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Ecological Dynamics in Compost-Amended Soils and the Resulting Effects on Escherichia coli Survival

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ECOLOGICAL DYNAMICS IN COMPOST-AMENDED SOILS AND THE RESULTING EFFECTS ON *ESCHERICHIA COLI* SURVIVAL

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ABSTRACT

Escherichia coli (*E. coli*) are common and typically innocuous copiotrophic bacteria found in the mammalian gut microbiome. However, over the past 30 years, pathogenic *E. coli* have been responsible for several outbreaks of foodborne illness linked to contaminated produce. The introduction of *Escherichia coli* to an agricultural soil, via contaminated water, compost, or raw manure, exposes the bacterium to a medley of ecological forces not found in a mammalian gut environment. This study assesses a variety of abiotic and biotic soil factors that influence the ability of an "invasive" copiotrophic coliform bacterium to survive in compost-amended agricultural soil. The study included both field and laboratory components. In the lab experiment, a cocktail of rifampicin-resistant generic *E.coli* strains was added to sterile and non-sterile extracts of eight different composts and one soil sample from the field sites. *E. coli* abundance was monitored over a one-week period and composts were analyzed for their nutrient profile. In the field experiment, the same *E. coli* cocktail was sprayed on plots with the following treatments: 1) dairy windrow compost, 2) dairy vermicompost, 3) poultry windrow compost, or 4) no compost. *E. coli* abundance, soil water potential, soil temperature, extracellular enzyme activity, microbial respiration, phospholipid fatty acid biomarker abundance, and genetic sequencing of the microbial community were measured over a six-month field season.

The lab experiment showed that *E. coli* were able to grow well in sterile compost extracts, without microbial competition for nutrients. Conversely, *E. coli* populations were only able to survive in non-sterile soil extracts. These results suggest that copiotrophic organisms adapted for high-nutrient environments may depend on the extracellular enzyme activity of native oligotrophic organisms to acquire sufficient nutrients to survive in soils. Results of the field experiment showed clear and interdependent effects of soil moisture and nutrient availability on microbial community dynamics and *E. coli* survival. Data suggest that saturated soils cause a decrease in microbial extracellular enzyme activity, and drying-rewetting cycles can cause respiration bursts, nutrient mineralization, and shifts in community composition. The saturation of soils, which mobilizes nutrients and may result in a decrease in competition from aerobic organisms, correlated directly with increased survival of *E. coli*. Additionally, amendment with ammonium-rich poultry compost resulted in the maintenance of high levels of *E. coli* throughout the field season. Despite an increase in microbial biomass from dairy vermicompost amendment, poultry compost was the only compost that had a significant effect on *E. coli* survival. The results suggest that nitrogen availability and water potential are strong drivers of *E. coli*'s survival in soils. Correlations among abiotic factors, community composition, and *E. coli* survival reveal insights into the complex relationships that occur in disturbed agricultural soil environments. Further research on *E. coli*'s response to targeted organisms, abiotic soil properties, and nutrient inputs could have implications for agricultural considerations in food safety and microbial ecology.

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CHAPTER 1. INTRODUCTION

1.1. *Escherichia coli* Characteristics and Environment

Escherichia coli (*E. coli*) are rod-shaped Gram negative bacteria primarily found in the lower intestine of warm-blooded animals (Smith 1965). *E. coli* is widely recognized as a model organism for microbiology, an indicator organism for public health research, and a potentially dangerous human pathogen. Despite being one of the most widely studied organisms on the planet, its ecological functions and biological interactions in natural habitats are poorly characterized (Winfield and Groisman 2003). Due to its rapid growth and easy culturability, the use of *E. coli* as a model organism in studies of ecological microbiology provides a useful tool for understanding the interactions between microbes and their surrounding environment. As genetic sequencing has become increasingly prevalent in scientific studies, microbial populations are now recognized as an important system for understanding ecological theory. Adaptive dynamics, such as the development of discrete niches within an ecosystem, are postulated by using links between quantitative information on microbial community structure and function (Prosser *et al.* 2007). To understand how *E. coli* can be used in such ecological applications, its primary and secondary habitats must first be discussed.

E. coli's primary, or natural, habitat is the mammalian gut. *E. coli* generally enter mammalian colons during birth, with only a few strains colonizing the colon during a mammal's lifetime (Sears *et al.* 1950). The mammalian gut provides a stable temperature

and osmolarity, with high levels of free amino acids and sugars that are broken down by intestinal enzymes but poorly absorbed by the mammalian system (Savageau 1983). The abundant supply of monomeric nutrients and warm temperatures sustain *E. coli* survival in the gut (Winfield and Groisman 2003). *E. coli* are considered copiotrophic *r*-strategists, because they grow rapidly in nutrient-rich environments but are relatively poor competitors when nutrients are limited. Competition for nutrients in the mammalian gut microbiome is partially limited by the absence of oxygen, which restricts inhabitation to only obligate and facultative anaerobes (Gao *et al.* 2014). *E. coli* is a facultative anaerobe, allowing it to respire in the absence of oxygen using nitrate, nitrite, fumarate, dimethyl sulfoxide (DMSO), and trimethylamine N-oxide as electron acceptors or by fermentation (Unden *et al.* 1994). The conditions found in a mammalian colon provide the environment necessary for *E. coli* to maintain high population levels, and mammalian excretions typically contain between 10^4 to 10^9 colony forming units (CFUs) of *E. coli* per gram of feces (Tenaillon *et al.* 2010). Once excreted, however, *E. coli* will enter secondary habitats such as soil, sediments, and water, in which fluctuating environmental conditions can have varying effects on *E. coli* survival (Savageau 1974). The dynamics between the abiotic and biotic factors of such secondary environments and *E. coli* survival provides a framework for ecological analysis.

Soil ecology is a rapidly growing field, using a combination of advanced sequencing techniques and molecular assays to understand the complex interactions that occur in heterogeneous and constantly changing soil environments. Fecal contamination of soils introduces *E. coli* to these dynamic systems, which can support *E. coli*

populations from only a few days (Savageau 1983) to many months (Jiang *et al.* 2002, Islam *et al.* 2004). The soil environment has many more fluctuating environmental variables that can affect *E.* coli's survival than the primary mammalian gut habitat. First, the aggregate soil structure creates physical barriers that result in heterogeneous hot spots of nutrient availability and creates infinite combinations of niche habitats for particular ecotypes (Six *et al.* 2004). Second, soil environments are generally aerobic and can sustain the growth of obligate aerobe populations that are absent in the colon, increasing competition and predation for *E. coli*. Third, the nutrients in secondary environments are found in complex organic substrates that require the extracellular secretion of microbial enzymes to be converted into biologically available forms (Sinsabaugh and Shah 2012). This energy-intensive and tightly-regulated process is unnecessary in a host gut environment. Additionally, fluctuations in temperature, pH, and osmotic stress can hamper *E. coli* survival in soil environments. However, niche environments that mimic mammalian gut conditions can extend *E. coli* survival. For example, tropical soils can sustain high levels of *E. coli* due to their warm, moist, and nutrient-rich conditions (Jimenez *et al*. 1989). In flooded soils, oxygen becomes depleted and microbial communities shift towards facultative and obligate anaerobes. *E. coli* have shown greater survival abilities in flooded soils that become anaerobic compared with aerobic soils (Tate 1978), likely due to the reduction in competition from obligate aerobes. Although studies have repeatedly shown differences in *E. coli* survival with varying soil environments, the links between *E. coli* survival and the soil environment have never been used to gain information on underlying ecological trends and community theory.

While *E. coli* serves as an excellent model organism for such a study because of its growth characteristics, research on its survival in soil is also critically important for public health. This work, although focused on advancing knowledge of soil ecology trends, has important implications for managing pathogenic *E. coli* strains.

1.2. Pathogenic *E. coli* and Implications in Food Safety

1.2.1. The Origin of Pathogenic *E. coli*

In 1982, two outbreaks of hemorrhagic colitis were linked to fast-food hamburgers containing a Shiga-toxin producing strain of *E. coli* (Mead and Griffin 1998). Over the past 30 years, enterohemorrhagic *E.coli* (EHEC) strains have become a recurring public health concern, accounting for more than 90% of haemolytic uraemic syndrome cases in developed countries and causing 73,000 related cases in the United States annually (Mead and Griffin 1998, Rangel *et al*. 2005). Enterohemorrhagic *E. coli* produces a Shiga toxin which, along with accessory virulence factors, can cause symptoms ranging from diarrhea to death depending on host-bacterial interactions (Paton and Paton 1998). Multiple studies have shown that cattle are the principle reservoir of pathogenic *E. coli* strains (Wang *et al.* 1996). Concentrated animal feeding operations (CAFOs), used in the meat and dairy industry in developed countries, rear cattle in high density environments and typically give sub-therapeutic levels of antibiotics to the cattle to stimulate growth (Alexander *et al.* 2008). CAFOs account for approximately 2% of farms in the United States, but produce over 40% of the livestock (Copeland 2010).

EHEC strains have high mutation rates (LeClerc *et al.* 1996), allowing populations to quickly evolve resistance to the antibiotics used in CAFOs and to proliferate rapidly in a nutrient-rich and uncompetitive environment (Alexander *et al.* 2008). Cattle lack Shiga toxin receptors and are primarily asymptomatic carriers of *E. coli*. Thus, EHEC cannot be eradicated from feedlots by removing symptomatic cattle (PruimBroom-Brees *et al.* 2000). Hussein (2007) found the prevalence of *E. coli* O157:H7, one of the most common and dangerous strains of hemorrhagic *E. coli*, to be 0.1-54.2% in ground beef, 0.1-4.4% in sausages, 1.1-36% in retail cuts, and 0.01 to 43.4% in whole carcasses. While feedlots are the most common reservoir of pathogenic *E. coli*, 0.7-23.7% of pastured cattle farms contain *E. coli* O157:H7 (Hussein 2007).

1.2.2. Pathogenic *E. coli* in Contaminated Produce

Unfortunately, the threat of pathogenic *E. coli* contamination does not end with beef and dairy products. In 2006, a large *E. coli* O157:H7 outbreak was traced to Dole® bagged spinach. The spinach came from four farms in Salinas Valley, California (Gelting 2007). The U.S. Centers for Disease Control and Prevention (CDC) investigated potential sources of contamination, including soil amendments, irrigation water, and runoff. A thorough study of the watershed revealed that Salinas Valley restores groundwater levels using imported surface water from nearby reservoirs and stored winter runoff. Such restoration methods may introduce pathogens into the groundwater used for irrigation in Salinas Valley (Gelting 2007). *E. coli* is relatively stable in groundwater compared to laboratory and soil conditions (Bitton *et al.* 1983). The investigation found that the farms

linked to contaminated produce would pump groundwater for irrigation, creating a gradient that draws surface contaminants downward into the groundwater (Gelting 2011). Furthermore, the *E. coli* O157:H7 strain linked to the outbreaks was also present in cattle manure suspended in surface water from nearby rivers. The investigation concluded that the irrigation water used for the Salinas Valley farms was a likely cause of the 2006 outbreaks. In addition to the 2006 outbreaks, illnesses from *E. coli* have been linked to the contamination of a variety of fresh produce products, shifting attention away from cattle farms (Ackers *et al.* 1998). *E. coli* O157:H7 can migrate into internal plant tissue, rendering surface sterilization ineffective (Solomon *et al* 2002). Because many of the crops linked to *E. coli* outbreaks are typically eaten raw, this particular method of contamination is a likely and alarming cause of widespread illness.

1.2.3. National Regulation of Pathogenic *E. coli*

In 2015, the United States Food and Drug Administration (FDA) released newly revised Food Safety and Modernization Act (FSMA) regulations to decrease the prevalence of pathogenic microorganisms in produce. The regulations include stringent testing for *E. coli* contamination of any ground or surface water used for the irrigation of crops (USDA 2015). The FSMA rule requires that the mean *E. coli* population in water sources occurs below 126 colony forming units (CFUs) per milliliter of water. Surface water must have 20 samples tested at the beginning of use by a farmer, with an annual testing of five samples every year after the initial survey. Ground water, because it is better protected from contamination than surface water, requires four samples at the beginning of use, and one sample each following year. Additionally, the FSMA allows

farmers to abstain from testing if they use treated public water. The FSMA also encourages farmers to use drip irrigation, which results in substantially lower levels of contamination than overhead irrigation (Stine *et al.* 2006). Although the Food Produce Rule appropriately focuses on reducing water contamination, it also includes limitations on soil amendment use. This is despite the fact that very few cases linking a foodborne outbreak to soil amendment use exist (FDA 2015). Past versions of the FSMA have required a 120-day interval between raw manure application and harvest for crops in contact with the soil and a 90 day interval between raw manure application and harvest for crops not in contact with the soil (USDA 2015). The updated FSMA guidelines have increased the required holding period to nine months between the application of raw manure and harvest. The cited studies that guided the reasoning for the increase in holding period showed that *E. coli* O157:H7 can survive up to 217 days in parsley when the *E. coli* is introduced by contaminated compost and up to 177 days when the *E. coli* is introduced by contaminated water (FDA 2015, Islam *et al.* 2004). However, these studies were performed in Georgia, USA, where the climate and soil type is not representative of many other growing regions. Indeed, a host of studies have shown differential survival of *E. coli* O157:H7 based on discrete soil characteristics. *E. coli* O157:H7 are able to survive longer in rhizosphere soil than in non-rhizosphere soil and phyllospheres (Ibekwe *et al.* 2004). Because rhizospheres can vary dramatically based on the plant species and cultivar and, in turn, have strong effects on the rhizosphere microbiome (Philpott *et al.* 2013), one would imagine that survival of *E. coli* O157:H7 would also vary depending on the crop species and planting density. Other studies show that clay soils increase *E. coli*

O157:H7 survivability compared to silty loam soils (Ingham *et al.* 2005). This could potentially be due to a greater cation carrying capacity and therefore larger available nutrient pool or a smaller pore density that excludes predation by other organisms, holds water more tightly, and limits oxygen exchange. *E. coli* O157:H7's survival rates vary with different soil temperatures, different soil carbon quality, and in sterile versus non-sterile soil (Vidovic *et al.* 2007). To better understand how to manage pathogenic *E. coli,* empirically testing the effects of ecological trends and soil community dynamics on *E. coli* survival would provide more predictive groundwork on which to base government regulations for protecting the soil from pathogen survival.

1.2.4. Regulating Pathogenic *E. coli* in Northern States

The use of raw manure and composted manure amendments is a common agricultural practice for increasing the nutrient content of soil. However, these amendments have the potential to introduce pathogenic *E. coli* (Islam *et al*. 2004). The recent FSMA recommendation of a 270-day holding interval from amendment to crop harvest caused a backlash from northern state farmers, where the growing season is rarely over five months long (Vermont Agency of Agriculture Food & Markets 2013). A 75-day public hearing pushed the FDA to recruit the Agricultural Research Service (ARS) and the University of Vermont to further assess site-specific survival patterns of *E. coli* in Vermont soils (Kahler 2014). The study uses two field sites located in South Burlington, Vermont. Plots are either inoculated with *E.coli*-contaminated manure or water and monitored until the *E. coli* are no longer detectable in the soil or in spinach (Lekkas *et al.* 2015). The sites used for the Vermont study have been in managed hay production for the

past several years. While most studies assessing *E. coli* survival were on land in continuous vegetable production, the sites used for this study have not supported vegetable crops in recent history. Vegetable production is the primary non-mammalian source of *E. coli* outbreaks (USDA 2015). If the sites were to be converted to vegetable production, soils would typically be amended by farmers to increase the nutrient concentrations. Although one of the treatments included raw manure in the ARS study, compost would be a much more common amendment for organic vegetable production because of the organic certification requirements by the Northeast Organic Farming Association of Vermont. Additionally, given that a 120-day holding interval exceeds the time that it takes for greens to mature after planting, farmers would not be able to wait for the required period of time if using raw manure as an amendment. To keep this study relevant to concerns of pathogenic *E. coli* survival, compost amendment was used as a treatment variable. Furthermore, compost amendment changes the microbial and nutritional profiles of soils (Insam *et al.* 1996, Goyal *et al.* 2005), therefore providing shifts in ecological dynamics from which links to *E. coli* survival can be extrapolated.

1.3. Composting Process and Effects

1.3.1. The Effects of Compost on Soil Properties

Compost amendment can be used as a tool in soil ecology research to dramatically alter soil properties and the soil microbiome, while providing information on soil management techniques relevant to agriculture practices. In agricultural settings,

composted manure often replaces raw manure as a soil amendment due to its decreased threat of pathogenic contamination, reduced phosphorous load, and beneficial effects on soil physical properties (Evanylo *et al*. 2008). Composting involves a controlled decomposition of organic waste under aerobic environments. The resulting product increases soil organic matter content, improves aggregation, reduces soil erosion and runoff, increases nutrient availability to the microbial and plant community, and increases biological activity (Blanco *et al* 2015, Giusquiani *et al* 1995, Paglai and De Nobili 1993). Compost production, however, is extremely diverse and can yield compost products with profoundly different effects on soil depending on the recipe and production methods used. As a general rule, the starting substrates affect the nutrient balance, pH, particle size, and porosity of the compost, and the processing method affects the oxygen concentration, temperature, and water content of the compost (Bernal *et al*. 2009). The nutrient availability of the compost depends on the extent to which is it degraded by the endemic microbes and colonized during the curing after thermophilic requirements are reached. In the early phase of composting, labile organic compounds, such as monosaccharides, fats, and amino acids, are fully degraded and the more complex organic compounds like lignin and hemicellulose are partially degraded (Haug 1993). Additional processing, such as vermicomposting, which uses earthworms to further transform compost products, can alter the nutritional profile (Frederickson *et al*. 2003) as well as the microbial profile (Neher *et al*. 2013) of the compost. Because of the heterogeneity among different compost products, the subsequent effects of compost amendment on soils can vary dramatically. Linking the different effects of compost

amendment on soil properties to *E. coli* survival provides a useful tool for understanding how organisms introduced to soils are affected by soil variables.

1.3.2. *E. coli* Survival in Compost-Amended Soil

The National Organic Program standards under the USDA require that windrow compost be held between 55°-77°C for fifteen days (Cornell Cooperative Extension 2004). While this would theoretically be sufficient to kill any contaminating pathogenic organism, the heterogeneity within windrow compost piles may not sustain sufficiently high temperatures uniformly throughout the pile (Islam *et al*. 2005). Additionally, compost can come in contact with pathogens by contamination with raw manure, contaminated water, or by wind dispersal. A number of studies have shown that compost amendments can increase the ability of *E. coli* to survive in the soil (Islam *et al*. 2005). In addition to soil amendments, *E. coli* survival can also be affected by soil type, temperature, pH, and microbial community composition (Van Veen *et al*. 1997). A study by Franz *et al*. (2008) found that variation in *E. coli* survival across 36 different soil types is correlated positively with dissolved organic carbon, ammonium content, and the number of years the soil had been in organic management. Because *E. coli* are copiotrophic organisms, adapted to high-nutrient gut environments, they would be expected to decline along a one-phase decay model when introduced to low-nutrient soil environments. Instead, they have been found to follow a biphasic model in many soil and water environments, in which an initial rapid decay is followed by a second slower decay (Phaiboun *et al*. 2015). The second phase may be due to several factors: The *E. coli* reach a carrying capacity, the organisms are regulating their population size by quorum sensing,

or a smaller subpopulation exists with more resilient adaptations to environmental stress and will therefore have a slower decay rate (Rogers *et al*. 2011). The kinetics of *E. coli* survival is a function of both environmental abiotic and biotic factors and their interactions. This study attempts to elucidate which of these environmental factors are prominent drivers of *E. coli* survival.

1.4. Biological Dynamics in Soils

1.4.1. *E. coli* as an Invasive Soil Organism and Potential Interactions

While nutritional inputs and climactic variables may have a strong influence on *E. coli* survival in soil, their effects can only be understood in context of the microbial community. Nutrient availability is the primary driver of soil microbial composition and dominating taxa (Hibbing *et al*. 2010). However, complex interactions between members of the soil community distort the relationship between bacterial abundance and nutrient levels. Biological interactions, including commensalism, mutualism, and parasitism, regulates community structure and function (Nemergut 2013). Microbial dynamics are stabilized by the co-evolution of competitive interactions between and among species (Hibbing *et al.* 2010). Soil microbial communities are over-dispersed due to high levels of competition, meaning that the dispersal of species is greater than what would occur if dispersal were random (Horner-Devine *et al.* 2007). An invasive organism such as *E. coli* could either benefit or suffer from biological interactions occurring within soil communities. Of course, the equilibrium of such biological interactions is influenced by

abiotic soil properties. Bacteria are maintained at threshold levels by protozoan grazing (Alexander 1981), but that threshold level can increase with an excess of nutrients, smaller soil pore size, and rhizosphere exudates (Recorbet *et al.* 1992, Van Elsas *et al.* 1986). Microbial dynamics are further complicated by horizontal gene transfer, which can transfer beneficial adaptations between disparate species (Papke and Gogarten 2012). The assessment of the microbial community in *E. coli* invaded soils could reveal potential biological interactions and ecotypes that influence the success of *E. coli* survival.

1.4.2. Extracellular enzymes

Most nutrients that enter the soil are found in polymeric organic matter and are, therefore, unavailable for direct bacterial consumption. Microbes secrete extracellular enzymes into the soil matrix to degrade complex substrates into monomeric biologically-available forms (Burns 1982). Enzyme synthesis and secretion is energy-intensive and tightly regulated (Schimel 2007). In general, microbes benefit by secreting enzymes that will increase the availability of limited nutrients and decreasing the synthesis of unnecessary enzymes. The addition of phosphorous fertilizers, for example, inhibits the secretion of the extracellular enzyme phosphatase, which catalyzes the hydrolysis of ester-phosphate bonds and releases phosphate (Allison and Vitousek 2005). However, there are several biological and environmental conditions which can alter the relationship between nutrient availability and enzyme secretion (Burns 1982). The activity of extracellular enzymes in soils can be measured spectrophotometrically after incubation of the soil with a fluorophore-tagged substrate that is cleaved by a specific enzyme (Sinsabaugh *et al.* 2008). The evaluation of enzyme activity in soil provides insight into

the functional link between resource availability, microbial composition, and ecosystem processes (Caldwell 2005).

1.4.3. Compositional analysis through sequencing and PLFA

Genetic sequencing has transformed scientific understanding of soil microbial communities (Paul 2015). Many organisms once thought to be abundant in most soils because of their easy culturability in the lab are now known to be rare in comparison to common soil taxa found by modern sequencing methods (Rappe and Giovannoni 2003). While only approximately 5,000 microbial species are culturable in total, genetic analysis has revealed approximately 500,000 species exist in a single 30 gram soil sample (Daniel 2004). Although sequencing has revealed a number of unknown species in recent years, the dynamics and functions of these communities are difficult to identify because of variable α-diversity and functional redundancy. For example, Buerger *et al.* (2012) found that 2-12% of 16S sequencing reads from soil communities were associated with unknown genera. However, because these taxa could not be cultured and there were no obvious functional differences between soil communities, their ecological roles remain unknown. Ecological functioning of a handful of common soil taxa have been proposed, mostly by defining them as *r*-strategist copiotrophs or *k*-strategist oligotrophs (Fierer *et al.* 2007).

Phospholipid fatty acid analysis (PLFA) has become one of the most popular methods for measuring microbial biomass and broad community structure (Frostegård *et al.* 2011). Although the classification of fatty acid biomarkers with particular taxa is somewhat debated, the technique provides a relatively inexpensive analysis of microbial

quantity. Combined with sequencing analysis, enzyme activity, and respiration measurements, complex dynamics in soil microbial structure and the links to fluctuations in abiotic soil factors can be inferred. The linking of such patterns with *E. coli* survival produces quantitative data from which ecological theory can be applied to soil systems.

1.5. Objectives and Hypotheses

The purpose of this study was to evaluate the effects of varying abiotic and biotic soil factors, driven by different compost amendments and climactic fluctuations, on *E. coli* survival in laboratory extracts and field environments. A laboratory experiment was designed to determine the: 1) difference in *E. coli* survival when the compost's endemic microbial community is present or absent; 2) correlation between *E. coli* survival and the compost's nutrient composition; 3) difference between *E. coli* survival in nutrient-rich compost extracts versus nutrient-poor soil extracts. A field experiment was designed to determine the: 1) effect of different compost amendments on *E. coli* survival in a soil environment; 2) most predictive model for *E. coli* survival and decay rates in compost-amended soil; 3) contribution of soil water potential and temperature to variation in *E. coli* survival kinetics; 4) effect of compost amendment on microbial community composition, enzyme activity, and respiration; 5) relationship between *E. coli* survival and the endemic community dynamics. We hypothesized that nutrient content in composts would drive differences in *E. coli* survival and microbial composition and their two-way interactions, water and temperature would cause *E. coli* to deviate from a standard decay model, and inputs of biologically available nutrients through compost

amendment would cause a decrease in extracellular enzyme activity. The study was constructed to identify the components of the complex soil system that can drive an introduced microbe to successfully inhabit a secondary environment.

CHAPTER 2. METHODS

2.1. Laboratory Experiment

2.1.1. Samples Used

Eight composts were collected from commercial composters in Vermont, New York, and Maryland with varying starting substrates and processing methods (Table 2.1). Compost samples were sent to the University of Maine Soil Testing Lab for nutrient analysis. Total carbon, potassium, nitrogen, phosphorous, ammonium, and nitrate were determined using the methods described by Peters et al. (2003). Additionally, a composite soil sample from the two fields used in the field experiment was obtained (see Field Experiment methods). Subsamples of soil and composts were dried at 90^{o}C to compute a gravimetric moisture for converting all measures to per gram of dry soil.

2.1.2. Extract Preparation

Compost and soil extracts were used as a growing medium for *E. coli* to determine the relationship between *E. coli* growth, nutrient levels, and the presence of endemic microbes. Extracts of each compost and soil sample were prepared by diluting 250 grams of sample with 500 mL of distilled water, shaking for 24 hours at 22^{o}C and centrifuging at 5,000g for 20 minutes. The supernatant was collected, half was reserved as non-sterile extract and half was filtered through 0.2µm pore diameter vacuum filters to prepare sterile extracts. All extracts were stored at -20^{o}C until use.

Table 2.1 Compost test results from the University of Maine Soil Testing Lab. Results are from single composite compost samples.

Compost	**Abb.**	**Starting Materials**	**Processing**	**C (%)**	**N (%)**	**K (%)**	**P (%)**	**NH_4-N (ppm)**	**NO_3-N (ppm)**
Worm Power Dairy Windrow	WP-MD	Dairy manure	Aerated Static Pile	43.6	2.98	1.71	0.35	46.6	5711
Worm Power Dairy Vermicompost	WP-MDV	WP-T Compost	Vermicompost, mesophilic	39.6	3.82	2.83	0.55	23.5	1.77
Black Dirt Poultry Mature Vermicompost	BD-MPV	Food Scraps picked through by poultry	Vermicompost mesophilic	17.2	1.42	0.8	0.4	0.74	1093
Black Dirt Immature Vermicompost	BD-IPV	Food Scraps picked through by poultry	Vermicompost mesophilic	23.8	1.73	1.06	0.44	2.42	1845
Black Dirt Food Poultry	BD-MP	Food scraps, poultry manure, poultry bedding	Aerated Static Pile	28.5	2.15	0.96	0.88	6.08	671
Someday Farm Mature Mixed	SF-MP	Dairy manure, poultry manure, poultry butchering products	Windrow	16.0	1.66	0.76	1.01	2.44	1497
Someday Farm Immature Mixed	SF-IP	Dairy manure, poultry manure, poultry butchering products	Windrow	18.9	1.52	0.51	0.6	1.7	433
Maryland Poultry	MD-MP	Poultry manure, poultry bedding	Windrow	27.9	3.79	4.66	2.57	1855	1001

2.1.3. *E. coli* Inoculation and Enumeration

A three-strain mixture of Rifampicin-resistant *E. coli* isolated from Salinas Valley, California was used as the inoculum (TVS 353, 354, and 355). The isolate cocktail was chosen by the USDA as a representative sample of generic *E. coli*, with survival patterns similar to *E. coli* O157:H7 (Graham *et al*. 2014). Individual strains were stored in a 20% glycerol solution at -80^{o}C. Frozen stocks were streaked onto MacConkey agar with 80mg/mL of rifampicin and incubated at 35^{o}C for 24 hours. Single colonies of each strain were added to 50mLs of 0.1X TSB and shaken at 35^{o}C. After 24 hours, cultures were centrifuged at 5,000g for 20 minutes, washed twice with 0.85% saline and resuspended in 1 mL of 0.85% saline. Each strain was then adjusted to an OD_{600} value of 0.5 (approximately 10^8 CFU/mL) and serially diluted to 10^4 CFU/mL. Three replicates of each sterile and non-sterile extract were added to test tubes in 5 mL aliquots. The three *E. coli* strains were added to each test tube at a 1:100 ratio. For sterile extracts, *E. coli* were enumerated at 0, 4, 8, 20, 50, 72, 110, and 150 hours after inoculation by spread plating on MacConkey agar with 80 mg/mL Rifampicin and incubating at 35^{o}C for 24 hours. *E. coli* in non-sterile extracts were enumerated at 0, 24, 72, 110, and 158 hours.

2.1.4. Statistical Analyses

To compare statistical differences in *E. coli* growth between extracts, the area under the curve for *E. coli* abundance through time of each replicate was calculated using Graph Pad Prism v.6.05 and statistical differences in treatments were determined by an ANOVA followed by a Tukey's multiple comparison *t*-test.

An exponential growth model ($Y=Y_0^{kx}$) was fit to the log growth phase for *E. coli* in each treatment type with Graph Pad Prism and the growth rate constant (*k*) values were compared between the sterile and nonsterile extracts of each compost type using a paired *t*-test. A linear regression was run between the *k*-values from the non-sterile extracts and the compost's nutrient content to analyze the effect of discrete nutrients on *E. coli* growth potential.

2.2. Field Experiment

2.2.1. Field Sites

Two fields in South Burlington, Vermont (44°26'37.4"N, 73°11'23.2"W) with sandy loam soil ("Wheelock" and "Lilac") were used for the field trial, which ran from May through November of 2015. Both fields have been utilized for hay production for the past 10 years. Prior to treatment application, baseline soil samples from each field were obtained by taking 10 cm soil cores from four 2 square meter untreated control plots. Replicates of four plots were pooled and sent to the University of Maine Soil Testing lab as a single composite sample for analysis. Nutrient content and pH were determined by the methods outlined in NEC-1012 (2011). Briefly, a modified Morgan extract was used for nutrient extraction and pH was tested in a 1:1 water solution with Modified Mehlich buffer. Lilac has a Hinesburg B Fine Sandy Loam soil, with a pH of 6.4 and organic matter content of 2.9%. Wheelock is an Adams B Loamy Sand soil with a pH of 6.3 and an organic matter content of 2.6%.

2.2.2. Experimental Design

For each field, fifteen 1x2m plots were tilled to a depth of 30 centimeters using a rototiller. Plot treatments were assigned in a completely randomized design within each field with 1.5 meter buffer strips between each plot. Treatment combinations included three types of compost with *E. coli, E. coli* only, or untreated with three replications per treatment per field. Plots were either treated with no compost (6 plots per field), 1.36 kg of Worm Power dairy windrow compost, 1.36 kg of Worm Power dairy vermicompost, or 2.7 kg of Maryland poultry compost per the manufacturer's recommendations. The poultry compost was made by the Agricultural Research Service (ARS) and used at an application rate of 13.4 tons/acre (30,038.8 kg/ha) to match the rate used in the ARS studies. The Worm Power vermicompost, however, is much more expensive than standard windrow compost and is used in much smaller amounts. Therefore, both dairy composts were applied at a rate of 6.72 metric tons/acre (15,064.2 kg/ha) so that application rates were within a realistic range of what farmers would use for vermicompost (United States Composting Council 2001), and so a comparison between the dairy vermicompost and dairy windrow compost processing methods could be made. Compost was spread evenly across the surface of each plot and tilled in to a depth of 10 cm using a rototiller with 75% ethanol sterilization of the blades between treatments.

2.2.3. *E. coli* Inoculation of Field Plots

The same three-strain cocktail of rifampicin-resistant *E. coli* was used for the field study as the laboratory study (TVS 353, 354, and 355). The use of a cocktail mimicked the variability in environmental resistance often found within fecal microbiomes. Each

strain was streaked onto MacConkey agar with 80mg/mL rifampicin from a frozen stock and incubated at 35°C for 24 hours. A single colony of each strain was cultured individually in 100 mL of TSB with 80mg/mL of Rifampicin at 35°C for 24 hours with shaking. Cultures were added in a 1:70 ratio with sterile manure extract and incubated at 35°C for 48 hours. Sterile manure extract was prepared by diluting dairy manure 1:10 with distilled water, filtering through a cheesecloth, further diluting 1:2 with distilled water, and autoclaving for 1 hour at 121°C. After *E. coli* had been cultured in manure extract, it was enumerated on MacConkey agar with 80mg/mL of rifampicin and cultures were stored at 4°C during enumeration. Appropriate volumes of the individual strains in manure extract were added to a Hudson backpack sprayer and diluted with distilled water so that the sprayer contained 20L of 1.67×10^5 CFUs/mL of each strain.

E. coli was sprayed onto half of the replicate plots without compost and all plots with compost. The sprayer delivered 1 L of the inoculum evenly over each plot, equivalent to 1.67×10^8 CFUs per plot. Inoculation levels were chosen by the Agricultural Research Service based on the ability to easily measure a five-log reduction in *E. coli* from these levels. A five-log reduction from inoculation levels is the standard requirement of a kill-step in the treatment of *E. coli* contaminated food products. After inoculation, all plots were re-tilled to a 10 cm depth using a rototiller. Tilling of plots occurred in the order of treatments, with the untreated plots tilled first, followed by the *E. coli* only plots, dairy windrow compost plots, dairy vermicompost plots, and poultry plots to avoid cross-contamination of compost and *E. coli*. Rototiller blades were surface sterilized with 75% ethanol between each treatment.

After *E. coli* and composts had been tilled into plots, approximately 390 Hybrid Savoyed Spinach Reflect F1 seeds from Johnny's Selected Seeds (Windslow, ME) were planted by hand-broadcasting across each plot. Spinach was chosen because of its frequent connection to pathogenic *E. coli* contamination. In addition to spinach plants, weeds were allowed to grow on all plots to emulate the effect of the plant rhizosphere on soil community dynamics. Although the abundance of weeds was similar among all plots, the species tended to vary between fields. Because the study was conducted during an unusually rainy period, plots did not need to be irrigated.

2.2.4. *E. coli* Population Enumeration

To enumerate the *E. coli* population within the rhizosphere, three 10 cm deep soil cores were taken from each plot in a stratified random pattern on days 0, 1, 3, 6, 10, 15, 23, 29, 37, 49, 63, 78, 105, and 161 post inoculation. Samples were never taken from the same location within a plot twice. Sampling occurred with more frequency during the initial half of the study to capture variability in survival during the exponential decay phase. Twenty grams of each sample were diluted 1:5 with buffered peptone in a filter Whirlpak bag. Samples were further diluted as needed in buffered peptone, streaked onto MacConkey agar with 80mg/mL rifampicin in triplicate, and incubated at 35°C for 24 hours for *E. coli* enumeration. Once colony counts were below 20 colonies per plate, *E. coli* was enumerated by Most Probable Number (MPN). MPN counts were measured by adding 1 mL of 2x MacConkey broth with 160 mg/mL rifampicin to the first column of a 24 well plate, and 1 mL of 1x MacConkey broth with 80mg/mL rifampicin was added to the remaining 5 columns. One mL of soil sample, diluted 1:5 with buffered peptone, was

added to the first column and serially diluted down each row by a factor of 2 per column. Cells that turned yellow in color were considered positive for *E. coli.* The number of positive cells per dilution was entered into an MPN calculator to determine CFUs/gram of dry soil (Lekkas *et al.* 2015).

2.2.5. Soil Temperature and Moisture

To record fluctuations in abiotic soil conditions and to relate these fluctuations to changes in *E. coli* survival and community structure and function, soil temperature and water potential were recorded every hour in each field at 2 cm and 10 cm depths during the duration of the field experiment with Campbell Scientific 10x dataloggers. Thermister probes and Watermark probes for used to quantify soil temperature and water matric potential, respectively.

2.2.6. Enzyme Activity of Soil Microbes

To measure the extracellular enzyme activity of the soil microbial community, composite soil samples from each plot were obtained as described above for *E. coli* enumeration on days 8, 16, 23, 30, 50, and 65 post inoculation. Samples were sifted through a 1 mm mesh sieve prior to enzyme, PLFA, respiration, and sequencing analysis. One gram of each sample was diluted 1:100 in citrate buffer (pH = 6.1) and homogenized for 90 seconds at 6,000 rpm using a PolyTron. 200µL of soil sample was added to 96 well plates with 50 µL of 40µM fluorescently tagged enzyme substrate or a positive fluorophore control (Table 2.2). The enzyme substrates were selected because of their frequent use in soil studies and specificity for the major enzymes that go after carbon,

nitrogen, and phosphorous in soils. Plates were incubated at room temperature for 6 hours and read at 450nm on a BioTek FLx800 plate reader (Williston, Vermont, USA). Fluorescence was converted to nmols of substrate used/ (hrs incubated * grams of dry soil * PLFA abundance) to yield enzyme activity per hour per unit of biomass, allowing for the determination of changes in enzyme activity per microbe and standardizing for fluctuations in overall microbial biomass. This provides data on the allocation of energy by microbes for the synthesis of particular enzymes, rather than reflecting overall growth and decay of the microbial population. The ratio of BG/(NAG+LUC): BG/(AP) was graphed to compare the relative microbial need for acquisition of carbon, nitrogen, and phosphorous in the soil through time (Sinsabaugh *et al.* 2010).

Table 2.2 Enzymes tested and associated soil substrates, experimental substrates, and positive controls

Enzyme	**Organic Substrate (Target Nutrient)**	**Substrate Used**	**Positive Control**
β-1,4-glucosidase (BG)	Cellulose (Carbon)	4-MUB- β-D-glucoside (Sigma #M2133)	4-methylumbilliferyl (Sigma #M1381)
Phosphatase (AP)	Phosphomonoesters (Phosphorous)	4-MUB-phosphate (Sigma #M8883)	4-methylumbilliferyl (Sigma #M1381)
β-1,4-N-acetylglucosaminidase (NAG)	Chitin (Carbon and Nitrogen)	4-MUB-N-acetyl-β-glucosaminide (Sigma #2133)	4-methylumbilliferyl (Sigma #M1381)
Leucine (LUC)	L-leucine aminopeptidase (Nitrogen)	L-leucine-7-amido-4-methylcoumarin (Sigma #L2145)	7-amido-4-methylcoumarin (Sigma #A9891)

2.2.7. Microbial Activity Measurements by Respiration

To measure changes in overall microbial activity and survival, samples were collected and prepared for respiration measurements as described in Enzyme Activity (Section 2.2.5). From the sifted bulk samples, ten 0.5g replicates of each sample were reserved for measuring the reduction of iodonitrotetrazolium chloride (INT) as an indicator of microbial respiration. Half of the samples were autoclaved to kill endemic microbes and all samples were prepped for INT readings (Von Mersi and Schinner 1991). Samples were read at 460nm on a Biotek FLx800 spectrophotometer. Readings from autoclaved "dead" soils were subtracted from the readings from non-autoclaved "living" soils to obtain nmols of INT reduced per hour per gram of dry soil.

2.2.8. Microbial Biomass Measurements by PLFA

To measure changes in overall microbial biomass as a measure of growth and to standardize enzyme activity to per unit biomass, samples were collected and prepared for PLFA as described in Enzyme Activity (Section 2.2.5). From the sifted bulk samples, 30 g subsamples were frozen at -80^{0}C until shipment to the ARS Sustainable Agricultural Systems Laboratory in Beltsville, Maryland, where they were analyzed for PLFA biomarkers using a high throughput method described (Buyer and Sasser 2012). PLFA biomarkers were categorized into one of the following major taxonomic groups: general FAME (unusable as biomarkers), arbuscular mycorrhizae, gram negative bacteria, gram positive bacteria, fungi, anaerobe, actinobacteria, and protozoa (Table 2.3). PLFA data was collected to standardize microbial enzyme activity to per unit microbial biomass (unit PLFA).

Table 2.3 Taxonomic classifications of PLFA biomarkers used

Taxonomic Group	PLFA Biomarker
General FAME	10:0, 11:0, 12:0, 11:0 iso 3OH, 13:0, 15:0 aldehyde, 14:0, 14:0 iso 3OH, 16:1 w9c aldehyde, 16:0 aldehyde, 18:1 w9c, 15:0 16:1 w7c alcohol, 16:0 N alcohol, 16:0, 16:0 DMA, 17:1 anteiso w9c, 17:1 anteiso w7c, 17:0, 18:0 cyclo w6c, 20:0, 21:0, 22:0, 22:0 10-methyl, 23:0, 24:0, 10:0 2OH, 10:0 3OH, 16:0 2OH, 15:4 w3c, 15:3 w3c, 16:4 w3c, 16:3 w6c, 18:3 w6c, 19:4 w6c, 19:3 w6c, 19:3 w3c, 20:5 w3c, 20:2 w6c, 21:3 w6c, 21:3 w3c, 22:5 w6c, 22:6 w3c, 22:4 w6c, 22:5 w3c, 22:2 w6c, 23:4 w6c, 23:3 w6c, 23:3 w3c, 23:1 w5c. 23:1 w4c, 24:4 w6c, 24:3 w6c , 24:3 w3c, 24:1 w3c
AM Fungi	16:1 w5c
Gram negative	12:1 w8c, 12:1 w5c, 13:1 w5c, 13:1 w4c, 13:1 w3c, 12:0 2OH, 14:1 w9c, 14:1 w8c, 14:1 w7c, 14:1 w5c, 15:1 w9c, 15:1 w8c, 15:1 w7c, 15:1 w6c, 15:1 w5c, 14:0 2OH, 16:1 w9c, 16:1 w7c, 16:1 w6c, 16:1 w4c, 16:1 w3c, 17:1 w9c, 17:1 w8c, 17:1 w7c, 17:1 w6c, 17:0 cyclo w7c, 17:1 w5c, 17:1 w4c, 17:1 w3c, 18:1 w8c, 18:1 w7c, 18:1 w6c, 18:1 w5c, 18:1 w3c, 19:1 w9c, 19:1 w8c, 19:1 w7c, 19:1 w6c, 19:0 cyclo w9c, 19:0 cyclo w7c, 19:0 cyclo w6c, 20:1 w9c, 20:1 w8c, 20:1 w6c, 20:1 w4c, 20:0 cyclo w6c, 21:1 w9c, 21:1 w8c, 21:1 w6c, 21:1 w5c, 21:1 w4c, 21:1 w3c, 22:1 w9c, 22:1 w8c, 22:1 w6c , 22:1 w5c, 22:1 w3c, 22:0 cyclo w6c, 24:1 w9c, 24:1 w7c
Fungi	18:2 w6c
Gram positive	11:0 iso, 11:0 anteiso, 12:0 iso, 12:0 anteiso, 13:0 iso, 13:0 anteiso, 14:1 iso w7c , 14:0 iso,14:0 anteiso, 15:1 iso w9c, 15:1 iso w6c, 15:1 anteiso w9c, 15:0 iso , 15:0 anteiso, 16:0 iso, 16:0 anteiso, 17:1 iso w9c, 17:0 iso, 17:0 anteiso, 18:0 iso, 19:0 iso , 19:0 anteiso, 20:0 iso, 22:0 iso
Anaerobe	12:0 DMA, 13:0 DMA, 14:1 w7c DMA, 14:0 DMA, 15:0 iso DMA, 15:0 DMA , 16:2 DMA, 17:0 DMA, 16:1 w9c DMA, 16:1 w7c DMA, 16:1 w5c DMA, 19:0 cyclo 9,10 DMA, 18:2 DMA, 18:1 w9c DMA, 18:1 w7c DMA, 18:1 w5c DMA, 18:0 DMA
Actinobacteria	16:0 10-methyl, 17:1 w7c 10-methyl, 17:0 10-methyl, 18:1 w7c 10-methyl, 18:0 10-methyl, 19:1 w7c 10-methyl, 20:0 10-methyl
Protozoa	20:3 w6c, 20:4 w6c

2.2.9. Sequencing

Samples were collected and prepared as described in Enzyme Activity (Section 2.2.5), with the exception of an additional sample collection on day 105 after *E. coli* inoculation. From the sifted bulk samples, 1 g composite subsamples were frozen at -80^{0}C until DNA extraction. During extraction, 0.5 grams from each sample was added to the spin columns of the MoBio PowerSoil DNA Isolation kit (Carlsbad, CA) with ethanol-flamed forceps. DNA was extracted following the manufacturer's instructions,

using the methods described by Lauber *et al.* (2006). Samples were amplified at the University of Colorado Boulder using 515f/806r primers targeted for the V4 region of the 16S rRNA gene for bacteria and archaea and ITS-1/ITS-2 primers to amplify the ITS-1 spacer gene of 18S rRNA for fungi. Samples were amplified in triplicate and adjusted to equimolar concentrations. One μL of genomic DNA was added to 13 μL of PCR-grade water, 10 μL of Prime Hot Master Mix, 0.5 μL of reverse primers, and 0.5 μL of forward primers. PCR was carried out in 35 thermocycles of 94^0C for 45 seconds, 50^0C for 60 seconds, and 72^0C for 90 seconds. Primers contained 12-bp barcodes unique to each sample and the appropriate adapters to permit sequencing on the Illumina Miseq platform. Quality filtering and clustering of sequences into Operational Taxonomic Units (OTUs) was done using the UPARSE pipeline as described by Edgar (2013). Clustering was conducted at the 97% similarity level using Greengenes for 16S (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) and UNITE for ITS (http://www2.dpes.gu.se/project/unite/UNITE_intro.htm).

2.2.10. Statistical Analyses

To compare statistical differences in *E. coli* growth between different treatments, the area under the curve (AUC) for *E. coli* abundance through time of each replicate was calculated. Statistical differences of AUC among treatments were analyzed by one-way ANOVA followed by a Tukey's multiple comparison *t*-test.

One-phase and biphasic decay models were fit to each survival curve to determine the most representative model of *E. coli* survival through time for each treatment. Once

the model was fit, the deviation of the slope of the data from a standard decay model (z) for each interval of time between consecutive sampling dates (x_1, x_2) was determined using the following formula:

$$z = \frac{\text{Obs } E.coli \text{ population at } x_2 - \text{ Obs } E.coli \text{ population at } x_1}{\text{Model } E.coli \text{ population at } x_2 - \text{ Model } E.coli \text{ population at } x_1}$$

Residual values (z) were correlated to the mean soil water potential and temperature between times x_1 and x_2 using a linear regression.

PLFA biomarker abundances were converted to proportion of total biomarkers to calculate the Bray-Curtis pairwise dissimilarity matrix and to analyze compositional differences by principal coordinate analysis. Treatment effects and temporal differences in PLFA abundance were analyzed using a 2-way ANOVA, with a subsequent Tukey's *t*-test for multiple comparisons of sampling dates or treatments. A linear regression was performed between the total PLFA abundance and INT to quantify the relationship between biomass and respiration. All ANOVAs, *t*-tests, and linear regressions were performed using GraphPad Prism v.6.05. Principal coordinate analysis was performed using PRIMER v.6.

16S sequences were rarefied to a depth of 19,600 reads per sample and ITS sequences were rarefied to a depth of 18,012 reads per sample so that all samples were analyzed using the same number of sequences. Analyses were limited to OTUs that had a total abundance of 200 or more copies when all samples were combined to limit the effect of rare taxa on compositional analysis. ITS sequences were limited to OTUs with a

total abundance of 10 or more copies in total. OTU abundance was converted to a proportion of the total number of sequences per sample. A redundancy analysis (RDA) was performed with a linear method to determine the contribution of environmental and treatment variables on community variation. Contributing variables were determined by forward selection of the variables with significant explanatory *p*-values ($p<0.05$), with a false discovery rate used to protect against Type I error. Principal response curves (PRC) were performed to assess the treatment effect on variation in community composition through time, with the baseline standardized to community composition of untreated plots. Significant effects of treatments were determined by Monte Carlo permutation tests. The top fifteen OTUs that most closely corresponded to principal response curves were also identified and illustrated. Pair-wise Bray Curtis Dissimilarity indices and principal coordinate analysis were run using PRIMER v.6 software. All RDA and PRC analyses were performed with CANOCO version 5 software (Ter braak and Smilauer 2012, Van den Brink and Ter Braak 1999).

CHAPTER 3. RESULTS

3.1. Extract Experiment

In contrast to the compost extracts, the *E. coli* population was eliminated within 50 hours in the sterile soil extract, but was sustained at approximately 10^4 CFUs/mL in the non-sterile soil extract. *E. coli* growth followed a logarithmic growth pattern within the first 50 hours in all sterile compost extracts (Figure 3.1), with the greatest growth in Maryland Poultry extract (MD/P-S). The sterile Maryland Poultry extract continued to promote growth, albeit at a slower rate, for the remainder of the experiment, whereas the other sterile compost extracts sustained asymptotic *E. coli* levels at approximately 10^{10} CFUs/mL after 50 hours. The non-sterile compost extracts sustained the *E. coli* population at $10^4 – 10^6$ CFUs/mL for the duration of the experiment, with the exception of the two dairy composts made by Worm Power, which both decreased the *E. coli* population to approximately 10^1 to 10^2 CFUs/mL.

There were no significant differences between the AUC of *E.coli* survival in sterile compost extract treatment types, although the sterile poultry compost tended to have a larger mean AUC than the other extracts *($p=0.1432$-0.2399)*. In contrast, the AUC of *E. coli* survival in non-sterile Maryland poultry extract was greater than in the Black Dirt immature vermicompost non-sterile extract (*$p=0.0489$*) and both Worm Power dairy compost non-sterile extracts (*$p=0.0191$*, *$p=0.0196$* for windrow and vermicompost, respectively)(**Figure 3.2**). Survival of *E. coli* in Worm Power extracts were less than both

Someday Farm poultry extracts in addition to the Maryland poultry compost ($p=0.0198 – 0.0328$).

The growth rate constant (k) values of the *E. coli* survival curves in sterile compost extracts were greater than the growth rate constant of the curves in the paired non-sterile compost extracts ($p = 6.8x10^{-5}$, Table 3.1). Phosphorous, potassium, and ammonium all have significant direct relationships with growth rate in sterile extracts (**Figure 3.3**). Growth rate was unaffected by carbon ($p=0.7407$), total nitrogen ($p=0.2571$), and nitrate ($p=0.8879$).

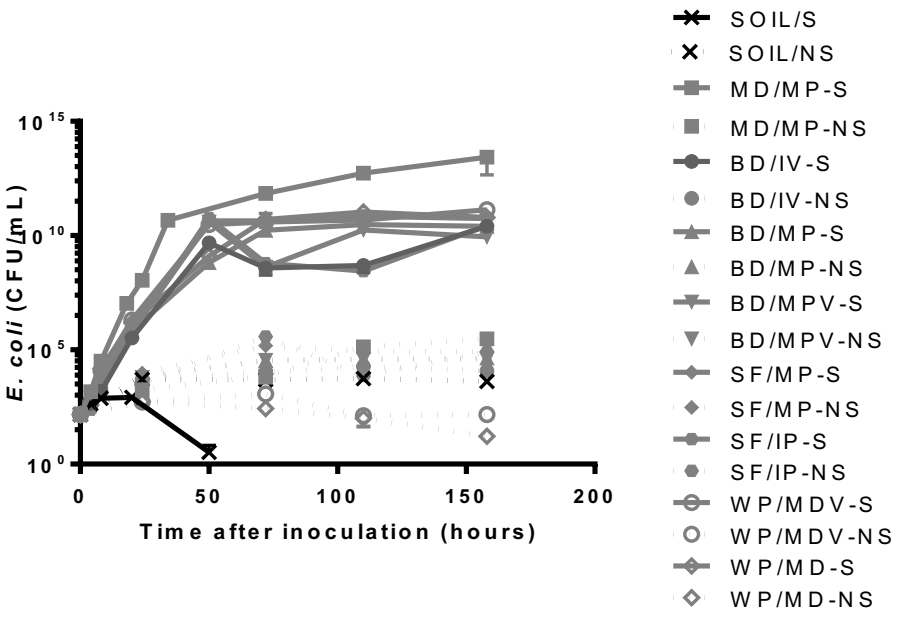


Figure 3.1 *E. coli* regrowth in non-sterile and sterile compost and soil extracts. Letters before the slash indicates the source (MD = Maryland, BD = Black Dirt Farm, SF = Someday Farm, WP = Worm Power). Letters after the slash indicate the compost type (I = immature, M = mature, P = poultry, D = dairy, v = vermicompost). Nonsterile extracts are denoted with "-NS" (dashed lines) and sterile extracts are denoted with "-S" (solid lines). Composts are in grey and the soil extracts are in black. Standard error bars are included, but are too small to see with the exception of the sterile poultry extract in the last time point.

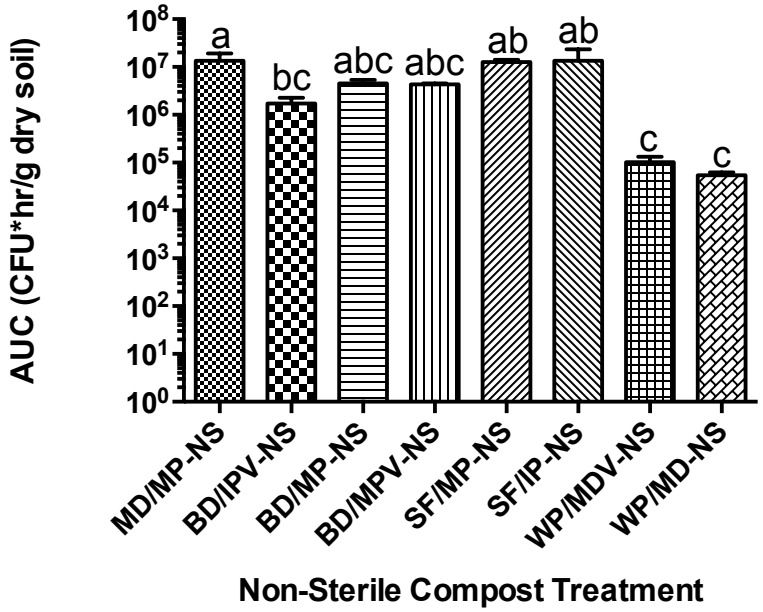


Figure 3.2 Area under the curve (AUC) comparison of *E. coli* survival curves in non-sterile extracts for 158 hours. Significant differences ($p<0.05$) are present between two treatments when they do not have any lower case letters in common. Uppercase letters before the slash indicates the source (MD = Maryland, BD = Black Dirt Farm, SF = Someday Farm, WP = Worm Power). Uppercase Letters after the slash indicate the compost type (I = immature, M = mature, P = poultry, D = dairy, v = vermicompost).

Table 3.1 The growth rate constant (*k*) of the log phase (0-24 hours for non-sterile samples and 0-50 hours for sterile samples) for all compost extract treatments. The *k*-values were determined from exponential growth models fit to the log phase of the mean *E. coli* survival curve for each treatment. Uppercase letters before the slash indicates the source (MD = Maryland, BD = Black Dirt Farm, SF = Someday Farm, WP = Worm Power). Uppercase Letters after the slash indicate the compost type (I = immature, M = mature, P = poultry, D = dairy, v = vermicompost). All *k*-value estimates had an R^2=1.0.

Sample	**Sterile**	**Unsterile**
	***k* value**	***k* value**
MD/P	0.5757	0.1046
BD/IV	0.3461	0.1471
BD/FP	0.3063	0.163
BD/MV	0.3881	0.1566
SF/MP	0.3903	0.1736
SF/IP	0.3961	0.1318
WP/V	0.3827	0.05237
WP/W	0.3198	0.0583

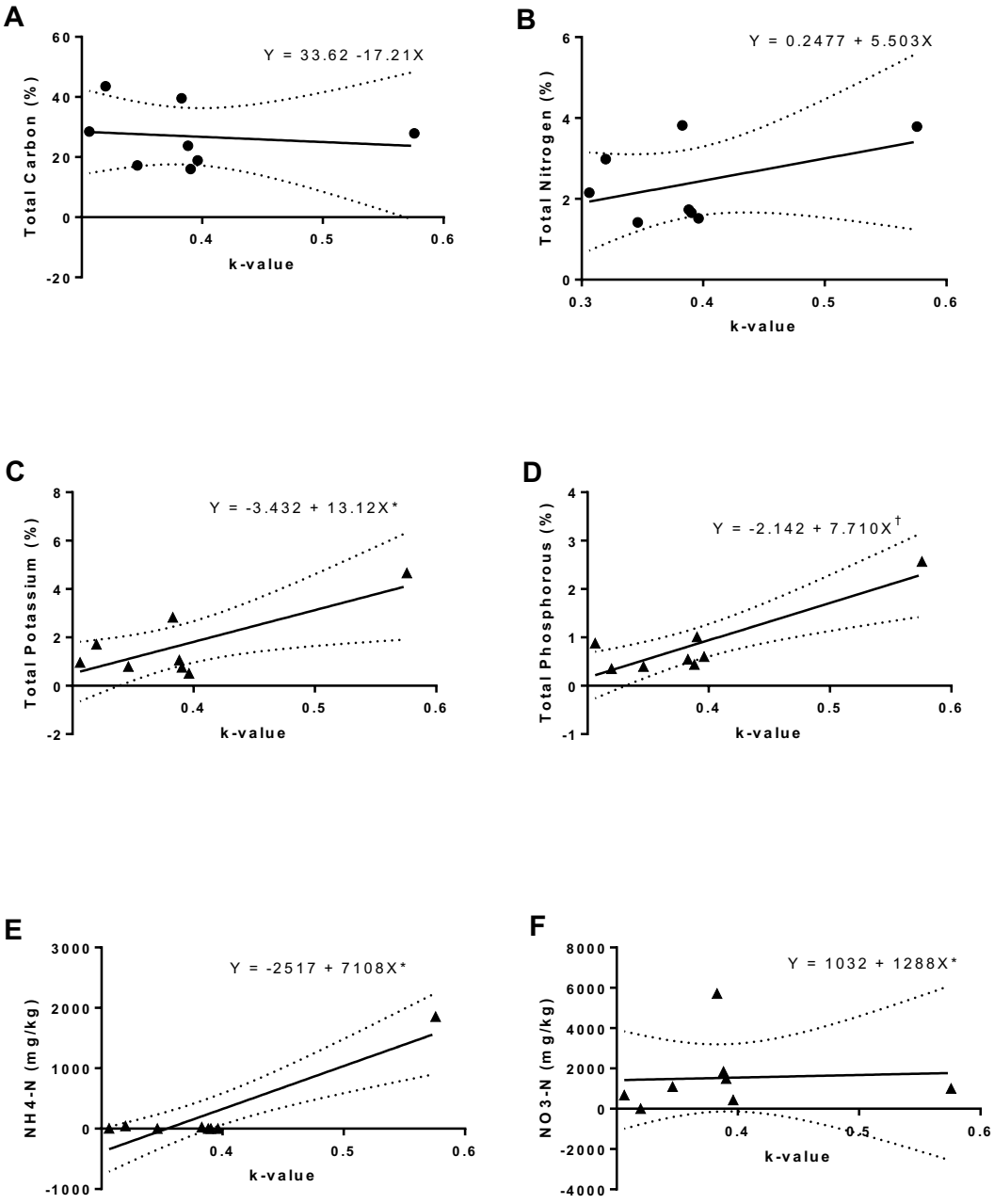


Figure 3.3 Linear regression between the growth rate constants (*k*-value) and the nutrient content of sterile compost extracts. *K*-values are compared to percent carbon (A), percent nitrogen (B), percent potassium (C), percent phosphorous (D), mg/kg ammonium (E), and mg/kg nitrate (F). Significant correlations are denoted by * ($0.01<p<0.05$) or † ($p<0.01$). Dashed lines represent the 95% confidence bands of the best-fit line.

3.2. Field Experiment

3.2.1. *E. coli* survival

E. coli survival trends were similar between field sites (Figure 3.4). *E. coli* was absent in non-inoculated plots, verifying that there was no cross contamination of the plots and no rifampicin-resistant *E. coli* endemic to the soil. The *E. coli* populations in plots with either of the Worm Power composts or no compost showed similar declining trends over the 6-month testing period. In plots with poultry compost, *E. coli* populations increased within the first seven days after inoculation, and then decreased to the inoculation levels by day 15, at which point the population stabilized until 105 days after inoculation. The last sampling date, which occurred 161 days after inoculation, showed lower *E. coli* population levels in the poultry compost plots than previous sampling dates, particularly at Wheelock field.

E. coli populations were no longer detectable by plating or MPN in any of the other plots by day 105 (lowest threshold of detection = 0.36 CFU/g). AUC values for *E. coli* survival were greater with poultry compost than other treatments (Figure 3.5) ($p=0.0159 – 0.0160$ in Lilac, $p=0.0337 – 0.0344$ in Wheelock).

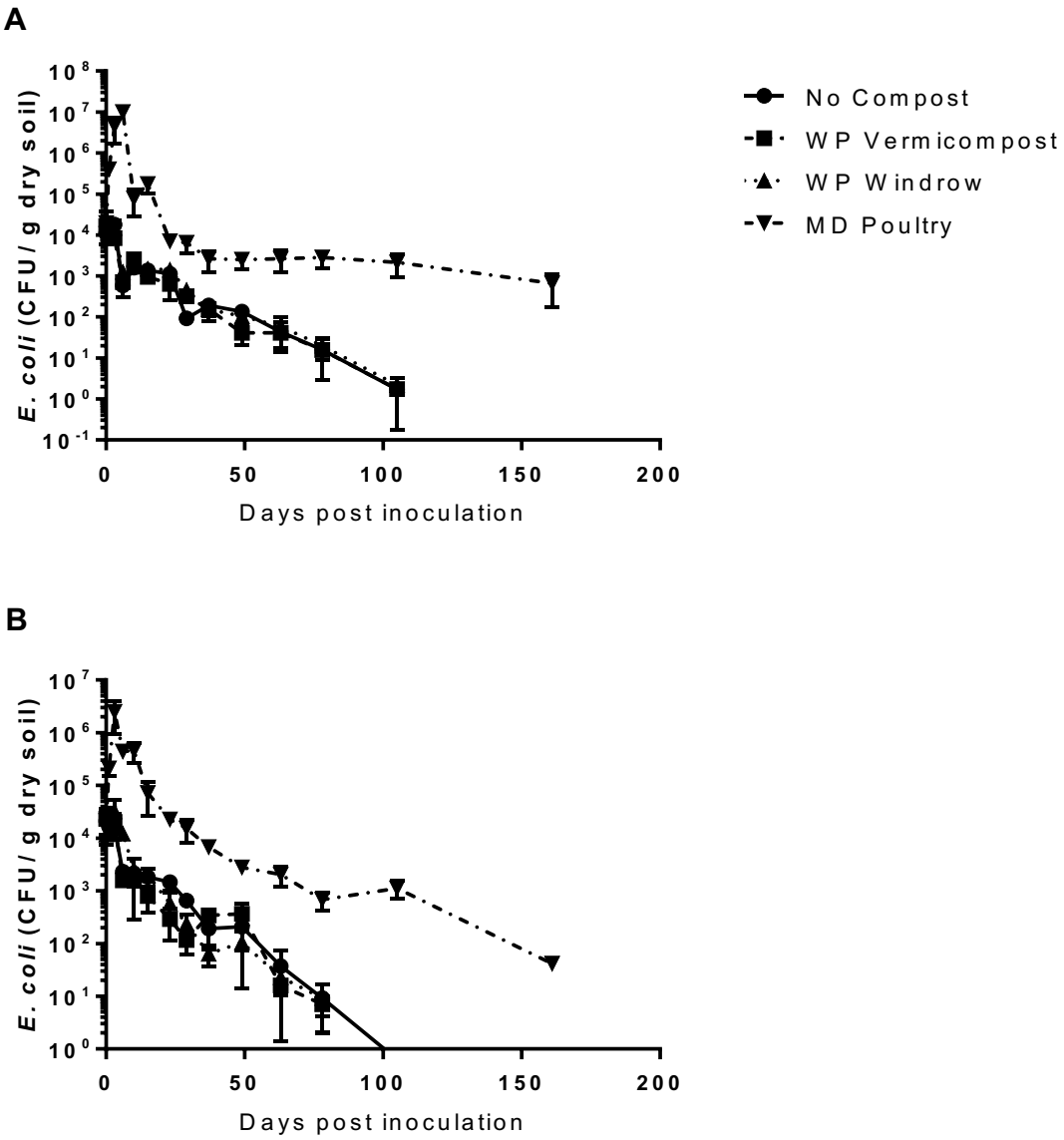


Figure 3.4 *E. coli* counts through time in Lilac (A) and Wheelock (B). Treatments include plots without compost, plots with mature windrow poultry compost from Maryland (MD Poultry), mature dairy windrow compost from Worm Power (WP Windrow), and mature dairy vermicompost from Worm Power (WP Vermicompost).

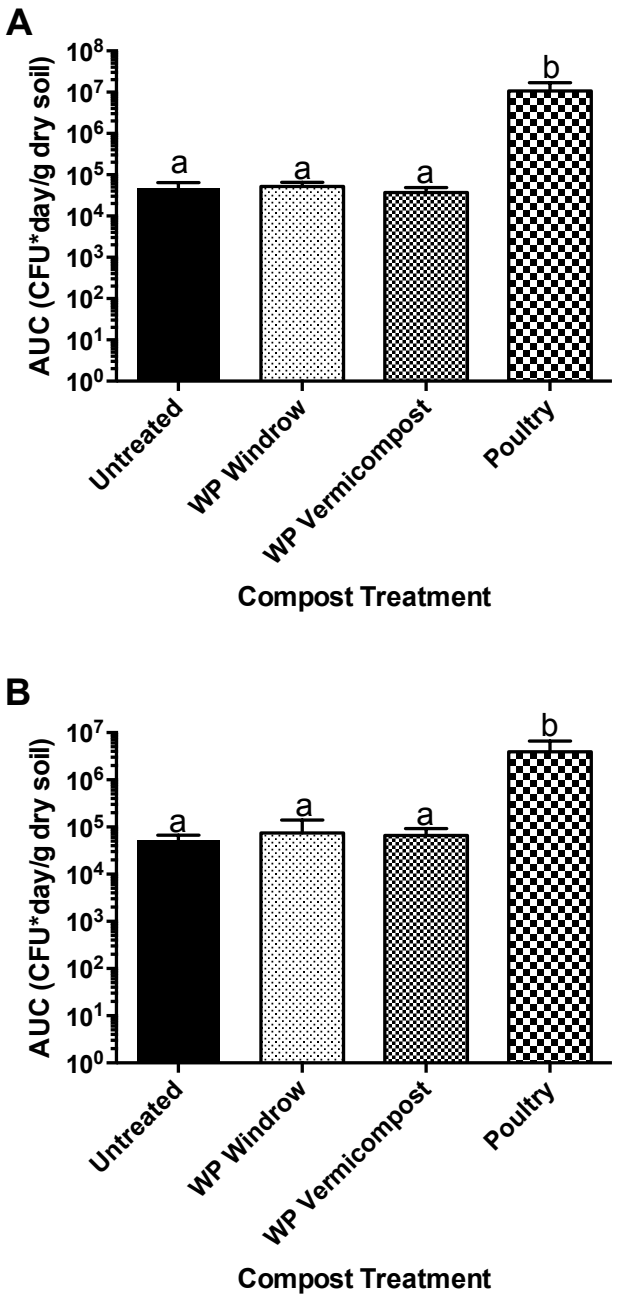


Figure 3.5 Area Under the Curve comparison of *E. coli* survival curves in Lilac (A) and Wheelock (B). Treatments include plots without compost, plots with mature windrow poultry compost from Maryland (MD Poultry), mature dairy windrow compost from Worm Power (WP Windrow), and mature dairy vermicompost from Worm Power (WP Vermicompost). Significant differences ($p<0.05$) are present between two treatments when they do not have any lower case letters in common.

E. coli survival best fit a one-phase decay model (Figure 3.6). *E. coli* populations declined at a faster rate than the decay model when conditions were drier than field capacity (water potential < -33 kPa) in plots without compost treatment or with either dairy compost. Likewise, the *E. coli* declined at a slower rate than the decay model when the conditions were wetter than field capacity (water potential > -33 kPa, **Figure 3.7**). In contrast, *E. coli* survival kinetics did not correlate to soil moisture in poultry. No plots correlated with temperature, with the exception of poultry compost plots in Wheelock (Table 3.2).

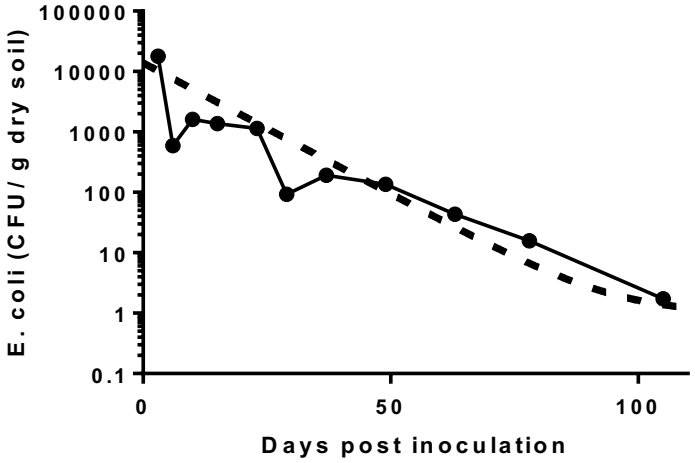


Figure 3.6 One phase decay model fit to the survival curve of *E. coli* through time in untreated plots. Residual values were calculated as the difference in *E. coli* population between two consecutive time points divided by the difference in the modelled population values between the same two time points. The model is illustrated as a dashed line and the observed data is illustrated as a solid line.

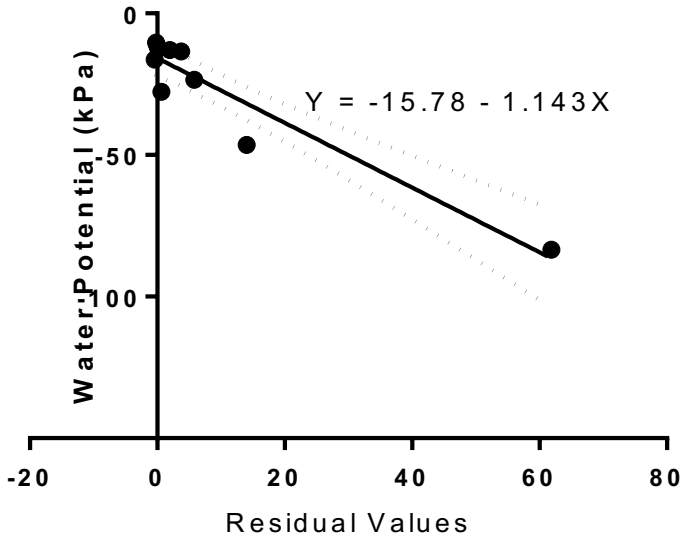


Figure 3.7 Linear regression between the average water potential at 2 cm and the residual values of *E. coli* survival kinetics compared to a one-phase decay model in plots without compost. Residual values were calculated as the difference in *E. coli* population between two consecutive time points divided by the difference in the modelled population values between the same two time points. Residual values greater than one indicate that the *E. coli* were decaying at a rate faster than the model predicted between two consecutive time points, residuals between 0 and 1 indicate that the *E. coli* were decaying at a rate slower than the model predicted, and residuals less than 0 indicate that the *E. coli* population was growing during that time interval. The *y*-axis represents the mean water potential for the corresponding time interval. Dashed lines represent the 95% confidence bands of the best-fit line.

Table 3.2 Linear regression equations and *p*-values between residual values of the *E. coli* survival curve compared to the model and water moisture or temperature.

Field	Treatment	2 cm moisture (kPa)		10 cm moisture (kPa)		Temperature (^{o}C)	
		Regression	p-value	Regression	p-value	Regression	p-value
L	NC	**Y= -15.78 -1.14X**	**<0.0001**	**Y= -10.96 -0.54X**	**<0.0001**	Y = 21.37 + 0.01X	0.7154
L	MD-P	Y = -27.48 + 4.17(10^{-5})X	0.6527	Y = -16.47 + 2.12(10^{-5})X	0.6161	Y = 20.78 + 1.13(10^{-6})	0.9291
L	WP-V	**Y = -16.17 -0.75X**	**<0.0001**	**Y = -11.17 -0.35X**	**<0.0001**	Y = 21.38 + 0.006X	0.7149
L	WP-W	**Y = -16.85 -0.54X**	**<0.0001**	**Y = -11.48 -0.25X**	**<0.0001**	Y = 21.38 + 0.004X	0.7059
W	NC	**Y = -15.29 - 0.59X**	**<0.0001**	**Y = -12.49 - 1.66X**	**<0.0001**	Y = 20.83 + 0.01X	0.2949
W	MP-P	Y = 32.39 + 3.96(10^{-5})X	0.7676	Y = -60.81 + 0.0001X	0.7186	**Y = 21.10 -3.29(10^{-5})X**	**0.0002**
W	WP-V	**Y = -16.06 - 0.89X**	**0.0003**	**Y = -16.17 - 2.44X**	**0.0002**	Y = 20.82 + 0.02X +	0.2889
W	WP-W	**Y = -17.09 - 0.70X**	**0.0004**	**Y = -14.35 - 2.02X**	**<0.0001**	Y = 21.27 + 0.008X	0.3333

No compost (NC), Maryland poultry compost (MD-P), Worm Power vermicompost (WP-V), and Worm Power windrow compost (WP-W) in Lilac (L) and Wheelock (W). Significant relationships ($p<0.05$) are in bold. N=3 for all treatments per field.

3.2.2. Moisture and temperature measurements

The early portion of the field season was unusually rainy, raining most days for a period of three weeks. During this period, soils stayed wetter than field capacity (0 to -33 kPa) at the 10 cm depth for the first 50 days after *E. coli* inoculation, and were close to saturation (0 kPa) at the 2 cm depth in both fields (Figure 3.8). Fifty days after inoculation, rain became less frequent and soils were drier. In Wheelock, the water potential reached -400 kPa at both the 2 cm and 10 cm depth in the later part of the season. In Lilac, only the 2 cm depth became much drier than field capacity. Both fields went above field capacity at both depths after 100 days post inoculation, when the rains again became frequent. The soil temperature remained relatively constant for the first 100 days and quickly dropped after as the fall season started.

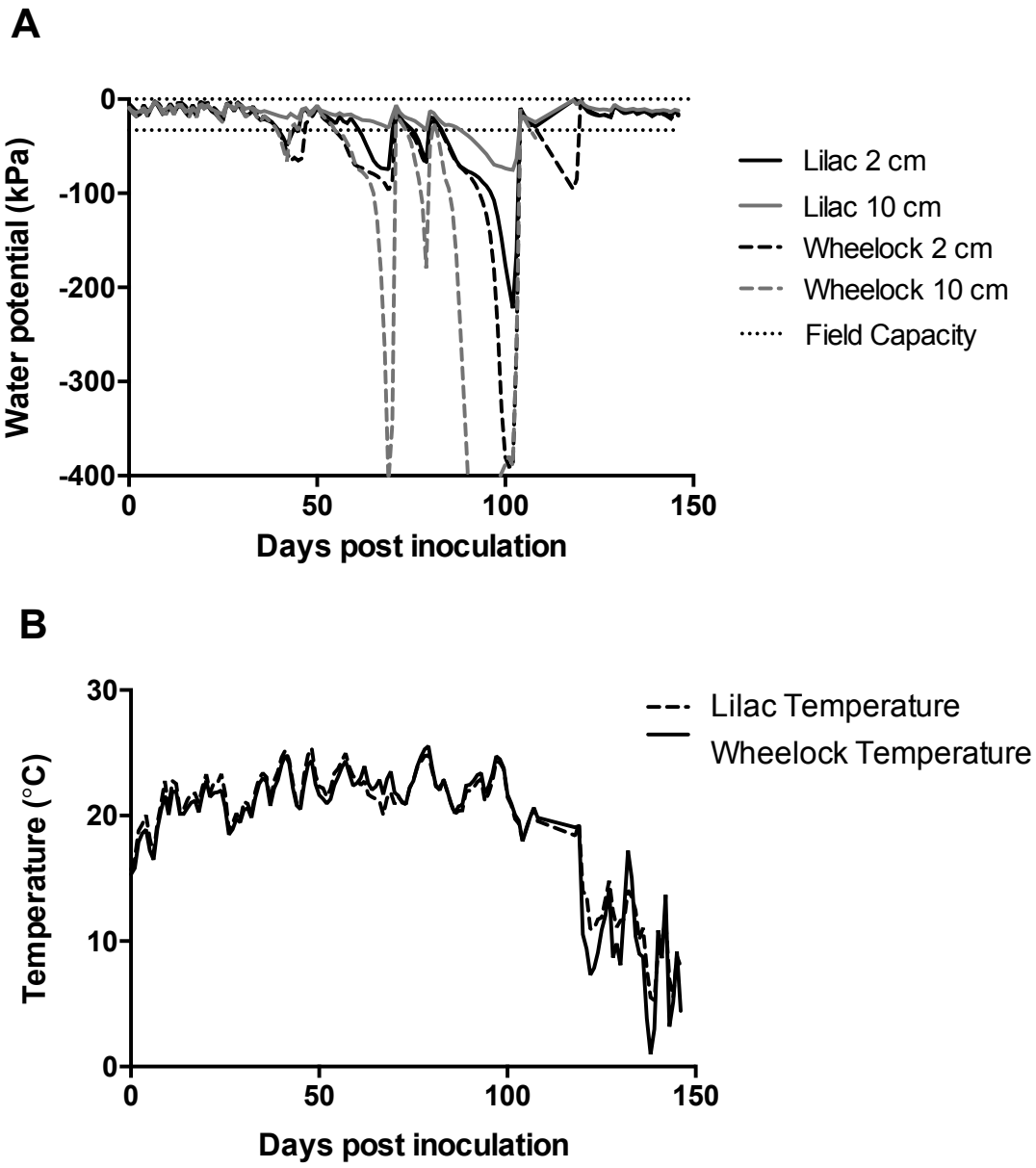


Figure 3.8 Water potential (A) and soil temperature (B) through time. Water potential at field capacity is shown as a dotted line. Water potential at the 2 cm depth is illustrated in black and water potential at the 10 cm depth is illustrated in grey. Data are illustrated as a dashed line and solid line for Lilac and Wheelock, respectively.

3.2.3. Enzyme Activity

The addition of composts did not significantly alter enzyme activity, but rather enzyme activity tracked fluctuations in soil moisture. Enzymatic activity was similar among different treatments at any individual sampling date, although the greatest variation in enzyme activity occurred on day 8 after *E. coli* inoculation (Figure 3.9). Temporal trends in enzymatic activity were essentially uniform among all treatment types. All enzyme activity declined in the first 30 days after inoculation as soils remained saturated with water, with the exception of β-glucosidase (BG) in Wheelock, which dropped dramatically between day 8 and 16 and then increased between day 16 and 23. Between 30 and 50 days after inoculation, microbial acquisition of cellulose carbon (BG activity), phosphorous (AP activity), and chitin nitrogen and carbon (NAG activity) increased in both fields, after which enzyme activity remained relatively constant between day 50 and 65 post inoculation. In contrast, acquisition of amino nitrogen (leucine activity) continued to decline for the duration of the experiment. Activity of BG tended to be greater than NAG + LUC or AP on all sampling dates, with the exception of samples in Lilac on day 30 post inoculation (Figure 3.10). In other words, microbes allocated more energy into acquiring cellulose carbon than nitrogen and phosphorous for the majority of the study. Additionally, all samples had greater activity of AP than NAG and LUC, allocating more energy to phosphorous acquisition than nitrogen acquisition. Principal coordinate analysis of the ratios revealed that samples on days 23 and 30 post inoculation, which had the wettest soils of the sampling dates, had distinctly different enzyme activity profiles than samples on the remaining sampling dates due to the relative

increase in NAG, LUC, and AP activity over BG activity (Figure 3.11). Ratios between treatment types were similar. The only difference in sites occurred on day 30 post inoculation, when almost all Wheelock samples had lower NAG and LUC activity than BG, while most Lilac samples had BG/(NAG+LUC) values close to 1.

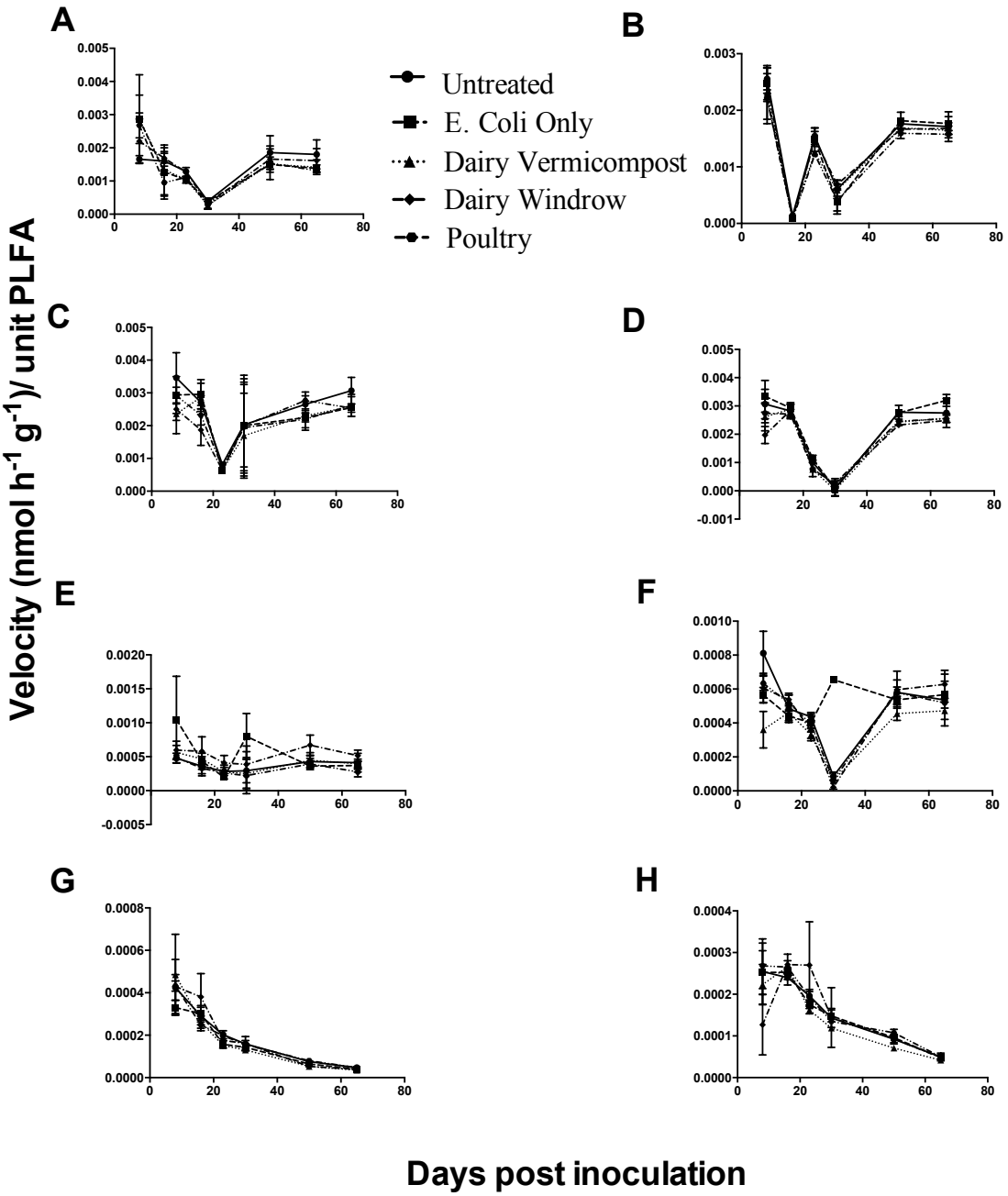


Figure 3.9 Enzyme activity in nmol substrate used (hr^{-1})(gram of dry $soil^{-1}$)(unit $PLFA^{-1}$) in the presence of 40μM substrate. Graphs are separated by site, enzyme, and treatment. Lilac samples are in the left column and Wheelock samples are in the right column. Microbial activity on enzyme substrates are illustrated for β-1,4-glucosidase activity (A,B), phosphatase activity (C,D), β-1,4-N-acetylglucosaminidase activity (E,F), and leucine activity (G,H). Standard error bars for each treatment illustrated.

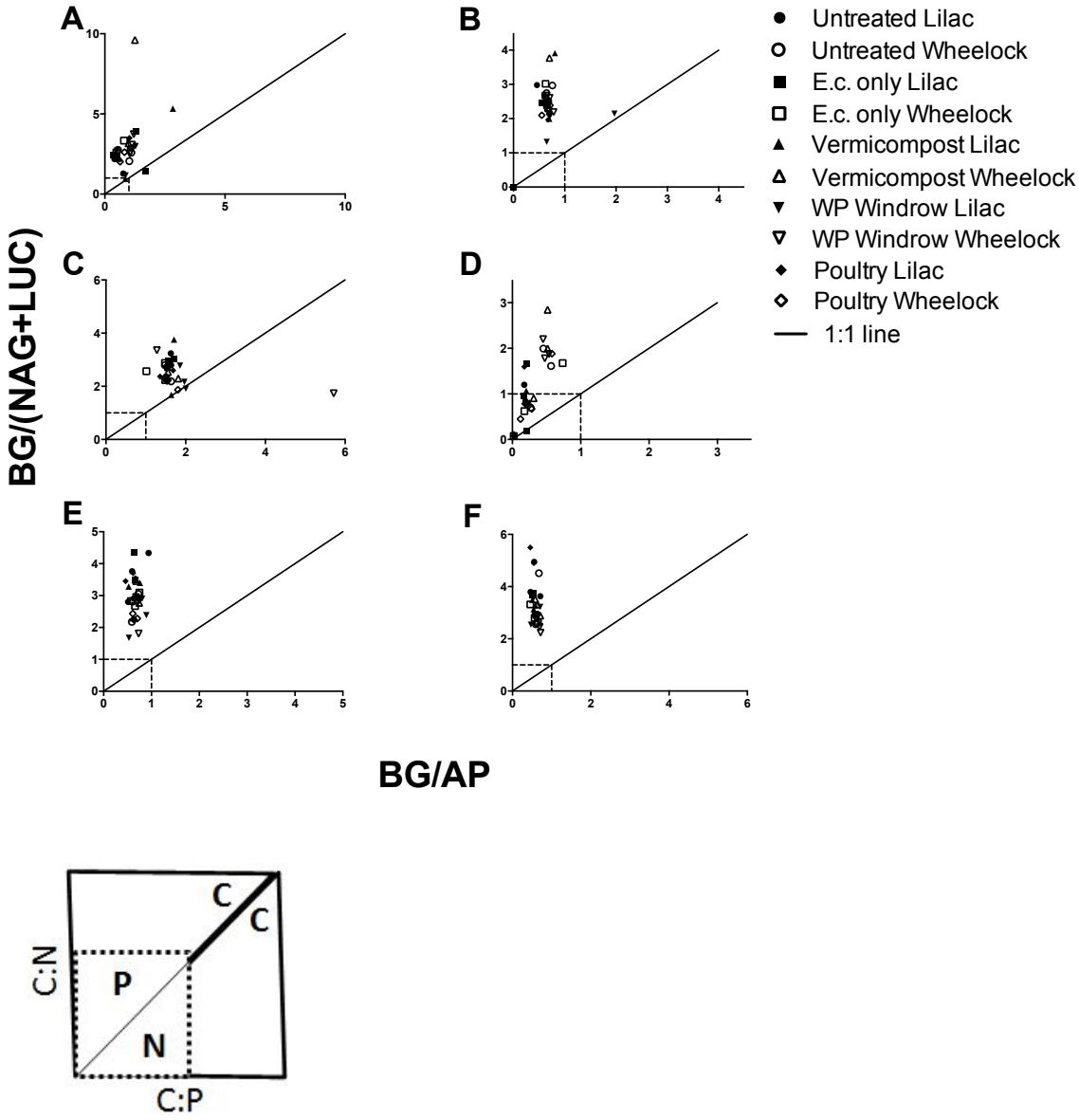


Figure 3.10 The ratio of β-1,4-glucosidase/(β-1,4-N-acetylglucosaminidase+leucine):β-1,4-glucosidase/ phosphatase [BG/(NAG+LUC):BG/AP] enzyme activity with 40 µM substrate on days 8(A), 16(B), 23(C), 30(D), 50(E), and 65(F) post *E. coli* inoculation. Circles represent Lilac samples and diamonds represent Wheelock samples. The solid line represents a 1:1 ratio. The horizontal dashed line outlines where BG is equivalent to NAG+LUC and the vertical dashed line outlines where BG is equivalent to AP. The graph underneath the data indicates the nutrient that the microbial community is allocating the most energy to acquire based on where the sample falls on the graph.

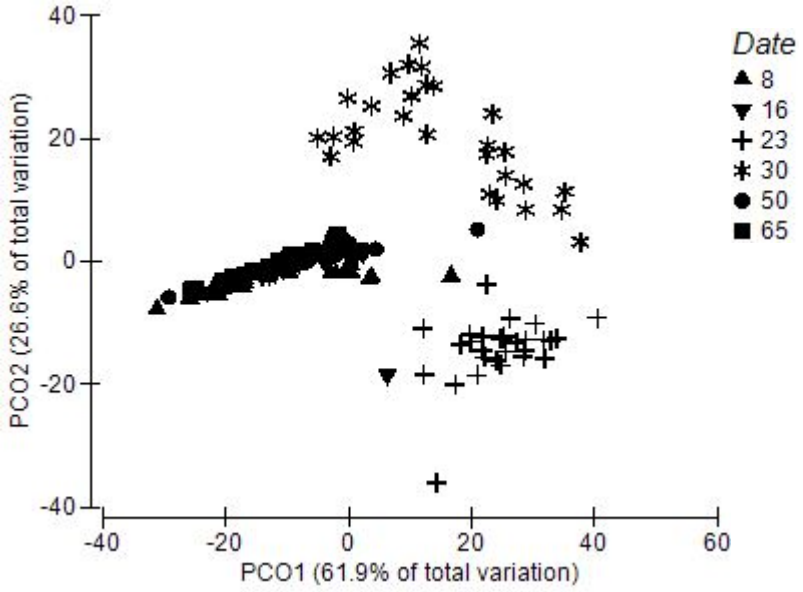


Figure 3.11 Principal coordinate analysis of the β-1,4-glucosidase/(β-1,4-N-acetylglucosaminidase+leucine):β-1,4-glucosidase/ phosphatase enzyme activity ratio. Samples are labelled by the sampling day they were taken on after *E. coli* inoculation. The number represents days after inoculating soil with *E. coli.*

3.2.4. Respiration

There was an overall decrease in Iodonitrotetrazolium chloride (INT) reduction on day 50 post inoculation at both fields (Figure 3.12). INT reduction was similar among treatments ($p>0.05$ by ANOVA). The decrease in INT reduction on day 50 mirrored the drying pattern of the soils at both the 2 cm and 10 cm depths (Figure 3.13).

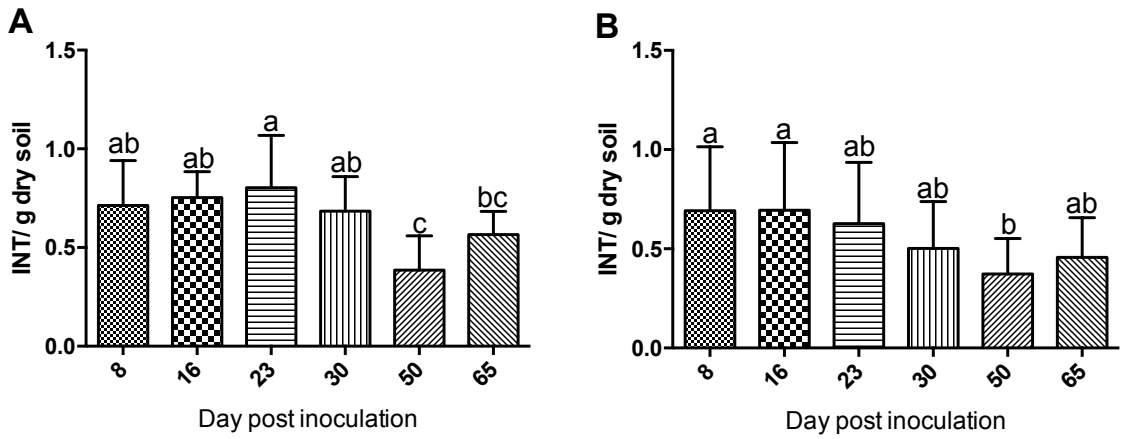


Figure 3.12 Iodonitrotetrazolium chloride reduction on separate sampling dates in Lilac (A) and Wheelock (B). Different letters represent statistical differences in INT reduction between sampling dates ($p<0.05$). Standard error bars for each date are illustrated.

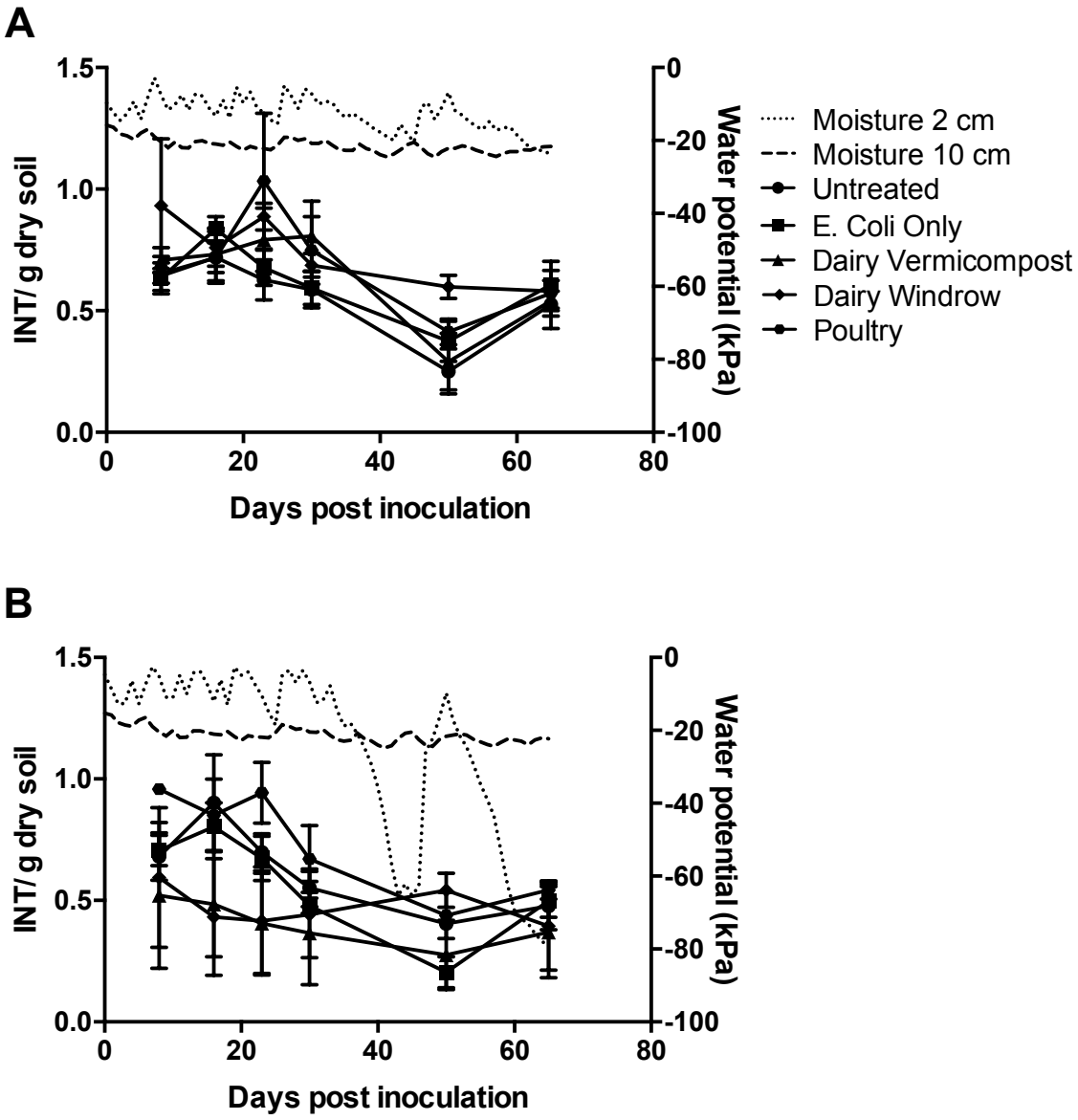


Figure 3.13 Iodonitrotetrazolium chloride (INT) reduction through time (left *y*-axis) and water potential through time (right *y*-axis) in Lilac (A) and Wheelock (B). Solid lines represent INT reduction separated by treatment with standard error bars. Dotted lines represent water potential taken at a 2 cm depth and dashed lines represent water potential taken at a 10 cm depth.

3.2.5. Phospholipid Fatty Acid Analysis

Phospholipid fatty acid (PLFA) abundance was correlated positively with INT reduction (Figure 3.14). Composition of PLFA was generally uniform throughout sampling dates and between fields and treatment types. Gram negative bacteria were the most abundant taxonomic group represented by PLFA analysis, followed by gram positive bacteria and actinobacteria (Figure 3.15). Microbial composition by PLFA was distinguished mostly by sampling date, and was otherwise consistent between treatment types and sites (Figure 3.16). There were no temporal differences in total PLFA abundance, with the exception of a lower abundance 23 days post inoculation compared to 65 days post inoculation in Lilac ($p=0.0157$, Figure 3.17 A,B). There was no difference in total PLFA among treatments for either field ($p=0.3271$, $p=0.1847$ in Lilac and Wheelock, respectively). Although not statistically significant, there was a trend that total PLFA abundance was greater in vermicompost plots at both fields and in poultry plots at Wheelock (Figure 3.17C,D). In Lilac, the general FAME group, arbuscular mycorrhizal fungi (AM fungi), fungi, gram positive bacteria, actinobacteria, and protozoa were significantly more abundant at 65 days post inoculation than earlier (Table 3.3). In contrast, anaerobes were most abundant at 16 days post inoculation and decreased thereafter. Differences among treatments within taxonomic groups occurred. For example, general FAME, AM fungi, gram negative bacteria, gram positive bacteria, actinobacteria, and protozoa increased with dairy compost amendment in Lilac (Table 3.4) and with dairy vermicompost or poultry compost in Wheelock (Table 3.6). In Lilac,

poultry and vermicompost also increased the abundance of anaerobes and fungi. In Wheelock, AM fungi, fungi, gram positive bacteria, actinobacteria, and protozoa were significantly higher either at 50 or 65 days post *E. coli* inoculation than the earliest sampling dates (Table 3.5). Similar to Lilac, anaerobes in Wheelock were highest at 8 and 16 days post inoculation and decreased in later sampling dates.

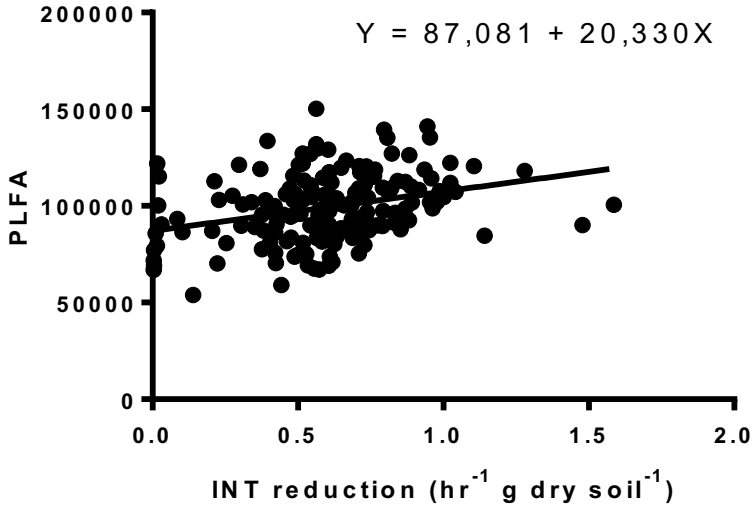


Figure 3.14 Linear regression between total PLFA abundance and INT reduction ($p<0.0001$, n=176). The linear regression equation is shown on the graph.

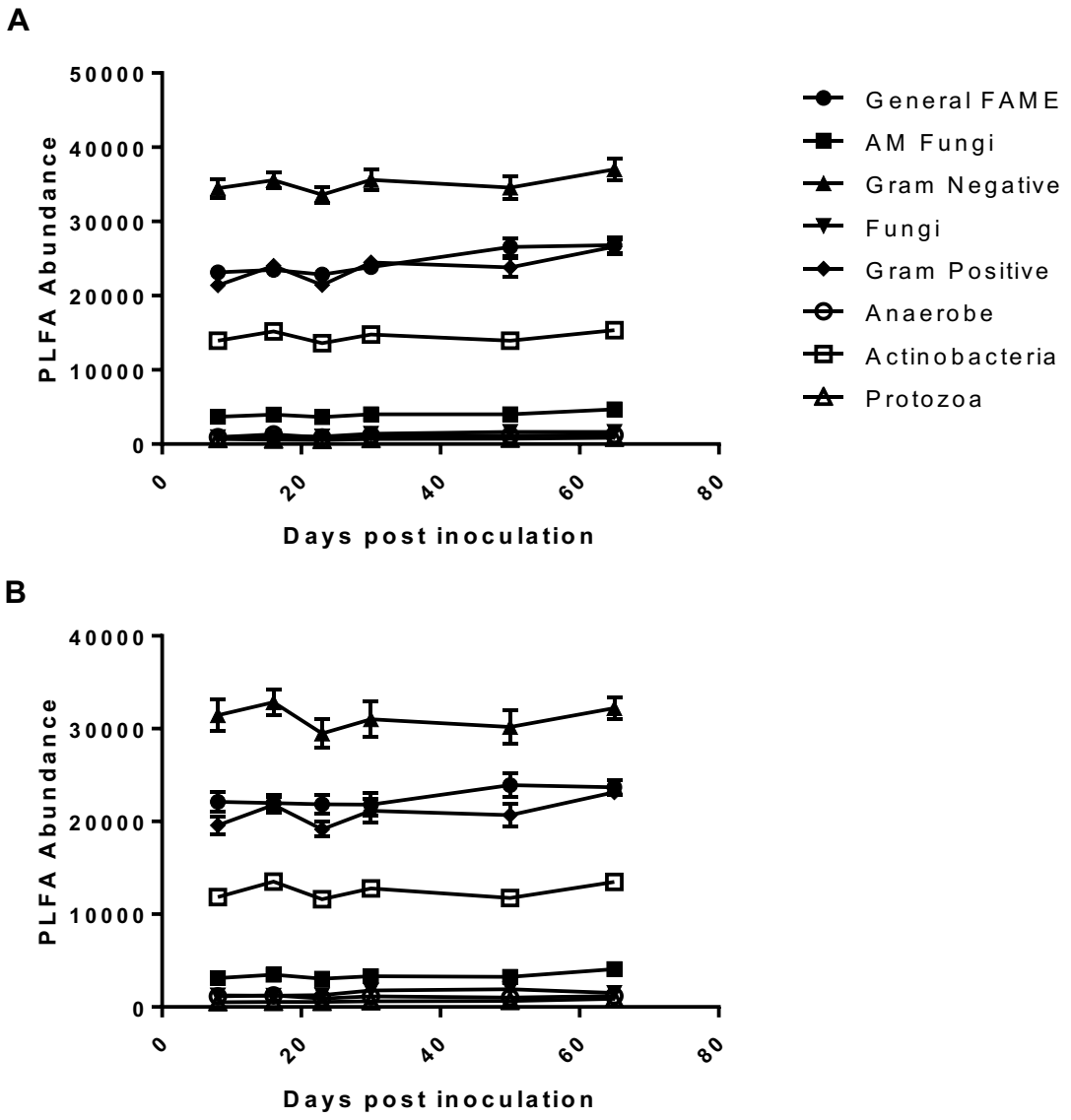


Figure 3.15 PLFA abundance of taxonomic groups and unknown markers through time (General FAME) in Lilac (A) and Wheelock (B). Means (±1 standard error) are illustrated.

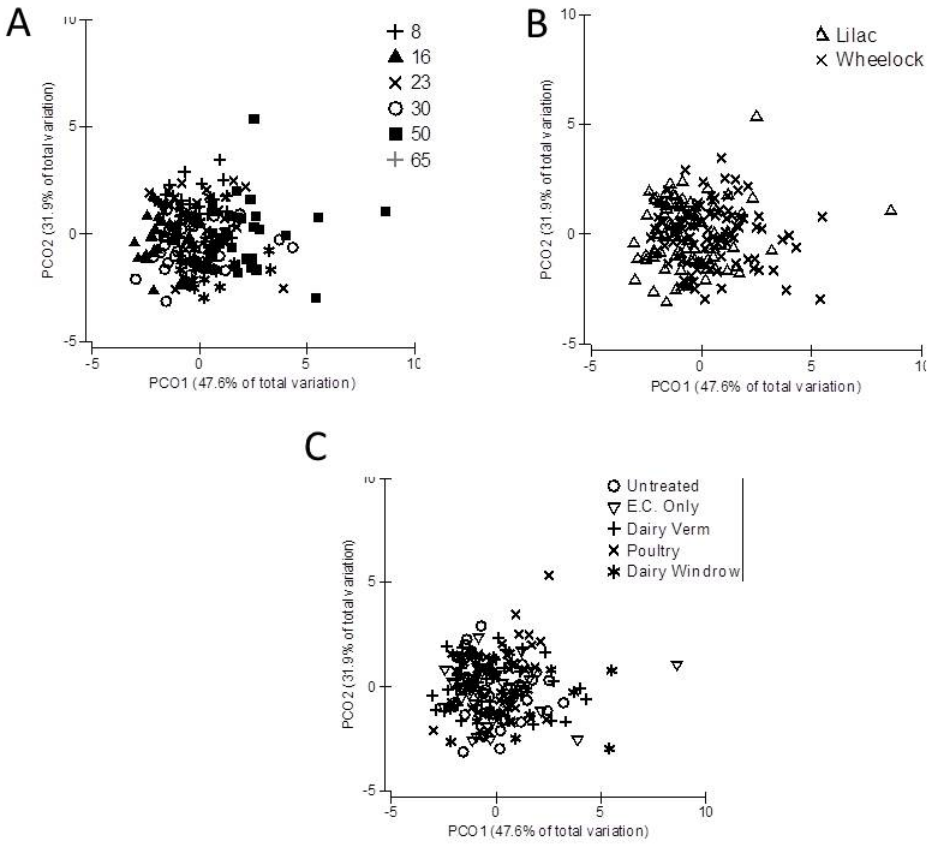


Figure 3.16 Principal coordinate analysis of PLFA taxonomic proportions, labelled by sampling day (A), field (B), and treatment (C).

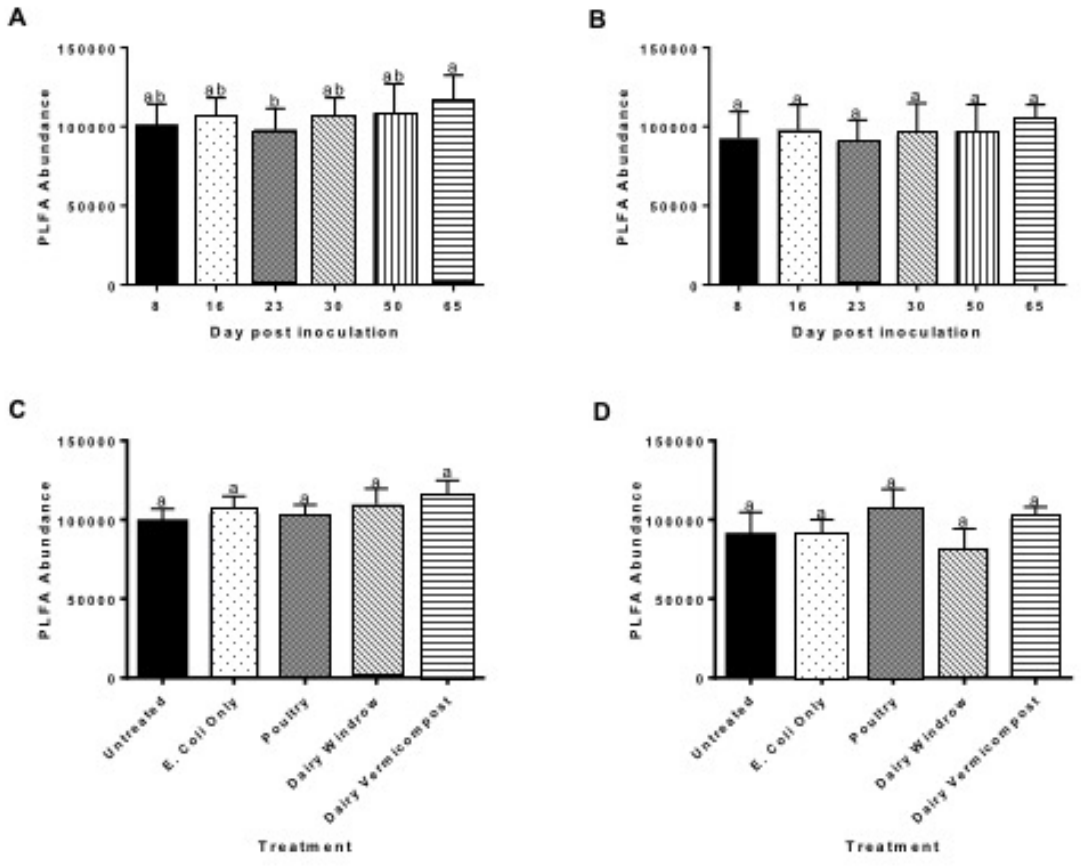


Figure 3.17 Total PLFA abundance through time in Lilac (A) and Wheelock (B) and between treatments in Lilac (C) and Wheelock (D). Contrasting letters signify statistical differences between means ($p<0.05$). Standard error (±1) bars are illustrated.

Table 3.3 Individual taxa PLFA mean ± 1 SE through time in Lilac

Day post inoculation	**General FAME (p<0.0001)**	**AM Fungi (p<0.0001)**	Gram negative	**Fungi (p<0.0001)**	**Gram positive (p<0.0001)**	**Anaerobe (p=0.0035)**	Actino-bacteria	**Protozoa (p=0.0130)**
8	23,153.45 ± 1,064^{A}	3,676.25 ± 250^{A}	34,486.82 ± 1,384	956.34 ± 56^{A}	21,387.59 ± 871^{A}	937.81 ± 45^{A}	13,947.83 ± 624	505.80 ± 5^{ABC}
16	23,142.03 ± 926^{AB}	3,959.88 ± 205^{A}	35,368.41 ± 1,282	1,023.07 ± 110^{AB}	23,906.85 ± 747^{AB}	1,292.50 ± 58^{B}	15,067.85 ± 482	543.25 ± 35^{AB}
23	22,840.26 ± 574^{A}	3,653.75 ± 186^{A}	33,587.16 ± 942	1,011.78 ± 94^{AB}	21,425.84 ± 622^{A}	894.95 ± 68^{A}	13,614.66 ± 486	533.71 ± 61^{A}
30	23,855.45 ±1,026^{ABC}	4,001.55 ± 279^{A}	35,588.07 ± 1,980	1,389.52 ± 121^{BC}	24,482.08 ± 1,126^{AB}	1,149.47 ± 85^{AB}	14,763.81 ± 757	719.11 ± 107^{B}
50	26,747.58 ±1,749^{BC}	3,947.95 ± 381^{A}	34,916.82 ± 2,407	1,671.67 ± 208^{BC}	24,200.32 ± 1,546^{AB}	1,129.95 ± 200^{AB}	14,292.32 ± 699	596.66 ± 37^{ABC}
65	26,870.09 ±1,286^{C}	4,720.18 ± 394^{B}	37,103.53 ± 2,312	1,634.42 ± 129^{C}	26,694.66 ± 1,462^{B}	1,215.71 ± 80^{AB}	15,400.06 ± 732	724.61 ± 185^{C}

Taxa in bold had significantly different means through time (ANOVA p-values shown under taxa with significant temporal effects). For taxa with significant temporal effects, a Tukey's t-test was run for multiple comparisons. Contrasting letters signify significant differences within a particular taxonomic group through time ($p<0.05$). Statistical groups are lettered from lowest to highest means, starting with A. Letters later in the alphabet indicate means with high values.

Table 3.4 Individual taxa PLFA mean ± 1 SE by treatment in Lilac

Day post inoculation	**General FAME (p=0.0019)**	**AM Fungi (p<0.0001)**	**Gram negative (p<0.0001)**	Fungi	**Gram positive (p=0.0018)**	Anaerobe	**Actino-bacteria (p=0.0010)**	**Protozoa (p=0.0288)**
Untreated	22,513.51 ± 528^{A}	3,349.51 ± 96^{A}	31,857.72 ± 764^{A}	1063.79 ± 135	21,516.22 ± 709^{A}	1,041.71 ± 98	13,351.83 ± 341^{A}	603.86 ± 39^{A}
E. coli only	24,222.84 ± 855^{B}	3,948.85 ± 183^{B}	34,273.61 ± 1,419^{AB}	1,189.78 ± 149	23,374.54 ± 795^{AB}	1,023.06 ± 62	14,535.87 ± 561^{AB}	637.92 ± 43^{AB}
Poultry	23,549.10 ± 945^{AB}	3,615.65 ± 219^{AB}	34,148.29 ± 1,073^{AB}	1,488.15 ± 183	23,121.54 ± 1,136^{AB}	1,070.60 ± 108	13,699.78 ± 554^{A}	649.70 ± 51^{AB}
Dairy Windrow	25,385.28 ± 1,088^{C}	4,465.25 ± 254^{C}	37,164.16 ± 1,210^{BC}	1,319.50 ± 115	24,635.60 ± 1,340^{B}	1,143.42 ± 86	15,044.53 ± 439^{AB}	702.32 ± 59^{AB}
Dairy Vermi-compost	26,503.33 ± 1,089^{C}	4,587.03 ± 297^{C}	38,431.91 ± 1,716^{C}	1,344.45 ± 217	25,766.57 ± 1,451^{B}	1,231.20 ± 156	15,940.10 ± 477.78^{B}	831.35 ± 111^{B}

Taxa in bold had significantly different means between treatments (ANOVA p-values shown under taxa with significant temporal effects). For taxa with significant temporal effects, a Tukey's t-test was run for multiple comparisons. Contrasting letters signify significant differences within a particular taxonomic group through time ($p<0.05$). Statistical groups are lettered from lowest to highest means, starting with A. Letters later in the alphabet indicate means with high values.

Table 3.5 Individual taxa PLFA means ± SE through time in Wheelock

Day post inoculation	General FAME	**AM Fungi (p=0.0014)**	Gram negative	**Fungi (<0.0001)**	**Gram positive (p=0.0397)**	Anaerobe	**Actino-bacteria (p=0.0044)**	**Protozoa (p<0.0001)**
8	22,788.34 ± 1,090	3,161.78 ± 181^{A}	32,485.63 ± 1,581	1,310.21 ± 92^{A}	21,387.59 ± 871^{A}	1,164.62 ± 93	12,067.40 ± 524^{ABC}	542.00 ± 29^{A}
16	22,051.85 ± 921	3,533.87 ± 178^{AB}	33,691.80 ± 1,559	1,230.63 ± 107^{AB}	23,906.85 ± 747^{AB}	1,297.88 ± 87.37	13,613.04 ± 557^{BC}	575.29 ± 31^{A}
23	22,577.66 ± 1,063	3,110.34 ± 157^{A}	30,498.25 ± 1,392	1,348.04 ± 128^{AB}	21,425.84 ± 622^{A}	927.30 ± 74	11,970.58 ± 349^{A}	572.34 ± 53^{A}
30	22,099.15 ± 1405	3,331.28 ± 222^{AB}	30,908.00 ± 1,981	1,805.00 ± 169^{BC}	24,482.08 ± 1,126^{AB}	1,166.21 ± 104	12,595.58 ± 788^{ABC}	607.90 ± 46^{A}
50	24,167.42 ± 1,000	3,363.94 ± 227^{A}	31,744.32 ± 1,420	1,965.01 ± 132^{C}	24,200.32 ± 1,546^{AB}	1,029.75 ± 130	12,063.51 ± 517^{AB}	682.36 ± 54^{A}
65	23,498.82 ± 847	4,113.70 ± 209^{B}	32,063.91 ± 1,282	1,527.78 ± 69^{AB}	26,694.66 ± 1,462^{B}	1,218.64 ± 105	13,876.79 ± 326^{C}	868.87 ± 56^{B}

Taxa in bold had significantly different means through time (ANOVA p-values shown under taxa with significant temporal effects). For taxa with significant temporal effects, a Tukey's t-test was run for multiple comparisons. Contrasting letters signify significant differences within a particular taxonomic group through time ($p<0.05$). Statistical groups are lettered from lowest to highest means, starting with A. Letters later in the alphabet indicate means with high values.

Table 3.6 Individual taxa PLFA means ± SE by treatment in Wheelock

Day post inoculation	**General FAME (p=0.0004)**	**AM Fungi (p=0.0005)**	**Gram negative (p=0.0003)**	**Fungi (p<0.0001)**	**Gram positive (p=0.0004)**	**Anaerobe (p=0.0025)**	**Actino-bacteria (p=0.0044)**	**Protozoa (p=0.0022)**
Untreated	21,714.95 ± 832^{AB}	3,241.07 ± 229^{AB}	30,155.53 ± 1,475^{AB}	1,326.67 ± 101^{A}	20,187.71 ± 1,244^{AB}	1,041.07 ± 124^{AB}	12,664.79 ± 708^{AB}	576.21 ± 52^{AB}
E. coli only	21,601.52 ± 626^{AB}	3,050.96 ± 191^{A}	30,916.63 ± 1,455^{ABC}	1,324.05 ± 50^{A}	20,141.89 ± 791^{AB}	1,125.68 ± 92^{AB}	12,541.70 ± 494^{AB}	554.64 ± 34^{A}
Poultry	25,282.64 ± 1,153^{C}	3,820.26 ± 103^{B}	35,510.95 ± 1,018^{C}	1,852.43 ± 195^{B}	23,637.48 ± 551^{C}	1,292.00 ± 57^{B}	13,665.82 ± 327^{B}	726.87 ± 45^{C}
Dairy Windrow	20,037.93 ± 751^{A}	2,999.53 ± 223^{A}	26,843.31 ± 1,020^{A}	1,428.56 ± 197^{A}	18,222.62 ± 917^{A}	866.16 ± 53^{A}	10,819.98 ± 420^{A}	599.52 ± 90^{ABC}
Dairy Vermicompost	24,061.18 ± 1,241^{BC}	3,788.58 ± 246^{B}	33,336.65 ± 961^{BC}	1,652.99 ± 209^{AB}	22,230.22 ± 978^{BC}	1,279.40 ± 76^{B}	12,837.41 ± 279^{B}	715.80 ± 91^{BC}

Taxa in bold had significantly different means between treatments (ANOVA p-values shown under taxa with significant temporal effects). For taxa with significant temporal effects, a Tukey's t-test was run for multiple comparisons. Contrasting letters signify significant differences within a particular taxonomic group through time ($p<0.05$). Statistical groups are lettered from lowest to highest means, starting with A. Letters later in the alphabet indicate means with high values.

3.2.6. Genetic Sequencing

Community composition of bacteria and fungi were distinct between field locations (Figure 3.18). Microbial composition of the composts alone was also dramatically different than soil composition, even with compost amendments (Figure 3.18). Microbial composition within each field clustered by treatment and through time. In Lilac, PCO analysis showed similar bacterial composition of plots with either dairy treatment, which differed from the composition of plots with poultry compost, and untreated plots overlapped between the two (Figure 3.19A). Clustering between treatment types occurs mostly along the *x*-axis, indicating that the starting compost substrate (poultry litter vs. dairy manure) has the greatest contribution to variation in bacterial community. In Wheelock, plots with either dairy composts and untreated plots had similar composition and poultry plots were more distinct (Figure 3.19B). In both fields, samples from the last sampling date had a more distinct composition than the earlier samples (Figure 3.19C,D). Effects of time and treatment on fungal composition varied slightly between fields. In Lilac, plots with either dairy compost had similar fungal composition, which differed from the overlapping fungal composition between plots with either untreated or poultry compost (Figure 3.20A). In Wheelock, compost treatments did not result in distinct fungal compositions. Instead of plots with dairy compost treatments overlapping, the dairy vermicompost and poultry compost treated plots had similar composition and the dairy windrow and untreated plots had similar composition, with some overlap between all treatments (Figure 3.20B). There was a temporal gradient in the PCO analysis of ITS sequences in both field sites, with complete overlap between plots

from the earliest sampling dates and increasing compositional dissimilarity of plots from the later sampling dates (Figure 3.20C,D).

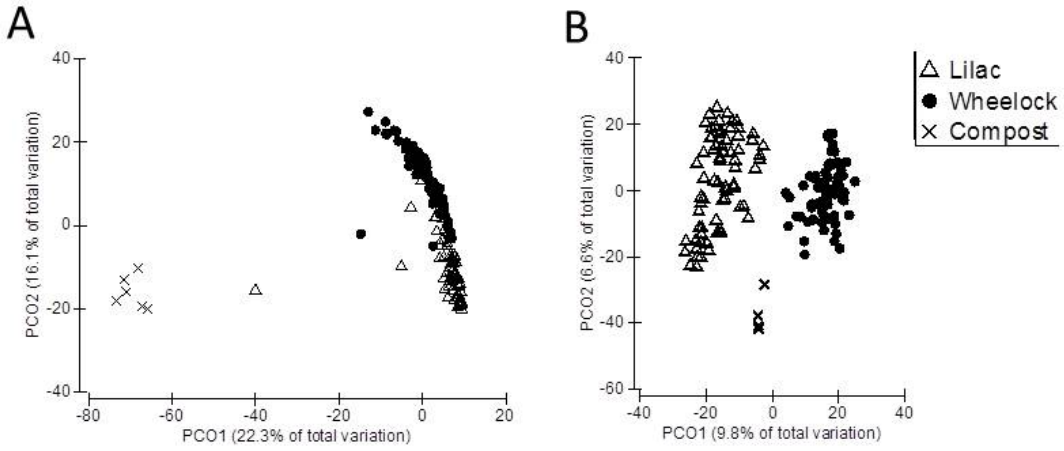


Figure 3.18 Principal coordinate analysis of 16S sequences for bacteria and archaea (A) and ITS sequences for fungi (B). All samples taken from Lilac are triangles, all samples taken from Wheelock are circles and sequences from the compost samples alone are crosses.

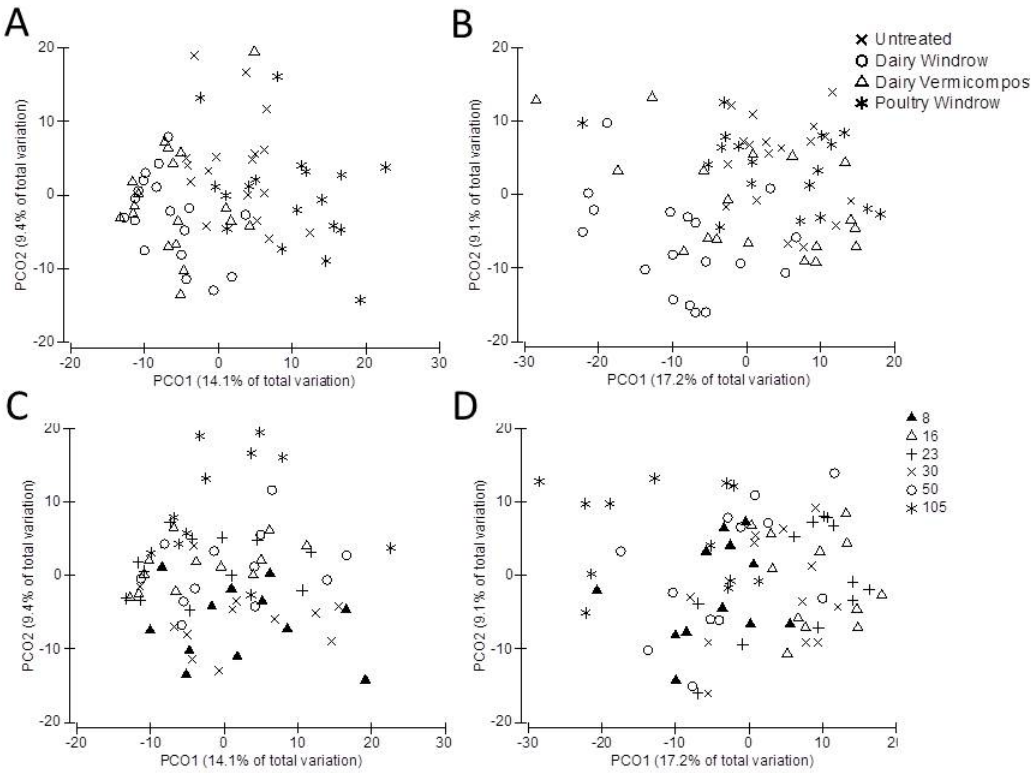


Figure 3.19 Principal Coordinate Analysis of 16S sequences in Lilac and Wheelock field plots. Figures are labelled by treatment (A for Lilac, B for Wheelock) and sampling date (C for Lilac, D for Wheelock).

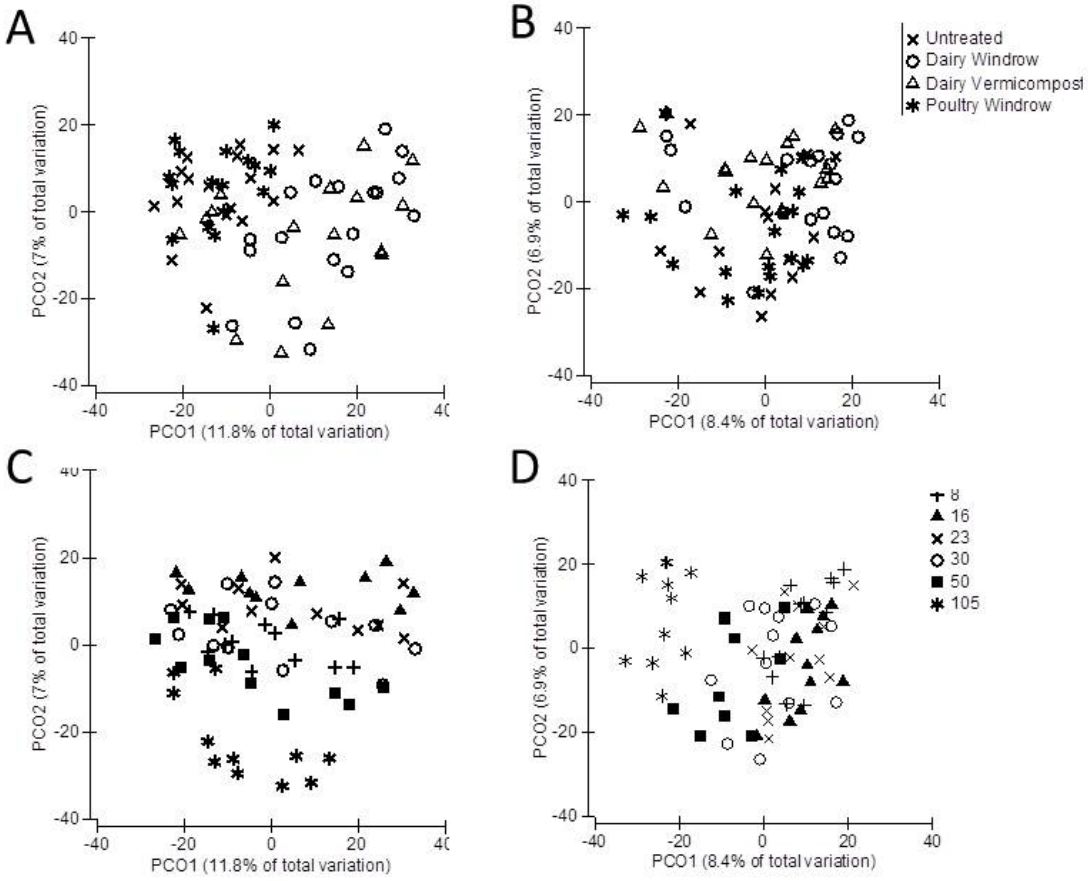


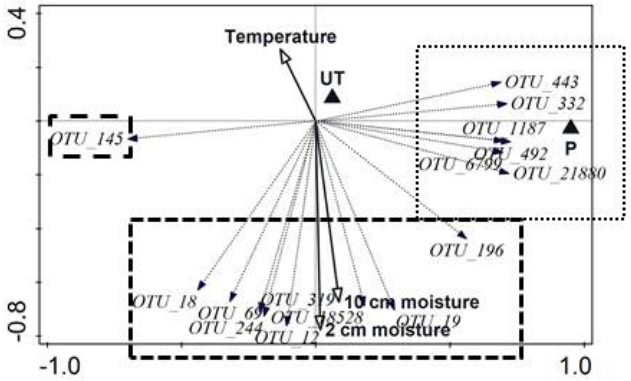
Figure 3.20 Principal Coordinate Analysis of ITS sequences in Lilac and Wheelock field plots. Figures are labelled by treatment (A for Lilac, B for Wheelock) and sampling date (C for Lilac, D for Wheelock).

At Lilac, poultry compost, moisture, untreated plots, and temperature had the highest contribution to variation in bacterial and archaea community composition (Table 3.7). Several operational taxonomic units (OTUs) correlated with poultry compost treatment or moisture (Figure 3.21). Members of *Verrucomicrobia*, *γ-proteobacteria*, *δ-proteobacteria*, *α-proteobacteria, Bacteriodetes, and Firmucutes* were the distinguishing taxa associated with poultry compost. Higher water potentials (wetter soils), in contrast, correlated with multiple OTUs matched to the iii1-15 order within the *Acidobacteria* phyla, as well as members of the *α-proteobacteria*, *β-proteobacteria*, and *Fibrobacteres* phyla. Principal response curve analysis of bacterial composition through time showed significant treatment effects (*p=0.002)*. The PRC of poultry-treated plots had positive canonical coefficients at each time point, whereas the PRC of dairy-treated plots had negative canonical coefficients, demonstrating opposite influences on variation in bacterial composition from untreated plots (Figure 3.22). The deviation in bacterial composition in poultry-treated plots was greater in later sampling dates than earlier sampling dates. In contrast, bacterial composition of plots with dairy compost treatment became more similar to that of untreated plots through time. The majority of OTUs that most closely fit to the principal response curves had scores between 0 and 1, indicating ubiquitous distribution among all plots. Three OTUs had scores between 1 and 4 and fell within the range of the poultry PRC during the first four sampling dates. Two OTUs had negative species scores and therefore correspond to dairy compost treatment.

Table 3.7 Variables with statistically significant contributions to explained variation in 16S composition in Lilac plots.

Variable	Contribution to explained variation	*p*-Value
Poultry treatment	31.1%	0.0028
Untreated treatment	19.4%	0.0023
Moisture at 2 cm depth	17.2%	0.0028
Moisture at 10 cm depth	14.3%	0.0028
Temperature	12.6%	0.0028

Explanatory variables accounted for 23.1% of the variation.

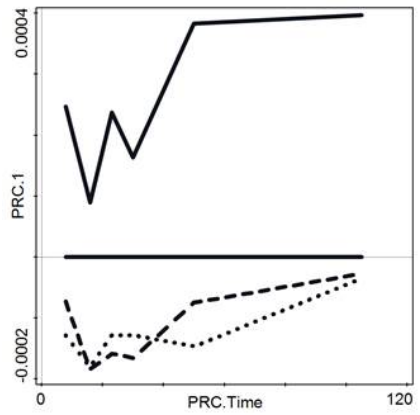


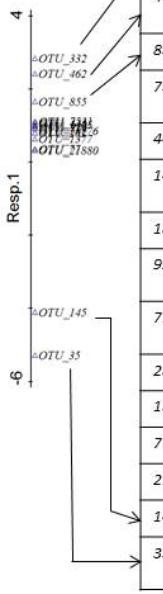
OTU #	Taxonomic Identity
443	Verrucomicrobia (P) Pedosphaerae (C) Pedosphaerales (O)
332	Proteobacteria (P) Gammaproteobacter (C) Xanthomonadales (O) Sinobacteraceae (F)
1187	Proteobacteria (P) Deltaproteobacteria (C) Bdellocibrionales (O) Bdellovibrionaceae (F) Bdellovibrio (G) Bacteriovorus (S)
492	Proteobacteria (P) Alphaproteobacteria (C) Rhodospirillales (O) Rhodospirillaceae (F)
6799	Bacteroidetes (P) Saprospirae (C) Saprospirales (O) Chitinophagaceae (F)
21880	Firmicutes (P) Bacilli (C) Bacillales (O) Bacillaceae (F) Virgibacillus (G)

OTU #	Taxonomic Identity
196	Fibrobacteres (P) Fibrobacteria (C) 258ds10 (O)
19	Proteobacteria (P) Betaproteobacteria (C) Burkholderiales (O) Oxalobacteraceae (F) Jathinobacterium (G)
18528	Proteobacteria (P) Betaproteobacteria (C) Burkholderiales (O) Oxalobacteraceae (F) Janthinobacterium (G)
319	Acidobacteria (P) Acidobacteria-6 (C) iii1-15 (O)
12	Proteobacteria (P) Alphaproteobacteria (C) Rhizobiales (O) Hyphomicrobiaceae (F) Rhodoplanes (G)
69	Acidobacteria (P) S035 (C)
244	Acidobacteria (P) Acidobacteria-6 (C) iii1-15 (O)
18	Acidobacteria (P) Acidobacteria-6 (C) iii1-15 (O)

OTU #	Taxonomic Identity
145	Proteobacteria (P) Betaproteobacteria (C) MND1 (O)

Figure 3.21 Redundancy analysis on Lilac 16S samples with forward selection of variables.
Variables with significant contributions to variation included temperature, plots without compost (UT), poultry compost treatment (P), and moisture taken at a 2 cm depth and 10 cm soil depth. The 15 OTUs with the best fit to explanatory variables are shown on the graph. Their taxonomic identity is given in the table outlined by the same pattern as the box that they are clustered in. P = phylum, C = class, O = order, F = family, G = genus, S = species. Missing taxonomic information occurs if higher resolution was not available for the OTU.





OTU #	Taxonomic Identity
332	*Proteobacteria (P) Gammaproteobacter (C) Xanthomonadales (O) Sinobacteraceae (F)*
462	*Verrucomicrobia (P) Opitutae (C) Opitutales (O) Opitutaceae (F) Opitutus (G)*
855	*Acidobacteria (P) Chloracidobacteria (C) RB41 (O) Ellin6075 (F)*
7531	*Bacteroidetes (P) Saprospirae (C) Saprospirales (O) Chitinophagaceae (F) Ferruginibacter (G) Lapsinanis (S)*
443	*Verrucomicrobia (P) Pedosphaerae (C) Pedosphaerales (O) Ellin517 (F)*
1405	*Bacteroidetes (P) Sphingobacteriia (C) Spingobacteriales (O) Sphingobacteriaceae (F)*
18176	*Bacteroidetes (P) Sphingobacteriia (C) Spingobacteriales (O)*
923	*Proteobacteria (P) Alphaproteobacteria (C) Sphingomonadales (O) Spingomonadaceae (F)*
719	*Proteobacteria (P) Alphaproteobacteria (C) Sphingomonadales (O) Spingomonadaceae (F) Novosphingobiu (G)*
282	*Acidobacteria (P) Chloracidobacteria (C) RB41 (O) Ellin 6075 (F)*
1377	*Verrucomicrobia (P) Opitutae (C) Opitutales (O) Opitutacaea (F) Opitutus (G)*
77	*Firmicutes (P) Bacilli (C) Bacillales (O) Bacillaceae (F)*
21880	*Firmicutes (P) Bacilli (C) Bacillales (O) Bacillaceae (F) Virgibacillus (G)*
145	*Proteobacteria (P) Betaproteobacteria (C) MND1 (O)*
35	*Proteobacteria (P) Gammaproteobacter (C) Xanthomonadales (O) Sinobacteraceae (F)*

OTU #	Taxonomic Identity
145	*Proteobacteria (P) Betaproteobacteria (C) MND1 (O)*
35	*Proteobacteria (P) Gammaproteobacter (C) Xanthomonadales (O) Sinobacteraceae (F)*

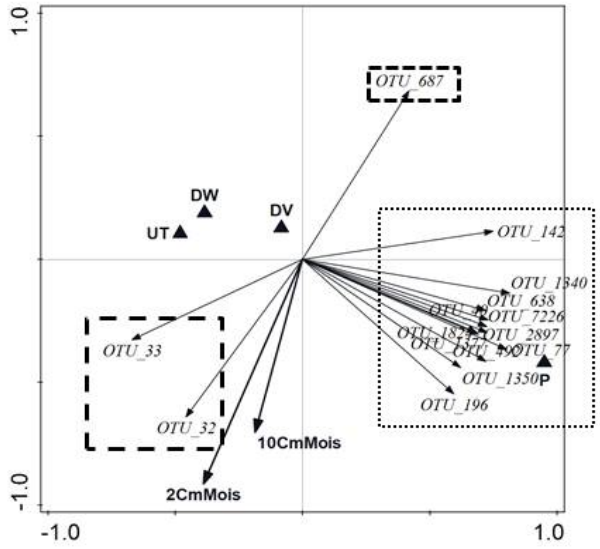
Figure 3.22 Principal response curves of Lilac 16S samples with the composition of untreated plots used as the baseline. The scores of the 15 best fit OTUs are shown on the right axis. The taxonomic identity of the OTUs are listed in the table in descending order from highest to lowest scores. Arrows indicate the corresponding OTU on the score axis with the identity in the table. P = phylum, C = class, O = order, F = family, G = genus, S = species. Missing taxonomic information occurs if higher resolution was not available for the OTU.

At Wheelock, all compost treatments and moisture had the greatest contribution to variation in bacterial and archaea community composition (Table 3.8). The majority of the top 15 OTUs that best fit to the explanatory variables were clustered with the poultry treatment (Figure 3.23). These included *Bacteriodetes*, *α-* and *γ-Proteobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Fibrobacteres*, and the iii1-150 order of *Acidobacteria-6*. An OTU identified as belonging to the iii1-150 order of *Acidobacteria-6* correlated with moisture, as did a member of the *Nitrospirae* phylum. One OTU, matched to the *Sphingobacteriaceae* family, had PCO scores that opposed moisture and was therefore correlated with dry conditions. The PRCs of all treatments had the same general pattern (Figure 3.24), with significant effects of treatments on bacterial composition ($p=0.002$). Deviation from the bacterial composition of untreated plots was greatest on day 50 post-inoculation for all treatment types. OTU scores ranged from 0 to 35. The three OTUs that had much higher scores than the others were also correlated with poultry treatment in the redundancy analysis. These OTUs matched to members of the *Spingobacteriales* order within *Bacteriodetes* and the *iii1-15* order of *Actinobacteria-6*. The remaining OTUs had low scores and were uniformly abundant in untreated soils.

Table 3.8 Variables with statistically significant contributions to explained variation in 16S composition in Wheelock plots.

Variable	Contribution to explained variation	*p*-Value
Poultry treatment	37.2%	0.0028
2 cm moisture	30.4%	0.0035
10 cm moisture	12.7%	0.0028
Untreated	11.5%	0.0035
Dairy windrow treatment	8.3%	0.049

Explanatory variables accounted for 20.1% of variation

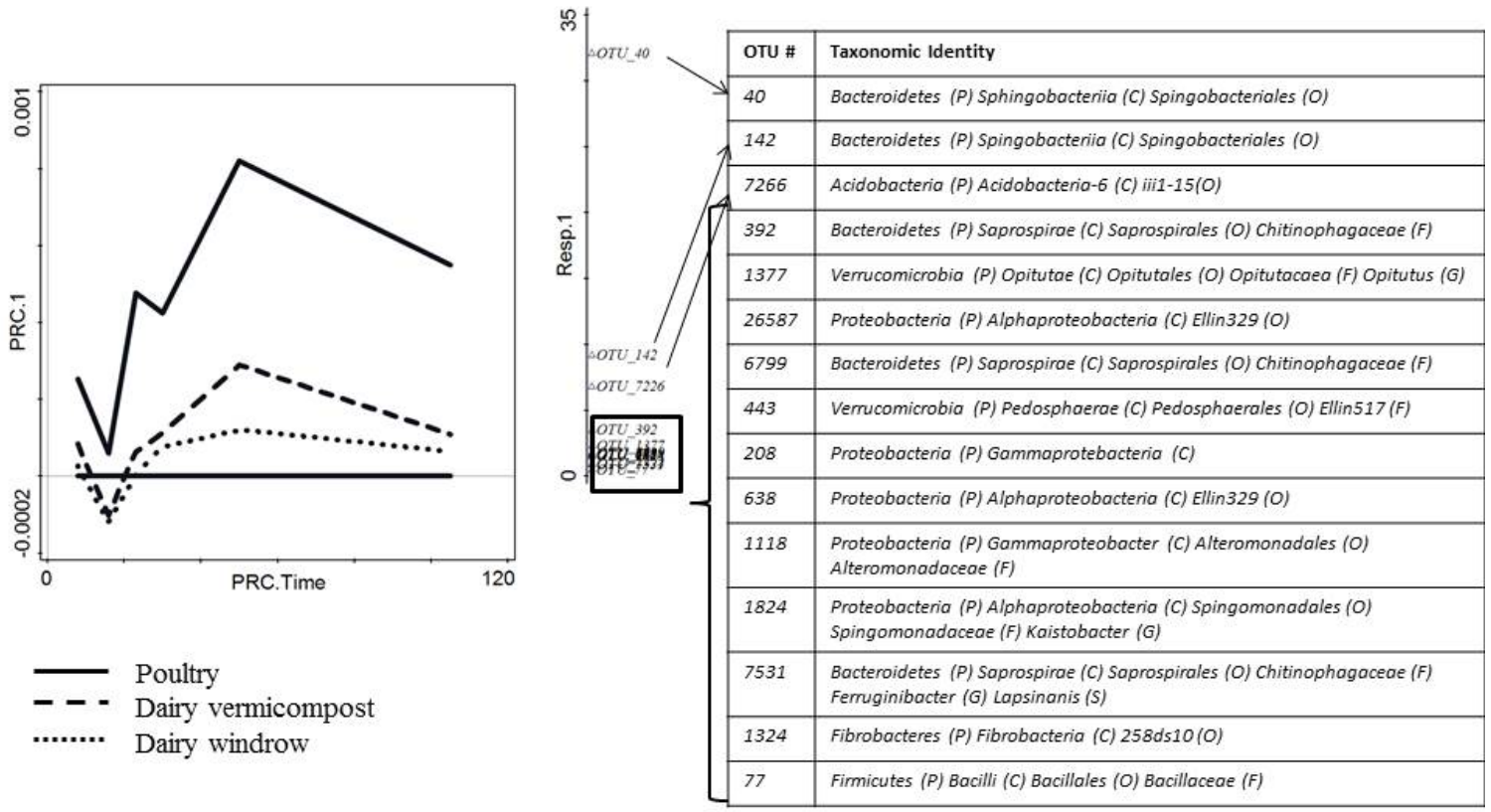


OTU #	Taxonomic Identity
142	Bacteroidetes (P) Spingobacteriia (C) Spingobacteriales (O)
1340	Bacteroidetes (P) Saprospirae (C) Saprospirales (O) Chitinophagaceae (F)
638	Proteobacteria (P) Alphaproteobacteria (C) Ellin329 (O)
40	Bacteroidetes (P) Spingobacteriia (C) Spingobacteriales (O)
7266	Acidobacteria (P) Acidobacteria-6 (C) iii1-15(O)
2897	Chloroflexi (P) Anaerolineae (C) envOPS12 (O)
1824	Proteobacteria (P) Alphaproteobacteria (C) Spingomonadales (O) Spingomonadaceae (F) Kaistobacter (G)
492	Proteobacteria (P) Alphaproteobacteria (C) Rhodospirillales (O) Rhodospirillaceae (F)
1377	Verrucomicrobia (P) Opitutae (C) Opitutales (O) Opitutacaea (F) Opitutus (G)
77	Firmicutes (P) Bacilli (C) Bacillales (O) Bacillaceae (F)
1350	Proteobacteria (P) Gammaproteobacteria (C)
196	Fibrobacteres (P) Fibrobacteria (C) 258ds10 (O)

OTU #	Taxonomic Identity
687	Bacteroidetes (P) Spingobacteriia (C) Spingobacteriales (O) Spingobacteriaceae (F)

OTU #	Taxonomic Identity
33	Nitrospirae (P) Nitrospira (C) Nitrospirales (O) 0319-6A21 (F)
32	Acidobacteria (P) Acidobacteria-6 (C) iii1-15 (O)

Figure 3.23 Redundancy analysis on Wheelock 16S samples with forward selection of variables.
Variables with significant contributions to variation included plots without compost (UT), poultry compost treatment (P), dairy windrow compost treatment (DW), dairy vermicompost treatment (DV), and moisture taken at a 2 cm depth and 10 cm soil depth. The 15 OTUs with the best fit to explanatory variables are shown on the graph. Their taxonomic identity is given in the table outlined by the same pattern as the box that they are clustered in. P = phylum, C = class, O = order, F = family, G = genus, S = species. Missing taxonomic information occurs if higher resolution was not available for the OTU.



OTU #	Taxonomic Identity
40	*Bacteroidetes (P) Sphingobacteriia (C) Spingobacteriales (O)*
142	*Bacteroidetes (P) Spingobacteriia (C) Spingobacteriales (O)*
7266	*Acidobacteria (P) Acidobacteria-6 (C) iii1-15(O)*
392	*Bacteroidetes (P) Saprospirae (C) Saprospirales (O) Chitinophagaceae (F)*
1377	*Verrucomicrobia (P) Opitutae (C) Opitutales (O) Opitutacaea (F) Opitutus (G)*
26587	*Proteobacteria (P) Alphaproteobacteria (C) Ellin329 (O)*
6799	*Bacteroidetes (P) Saprospirae (C) Saprospirales (O) Chitinophagaceae (F)*
443	*Verrucomicrobia (P) Pedosphaerae (C) Pedosphaerales (O) Ellin517 (F)*
208	*Proteobacteria (P) Gammaprotebacteria (C)*
638	*Proteobacteria (P) Alphaproteobacteria (C) Ellin329 (O)*
1118	*Proteobacteria (P) Gammaproteobacter (C) Alteromonadales (O) Alteromonadaceae (F)*
1824	*Proteobacteria (P) Alphaproteobacteria (C) Spingomonadales (O) Spingomonadaceae (F) Kaistobacter (G)*
7531	*Bacteroidetes (P) Saprospirae (C) Saprospirales (O) Chitinophagaceae (F) Ferruginibacter (G) Lapsinanis (S)*
1324	*Fibrobacteres (P) Fibrobacteria (C) 258ds10 (O)*
77	*Firmicutes (P) Bacilli (C) Bacillales (O) Bacillaceae (F)*

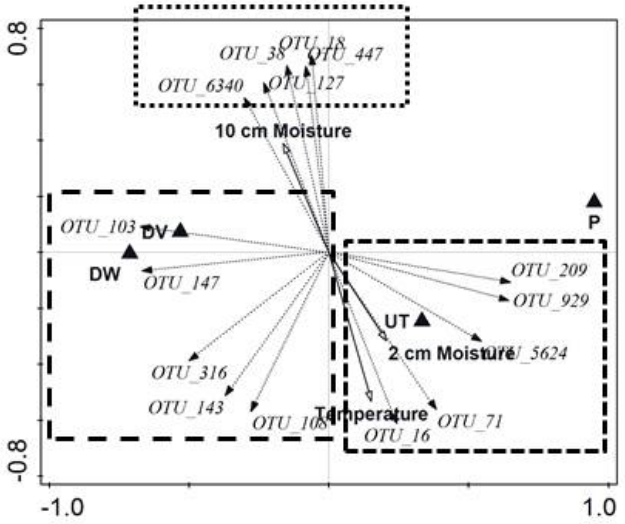
Figure 3.24 Principal response curves of Wheelock 16S samples with the composition of untreated plots as the baseline. The scores of the 15 best fit OTUs are shown on the right axis. The taxonomic identity of the OTUs are listed in the table in descending order from highest to lowest scores. Arrows indicate the corresponding OTU on the score axis with the identity in the table. P = phylum, C = class, O = order, F = family, G = genus, S = species. Missing taxonomic information occurs if higher resolution was not available for the OTU.

At Lilac, moisture had a stronger contribution to variation in fungal composition than compost treatments (Table 3.9). While bacterial composition was heavily influenced by poultry treatment, fungal OTUs were closely correlated to moisture, temperature, and dairy treatment (Figure 3.25). PCO scores of the 2 cm depth moisture measurement and 10 cm depth moisture measurement fell on opposite ends of the axes, indicating a soil depth effect of moisture on fungal composition. The majority of ITS OTUs did not have taxonomic resolution greater than the phylum, making it difficult to identify potential ecological roles of the fungal community. Principal response curves of Lilac ITS sequences showed a greater deviation in the fungal community from dairy compost treatment, particularly dairy windrow compost, than poultry compost with significant treatment effects ($p=0.004$) (Figure 3.26). Dairy windrow compost treatment had the greatest separation in fungal composition from untreated plots at day 50 post-inoculation. All treatments had positive canonical coefficients, and thus influenced deviation from the untreated plots in the same direction. The majority of ITS OTUs clustered around the untreated baseline plot. One OTU, which matched to the species *Mortierella camargensis*, was present in high numbers in all Lilac plots and had a species score of approximately -50. Several other OTUs matched to the same species, but were much less abundant.

Table 3.9 Variables with statistically significant contributions to explained variation in ITS composition in Lilac plots.

Variable	Contribution to explained variation	P-Value
Poultry treatment	23.1%	0.0028
Untreated	19.0%	0.0023
Temperature	17.7%	0.0028
10 cm moisture	20.5%	0.0028
2 cm moisture	11.1%	0.0028
Dairy windrow	8.7%	0.0080

Explanatory variables accounted for 19.9% of the variation.

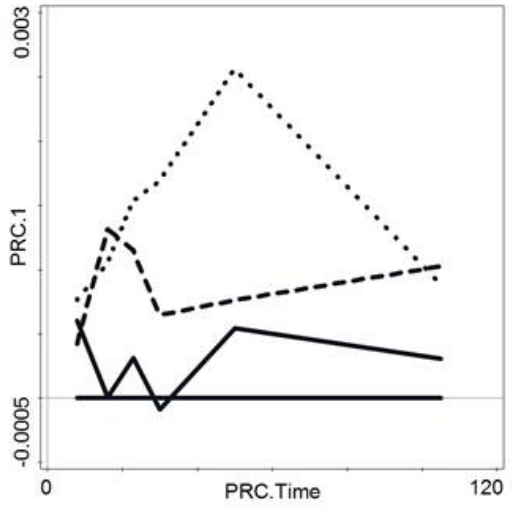


OTU #	Taxonomic Identity
6340	Fungi (K) Ascomycota (P) Sordariomycetes (C) Hypocreales (O) Nectriaceae (F)
38	Fungi (K) Ascomycota (P) Eurotiomycetes (C) Eurotiales (O) Trichocomaceae (F) Penicillium (G)
18	Fungi (K) Ascomycota (P)
447	Fungi (K) Basidiomycota (P)
127	Fungi (K) Ascomycota (P) Sordariomycetes (C) Hypocreales (O)

OTU #	Taxonomic Identity
108	Fungi (K)
143	Fungi (K) Basidiomycota (P)
316	Fungi (K) Basidiomycota (P)
147	Fungi (K) Ascomycota (P) Ascomycota sp r433 (S)
103	Fungi (K) Zygomycota (P) Incertae sedis (C) Mortierellales (O) Mortierellaceae (F) Mortierella (G) Mortierella camargensis (S)

OTU #	Taxonomic Identity
209	Fungi (K) Basidiomycota (P) Agaricomycetes (C)
929	Fungi (K)
5624	Fungi (K) Ascomycota (P)
71	Fungi (K)
16	Fungi (K) Ascomycota (P)

Figure 3.25 Redundancy analysis of Lilac ITS samples with forward selection of variables.
Variables with significant contributions to variation included temperature, plots without compost (UT), poultry compost treatment (P), dairy windrow compost treatment (DW), dairy vermicompost treatment (DV), and moisture taken at a 2 cm depth and 10 cm soil depth. The 15 OTUs with the best fit to explanatory variables are shown on the graph. Their taxonomic identity is given in the table outlined by the same pattern as the box that they are clustered in. P = phylum, C = class, O = order, F = family, G = genus, S = species. Missing taxonomic information occurs if higher resolution was not available for the OTU.



OTU #	Taxonomic Identity
224	Fungi (K) Ascomycota (P)
1257	Fungi (K)
1454	Fungi (K) Ascomycota (P) Sordariomycetes (C) Xylariales (O) Incertae sedis (F) Monographella (G) Monographella nicalis (S)
1397	Fungi (K) Ascomycota (P)
2254	Fungi (K)
16950	Fungi (K) Zygomycota (P) Incertae sedis (C) Mortierellales (O) Mortierellaceae (F) Mortierella (G) Mortierella camargensis (S)
1143	Fungi (K) Basidiomycota (P)
16464	Fungi (K) Zygomycota (P) Incertae sedis (C) Mortierellales (O) Mortierellaceae (F) Mortierella (G) Mortierella camargensis (S)
18771	Fungi (K) Zygomycota (P) Incertae sedis (C) Mortierellales (O) Mortierellaceae (F) Mortierella (G) Mortierella camargensis (S)
1976	Fungi (K) Zygomycota (P) Incertae sedis (C) Mortierellales (O) Mortierellaceae (F) Mortierella (G) Mortierella camargensis (S)
166	Fungi (K) Ascomycota (P) Sodariomycetes (C) Microascales (O) Microascaceae (F)
593	Fungi (K)
195	Fungi (K) Ascomycota (P)
49	Fungi (K)
1	Fungi (K) Zygomycota (P) Incertae sedis (C) Mortierellales (O) Mortierellaceae (F) Mortierella (G) Mortierella camargensis (S)

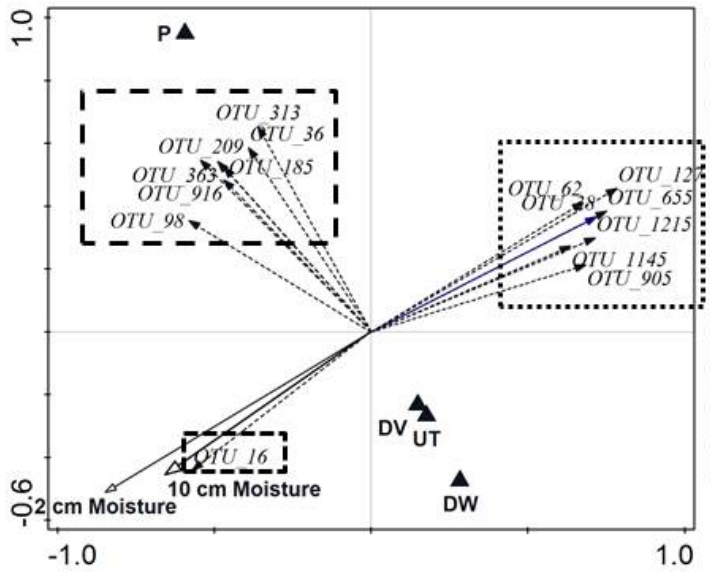
Figure 3.26 Principal response curves of Lilac ITS samples with composition of untreated plots as the baseline.
The scores of the 15 best fit OTUs are shown on the right axis. The taxonomic identity of the OTUs are listed in the table in descending order from highest to lowest scores. Arrows indicate the corresponding OTU on the score axis with the identity in the table. P = phylum, C = class, O = order, F = family, G = genus, S = species. Missing taxonomic information occurs if higher resolution was not available for the OTU.

At Wheelock, all compost treatments and moisture had the highest contribution to variation in bacterial and archaea community composition (Table 3.10). Three distinct clusters of OTUs were present (Figure 3.27). Only one OTU, which matched to a member of the *Ascomycota* phylum, grouped with moisture at both 2 cm and 10 cm depths. Another cluster, which contained seven OTUs, grouped directly opposite from moisture and, therefore, correlated positively with dry conditions. The third cluster, also containing seven OTUs, correlated positively with the application of poultry compost. PRC curves of Wheelock ITS samples varied considerably (Figure 3.28), but treatment effects were still significant ($p=0.04$). Poultry treatment had the greatest deviation from the fungal composition of untreated plots, but mostly at 8, 23, and 30 days after inoculation. The PRCs of the dairy compost treatments fluctuated around the untreated baseline axis, without any strong deviations from the untreated fungal composition at any time point. Most OTUs had low species scores, with the exception of one OTU which matched to an *Ascobolus* species and had a species score of approximately 50.

Table 3.10 Variables with statistically significant contributions to explained variation in ITS composition in Wheelock plots.

Variable	Contribution to explained variation	*p*-Value
2 cm moisture	29.8%	0.0028
Poultry treatment	29.3%	0.0023
Untreated	15.0%	0.0020
10 cm moisture	13.6%	0.0028
Dairy vermicompost	12.2%	0.0047

Explanatory variables accounted for 15.8% of variation.

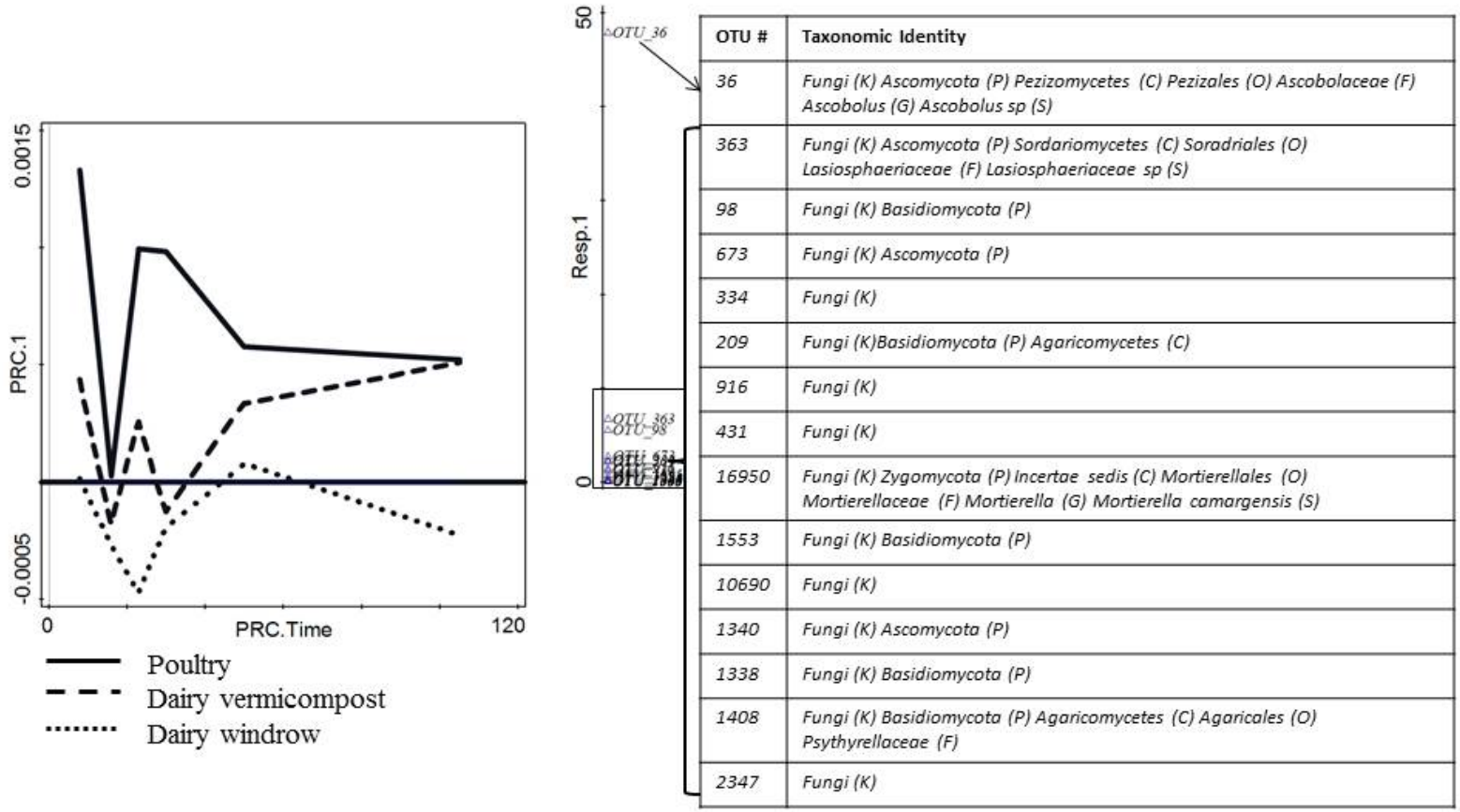


OTU #	Taxonomic Identity
127	Fungi (K) Ascomycota (P) Sordariomycetes (C) Hypocreales (O)
655	Fungi (K) Ascomycota (P) Dothideomycetes (C) Capnodiales (O) Mycosphaerellaceae (F) Dissoconium (G) Dissoconium proteae (S)
1215	Fungi (K) Ascomycota (P) Sordariomycetes (C) Glomerellales (O) Glomerellaceae (F) Colletotrichum (G)
62	Fungi (K) Ascomycota (P) Sordariomycetes (C) Incertae sedis (O) Plectosphaerellaceae (F)
38	Fungi (K) Ascomycota (P) Eurotiomycetes (C) Eurotiales (O) Trichocomaceae (F) Penicillium (G)
1145	Fungi (K) Basidiomycota (P) Microbotryomycetes (C) Sporidiobolales (O)
905	Fungi (K)

OTU #	Taxonomic Identity
313	Fungi (K) Ascomycota (P)
36	Fungi (K) Ascomycota (P) Pezizomycetes (C) Pezizales (O) Ascobolaceae (F) Ascobolus (G) Ascobolus sp (S)
209	Fungi (K) Basidiomycota (P) Agaricomycetes (C)
185	Fungi (K)
363	Fungi (K) Ascomycota (P) Sordariomycetes (C) Soradriales (O) Lasiosphaeriaceae (F) Lasiosphaeriaceae sp (S)
916	Fungi (K)
98	Fungi (K) Basidiomycota (P)

OTU #	Taxonomic Identity
16	Fungi (K) Ascomycota (P)

Figure 3.27 Redundancy Analysis on Wheelock ITS samples with forward selection of variables.
Variables with significant contributions to variation included temperature, plots without compost (UT), poultry compost treatment (P), dairy windrow compost treatment (DW), dairy vermicompost treatment (DV), and moisture taken at a 2 cm depth and 10 cm soil depth. The 15 OTUs with the best fit to explanatory variables are shown on the graph. Their taxonomic identity is given in the table outlined by the same pattern as the box that they are clustered in. P = phylum, C = class, O = order, F = family, G = genus, S = species. Missing taxonomic information occurs if higher resolution was not available for the OTU.



OTU #	Taxonomic Identity
36	Fungi (K) Ascomycota (P) Pezizomycetes (C) Pezizales (O) Ascobolaceae (F) Ascobolus (G) Ascobolus sp (S)
363	Fungi (K) Ascomycota (P) Sordariomycetes (C) Soradriales (O) Lasiosphaeriaceae (F) Lasiosphaeriaceae sp (S)
98	Fungi (K) Basidiomycota (P)
673	Fungi (K) Ascomycota (P)
334	Fungi (K)
209	Fungi (K)Basidiomycota (P) Agaricomycetes (C)
916	Fungi (K)
431	Fungi (K)
16950	Fungi (K) Zygomycota (P) Incertae sedis (C) Mortierellales (O) Mortierellaceae (F) Mortierella (G) Mortierella camargensis (S)
1553	Fungi (K) Basidiomycota (P)
10690	Fungi (K)
1340	Fungi (K) Ascomycota (P)
1338	Fungi (K) Basidiomycota (P)
1408	Fungi (K) Basidiomycota (P) Agaricomycetes (C) Agaricales (O) Psythyrellaceae (F)
2347	Fungi (K)

Figure 3.28 Principal response curves of Wheelock ITS samples with composition of untreated plots as the baseline.
The scores of the 15 best fit OTUs are shown on the right axis. The taxonomic identity of the OTUs are listed in the table in descending order from highest to lowest scores. Arrows indicate the corresponding OTU on the score axis with the identity in the table. P = phylum, C = class, O = order, F = family, G = genus, S = species. Missing taxonomic information occurs if higher resolution was not available for the OTU.

CHAPTER 4. DISCUSSION

4.1.Extract Experiment

E. coli were able to grow rapidly in compost extracts in the absence of competition, whereas the presence of endemic microbes limited the *E. coli* population to remain at inoculation levels or decay. The nutrient content of each compost, particularly ammonium, phosphorous, and potassium, also related to *E. coli*'s growth potential when endemic microbes were absent. Sterilization of the compost extracts allowed the *E. coli* to access the existent nutrients without competition from the native. When competition is absent and nutrients are available for direct consumption, *E. coli* will follow a log growth phase until a carrying capacity is reach, which, in this study, occurred approximately 50 hours after inoculation. The Maryland poultry compost had the most profound *E. coli* growth, likely due to its very high ammonium levels, which is *E.* coli's preferred nitrogen source (Reitzer 2003). The Maryland poultry compost also had the highest phosphorous and potassium levels, which, along with ammonium, correlated positively with the growth rate constants during the log growth phase. Nitrogen has been shown to be a strong driver of *E. coli* survival (Franz *et al*. 2008), and likely had more of an effect on *E. coli*'s success in the Maryland poultry compost than phosphorous or potassium.

The two dairy composts from Worm Power were the only two compost extracts that caused a decrease in the *E. coli* population when the native microbes were present. With the exception of the Maryland poultry extract, the two dairy extracts had greater levels of carbon, nitrogen, potassium, and ammonium than any of the other composts tested. While some of these nutrients were correlated with increased growth rate

constants in sterile extracts, the slightly higher nutrient levels in the dairy composts than the other composts clearly did not give the *E. coli* an advantage in non-sterile extracts. In fact, the higher nutrient levels may increase the overall microbial abundance in the dairy composts, resulting in strong competition and predation against *E. coli*. These results suggest that excessive nutrients, such as those seen in Maryland poultry, are sufficient to sustain both the endemic microbial population and increase *E. coli* survival. However, moderate levels of nutrients, such as those seen in the dairy composts, may be enough to encourage endemic microbial growth and create a more competitive environment for *E. coli* without providing enough nutrients to be able to sustain both the endemic and *E. coli* populations. Additionally, given that *E. coli* is typically found in dairy manure, the microbial community that develops through the composting of dairy manure may be better adapted to compete with and prey on *E. coli* than those found in poultry manure. The vermicomposting process also alters the microbial and nutritional profile. Worm Power vermicompost is created by the worm species *Eisenia fetida* digesting the Worm Power dairy compost after it been pre-treated with a thermophilic phase to meet pathogen reduction standards. The worm digestion occurs after the pile has been cooled so that the worms can survive. There are no additional substrates added to alter the microbial or nutritional profile other than the worm castings. While the vermicomposting process has been shown to alter the microbial community of the compost substrate (Neher *et al.* 2013), the Worm Power windrow and vermicompost microbial communities exhibited similar suppressive effects on *E. coli* survival in this study.

The presence of endemic microbes had the opposite effect on *E. coli* survival in mineral soil extract than it did in compost extracts. Composts have substantially greater concentrations of bioavailable carbon, nitrogen, and phosphorous, as well as increased microbial biomass and activity compared to mineral soils (Debosz *et al.* 2002). While the nutrients in the compost extracts were sufficiently abundant to support regrowth of the *E. coli* population, levels of bioavailable nutrients in the sterile soil extract were inadequate to sustain the *E. coli* population. Nutrients in mineral soil are typically tied up in polymeric organic material and require enzymatic breakdown for microbial ingestion. While *E. coli* are poorly adapted for environments in which nutrients are not readily available, endemic soil microbes are well adapted for extracellular enzyme secretion where bioavailable nutrients are scarce (Allison and Vitousek 2005). The increased survival of *E. coli* in non-sterile soil extract is potentially due to increased levels of bioavailable nutrients from the extracellular enzymes secreted by the native microbes. Competition with the native microbes, however, may have prevented the *E. coli* from increasing its population size beyond the inoculum level. The majority of non-sterile compost extracts also had sufficient nutrient levels to sustain the *E. coli* population, but the bioavailable nutrients were naturally present in high enough levels in the composts that the competition from the microbes was suppressive rather than augmentative for the *E. coli* population.

4.2. Field Experiment

4.2.1. *E. coli* Survival

Compost amendments had variable effects on *E. coli* survival, likely due to the differences in nutritional profiles and the interactions between nutrient availability and soil moisture. The nitrogen and phosphorous content in the Wheelock and Lilac soils before compost amendment were within an ideal range for crop growth, so compost amendment in this situation added an excess of nutrient sources that could be accessed by the microbial community without competition by plant growth. However, both Lilac and Wheelock had high sand contents (88-90% sand and 80-82% sand, respectively). Because the field season was unusually rainy, any nitrogen in the form of nitrate was likely quickly leached into the ground water. Ammonium, in contrast, is positively charged and binds to negative charges on organic matter and clay particles, and is thus more resistant to leaching (Paul 2015). Because the poultry manure had exceptionally high ammonium levels, the nitrogen may have been able to reside within the soil for longer than nitrate forms of nitrogen from other composts. The net result is a sustained *E. coli* population in poultry compost-treated plots. Additionally, Lilac soil had 0.4% higher organic matter content and 5% higher clay content than Wheelock soil, and, therefore, contained more cation exchange attachment sites for ammonium. These additional attachment sites may have maintained higher nitrogen levels in Lilac than Wheelock, accounting for at least a portion of the difference in *E. coli* population decline between Lilac and Wheelock poultry compost-treated plots during the bout of rains in the last two months of the field trial. In contrast, treatment by the other composts exhibited no difference in *E. coli*

survival between fields. Because nitrogen was mostly in the nitrate form in the dairy composts, which leaches easily in sandy soils during rainy seasons, it is possible that the two dairy composts would have had a larger effect on *E.* coli survival in less sandy soils with fewer rains.

During the first 50 days after inoculation, both fields remained close to full saturation. Despite the fields containing well-drained sandy soils, the sites at the end of this 50-day period were water-logged. When soil pores are completely saturated with water, the soil may become anaerobic (Tiedje *et al*. 1984). *E. coli* are facultative anaerobes, making them capable of metabolic respiration in the absence of oxygen. This gives them a competitive advantage over the large number of obligate aerobes that live in the soil when pores are saturated. The one-phase decay model, which represents a standard microbial population decline under nutrient-starved conditions, fit well with the *E. coli* survival rate when conditions were wet. Dryer periods correlated to intervals of time when the *E. coli* populations decreased at a much more rapid rate than a one-phase decay model would predict. Under dryer conditions, *E. coli* may encounter much fiercer competition and predation from aerobic organisms than in saturated conditions. Saturated conditions not only give *E. coli* a metabolically competitive advantage, but may also mobilize nutrients in the soil and temporarily relieve nutrient starvation.

4.2.2. Enzyme Activity

Overall, there were no clear long-term effects of compost on enzyme activity. Any observed effect was temporary, lasting no more than two weeks after composts were

added to soil, at which point enzyme activity returned to that of the endemic microbial signature. Activity of all extracellular enzymes were the most variable between plots on the first sampling date, which was eight days after *E. coli* inoculation. Because it was the beginning of the field trial, plots had been recently tilled and amended with the appropriate compost treatment. Tilling of soil disrupts soil aggregate structure and redistributes nutrients, creating a new set of dynamics among the biotic community (Six *et al.* 1999). This initial variation in extracellular enzyme activity (EEA) is likely a function of such disruption and addition of organic inputs. Additionally, the soil temperature increased by approximately 10^{o}C during the ten days prior to the first sampling date. High temperatures lead to increased enzyme activity in soil (German *et al.* 2008), which also could have caused a sudden burst in enzyme production and contributed to the early EEA variation. Overall, enzyme release was affected more by time than by compost application. Contrary to the hypothesis that nutrient inputs by compost amendment would drive changes in enzyme activity, the input of both organic and inorganic substrates by compost amendment did not lead to significant differences in EEA. The majority of studies showing decreased activity of EEA with addition of simple substrates were conducted in wetland soils or aquatic systems (Chrost 1991, Chlarholm 1993). Interactions between microbes and nutrients in such aquatic environments are much more homogenous than agricultural soils, given increased bacterial and nutrient mobility in water and less niche segregation by aggregates. The relationship between nutrient inputs and EEA in terrestrial soils may be much more complicated. Microbes likely have constitutive enzyme production, which would alter the relative need for

further enzyme production (Allison and Vitousek 2005). Additionally, the ability to produce particular enzymes can be confounded by the carbon and nitrogen requirements of enzyme synthesis alone. For example, even if the microbe would benefit from producing extracellular enzymes to obtain carbon, the carbon levels are so limited in the soil that enzyme synthesis is not possible (Burns 1982). The release of enzymes in soil can be affected by an infinite number of abiotic and biotic trigger combinations, making predictions about EEA responses extremely difficult.

Enzyme activity, did however, exhibit paralleling trends with soil moisture. Several studies have demonstrated a strong relationship between soil moisture and EEA. Henry (2013) proposed general relationships of soil moisture and EEA depending on the soil drainage properties. Poorly drained soils exhibit a parabolic curve of EEA with increasing soil moisture, in which dry conditions are associated with low EEA, intermediate rainfall causes the greatest EEA, and high rainfall leads to anaerobic conditions and also reduces EEA. In well-drained soils, EEA continues to rise with increasing moisture until it reaches a plateau, but the soil never becomes anaerobic and, therefore, never decreases. Although the soils in this study have high sand content and would be considered well-drained, the rains were heavy enough that plots were completely saturated by day 30 post *E. coli* inoculation. Additionally, Lilac is near a stream and may have a high water table, resulting in frequent saturation. Enzyme activity decreased in both fields until day 30, potentially due to the soils becoming anaerobic. Obligate and facultative anaerobes use different enzymatic strategies for obtaining nutrients than obligate aerobes (Reguera and Leschine 2001). Therefore, the decrease in

EEA measured may have been due to a switch in enzyme production by the dominating anaerobic community. As seen in the extract experiment, *E. coli* growth was correlated with potassium, phosphorous, and nitrogen levels, but not carbon. As soils became saturated, AP and NAG activity increased relative to BG, thereby switching microbial nutrient acquisition from carbon to nitrogen and phosphorous. Anaerobes and facultative anaerobes, such as *E. coli,* may require more nitrogen and phosphorous than carbon for metabolism in anaerobic conditions. Rainfall also mobilizes nutrients, increasing their availability (Stark and Firestone 1995), which may have decreased need for synthesis of microbial enzymes. Alternatively, heavy rains can result in the leaching of enzymes (Bell and Henry 2011), resulting in lower levels of overall enzyme activity. Rains decreased briefly between days 30 and 40, and then became heavy again between days 40 and 50 post inoculation. Activity of β-glucosidase (BG), phosphatase (AP), and β-1,4-N-acetylglucosaminidase (NAG) increased in both fields between days 30 and 50. Drying and rewetting of soils causes mineralization bursts (Borken and Matzner 2009, Inglima *et al.* 2009), which may at least partially explain the increase in enzyme activity at day 50 post inoculation. An increase in soil saturation causes an increase in microbial phosphorous acquisition (increase in AP activity) and a decrease in microbial carbon acquisition (decrease in BG activity) (Sinsabaugh *et a.l* 2008). Bell and Henry (2011) showed that NAG activity increased with prolonged water addition, while BG activity was unaffected, suggesting the same pattern for NAG and AP. Between day 23 and 30 post inoculation, AP and (NAG + LUC) activity increased relative to BG activity, particularly in Lilac soils, which was the wetter of the two fields. These data reinforce the

same trends found by previous studies that nitrogen and phosphorous acquisition is greater in saturated soils than carbon acquisition.

4.2.3. Respiration

INT reduction followed a similar pattern as enzyme activity, in that compost amendment did not have obvious effects and soil moisture seemed to have a strong influence on microbial respiration. Both INT reduction and enzyme activity decreased during the initial part of the season and increased by the last sampling date. The primary difference between the two was enzyme activity started to increase after day 30 post inoculation, and INT reduction did not increase until after day 50. Enzyme activity increased after a brief gap in the rainfall when water potential dropped slightly and then resurged between days 30 and 50 post inoculation. Respiration, in contrast, was significantly less at day 50 than earlier in the season. Because extracellular enzyme secretion is energy-intensive and unnecessary for immediate survival, its regulation is likely to be more sensitive to small environmental changes (Schimel *et al.* 2007). INT reduction, however, is indicative of dehydrogenase activity, which is necessary for basic microbial respiration and survival. Therefore, it is beneficial for microbes to maintain constitutive dehydrogenase activity throughout a range of environmental conditions. High oxygen content in soil pores has been correlated with low INT reduction, whereas anaerobic conditions has been correlated directly with INT reduction (Trevors 1984), which may be due to higher respiration levels of anaerobic metabolism than aerobic metabolism. The drying of the soil between days 30-40 allowed for an influx of oxygen

into the soil, and may have, thereby, decreased levels of microbial respiration as the population switched from anaerobic to aerobic. However, the subsequent rewetting between days 40 and 50 post inoculation could have resulted in the mineralization burst by the extracellular enzymes. The mineralization burst would have resulted in a sudden increase in nutrients available for microbial growth and, therefore, stimulated the increase in respiration by 65 days after inoculation. Additionally, dry periods result in microbial accumulation of osmolytes to counteract the decreasing water potential of the soil and avoid dehydration by osmosis (Schimel *et al*. 2007). The rewetting of soil causes a need for rapid release of these osmolytes, which in turn increases respiration. This may also account for the increase in respiration observed after the drying and rewetting of the soil.

4.2.4. Phospholipid Fatty Acid Analysis

The results from the PLFA data suggest that compost amendment can alter soil microbial biomass in two ways. First, compost adds its own microbes to the soil, which, as long as they can survive in the soil's environment, will directly increase total biomass. Second, the nutritional content of the compost enhances the growth of the microbial community endemic to the soil. The effects of both compost amendment and the climate depend on the soil. In Lilac, both dairy composts increased the abundance of most taxonomic groups measured, even though the total biomass remained unchanged. Application of dairy vermicompost also increased the biomass of most taxonomic groups in Wheelock. However, in Wheelock, poultry compost had a similar effect as dairy vermicompost, and dairy windrow compost did not result in an increase in biomass for

any taxonomic group. Wheelock soil potentially contains more microbes that prefer ammonium as their nitrogen source than Lilac soil, and is, therefore, more influenced by poultry amendment. Conversely, vermicompost applications resulted in an increase of biomass of most groups in both fields. The extract experiment had the greatest *E. coli* suppression in non-sterile extracts made from the dairy vermicompost. The gut microbiome of the worms may add substantial microbial biomass to the dairy compost, which, in turn, increases the abundance of soil microbes when the compost is used as an amendment.

Many of the taxonomic groups had the greatest abundance on the last sampling date. Large numbers of these taxa are aerobic, and their growth may have been suppressed by the high water content of the soil. Given that respiration decreased gradually for the first 50 days post inoculation, it is unlikely that much growth occurred during this time. The respiration burst between the last two sampling dates coincides with the increase in abundance of many of the PLFA biomarkers, which likely drove the positive relationship between INT reduction and total biomass. Additionally, fungal biomass, which significantly increased through time, was likely affected by the tillage at the beginning of the field trial. Cultivation will interfere with fungal hyphae and decrease overall biomass (Schimel *et al.* 2007), and the increase in fungi may have simply been a function of recovery after disturbance. While anaerobes would have been expected to increase during the saturated period, the only significant difference in anaerobes was a statistically higher abundance on day 23 post inoculation than day 16 in Lilac. However,

taxonomic classifications of PLFA biomarkers are controversial, and sequencing serves as a much more accurate tool for microbial composition analysis (Frostegård *et al.* 2011).

4.2.5. Sequencing

Community composition characterized by genetic sequencing verified the interaction of field site by compost. In Wheelock soil, a greater proportion of the explained variation in bacterial composition was attributed to poultry treatment than in Lilac. Therefore, poultry compost not only had a greater influence on biomass abundance in Wheelock than Lilac as shown by the PLFA results, but also had a greater influence on bacterial composition. The combined contribution of water potential at the 2 cm and 10 cm depth to variation in bacterial and fungal composition was approximately 30% for both sites. Multiple studies have demonstrated shifts in bacterial and fungal composition with changing water potential (Barnard *et al*. 2013). A microbe's response to the drying and rewetting of soil could be a direct effect of the organism's adaptive responses to water stress (Schimel *et al*. 2007) or an indirect response from moisture's effect on nutrient availability (Jackson 2003).

Although the ecological roles of most soil microbes have yet to be determined, general life history strategies of large taxonomic groups have been correlated with shifts in soil water potential and nutrient cycling. For example, *Acidobacteria* are predominantly considered oligotrophic *k*-strategists (Ward *et al.* 2009). They are found in high abundances in bulk soils with low carbon content (Fierer *et al* 2007, Marilley and Aragno 1999). They also have a high tolerance for drying and rewetting cycles (Ward *et*

al 2007). Both *Acidobacteria* and *Verrucomicrobia* will increase in abundance in wet soils (Barnard *et al*. 2013) and decrease in abundance in soils amended with nitrogen (Ramirez *et al.* 2012). In contrast, copiotrophic taxa, such as *Firmicutes*, will increase with dry conditions and nitrogen amendments (Ramirez *et al*. 2012, Barnard *et al.* 2013). *Proteobacteria* and *Bacteroidetes* have also been observed to increase with high carbon availability (Fierer *et al.* 2007). However, *Proteobacteria* is an extremely diverse phylum with a wide range of habitats and ecological roles (Spain *et al.* 2009). The positive correlation between *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* with poultry treatment reinforces the effects of high nitrogen amendments seen in previous studies. All of these phyla are recognized as copiotrophic organisms, which likely increase in abundance from the high nitrogen content of the poultry compost along with *E. coli*. High moisture content was often correlated positively with members of the *Acidobacteria* and *Proteobacteria* phyla. This corroborates previous observations that *Acidobacteria* have high tolerance to wet soils. A member of the *Sphingobacteriales* order of *Bacteroidetes* increased in abundance in poultry-treated plots during the dry period of the field season. *Bacteroidetes* are copiotrophic Gram negative bacteria. Schimel *et al*. (2007) suggested that the broad life history pattern of Gram negative organisms would result in decreased survival in wet soils. Because they are copiotrophic organisms, it would make sense that they were found primarily in poultry plots during the dry period. In Lilac, a member of the *Opititus* genus of *Verrucomicrobia* correlated positively with poultry compost treatment during the rainy part of the season. *Opititus* is an obligate anaerobe, and was likely present because of its competitive advantage in fully saturated soils. Overall,

bacterial composition variation from poultry compost treatment was greatest during the dry period, whereas dairy compost treatment did not contribute to substantial variation and was close to the composition of untreated plots by the last sampling date. This implies that the high levels of nutrients from the poultry compost have the greatest effect on bacterial communities when the soil is dry and nutrients are therefore limited.

Fungal composition was also primarily affected primarily by poultry compost treatment and moisture. Fungi, in contrast to bacteria, are more uniform in their response to moisture. In general, fungi tend to increase with drying and decrease with wetting of soils, although the response can be site-dependent (Barnard *et al*. 2013). In Wheelock, seven ITS OTUs formed a tight cluster that directly opposed the PCO moisture scores in the Redundancy analysis. This implies a direct effect of drying on the increase in abundance of multiple fungal species in Wheelock. The relationship between fungi and moisture was less obvious in Lilac. Because fungi have long hyphal extensions, they are less limited by the immobility of nutrients during dry periods than bacteria (Orchard and Cook 1983). The decrease in competition from bacteria during dry periods likely gave fungi a competitive advantage.

Unlike bacterial composition, dissimilarity in fungal composition from treatment types did not show clear temporal trends. In Lilac, dairy compost plots had greater overall deviation in fungal composition from untreated plots than poultry compost treated plots, whereas the opposite was true in Wheelock. Similar to the biomass data and 16S composition, fungal composition seemed to be more influenced by poultry compost in Wheelock than Lilac soils. Fungal community composition can be heavily influenced by

nitrogen inputs, particularly ammonium (Paul 2015). However, as shown with both the biomass and composition data, the effects of compost amendment will differ drastically with changing environmental variables and endemic soil communities.

4.2.6. Conclusions

This study revealed that compost made from poultry litter can have drastic effects on microbial composition and invasive microbial survival in soils, whereas lower nutrient composts have a more nominal effect. High levels of proteins and amino acids in poultry litter result in substantial nitrogen availability. In fresh poultry manure, 60-80% of nitrogen is in organic form (Kelleher *et al*. 2002). Throughout the composting process, a large fraction of the organic nitrogen is converted to ammonia, ammonium, and nitrate (DeLaune *et al*. 2004). Although high concentrations of mineralized nitrogen are desirable for compost, because nitrogen is only plant-available in mineralized form, this study showed that excessive levels of ammonium may also cause the soil to harbor high levels of pathogenic *E. coli*. Mixing poultry litter with other substrates, as in the case with the poultry composts from sources other than Maryland, decreases ammonium levels dramatically and could provide enough mineralized nitrogen to support plant growth without augmenting *E. coli* survival. Additionally, the dairy manure compost provides sufficient nutrient levels for plant growth while also sustaining competitive endemic microbial communities and may provide the best nutritional amendments without compromising the safety of produce for human consumption.

The contrast between *E. coli* survival in poultry compost-treated plots and plots treated with other composts may partly be a function of soil type and the frequent rain. The high water potential of the soils likely led to leaching of nutrients and a shift in the microbial community composition from aerobes to anaerobes and facultative anaerobes. Because the source of *E. coli* outbreaks in agricultural soils is mostly contaminated water, flooding soils with surface water may not only introduce the pathogen, but give the pathogen a competitive advantage for survival against endemic obligate aerobes. The results provide further evidence that it is critically important to test surface and ground water for fecal contamination and that irrigation methods that prevent over-saturation of the soils may protect against pathogen survival.

Sequencing analysis revealed a strong influence of poultry compost amendments on overall microbial composition, particularly by increasing copiotrophic *r*-strategists that belong to the *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* phylas. Moisture, in contrast, decreased fungi and copiotrophic *r*-strategists, but increased oligotrophic *k*-strategists such as *Acidobacteria*. This implies that *E. coli* are able to co-survive in soil environments with a variety of microbes, provided that nutrient levels are high. The increase in soil microbial biomass by dairy vermicompost did not have an effect on *E. coli* survival. The combined analyses from this study suggests that moisture and nutrient availability are the driving factors in soil microbial composition, and that competition and predation by native soil organisms may not have a prominent influence on the ability of an invasive copiotrophic organism to survive in an oligotrophic soil.

LITERATURE CITED

Ackers ML,Mahon BE, Leahy E, Goode B, Damrow T, Hayes PS, Bibb WF, Rice DH, Barrett TJ, Hutwagner L, Griffin PM, and Slutsker L (1998) "An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption" *Journal of Infectious Disease* 177:1588–1593.

Alexander TW, Yanke LJ, Toppe E, Olson ME, Read RRR, Morck DW, and McAllister TA (2008) "Effect of Subtherapeutic Administration of Antibiotics on the Prevalence of Antibiotic-resistance *Escherichia coli* Bacteria in Feedlot Cattle" *Applied and Environmental Microbiology* 74(14): 4405-4416.

Allison SD and Vitousek PM (2005) "Responses of extracellular enzymes to simple and complex nutrient inputs" *Soil Biology and Biochemistry* 37(5): 937-944.

Anderson KL, Whitlock JE, and Harwood VJ (2005) "Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments" *Applied and Environmental Microbiology* 71(6): 3041-3048.

Barnard RL, Osborne CA, and Firestone MK (2013) "Responses of soil bacterial and fungal communities to extreme dessication and rewetting" *The ISME Journal* 7: 2229-2241.

Bell TH and Henry HAL (2011) "Fine scale variability in soil extracellular enzyme activity is insensitive to rain events and temperature in a mesic system" *Pedobiologia* 54: 141-146.

Bernal MP, Alburquerque JA, and Moral R (2009) "Composting of animal manures and chemical criteria for compost maturity assessment. A review." *Bioresource Technology* 100(22): 5444-5453.

Blanco JM, Lazcano C, Christensen TH, Munoz P, Rieradevall J, Moller J, Anton A, and Boldrin A (2013) "Compost benefits for agriculture evaluated by life cycle assessment. A Review." *Agronomy for Sustainable Development* 33(4): 721-732.

Borken W, Matzner E (2009) "Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils" *Global Change Biology* 15: 808–824.

Buerger S, Spoering A, Gavrish E, Leslin C, Ling L, and Epstein SS (2012) "Microbial scout hypothesis and microbial diversity" *Applied and Environmental Microbiology* 78(9): 3229-3233.

Burns RG (1982) "Enzyme activity in soil: Location and a possible role in microbial ecology" *Soil Biology and Biochemistry* 14(5): 423-427.

Buyer J and Sasser M (2012) "High throughput phospholipid fatty acid analysis of soils" *Applied Soil Ecology* 61: 127-130.

Caldwell BA (2005) "Enzyme activity as a component of soil biodiversity: A review" *Pedobiologia* 49: 637-644.

Chang D, Smalley DJ, Tucker DL, Leatham MP, Norris WE, Stevenson SJ, Anderson AB, Grissom JE, Laux DC, Cohen PS, and Conway TL (2004) "Carbon nutrition of *Escherichia coli* in the mouse intestine" *PNAS* 101(19): 7427-7432.

Chrost RJ (1991) "Environmental control of the synthesis and activity of aquatic microbial ectoenzymes" *Microbial Enzymes in Aquatic Environments*. Springer-Verlag, New York, pp. 29–59.

Clarholm M (1993) "Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizers" *Biology and Fertility of Soils* 16: 287-292.

Copeland C. (2010) "Animal Waste and Water Quality: EPA Regulation of Concentrated Animal Feeding Operations" *Congressional Research Service Report for Congress* 7-5700.

Cornell Cooperative Extension (2004) "Compost Fact Sheet #2: Regulation and Certification of Composts" *Cornell University Department of Crop and Soil Sciences* http://cwmi.css.cornell.edu/compostfs2.pdf

Debosz K, Petersen S, Kure L, and Ambus P (2002) "Evaluating effects of sewage sludge and household compost on soil physical, chemical and microbiological properties". *Applied Soil Ecology* 19: 237-248.

DeLaune PB, Moore PA, Daniel TC, and Lemunyon JL (2004) "Effect of chemical and microbial amendments on ammonia voltalization from composting poultry litter" *Journal of Environmental Quality* 33(2): 728-734.

Edgar RC (2013) "UPARSE: Highly accurate OTU sequences from microbial amplicon reads" *Nature Methods* 10(10): 996-999.

Evanylo G, Sherony C, Spargo J, Starner D, Brosius M, and Haering K (2008) "Soil and water environmental effects of fertilizer-, manure-, and compost-based fertility practices in an organic vegetable cropping system" *Agriculture, Ecosystems, and Environment* 127:50-58.

FDA (2015) "Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce: Chapter II. Production Practices as Risk Factors in Microbial Food

Safety of Fresh and Fresh-Cut Produce" *Food and Drug Administration* <http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm091083.htm>

Fierer N and Schimel JP (2003) "A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil" *Soil Science Society of America Journal* 67: 798–805.

Fierer N, Bradford MA, and Jackson RB (2007) "Toward an ecological classification of soil bacteria" *Ecology* 88(6): 1354-1364.

Franz E, Semenov AV, Termorshuizen AJ, De Vos OJ, Bokhorst JG, and Van Bruggen AHC (2008) "Manure amended soil characteristics affecting the survival of *E. coli* O157:H7 in 36 Dutch soils" *Environmental Microbiology* 10(2): 313-327.

Frederickson J, Howell G, and Hobson A (2003) "Effect of pre-composting and vermicomposting on compost characteristics" *European Journal of Soil Biology* 43: S320-326.

Freter R (1976) "Factors affecting the composition of the intestinal microflora" *Proceedings of the Microbial Aspects of Dental Carries Supplemental Microbiology Abstracts* Vol I: 109-120.

Frostegård A, Tunlid A, and Bååth E (2011) "Use and misuse of PLFA measurements in soils" *Soil Biology and Biochemistry* 43: 1621-1625.

Gao YD, Zhao Y, and Huang J (2014) "Metabolic modeling of common *Escherichia coli* strains in human gut microbiome" *Biomedical Research International* doi: 10.1155/2014/694967.

Gelting RJ (2007) "Irrigation water issues potentially related to 2006 *E. coli* O157:H7 in spinach outbreak" *Center for Disease Control and Prevention*

Gelting RJ (2007) "Irrigation water issues potentially related to 2006 *E. coli* O157:H7 in spinach outbreak" *Center for Disease Control and Prevention*

German DP, Marcelo KRB, Stone MM, and Allison SD (2012) "The Michaelis-Menten kinetics of soil extracellular enzymes in response to temperature: A cross-latitudinal study" *Global Change Biology* doi: 10.1111/j.1365-2486.2011.02615.x.

Goyal S, Dhull SK, and Kapoor KK (2005) "Chemical and biological changes during composting of different organic wastes and assessment of compost maturity" *Bioresource Technology* 96(14): 1584-1591.

Graham L, Wright D, Hashem F, Collins A, White K, Stonebraker R, Sharma M, and Millner P (2014) "Survival of *E. coli* in Biological Soil Amendments Applied to Soils in Southeastern Pennsylvania" *International Association of Food Protection 2014 Annual Meeting.* Des Moines, Iowa. August. Poster Presentation.

Haug RT (1993) "The practical handbook of compost engineering" *Lewis Publishers*.

Henry HAL (2013) "Reprint of 'Soil extracellular dynamics in a changing climate'" *Soil Biology and Biochemistry* 56: 53-59.

Hibbing ME, Fuqua C, Parsek MR, and Peterson SB (2010) "Bacterial competition: surviving and thriving in the microbial jungle" *Nature Reviews Microbiology* 8: 15-25.

Horner-Devine MC, Silver JM, Leibold MA, Bohannah BJM, Colwell RK, Fuhrman JA, *et al.* (2007) "A Comparison of Taxon Co-occurence Patterns for Macro- and Microorganisms" *Ecology* 88: 1345-1353.

Ibekwe AM, Watt PM, Shouse PJ, and Grieve CM (2004) "Fate of *Escherichia coli* O157:H7 in irrigation water on soils and plants as validated by culture method and real-time PCR" *Canadian Journal of Microbiology* 50: 1007–1014.

Ingham SC, Fanslau MA, Engel RA, Zhu J, Breuer JR, et al. (2005) "Evaluation of fertilization-to-planting and fertilization-to-harvest intervals for safe use of noncomposted bovine manure in Wisconsin vegetable production" *Journal of Food Protection* 68: 1134–1142.

Inglima I, Alberti G, Bertolini T, Vaccari FP, Gioli B, Miglietta F *et al* (2009) "Precipitation pulses enhance respiration of Mediterranean ecosystems: the balance between organic and inorganic components of increased soil CO_2 efflux" *Global Change Biology* 15: 1289–1301.

Insam H, Amor K, Renner M, and Crepaz C (1996) "Changes in functional abilities of the microbial community during composting of manure" *Microbial Ecology* 31(1): 77-87.

Islam M, Doyle MP, Phatak SD, Millner P, and Jiang X (2004) "Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water" *Journal of Food Protection* 7:1365-1370.

Islam M, Doyle MP, Phatak SD, Millner P, and Jiang X (2005) "Survival of *Escherichia coli* O157:H7 in soil and on carrots and onions grown in fields treated with contaminated manure composts or irrigation water" *Food Microbiology* 22(1): 63-70.

Jackson, C R (2003) “Changes in community properties during microbial succession” *Oikos* 101:444-448.

Jimenez L, Muniz I, Toranzos GA, and Hazen TC (1989) “Survival and activity of *Salmonella typhimurium* and *Escherichia coli* in tropical freshwater” *Journal of Applied Bacteriology* 67:61-69.

Kahler E (2014) “New FSMA draft rules released: 75 day public comment period underway” *Vermont Farm to Plate.*

< http://www.vtfarmtoplate.com/announcements/new-fsma-draft-rules-released-75-day-public-comment-period-underway#.VytbVfkrKCg<

Kelleher BP, Leahy JJ, Henihan AM, O’Dwyer TF, Sutton D, and Leahy MJ (2002) “Advances in poultry litter disposal technology – a review” *Bioresource Technology* 83: 27-36.

Kirchman, DL (2002) “The ecology of Cytophaga-Flavobacteria in aquatic environments” *FEMS Microbiology Ecology* 39:91-100.

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

LeClerc JE, Baoguang L, Payne WL, and Cebula TA (1996) “High Mutation Frequencies Among *Escherichia coli* and *Salmonella* Pathogens” *Science* 274: 1208-1211.

Lekkas P, Sharma M, Neher D, Weicht T, Millner P, and Donnelly C (2015) “Population dynamics of generic *E. coli* and naturally occurring *Listeria* in manure-amended soils” *Northeastern U.S. International Association for Food Protection*.

Lessard IA, Pratt SD, McCafferty DG, Bussiere DE, Hutchins C, Wanner BL, Katz L, and Walsh CT (1998) “Homologs of the vancomycin resistance D-Ala-D-Ala dipeptidase VanX in *Streptomyces toyocaensis*, *Escherichia coli* and *Synechocystis*: attributes of catalytic efficiency, stereoselectivity and regulation with implications for function” *Chemistry and Biology* 5:489-504.

Marilley L, and Aragno M (1999) “Phylogenetic diversity of bacterial communities differing in degree of proximity of *Lolium perenne* and *Trifolium repens* roots” *Applied Soil Ecology* 13:127-136

Mead P and Griffin P (1998) “*Escherichia Coli* O157:H7” *The Lancet* 352(9135): 1207-1212.

NEC-1012 (2011) “Recommended soil testing procedures for the Northeastern United States” *Northeastern Regional Publication* 493(3rd *ed*).

Neher DA, Weicht TR, Bates ST, Leff JW, and Fierer N (2013) "Changes in bacterial and fungal communities across compost recipes, preparation methods, and composting times" *PLoS ONE* 8(11): e79512. doi:10.1371/journal.pone.0079512.

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P, and Ferrenberg S (2013) "Patterns and Processes of Microbial Community Assembly" *Microbiology and Molecular Biology Reviews* 77(3): 342-356.

Orchard VA and Cook FJ (2008) "Relationships between soil respiration and soil moisture" *Soil Biology and Biochemistry* 40: 1013-1018.

Papke RT and Gogarten JP (2012) "How Bacterial Lineages Emerge" *Science* 336: 45-46.

Paul EA (2015) "Soil Microbiology, Ecology, and Biochemistry 4th ed." *Elsevier Publishing*

Paglai M and De Nobili M (1993) "Relationships between soil porosity, root development, and enzyme activity in cultivated soils" *Geoderma* 56: 243-256.

Paton JC and Paton AW (1998) "Pathogenesis and diagnosis of Shiga-toxin producing *Escherichia coli* infections" *Clinical Microbiology Reviews* 11(3): 450-479.

Peters J, Combs S, Hoskins B, Jarman J, Kovar J, Watson M, Wolf A, and Wolf N (2003) "Recommended methods of manure analysis" *University of Wisconsin* A3769.

Phaiboun A, Zhang Y, Park B, and Kim M (2015) "Survival kinetics of starving bacteria is biphasic and density-dependent" *PLOS Computational Biology* 11(4): e1004198.

Philppot L, Raaijmakers JM, Lemanceau P, and Van der Putten WH (2013) "Going back to the roots: the microbial ecology of the rhizosphere" *Nature Reviews Microbiology* 11: 789-799.

Prosser JI, Bohannan BJM, Curtis TP, Ellis RJ, Firestone MK, Freckleton RP, Green JL, Green LE, Killham K, Lennon JJ, Osborn AM, Solan M, Van der Gast CJ, and Young PW (2007) "The role of ecological theory in microbial ecology" *Nature Reviews Microbiology* 5: 384-392.

Pruimboom-Brees I, Morgan T, Ackermann M, Nystrom E, Samuel J, Cornick N, and Moon H (2000) "Cattle Lack Vascular Receptors for *Escherichia coli* O157:H7 Shiga Toxins" *PNAS* 97(19): 10325-10329.

Ramirez KS, Craine JM, and Fierer N (2012) “Consistent effects of nitrogen amendment on soil microbial communities and processes across biomes” *Global Change Biology* 18(6): 1918-1927.

Rangel JM, Sparling PH, Crowe C, Griffin P, and Swerdlow D (2005) “Epidemiology of *Escherichia coli* O157:H7 Outbreaks, United States, 1982-2002” *Emerging Infectious Diseases* 11(4): 603-609.

Rappe MS and Giovannoni SJ (2003) “The uncultured microbial majority” *Annual Reviews in Microbiology* 57: 369-394.

Recorbet G, Steinberg C, and Faurie G (1992) “Survival in soil of genetically engineered *Escherichia coli* as related to inoculum density, predation, and competition” *FEMS Microbiology Ecology* 101: 251-260.

Reguera G and Leschine SB (2001) “Chitin degradation by cellulolytic anaerobes and facultative aerobes from soils and sediments” *FEMS Microbiology Letters* 204(2): 367-374.

Reitzer L (2003) “Nitrogen Assimilation and Global Regulation in *Escherichia coli*” *Annual Review Microbiology* 57: 155-176.

Rogers SW, Donnelly M, Peed L, Kelty CA, Mondal S, Zhong Z, and Shanks OC (2011) “Decay of bacterial pathogens, fecal indicators, and real-time quantitative PCR genetic markers in manure-amended soils” *Applied and Environmental Microbiology* 77(14): 4839-4848.

Savageau, MA (1974) “Genetic regulatory mechanisms and the ecological niche of *Escherichia coli*” *Proceedings of the National Academy of Sciences*. USA 71:2453-2455.

Schimel J, Balser TC, and Wallenstein M (2007) “Microbial stress response physiology and its implications for ecosystem function” *Ecology* 88(6): 1386-1394.

Sears HI, Brownlee I, and Uchiyama JK (1950) “Persistence of individual strains of *E. coli* in the intestinal tract of man” *Journal of Bacteriology* 59:293-301.

Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR *et al.* (2008) "Stoichiometry of soil enzyme activity at global scale." *Ecology letters* 11(11): 1252-1264.

Sinsabaugh RL, Van Horn DJ, Shah JJF, and Findlay S (2010) “Ecoenzymatic stoichiometry in relation to productivity for freshwater biofilm and plankton communities” *Microbiology of Aquatic Systems* 60(4): 885-893.

Sinsabaugh RL and Shah JFF (2012) "Ecoenzymatic Stoichiometry and Ecological Theory" *Ecology, Evolution, and Systematics* 43: 313-343.

Six J, Bossuyt H, Degryze S, and Denef K (2004) "A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics" *Soil and Tillage Research* 79(1): 7-31.

Six J, Elliott ET, and Paustian K (1999) "Aggregate and soil organic matter dynamics under conventional and no-tillage systems" *Soil Science Society of America Journal* 63(5): 1350-1358.

Smith HW (1965) "Observations on the flora of the alimentary tract of animals and factors affectings its composition" *Journal of Pathology and Bacteriology* 89:95-102.

Spain AM, Krumholz LR, and Elshahed MS (2009) "Abundance, composition, diversity, and novelty of soil *Proteobacteria*" *Multidisciplinary Journal of Microbial Ecology* 3: 992-1000.

Stark JM, Firestone MK. (1995). Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Appl Environ Microbiol* **61**: 218–221.

Stine SW, Song I, Choi CY, and Gerba CP (2005) "Application of microbial risk assessment to the development and standards for enteric pathogens in water used to irrigate fresh produce" *Journal of Food Protection* 68: 913-918.

Tate RL (1978) "Cultural and environmental factors affecting the longevity of *Escherichia coli* in histosols" *Applied and Environmental Microbiology* 35(5): 925-929.

Tenaillon O, Skurnik D, Picard B, and Denamur E (2010) "The population genetics of commensal *Echerichia coli*" *Nature Reviews Microbiology* 8(3): 207-2017.

Ter braak CJF and Smilauer P (2012) "Canoco Reference Manual and User's Guide" *Software for Ordination* (version 5.0), Microcomputer Power (Ithaca, NY, USA).

Tiedje JM, Sexstone AJ, Parkin TB, Revsbech NP, and Shelton DR (1984) Anaerobic processes in soil" *Plant and Soil* 76(1): 197-212.

Unden G, Becker S, Bongaerts J, Schirawski J, and Six S (1994) "Oxygen regulated gene expression in facultatively anaerobic bacteria" *Antonie Van Leeuwenhoek* 66(1-3): 3-22.

United States Composting Council (2001) "Field guide to compost use" < http://compostingcouncil.org/admin/wp-content/plugins/wp-pdfupload/pdf/1330/Field_Guide_to_Compost_Use.pdf>

U.S. Depeartment of Health & Human Services/ U.S. Food & Drug Administration (2015) "Key Requirements: Final Rule on Produce Safety" <http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm247546.htm>

Van den Brink PJ and Ter Braak CJF (1999) "Principal response curves: Analysis of time-dependent multivariate responses of biological community to stress" *Environmental Toxicology and Chemistry* 18(2): 138-148.

Van Elsas JD, Dijkstra AF, Govaert JM, and Van Veen JA (1986) "Survival of *Pseudomonas fluorescens* and *Bacillus subfilis* introduced into two soil of different texture in field microplots" *FEMS Microbiology Ecolology* 38: 151-160.

Van Veen JA, Van Overbeek LS, and Van Elsas JD (1997) "Fate and activity of microorganisms introduced into soil" *Microbiology and Molecular Biology Reviews* 61:121-135.

Vermont Agency of Agriculture Food & Markets (2013) "Vermont Agency of Agriculture, Food, and Markets comment to the Food and Drug Administration on rules promulgated under the Food Safety Modernization Act" < http://agriculture.vermont.gov/sites/ag/files/VAAFM%20FSMA%20Finalized%20Comments%20Nov%2020132.pdf>

Von Mersi W and Schinner F (1991) "An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride" *Biology and Fertility of Soils* 11(3): 216-220.

Ward NL, Challacombe JF, Janssen PH, Henrissat B, Coutinho PM, Wu M *et al.* (2009) "Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils" *Applied and environmental microbiology*, *75*(7), 2046-2056.

Winfield MD and Groisman EA (2003) "Role of non-host environments in the lifestyles of *Salmonella* and *Escherichia coli*" *Applied Environmental Microbiology* 69(7): 3687-3694.

APPENDIX A

Introduction

Michaelis-Menten enzyme kinetics are commonly used to determine the maximum enzyme velocity (V_{max}) and the Michaelis constant (K_m) for enzymes of interest. The Michaelis constant, which is equivalent to the substrate concentration at one half of V_{max}, provides a measure of an enzyme's affinity for a substrate (Logan and Fleurry 1993). Enzyme kinetic analysis allows for the prediction of enzyme velocity (v) given a particular substrate concentration (S) by the equation:

$$v = \frac{V_{max}S}{K_m + S}$$

Determination of V_{max} and K_m is a useful tool for understanding enzyme activity in systems with ephemeral substrate inputs.

Methods

On days 8 and 30 post *E. coli* inoculation, samples were prepared and analyzed as described in Section 2.2.5. In addition to incubating samples with 40µM enzyme substrate, samples were also incubated with 5µM, 10µM, 15µM, 25µM, and 30µM substrate concentrations. The enzyme velocity was graphed against substrate concentration and a Michaelis-Menten model was fit to the data using GraphPad Prism v.6.05.

Results

Michaelis-Menten equations best fit to β-glucosidase activity ($R^2 = 0.7754 – 0.9964$). However, the calculated V_{max} and K_m values were ambiguous for over half of the samples and the confidence intervals were incalculable on GraphPad Prism. Both phosphatase and β-glucosidase exhibited multiphasic curves, with enzyme activity increasing dramatically between 30μM and 40μM substrate (Figures 1,2). β-1,4-N-acetylglucosaminidase activity exhibited a more linear curve, with activity increasing at a constant rate with substrate concentration and, therefore, never reaching a maximum velocity (Figure 3). Leucine activity had the least precise fit of the enzymes ($R^2 = 0.0222 – 0.9177$, Figure 4). Because V_{max} and K_m values could not be determined for many of the replicates, statistical analysis to determine treatment and site differences were not done.

Discussion

Natural soil and aquatic systems frequently exhibit multiphasic enzyme kinetics (Schmidt and Gier 1990, Lewis *et al.* 1984). This is largely due to mixed populations, which secrete enzymes with variable substrate affinities. Different subsets of microbes may be responsible for enzyme activity when substrates are present in low concentrations versus when substrates are present in high concentrations. Because soils contain highly diverse microbiomes, the enzyme kinetics in this experiment did not follow a predictable Michaelis-Menton model and thus could not be used to analyze treatment and site differences in enzyme activity.

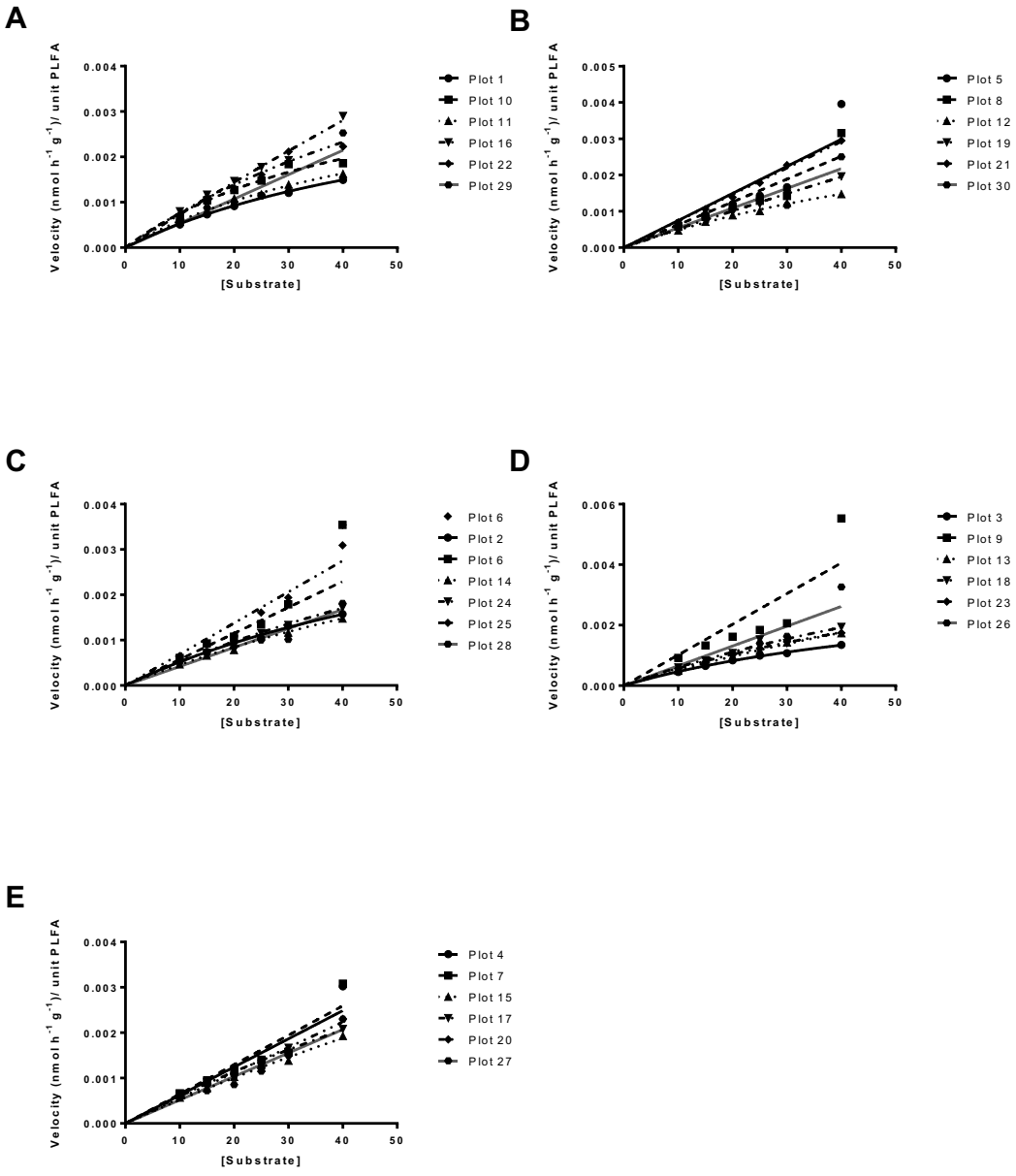


Figure 1: Activity of β-1,4-glucosidase on day 8 post *E. coli* inoculation in untreated plots (A), plots with *E. coli* only (B), plots with dairy windrow compost (C), plots with dairy vermicompost (D), and plots with poultry windrow compost (E). Symbols represent the measured velocity at a given substrate concentration. Lines represent the best fit Michaelis-Menten model. Plots 1 – 15 are from Lilac and plots 16 – 30 are from Wheelock.

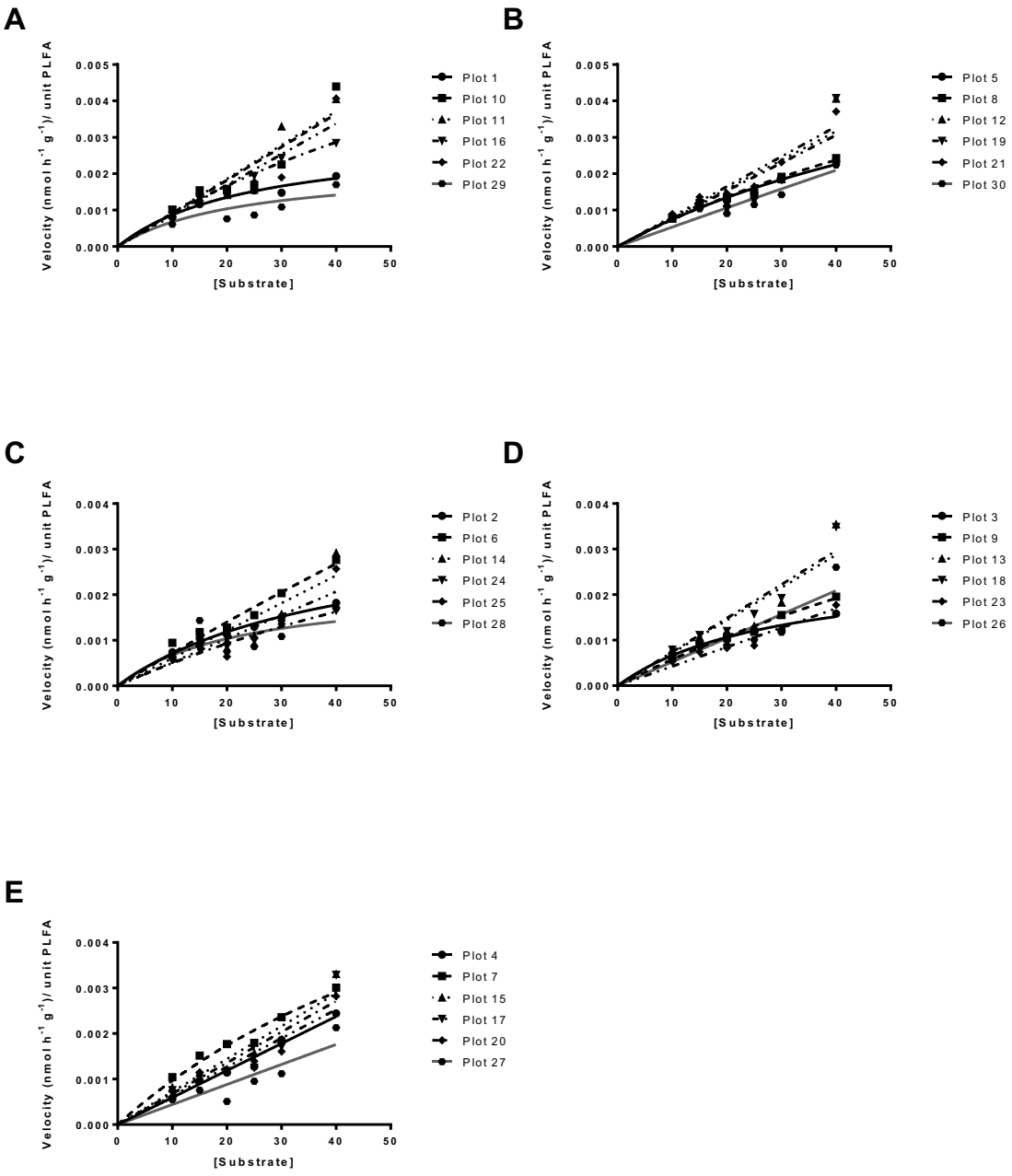


Figure 2: Activity of phosphatase on day 8 post *E. coli* inoculation in untreated plots (A), plots with *E. coli* only (B), plots with dairy windrow compost (C), plots with dairy vermicompost (D), and plots with poultry windrow compost (E). Symbols represent the measured velocity at a given substrate concentration. Lines represent the best fit Michaelis-Menten model. Plots 1 – 15 are from Lilac and plots 16 – 30 are from Wheelock.

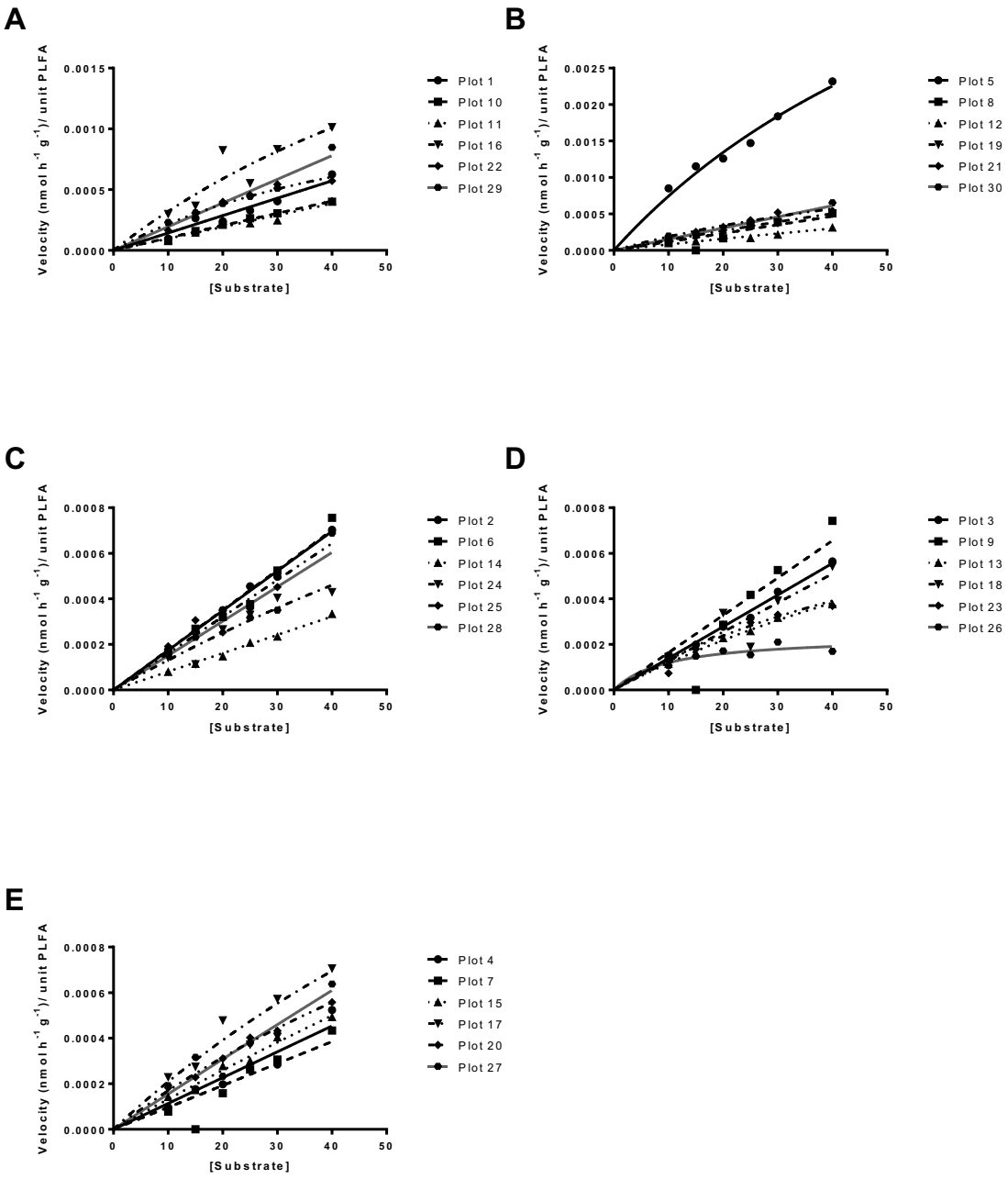


Figure 3: Activity of β-1,4-N-acetylglucosaminidase on day 8 post *E. coli* inoculation in untreated plots (A), plots with *E. coli* only (B), plots with dairy windrow compost (C), plots with dairy vermicompost (D), and plots with poultry windrow compost (E). Symbols represent the measured velocity at a given substrate concentration. Lines represent the best fit Michaelis-Menten model. Plots 1 – 15 are from Lilac and plots 16 – 30 are from Wheelock.

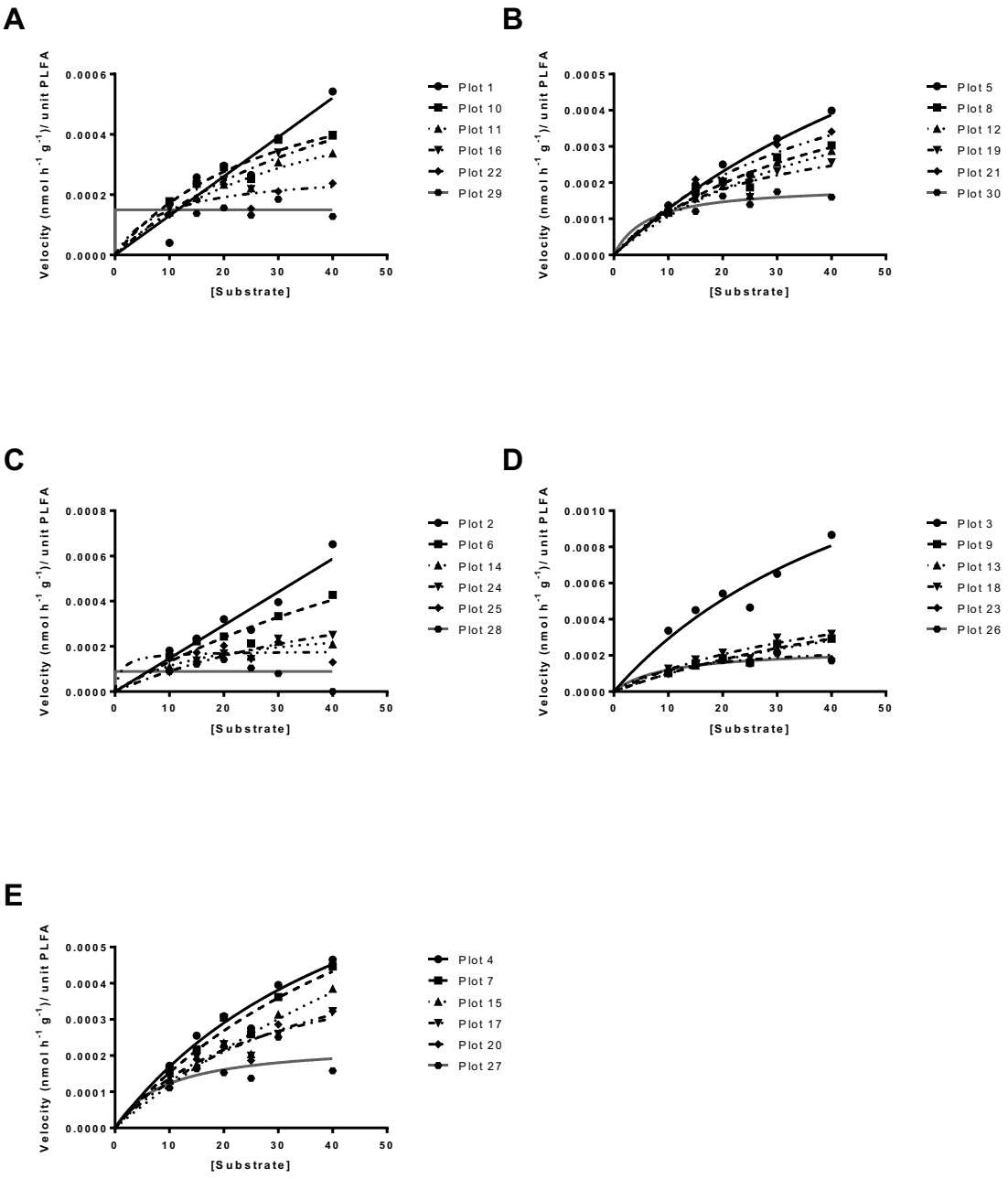


Figure 4: Activity of leucine on day 8 post *E. coli* inoculation in untreated plots (A), plots with *E. coli* only (B), plots with dairy windrow compost (C), plots with dairy vermicompost (D), and plots with poultry windrow compost (E). Symbols represent the measured velocity at a given substrate concentration. Lines represent the best fit

Michaelis-Menten model. Plots 1 – 15 are from Lilac and plots 16 – 30 are from Wheelock.

Literature Cited

Lewis DL, Holm HW, and Hodson RE (1984) "Application of single- and multiphasic Michaelis-Menten kinetics to predictive modeling for aquatic ecosystems" *Environmental Toxicology and Chemistry* 3(4): 563-574.

Logan BE and Fleury RC (1993) "Multiphasic kinetics can be an artifact of the assumption of saturable kinetics for microorganisms" *Marine Ecology Progress Series* 102: 115-124.

Schimidt SK and Gier MJ (1990) "Coexisting bacterial populations responsible for multiphasic mineralization kinetics in soil" *Applied and Environmental Microbiology* 56(9): 2692-2697.