EXC			ACC		
Phlyum	Count	Rel. Abundance (%)	Phlyum	Count	Rel. Abundance (%)
Proteobacteria	64,219	81.69	*Firmicutes*	21,684	96.74
Firmicutes	12,843	16.34	*Proteobacteria*	679	3.03
Actinobacteria	864	1.10	*Actinobacteria*	35	0.16
Bacteroidetes	658	0.84	*Bacteroidetes*	8	0.04
Fusobacteria	16	0.02	*Acidobacteria*	6	0.03
Tenericutes	4	0.01	*Nitrospira*	1	0.00
TM7	3	0.00	*Gemmatimonadetes*	1	0.00
Acidobacteria	2	0.00			
Chloroflexi	1	0.00			
N =	78,610		N =	22,414	

Table B2. Taxa and relative abundance at the class level (5% dissimilarity) for carcasses across decomposition day based on RDP classifications.

Day 0					
EXC			ACC		
Class	Count	Rel. Abundance (%)	Class	Count	Rel. Abundance (%)
Gammaproteobacteria	35,451	62.46	*Gammaproteobacteria*	32,007	69.51
Bacilli	15,239	26.85	*Bacilli*	7,950	17.27
Clostridia	2,494	4.39	*Actinobacteria*	2,274	4.94
Actinobacteria	1,833	3.23	*Bacteroidia*	1,129	2.45
Betaproteobacteria	596	1.05	*Clostridia*	1,053	2.29
Epsilonproteobacteria	297	0.52	*Betaproteobacteria*	560	1.22
Bacteroidia	286	0.50	*Flavobacteria*	517	1.12
Flavobacteria	192	0.34	*Fusobacteria*	229	0.50
Fusobacteria	143	0.25	*Epsilonproteobacteria*	125	0.27
Erysipelotrichia	137	0.24	*Erysipelotrichia*	95	0.21
Alphaproteobacteria	47	0.08	*Alphaproteobacteria*	60	0.13
Sphingobacteria	35	0.06	*Sphingobacteria*	39	0.08
Deltaproteobacteria	4	0.01	*Chloroplast*	2	0.00
Synergistia	2	0.00	*Deinococci*	2	0.00
Bacteroidetes incertae sedis	1	0.00	*Acidobacteria Gp4*	1	0.00
Mollicutes	1	0.00	*Acidobacteria Gp16*	1	0.00
			Planctomycetacia	1	0.00
			Mollicutes	1	0.00
	N = 56,758			N = 46,046	

Table B2. Continued

Day 1					
EXC			ACC		
Class	Count	Rel. Abundance (%)	Class	Count	Rel. Abundance (%)
Bacilli	11,202	35.26	*Gammaproteobacteria*	9,517	38.66
Gammaproteobacteria	10,618	33.42	*Bacilli*	7,586	30.81
Actinobacteria	4,744	14.93	*Actinobacteria*	2,777	11.28
Flavobacteria	2,322	7.31	*Flavobacteria*	2,568	10.43
Clostridia	1,750	5.51	*Clostridia*	973	3.95
Betaproteobacteria	609	1.92	*Betaproteobacteria*	835	3.39
Erysipelotrichia	150	0.47	*Alphaproteobacteria*	98	0.40
Sphingobacteria	146	0.46	*Erysipelotrichia*	80	0.32
Alphaproteobacteria	105	0.33	*Sphingobacteria*	67	0.27
Epsilonproteobacteria	62	0.20	*Fusobacteria*	49	0.20
Bacteroidia	49	0.15	*Bacteroidia*	38	0.15
Fusobacteria	5	0.02	*Epsilonproteobacteria*	20	0.08
Chloroplast	3	0.01	*Deinococci*	2	0.01
Planctomycetacia	2	0.01	*Spirochaetes*	1	0.00
Deltaproteobacteria	1	0.00	*Deltaproteobacteria*	1	0.00
Acidobacteria Gp4	1	0.00	*Chloroplast*	1	0.00
Acidobacteria Gp7	1	0.00	*Acidobacteria Gp4*	1	0.00
Synergistia	1	0.00	*Acidobacteria Gp1*	1	0.00
Mollicutes	1	0.00	*Synergistia*	1	0.00
Lentisphaeria	1	0.00	*Opitutae*	1	0.00
			Thermomicrobia	1	0.00
N =	31,773		N =	24,618	

Table B2. Continued

Day 3					
EXC			ACC		
Class	Count	Rel. Abundance (%)	Class	Count	Rel. Abundance (%)
Gammaproteobacteria	34,773	58.66	*Gammaproteobacteria*	17,274	44.23
Bacilli	13,699	23.11	*Bacilli*	17,135	43.87
Actinobacteria	4,292	7.24	*Actinobacteria*	3,235	8.28
Clostridia	2,111	3.56	*Clostridia*	985	2.52
Betaproteobacteria	1,674	2.82	*Betaproteobacteria*	209	0.54
Epsilonproteobacteria	1,200	2.02	*Erysipelotrichia*	112	0.29
Flavobacteria	1,080	1.82	*Flavobacteria*	47	0.12
Erysipelotrichia	163	0.27	*Alphaproteobacteria*	42	0.11
Alphaproteobacteria	142	0.24	*Bacteroidia*	10	0.03
Fusobacteria	91	0.15	*Sphingobacteria*	3	0.01
Bacteroidia	21	0.04	*Chloroplast*	2	0.01
Sphingobacteria	16	0.03	*Epsilonproteobacteria*	1	0.00
Deinococci	5	0.01	*Synergistia*	1	0.00
Chloroplast	3	0.01			
Deltaproteobacteria	1	0.00			
Acidobacteria Gp2	1	0.00			
Acidobacteria Gp1	1	0.00			
Synergistia	1	0.00			
Thermomicrobia	1	0.00			
N =	59,275		N =	39,056	

Table B2. Continued

Day 5					
EXC			ACC		
Class	Count	Rel. Abundance (%)	Class	Count	Rel. Abundance (%)
Gammaproteobacteria	61,561	78.64	*Bacilli*	14,653	71.44
Clostridia	6,860	8.76	*Clostridia*	5,122	24.97
Bacilli	5,766	7.37	*Gammaproteobacteria*	657	3.20
Betaproteobacteria	2,470	3.16	*Actinobacteria*	35	0.17
Actinobacteria	864	1.10	*Alphaproteobacteria*	13	0.06
Flavobacteria	495	0.63	*Erysipelotrichia*	10	0.05
Bacteroidia	147	0.19	*Betaproteobacteria*	8	0.04
Erysipelotrichia	49	0.06	*Acidobacteria Gp2*	4	0.02
Alphaproteobacteria	39	0.05	*Bacteroidia*	2	0.01
Fusobacteria	16	0.02	*Sphingobacteria*	2	0.01
Mollicutes	4	0.01	*Flavobacteria*	2	0.01
Epsilonproteobacteria	3	0.00	*Nitrospira*	1	0.00
Deltaproteobacteria	1	0.00	*Gemmatimonadetes*	1	0.00
Acidobacteria Gp1	1	0.00	*Acidobacteria Gp1*	1	0.00
Acidobacteria Gp17	1	0.00	*Acidobacteria Gp6*	1	0.00
Anaerolineae	1	0.00			
N =	78,278		N =	20,512	

Table B3. Taxa and relative abundance at the species level (3% dissimilarity) for carcasses across decomposition day based on RDP classifications.

Day 0					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Psychrobacter	11,475	28.99	*Psychrobacter*	9,320	29.20
Moraxella	9,072	22.92	*Moraxella*	7,886	24.71
Acinetobacter	4,238	10.70	*Acinetobacter*	6,348	19.89
Streptococcus	2,967	7.49	*Streptococcus*	1,429	4.48
Aerococcus	2,882	7.28	*Aerococcus*	1,022	3.20
Globicatella	1,561	3.94	*Globicatella*	694	2.17
Micrococcus	778	1.97	*Facklamia*	515	1.61
Facklamia	653	1.65	*Porphyromonas*	435	1.36
Haemophilus	606	1.53	*Haemophilus*	392	1.23
Lactobacillus	490	1.24	*Micrococcus*	292	0.91
Gemella	346	0.87	*Staphylococcus*	232	0.73
Caryophanon	311	0.79	*Jeotgalicoccus*	220	0.69
Jeotgalicoccus	295	0.75	*Lactobacillus*	202	0.63
Arcobacter	293	0.74	*Corynebacterium*	193	0.60
Staphylococcus	261	0.66	*Caryophanon*	179	0.56
Escherichia/Shigella	251	0.63	*Fusobacterium*	153	0.48
Parvimonas	251	0.63	*Comamonas*	152	0.48
Granulicatella	208	0.53	*Solibacillus*	141	0.44
Megasphaera	196	0.50	*Megasphaera*	132	0.41
Comamonas	136	0.34	*Riemerella*	125	0.39
Kocuria	129	0.33	*Arcobacter*	116	0.36
Clostridium sensu stricto	107	0.27	*Escherichia/Shigella*	99	0.31

Table B3. Continued

Day 0					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Solibacillus	98	0.25	*Clostridium sensu stricto*	96	0.30
Fusobacterium	97	0.25	*Gemella*	96	0.30
Peptostreptococcus	91	0.23	*Bacteroides*	72	0.23
Helcococcus	88	0.22	*Aeromonas*	70	0.22
Corynebacterium	84	0.21	*Helcococcus*	64	0.20
Actinobacillus	80	0.20	*Granulicatella*	62	0.19
Kurthia	79	0.20	*Clostridium XI*	60	0.19
Aeromonas	76	0.19	*Neisseria*	57	0.18
Porphyromonas	69	0.17	*Succinivibrio*	43	0.13
Succinivibrio	66	0.17	*Turicibacter*	43	0.13
Abiotrophia	62	0.16	*Kocuria*	41	0.13
Oscillibacter	60	0.15	*Prevotella*	40	0.13
Clostridium XI	59	0.15	*Pseudomonas*	33	0.10
Macrococcus	54	0.14	*Mannheimia*	30	0.09
Neisseria	51	0.13	*Luteimonas*	30	0.09
Turicibacter	49	0.12	*Macrococcus*	28	0.09
Erysipelotrichaceae incertae sedis	47	0.12	*Sphingobacterium*	27	0.08
Veillonella	42	0.11	*Oscillibacter*	26	0.08
Pseudomonas	38	0.10	*Chryseobacterium*	24	0.08
Rummeliibacillus	38	0.10	*Desemzia*	24	0.08
Trichococcus	36	0.09	*Kurthia*	22	0.07
Riemerella	35	0.09	*Rothia*	21	0.07
Luteimonas	30	0.08	*Abiotrophia*	21	0.07

Table B3. Continued

Day 0					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Prevotella	27	0.07	*Parabacteroides*	19	0.06
Enterococcus	26	0.07	*Alysiella*	19	0.06
Parabacteroides	24	0.06	*Trichococcus*	19	0.06
Alloiococcus	22	0.06	*Erysipelotrichaceae incertae sedis*	19	0.06
Rothia	20	0.05	*Enhydrobacter*	18	0.06
Pasteurella	18	0.05	*Stenotrophomonas*	18	0.06
Selenomonas	17	0.04	*Pasteurella*	17	0.05
Bacteroides	16	0.04	*Selenomonas*	17	0.05
Proteus	16	0.04	*Anaerovorax*	16	0.05
Enhydrobacter	15	0.04	*Proteus*	15	0.05
Mannheimia	15	0.04	*Rummeliibacillus*	15	0.05
Brevibacterium	14	0.04	*Arcanobacterium*	14	0.04
Tessaracoccus	14	0.04	*Actinobacillus*	14	0.04
Sphingobacterium	14	0.04	*Dietzia*	13	0.04
Anaerovorax	14	0.04	*Brevundimonas*	13	0.04
Subdoligranulum	14	0.04	*Dorea*	13	0.04
Salmonella	12	0.03	*Brevibacterium*	12	0.04
Eubacterium	12	0.03	*Acidaminococcus*	12	0.04
Brevundimonas	11	0.03	*Peptostreptococcus*	10	0.03
Proteiniclasticum	11	0.03	*Solobacterium*	10	0.03
Dorea	11	0.03	*Mogibacterium*	9	0.03
Arcanobacterium	10	0.03	*Proteiniclasticum*	9	0.03
Acidaminococcus	10	0.03	*Faecalibacterium*	9	0.03

Table B3. Continued

Day 0					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
TM7 genera incertae sedis	9	0.02	*Flavobacterium*	8	0.03
Roseburia	9	0.02	*Anaerococcus*	8	0.03
Faecalibacterium	9	0.02	*Filifactor*	8	0.03
Catenibacterium	9	0.02	*Atopostipes*	8	0.03
Ottowia	8	0.02	*Campylobacter*	7	0.02
Janibacter	7	0.02	*Enterococcus*	7	0.02
Alysiella	7	0.02	*Catenibacterium*	7	0.02
Dialister	7	0.02	*Capnocytophaga*	6	0.02
Syntrophococcus	7	0.02	*Wautersiella*	6	0.02
Anaerococcus	7	0.02	*Alishewanella*	6	0.02
Nosocomiicoccus	7	0.02	*Lachnospiracea incertae sedis*	6	0.02
Dietzia	6	0.02	*Parvimonas*	6	0.02
Sphingomonas	6	0.02	*Psychrobacillus*	6	0.02
Paracoccus	6	0.02	*Ornithinimicrobium*	5	0.02
Stenotrophomonas	6	0.02	*Ottowia*	5	0.02
Shewanella	6	0.02	*Lysobacter*	5	0.02
Anaerovibrio	6	0.02	*Planococcaceae incertae sedis*	5	0.02
Peptostreptococcaceae incertae sedis	6	0.02	*Nosocomiicoccus*	5	0.02
Desemzia	6	0.02	*Sphingomonas*	4	0.01
Allisonella	5	0.01	*Dialister*	4	0.01
Vagococcus	5	0.01	*Roseburia*	4	0.01
Atopostipes	5	0.01	*Subdoligranulum*	4	0.01
Gemmiger	4	0.01	*Proteocatella*	4	0.01

Table B3. Continued

Day 0					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Klebsiella	4	0.01	*Exiguobacterium*	4	0.01
Mogibacterium	4	0.01	*Salinicoccus*	4	0.01
Lachnospiracea incertae sedis	4	0.01	*Sharpea*	4	0.01
Gallicola	4	0.01	*Brachybacterium*	3	0.01
Solobacterium	4	0.01	*Leucobacter*	3	0.01
Campylobacter	3	0.01	*Tessaracoccus*	3	0.01
Lysobacter	3	0.01	*TM7 genera incertae sedis*	3	0.01
Oribacterium	3	0.01	*Vitreoscilla*	3	0.01
Ruminococcus	3	0.01	*Methylobacterium*	3	0.01
Weissella	3	0.01	*Gemmiger*	3	0.01
Exiguobacterium	3	0.01	*Paracoccus*	3	0.01
Salinicoccus	3	0.01	*Shewanella*	3	0.01
Sharpea	3	0.01	*Syntrophococcus*	3	0.01
SR1 genera incertae sedis	3	0.01	*Catonella*	3	0.01
Clostridium XIX	2	0.01	*Vagococcus*	3	0.01
Ornithinimicrobium	2	0.01	*Allofustis*	3	0.01
Yaniella	2	0.01	*Streptobacillus*	2	0.01
Brachybacterium	2	0.01	*Janibacter*	2	0.01
Leucobacter	2	0.01	*Arthrobacter*	2	0.01
Collinsella	2	0.01	*Collinsella*	2	0.01
Flavobacterium	2	0.01	*Helicobacter*	2	0.01
Oxalobacter	2	0.01	*Bergeriella*	2	0.01
Acidovorax	2	0.01	*Devosia*	2	0.01

Table B3. Continued

Day 0					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Vitreoscilla	2	0.01	*Pedomicrobium*	2	0.01
Propionivibrio	2	0.01	*Novosphingobium*	2	0.01
Pseudochrobactrum	2	0.01	*Legionella*	2	0.01
Gemmobacter	2	0.01	*Providencia*	2	0.01
Alkanindiges	2	0.01	*Citrobacter*	2	0.01
Providencia	2	0.01	*Ruminobacter*	2	0.01
Alishewanella	2	0.01	*Allisonella*	2	0.01
Catonella	2	0.01	*Anaerovibrio*	2	0.01
Butyricicoccus	2	0.01	*Veillonella*	2	0.01
Fastidiosipila	2	0.01	*Oribacterium*	2	0.01
Flavonifractor	2	0.01	*Cellulosilyticum*	2	0.01
Pseudoflavonifractor	2	0.01	*Clostridium XlVa*	2	0.01
Filifactor	2	0.01	*Gallicola*	2	0.01
Proteocatella	2	0.01	*Butyricicoccus*	2	0.01
Acetoanaerobium	2	0.01	*Peptostreptococcaceae incertae sedis*	2	0.01
Psychrobacillus	2	0.01	*Alloiococcus*	2	0.01
Arthrobacter	1	0.00	*Planococcus*	2	0.01
Microbacterium	1	0.00	*Deinococcus*	2	0.01
Curtobacterium	1	0.00	*Leptotrichia*	1	0.00
Kytococcus	1	0.00	*Iamia*	1	0.00
Trueperella	1	0.00	*Sanguibacter*	1	0.00
Actinomyces	1	0.00	*Trueperella*	1	0.00

Table B3. Continued

Day 0					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Atopobium	1	0.00	*Actinomyces*	1	0.00
Ohtaekwangia	1	0.00	*Rhodococcus*	1	0.00
Alistipes	1	0.00	*Aeromicrobium*	1	0.00
Xylanibacter	1	0.00	*Propionibacterium*	1	0.00
Paraprevotella	1	0.00	*Propionimicrobium*	1	0.00
Anaerorhabdus	1	0.00	*Olsenella*	1	0.00
Pedobacter	1	0.00	*Alistipes*	1	0.00
Wautersiella	1	0.00	*Paraprevotella*	1	0.00
Sutterella	1	0.00	*Anaerorhabdus*	1	0.00
Brachymonas	1	0.00	*Tannerella*	1	0.00
Bergeriella	1	0.00	*Dysgonomonas*	1	0.00
Bosea	1	0.00	*Ferruginibacter*	1	0.00
Devosia	1	0.00	*Fluviicola*	1	0.00
Cellvibrio	1	0.00	*Oxalobacter*	1	0.00
Serpens	1	0.00	*Brachymonas*	1	0.00
Rugamonas	1	0.00	*Thauera*	1	0.00
Rheinheimera	1	0.00	*Alkanindiges*	1	0.00
Raoultella	1	0.00	*Pantoea*	1	0.00
Citrobacter	1	0.00	*Morganella*	1	0.00
Suttonella	1	0.00	*Salmonella*	1	0.00
Megamonas	1	0.00	*Suttonella*	1	0.00
Schwartzia	1	0.00	*Zobellella*	1	0.00
Clostridium XlVa	1	0.00	*Streptophyta*	1	0.00

Table B3. Continued

Day 0					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Blautia	1	0.00	*Gp4*	1	0.00
Peptoniphilus	1	0.00	*Gp16*	1	0.00
Clostridium IV	1	0.00	*Mitsuokella*	1	0.00
Anaerotruncus	1	0.00	*Succinispira*	1	0.00
Murdochiella	1	0.00	*Succiniclasticum*	1	0.00
Ignavigranum	1	0.00	*Blautia*	1	0.00
Eremococcus	1	0.00	*Alkaliphilus*	1	0.00
Saccharibacillus	1	0.00	*Hydrogenoanaerobacterium*	1	0.00
Planococcus	1	0.00	*Ruminococcus*	1	0.00
Planococcaceae incertae sedis	1	0.00	*Eubacterium*	1	0.00
Erysipelothrix	1	0.00	*Pseudoramibacter*	1	0.00
Acholeplasma	1	0.00	*Catellicoccus*	1	0.00
			Erysipelothrix	1	0.00
			Mycoplasma	1	0.00
N =	39,589		N =	31,913	

Table B3. Continued

Day 1					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Aeromonas	4,116	18.15	*Providencia*	2,247	15.76
Aerococcus	3,324	14.66	*Aerococcus*	2,191	15.37
Micrococcus	2,729	12.03	*Micrococcus*	1,438	10.08
Jeotgalicoccus	1,209	5.33	*Acinetobacter*	1,108	7.77
Globicatella	1,189	5.24	*Jeotgalicoccus*	999	7.01
Acinetobacter	1,140	5.03	*Moraxella*	969	6.80
Shewanella	925	4.08	*Globicatella*	772	5.41
Moraxella	912	4.02	*Lactobacillus*	409	2.87
Psychrobacter	848	3.74	*Comamonas*	356	2.50
Staphylococcus	706	3.11	*Streptococcus*	348	2.44
Lactobacillus	550	2.43	*Psychrobacter*	316	2.22
Comamonas	395	1.74	*Staphylococcus*	249	1.75
Flavobacterium	386	1.70	*Facklamia*	238	1.67
Facklamia	371	1.64	*Flavobacterium*	154	1.08
Streptococcus	364	1.61	*Megasphaera*	154	1.08
Clostridium sensu stricto	250	1.10	*Caryophanon*	136	0.95
Caryophanon	239	1.05	*Proteus*	107	0.75
Kocuria	204	0.90	*Corynebacterium*	104	0.73
Proteus	170	0.75	*Morganella*	96	0.67
Corynebacterium	164	0.72	*Kocuria*	89	0.62
Megasphaera	163	0.72	*Stenotrophomonas*	88	0.62
Pseudomonas	150	0.66	*Vitreoscilla*	84	0.59
Providencia	109	0.48	*Clostridium sensu stricto*	83	0.58

Table B3. Continued

Day 1					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Sphingobacterium	108	0.48	*Myroides*	80	0.56
Escherichia/Shigella	104	0.46	*Wautersiella*	70	0.49
Clostridium XI	102	0.45	*Ignatzschineria*	68	0.48
Macrococcus	101	0.45	*Escherichia/Shigella*	57	0.40
Kurthia	92	0.41	*Kurthia*	54	0.38
Erysipelotrichaceae incertae sedis	73	0.32	*Aeromonas*	51	0.36
Stenotrophomonas	67	0.30	*Sphingobacterium*	50	0.35
Oscillibacter	67	0.30	*Macrococcus*	50	0.35
Rothia	65	0.29	*Helcococcus*	46	0.32
Arcobacter	62	0.27	*Clostridium XI*	43	0.30
Succinivibrio	49	0.22	*Fusobacterium*	39	0.27
Nosocomiicoccus	46	0.20	*Erysipelotrichaceae incertae sedis*	39	0.27
Wautersiella	41	0.18	*Riemerella*	35	0.25
Riemerella	39	0.17	*Rothia*	34	0.24
Pseudochrobactrum	39	0.17	*Acidaminococcus*	34	0.24
Chryseobacterium	38	0.17	*Shewanella*	33	0.23
Mogibacterium	37	0.16	*Solibacillus*	33	0.23
Vitreoscilla	32	0.14	*Haemophilus*	31	0.22
Haemophilus	31	0.14	*Pseudochrobactrum*	29	0.20
Trichococcus	31	0.14	*Oscillibacter*	29	0.20
Granulicatella	30	0.13	*Pseudomonas*	28	0.20
Turicibacter	30	0.13	*Mogibacterium*	24	0.17
Brevibacterium	29	0.13	*Vagococcus*	24	0.17

Table B3. Continued

Day 1					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Dorea	29	0.13	*Rummeliibacillus*	21	0.15
Helcococcus	29	0.13	*Arcobacter*	20	0.14
Rummeliibacillus	29	0.13	*Faecalibacterium*	18	0.13
Alloiococcus	28	0.12	*Peptostreptococcus*	18	0.13
Leucobacter	27	0.12	*Brevibacterium*	17	0.12
Rheinheimera	27	0.12	*Succinivibrio*	17	0.12
Solibacillus	27	0.12	*Atopostipes*	17	0.12
Blautia	26	0.11	*Nosocomiicoccus*	17	0.12
Enterococcus	24	0.11	*Granulicatella*	15	0.11
Anaerovorax	22	0.10	*Gemella*	15	0.11
Acidaminococcus	22	0.10	*Turicibacter*	15	0.11
Brevundimonas	20	0.09	*Enterobacter*	14	0.10
Zobellella	20	0.09	*Dorea*	14	0.10
Catenibacterium	18	0.08	*Brevundimonas*	13	0.09
Brachybacterium	17	0.07	*Luteimonas*	13	0.09
Vagococcus	17	0.07	*Enterococcus*	12	0.08
Anaerococcus	15	0.07	*Prevotella*	11	0.08
Ornithinimicrobium	14	0.06	*Chryseobacterium*	11	0.08
Faecalibacterium	14	0.06	*Anaerovorax*	11	0.08
Prevotella	13	0.06	*Alloiococcus*	11	0.08
Roseburia	13	0.06	*Catenibacterium*	11	0.08
Solobacterium	12	0.05	*Trichococcus*	10	0.07
Alishewanella	11	0.05	*Blautia*	9	0.06

Table B3. Continued

Day 1					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Dietzia	10	0.04	*Roseburia*	9	0.06
Neisseria	10	0.04	*Arcanobacterium*	8	0.06
Janibacter	9	0.04	*Dietzia*	8	0.06
Anaerovibrio	9	0.04	*Tessaracoccus*	8	0.06
Parvimonas	9	0.04	*Yokenella*	8	0.06
Subdoligranulum	9	0.04	*Alishewanella*	8	0.06
Tessaracoccus	8	0.04	*Brachybacterium*	7	0.05
Paracoccus	8	0.04	*Paracoccus*	7	0.05
Dialister	8	0.04	*Anaerococcus*	6	0.04
Proteiniclasticum	8	0.04	*Abiotrophia*	6	0.04
Atopostipes	8	0.04	*Lachnospiracea_incertae_sedis*	5	0.04
Lachnospiracea_incertae_sedis	7	0.03	*Solobacterium*	5	0.04
Arthrobacter	6	0.03	*Janibacter*	4	0.03
Parabacteroides	6	0.03	*Ornithinimicrobium*	4	0.03
Enhydrobacter	6	0.03	*Leucobacter*	4	0.03
Luteimonas	6	0.03	*Parabacteroides*	4	0.03
Peptoniphilus	6	0.03	*Porphyromonas*	4	0.03
Fusobacterium	5	0.02	*Empedobacter*	4	0.03
Kytococcus	5	0.02	*Selenomonas*	4	0.03
Selenomonas	5	0.02	*Proteiniclasticum*	4	0.03
Microbacterium	4	0.02	*Flavonifractor*	4	0.03
Bacteroides	4	0.02	*Planococcaceae incertae sedis*	4	0.03
Butyricicoccus	4	0.02	*Erysipelothrix*	4	0.03

Table B3. Continued

Day 1					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Psychrobacillus	4	0.02	*Mannheimia*	3	0.02
Gemella	4	0.02	*Allisonella*	3	0.02
Alcaligenes	3	0.01	*Syntrophococcus*	3	0.02
Actinobacillus	3	0.01	*Parvimonas*	3	0.02
Pasteurella	3	0.01	*Subdoligranulum*	3	0.02
Allisonella	3	0.01	*Arthrobacter*	2	0.01
Mitsuokella	3	0.01	*Kytococcus*	2	0.01
Syntrophococcus	3	0.01	*Olsenella*	2	0.01
Coprococcus	3	0.01	*Soonwooa*	2	0.01
Ruminococcus	3	0.01	*Neisseria*	2	0.01
Abiotrophia	3	0.01	*Rhodobacter*	2	0.01
Lysinibacillus	3	0.01	*Salmonella*	2	0.01
Erysipelothrix	3	0.01	*Pasteurella*	2	0.01
Citricoccus	2	0.01	*Dialister*	2	0.01
Rhodococcus	2	0.01	*Coprococcus*	2	0.01
Collinsella	2	0.01	*Filifactor*	2	0.01
Soonwooa	2	0.01	*Deinococcus*	2	0.01
Naxibacter	2	0.01	*Citricoccus*	1	0.01
Sphingomonas	2	0.01	*Cellulomonas*	1	0.01
Serpens	2	0.01	*Agrococcus*	1	0.01
Thermomonas	2	0.01	*Microbacterium*	1	0.01
Streptophyta	2	0.01	*Collinsella*	1	0.01
Guggenheimella	2	0.01	*TM7_genera_incertae_sedis*	1	0.01

Table B3. Continued

Day 1					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Oribacterium	2	0.01	*Treponema*	1	0.01
Hydrogenoanaerobacterium	2	0.01	*Dysgonomonas*	1	0.01
Fastidiosipila	2	0.01	*Nitrosospira*	1	0.01
Pseudoflavonifractor	2	0.01	*Sutterella*	1	0.01
Allofustis	2	0.01	*Oxalobacter*	1	0.01
Planococcus	2	0.01	*Alysiella*	1	0.01
Sharpea	2	0.01	*Rhizobium*	1	0.01
Coprobacillus	2	0.01	*Paenochrobactrum*	1	0.01
Serinicoccus	1	0.00	*Methylobacterium*	1	0.01
Ornithinicoccus	1	0.00	*Gemmiger*	1	0.01
Sanguibacter	1	0.00	*Devosia*	1	0.01
Gordonia	1	0.00	*Sneathiella*	1	0.01
Olsenella	1	0.00	*Sphingomonas*	1	0.01
Porphyromonas	1	0.00	*Thalassospira*	1	0.01
Flavisolibacter	1	0.00	*Enhydrobacter*	1	0.01
Myroides	1	0.00	*Serpens*	1	0.01
Pusillimonas	1	0.00	*Rheinheimera*	1	0.01
Delftia	1	0.00	*Raoultella*	1	0.01
Conchiformibius	1	0.00	*Citrobacter*	1	0.01
Devosia	1	0.00	*Wohlfahrtiimonas*	1	0.01
Rhodobacter	1	0.00	*Lysobacter*	1	0.01
Aquicella	1	0.00	*Zobellella*	1	0.01
Morganella	1	0.00	*Ruminobacter*	1	0.01

Table B3. Continued

Day 1					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Salmonella	1	0.00	*Bacillariophyta*	1	0.01
Mannheimia	1	0.00	*Gp4*	1	0.01
Ignatzschineria	1	0.00	*Veillonella*	1	0.01
Lysobacter	1	0.00	*Gallicola*	1	0.01
Pseudoxanthomonas	1	0.00	*Peptoniphilus*	1	0.01
Chlorarachniophyceae	1	0.00	*Butyricicoccus*	1	0.01
Gp4	1	0.00	*Ruminococcus*	1	0.01
Gp7	1	0.00	*Clostridium IV*	1	0.01
Megamonas	1	0.00	*Murdochiella*	1	0.01
Sarcina	1	0.00	*Anaerosphaera*	1	0.01
Clostridium XlVa	1	0.00	*Acetoanaerobium*	1	0.01
Gallicola	1	0.00	*Weissella*	1	0.01
Clostridium IV	1	0.00	*Desemzia*	1	0.01
Flavonifractor	1	0.00	*Salinicoccus*	1	0.01
Murdochiella	1	0.00	*Cloacibacillus*	1	0.01
Peptostreptococcus	1	0.00	*Opitutus*	1	0.01
Ignavigranum	1	0.00			
Eremococcus	1	0.00			
Weissella	1	0.00			
Desemzia	1	0.00			
Bacillus	1	0.00			
Salinicoccus	1	0.00			
Asteroleplasma	1	0.00			

Table B3. Continued

Day 1					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Victivallis	1	0.00			
N =	22,676		N =	14,259	

Table B3. Continued

Day 3					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Proteus	*23,029*	49.78	*Proteus*	8,034	26.56
Aerococcus	4,311	9.32	*Aerococcus*	5,668	18.74
Jeotgalicoccus	2,371	5.13	*Ignatzschineria*	5,093	16.84
Micrococcus	1,743	3.77	*Providencia*	2,641	8.73
Arcobacter	1,197	2.59	*Jeotgalicoccus*	1,792	5.93
Globicatella	1,028	2.22	*Corynebacterium*	943	3.12
Myroides	991	2.14	*Staphylococcus*	922	3.05
Ignatzschineria	984	2.13	*Globicatella*	868	2.87
Comamonas	940	2.03	*Lactobacillus*	759	2.51
Corynebacterium	937	2.03	*Micrococcus*	557	1.84
Providencia	742	1.60	*Psychrobacillus*	464	1.53
Psychrobacter	647	1.40	*Facklamia*	307	1.02
Acinetobacter	644	1.39	*Wohlfahrtiimonas*	279	0.92
Lactobacillus	584	1.26	*Vagococcus*	240	0.79
Morganella	542	1.17	*Streptococcus*	221	0.73
Aeromonas	532	1.15	*Thiobacillus*	197	0.65
Shewanella	522	1.13	*Clostridium sensu stricto*	155	0.51
Clostridium sensu stricto	487	1.05	*Kocuria*	107	0.35
Facklamia	444	0.96	*Macrococcus*	106	0.35
Staphylococcus	363	0.78	*Morganella*	95	0.31
Vitreoscilla	344	0.74	*Megasphaera*	67	0.22
Clostridium XI	257	0.56	*Nosocomiicoccus*	64	0.21
Streptococcus	167	0.36	*Clostridium XI*	58	0.19

Table B3. Continued

Day 3					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Vagococcus	147	0.32	*Brevibacterium*	50	0.17
Kocuria	140	0.30	*Psychrobacter*	46	0.15
Megasphaera	131	0.28	*Myroides*	45	0.15
Peptostreptococcus	120	0.26	*Erysipelothrix*	37	0.12
Moraxella	100	0.22	*Alloiococcus*	28	0.09
Escherichia/Shigella	98	0.21	*Dietzia*	22	0.07
Stenotrophomonas	96	0.21	*Caryophanon*	20	0.07
Macrococcus	90	0.19	*Oscillibacter*	19	0.06
Pseudomonas	89	0.19	*Enterococcus*	19	0.06
Helcococcus	83	0.18	*Acinetobacter*	16	0.05
Brevibacterium	61	0.13	*Erysipelotrichaceae incertae sedis*	16	0.05
Alloiococcus	61	0.13	*Rothia*	15	0.05
Erysipelotrichaceae incertae sedis	59	0.13	*Trichococcus*	14	0.05
Anaerococcus	58	0.13	*Catenibacterium*	13	0.04
Fusobacterium	55	0.12	*Turicibacter*	12	0.04
Pseudochrobactrum	47	0.10	*Tessaracoccus*	11	0.04
Kurthia	40	0.09	*Paracoccus*	10	0.03
Caryophanon	38	0.08	*Peptostreptococcus*	9	0.03
Erysipelothrix	38	0.08	*Moraxella*	8	0.03
Brachybacterium	35	0.08	*Acidaminococcus*	8	0.03
Rummeliibacillus	34	0.07	*Dorea*	8	0.03
Nosocomiicoccus	33	0.07	*Collinsella*	7	0.02
Enterococcus	32	0.07	*Dialister*	7	0.02

Table B3. Continued

Day 3					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Trichococcus	31	0.07	*Allofustis*	7	0.02
Alcaligenes	30	0.06	*Brachybacterium*	6	0.02
Proteocatella	30	0.06	*Brenneria*	6	0.02
Rothia	29	0.06	*Anaerococcus*	6	0.02
Acidaminococcus	28	0.06	*Rummeliibacillus*	6	0.02
Mogibacterium	27	0.06	*Solibacillus*	6	0.02
Tessaracoccus	25	0.05	*Mogibacterium*	5	0.02
Catenibacterium	25	0.05	*Helcococcus*	5	0.02
Dietzia	24	0.05	*Atopostipes*	5	0.02
Solibacillus	21	0.05	*Granulicatella*	5	0.02
Brevundimonas	20	0.04	*Gemmiger*	4	0.01
Atopostipes	20	0.04	*Escherichia/Shigella*	4	0.01
Paracoccus	19	0.04	*Succinivibrio*	4	0.01
Turicibacter	19	0.04	*Faecalibacterium*	4	0.01
Oscillibacter	18	0.04	*Desemzia*	4	0.01
Dorea	17	0.04	*Planococcaceae incertae sedis*	4	0.01
Wohlfahrtiimonas	16	0.03	*Janibacter*	3	0.01
Finegoldia	15	0.03	*Agrococcus*	3	0.01
Flavobacterium	14	0.03	*Mycobacterium*	3	0.01
Peptoniphilus	14	0.03	*Allisonella*	3	0.01
Salmonella	13	0.03	*Ruminococcus*	3	0.01
Neisseria	12	0.03	*Anaerosphaera*	3	0.01
Succinivibrio	12	0.03	*Planococcus*	3	0.01

Table B3. Continued

Day 3					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Collinsella	11	0.02	*Solobacterium*	3	0.01
Ornithinimicrobium	10	0.02	*Iamia*	2	0.01
Citrobacter	8	0.02	*Ornithinimicrobium*	2	0.01
Anaerofilum	8	0.02	*Trueperella*	2	0.01
Desemzia	8	0.02	*Enhydrobacter*	2	0.01
Janibacter	7	0.02	*Zymobacter*	2	0.01
Wautersiella	7	0.02	*Selenomonas*	2	0.01
Luteimonas	7	0.02	*Syntrophococcus*	2	0.01
Selenomonas	7	0.02	*Peptoniphilus*	2	0.01
Parvimonas	7	0.02	*Lactococcus*	2	0.01
Subdoligranulum	7	0.02	*Eremococcus*	2	0.01
Sphingobacterium	6	0.01	*Kurthia*	2	0.01
Anaerovorax	6	0.01	*Yaniella*	1	0.00
Granulicatella	6	0.01	*Dermatophilus*	1	0.00
Clostridium XIX	5	0.01	*Sanguibacter*	1	0.00
Veillonella	5	0.01	*Nocardioides*	1	0.00
Syntrophococcus	5	0.01	*Propionibacterium*	1	0.00
Blautia	5	0.01	*Atopobium*	1	0.00
Lachnospiracea incertae sedis	5	0.01	*Prevotella*	1	0.00
Fastidiosipila	5	0.01	*Porphyromonas*	1	0.00
Anaerosphaera	5	0.01	*Sediminibacterium*	1	0.00
Solobacterium	5	0.01	*Chitinophaga*	1	0.00
Deinococcus	5	0.01	*Planobacterium*	1	0.00

Table B3. Continued

Day 3					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Sanguibacter	4	0.01	*Arcobacter*	1	0.00
Olsenella	4	0.01	*Achromobacter*	1	0.00
Chryseobacterium	4	0.01	*Undibacterium*	1	0.00
Brachymonas	4	0.01	*Delftia*	1	0.00
Sphingomonas	4	0.01	*Acidovorax*	1	0.00
Gallicola	4	0.01	*Ochrobactrum*	1	0.00
Faecalibacterium	4	0.01	*Sphingomonas*	1	0.00
Salinicoccus	4	0.01	*Klebsiella*	1	0.00
Gemella	4	0.01	*Gallibacterium*	1	0.00
Arthrobacter	3	0.01	*Luteimonas*	1	0.00
Leucobacter	3	0.01	*Bacillariophyta*	1	0.00
Bacteroides	3	0.01	*Streptophyta*	1	0.00
Azospira	3	0.01	*Anaerovorax*	1	0.00
Enhydrobacter	3	0.01	*Succiniclasticum*	1	0.00
Haemophilus	3	0.01	*Proteiniclasticum*	1	0.00
Allisonella	3	0.01	*Oribacterium*	1	0.00
Proteiniclasticum	3	0.01	*Cellulosilyticum*	1	0.00
Roseburia	3	0.01	*Blautia*	1	0.00
Butyricicoccus	3	0.01	*Roseburia*	1	0.00
Pseudoflavonifractor	3	0.01	*Lachnospiracea incertae sedis*	1	0.00
Porphyromonas	2	0.00	*Tissierella*	1	0.00
Riemerella	2	0.00	*Butyricicoccus*	1	0.00
Rhodobacter	2	0.00	*Flavonifractor*	1	0.00

Table B3. Continued

Day 3					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Bacillariophyta	2	0.00	*Subdoligranulum*	1	0.00
Dialister	2	0.00	*Ignavigranum*	1	0.00
Oribacterium	2	0.00	*Exiguobacterium*	1	0.00
Coprococcus	2	0.00	*Lysinibacillus*	1	0.00
Alkaliphilus	2	0.00	*Salinicoccus*	1	0.00
Hydrogenoanaerobacterium	2	0.00	*Sharpea*	1	0.00
Flavonifractor	2	0.00			
Acetoanaerobium	2	0.00			
Exiguobacterium	2	0.00			
Planococcus	2	0.00			
Iamia	1	0.00			
Cellulosimicrobium	1	0.00			
Mobilicoccus	1	0.00			
Georgenia	1	0.00			
Cellulomonas	1	0.00			
Agrococcus	1	0.00			
Kytococcus	1	0.00			
Arcanobacterium	1	0.00			
Actinomyces	1	0.00			
Mycobacterium	1	0.00			
Atopobium	1	0.00			
TM7 genera incertae sedis	1	0.00			
Prevotella	1	0.00			

Table B3. Continued

Day 3					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Barnesiella	1	0.00			
Helicobacter	1	0.00			
Campylobacter	1	0.00			
Naxibacter	1	0.00			
Alysiella	1	0.00			
Bosea	1	0.00			
Rhizobium	1	0.00			
Camelimonas	1	0.00			
Mesorhizobium	1	0.00			
Hyphomicrobium	1	0.00			
Gemmiger	1	0.00			
Devosia	1	0.00			
Phenylobacterium	1	0.00			
Sphingopyxis	1	0.00			
Ponticoccus	1	0.00			
Rheinheimera	1	0.00			
Raoultella	1	0.00			
Pasteurella	1	0.00			
Thermomonas	1	0.00			
Alishewanella	1	0.00			
Streptophyta	1	0.00			
Gp2	1	0.00			
Gp1	1	0.00			

Table B3. Continued

Day 3					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Anaerovibrio	1	0.00			
Mitsuokella	1	0.00			
Succiniclasticum	1	0.00			
Sarcina	1	0.00			
Ruminococcus	1	0.00			
Isobaculum	1	0.00			
Allofustis	1	0.00			
Psychrobacillus	1	0.00			
Lysinibacillus	1	0.00			
Sphaerobacter	1	0.00			
N =	46,261		N =	30,244	

Table B3. Continued

Day 5					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Proteus	39,360	72.23	*Psychrobacillus*	1,907	57.98
Peptostreptococcus	2,767	5.08	*Ignatzschineria*	578	17.57
Ignatzschineria	2,328	4.27	*Clostridium sensu stricto*	329	10.00
Anaerosphaera	1,452	2.66	*Tissierella*	94	2.86
Wohlfahrtiimonas	1,169	2.15	*Vagococcus*	75	2.28
Morganella	878	1.61	*Clostridium XI*	66	2.01
Aerococcus	826	1.52	*Peptostreptococcus*	32	0.97
Clostridium sensu stricto	810	1.49	*Nosocomiicoccus*	32	0.97
Jeotgalicoccus	784	1.44	*Providencia*	25	0.76
Vagococcus	539	0.99	*Staphylococcus*	22	0.67
Myroides	489	0.90	*Corynebacterium*	16	0.49
Globicatella	408	0.75	*Streptococcus*	15	0.46
Clostridium XI	393	0.72	*Allofustis*	11	0.33
Corynebacterium	378	0.69	*Lactobacillus*	11	0.33
Facklamia	198	0.36	*Jeotgalicoccus*	8	0.24
Lactobacillus	179	0.33	*Pseudomonas*	7	0.21
Micrococcus	155	0.28	*Gp2*	4	0.12
Staphylococcus	150	0.28	*Comamonas*	3	0.09
Bacteroides	116	0.21	*Acinetobacter*	3	0.09
Psychrobacter	105	0.19	*Psychrobacter*	3	0.09
Streptococcus	78	0.14	*Tepidimicrobium*	3	0.09
Pseudomonas	68	0.12	*Enterococcus*	3	0.09
Tissierella	67	0.12	*Paenalcaligenes*	2	0.06

Table B3. Continued

Day 5					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Kocuria	64	0.12	*Marinobacter*	2	0.06
Anaerococcus	50	0.09	*Atopostipes*	2	0.06
Paenalcaligenes	47	0.09	*Erysipelothrix*	2	0.06
Helcococcus	46	0.08	*Turicibacter*	2	0.06
Cellulosilyticum	44	0.08	*Kocuria*	1	0.03
Kurthia	40	0.07	*Arthrobacter*	1	0.03
Providencia	34	0.06	*Trueperella*	1	0.03
Erysipelothrix	34	0.06	*Propionibacterium*	1	0.03
Brevibacterium	30	0.06	*Collinsella*	1	0.03
Alcaligenes	30	0.06	*Porphyromonas*	1	0.03
Acinetobacter	29	0.05	*Niastella*	1	0.03
Proteocatella	27	0.05	*Sediminibacter*	1	0.03
Macrococcus	21	0.04	*Sphingobium*	1	0.03
Enterococcus	17	0.03	*Moraxella*	1	0.03
Alloiococcus	17	0.03	*Cellvibrio*	1	0.03
Dietzia	14	0.03	*Alcanivorax*	1	0.03
Anaerofilum	14	0.03	*Escherichia/Shigella*	1	0.03
Trichococcus	13	0.02	*Proteus*	1	0.03
Nosocomiicoccus	13	0.02	*Morganella*	1	0.03
Aeromonas	12	0.02	*Klebsiella*	1	0.03
Alkaliphilus	11	0.02	*Citrobacter*	1	0.03
Brachybacterium	10	0.02	*Luteimonas*	1	0.03
Fusobacterium	9	0.02	*Nitrospira*	1	0.03

Table B3. Continued

Day 5					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Comamonas	8	0.01	*Gemmatimonas*	1	0.03
Escherichia/Shigella	7	0.01	*Gp1*	1	0.03
Ornithinimicrobium	6	0.01	*Gp6*	1	0.03
Moraxella	6	0.01	*Anaerococcus*	1	0.03
Mogibacterium	6	0.01	*Subdoligranulum*	1	0.03
Dorea	6	0.01	*Anaerosphaera*	1	0.03
Tessaracoccus	5	0.01	*Aerococcus*	1	0.03
Pseudochrobactrum	5	0.01	*Globicatella*	1	0.03
Paracoccus	5	0.01	*Leuconostoc*	1	0.03
Megasphaera	5	0.01	*Weissella*	1	0.03
Gallicola	5	0.01	*Carnobacterium*	1	0.03
Granulicatella	5	0.01	*Paenisporosarcina*	1	0.03
Erysipelotrichaceae incertae sedis	5	0.01	*Catenibacterium*	1	0.03
Catenibacterium	5	0.01			
Rothia	4	0.01			
Syntrophococcus	4	0.01			
TM7 genera incertae sedis	3	0.01			
Flavobacterium	3	0.01			
Arcobacter	3	0.01			
Paenochrobactrum	3	0.01			
Guggenheimella	3	0.01			
Finegoldia	3	0.01			
Peptoniphilus	3	0.01			

Table B3. Continued

Day 5					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Fastidiosipila	3	0.01			
Spiroplasma	3	0.01			
Clostridium XIX	2	0.00			
Janibacter	2	0.00			
Arthrobacter	2	0.00			
Marmoricola	2	0.00			
Burkholderia	2	0.00			
Altererythrobacter	2	0.00			
Blautia	2	0.00			
Butyricicoccus	2	0.00			
Subdoligranulum	2	0.00			
Filifactor	2	0.00			
Allofustis	2	0.00			
Desemzia	2	0.00			
Caryophanon	2	0.00			
Salinicoccus	2	0.00			
Georgenia	1	0.00			
Olsenella	1	0.00			
Collinsella	1	0.00			
Sorangium	1	0.00			
Vitreoscilla	1	0.00			
Pedomicrobium	1	0.00			
Sphingomonas	1	0.00			

Table B3. Continued

Day 5					
EXC			ACC		
Genus	*Count*	*Rel. Abundance (%)*	*Genus*	*Count*	*Rel. Abundance (%)*
Cosenzaea	1	0.00			
Citrobacter	1	0.00			
Luteimonas	1	0.00			
Stenotrophomonas	1	0.00			
Gp1	1	0.00			
Gp17	1	0.00			
Dialister	1	0.00			
Acidaminococcus	1	0.00			
Veillonella	1	0.00			
Proteiniclasticum	1	0.00			
Oscillibacter	1	0.00			
Acetoanaerobium	1	0.00			
Eremococcus	1	0.00			
Atopostipes	1	0.00			
Bacillus	1	0.00			
Rummeliibacillus	1	0.00			
Planococcus	1	0.00			
Psychrobacillus	1	0.00			
Turicibacter	1	0.00			
Clostridium XVIII	1	0.00			
N =	54,492		N =	3,289	

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