Understanding soil bacterial communities for sustainable agriculture

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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DEDICATION

This dissertation is dedicated to my mentor, Cynthia Cambardella. Thank you for providing valued insight during my scientific development. Thank you for showing me how to look at problems from a different perspective. Thank you for exposing me to the mysteries of the soil and the importance of sustaining soil health.

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NOMENCLATURE

OTU	Operational Taxonomic Unit
ASV	Amplicon Sequence Variant
ARG	Antibiotic Resistant Gene
ARB	Antibiotic Resistant Bacteria

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ABSTRACT

Nutrient loss from synthetic fertilizer use contributes to poor water quality, conversion of native landscapes to agricultural production reduces biodiversity, and antibiotic use in animal production contributes to antibiotic resistance. Therefore, agriculture can have adverse effects on local and global environments. Soil bacteria mediate processes that can influence the impact of agriculture on the environment. Characterizing soil bacterial communities in response to changes in agricultural management may inform scientific understanding of how management decisions can alter soil bacteria.

Several management practices aim to reduce the impact of agriculture on the environment and improve its sustainability. Offsetting synthetic fertilizer use with organic amendments can reduce nitrate losses and improve water quality. However, the microbially mediated and hard to predict the release of plant-available nutrients from organic amendments can limit their adoption and use. Installation of prairie strips as a conservation practice can reduce sediment loss and provide habitat for native animals, improving diversity. However, the impact of prairie strips on the soil bacterial community remains under characterized. Grasses can filter antibiotics and antibiotic resistance genes in runoff water from manure treated fields, though the ability of prairie strips to reduce antibiotic resistance in runoff water is not understood.

The experiments described in this dissertation contribute to a scientific understanding of soil bacteria's response to organic amendment and prairie strip installation, as well as the impact of prairie strips on attenuating the transport of manure associated bacteria and antibiotic resistance genes. Soil bacterial communities' response to organic amendments of varying quality was compared against soil receiving no amendment during an incubated microcosm study. Soil bacterial communities exhibited community similarity in two temporal groupings in all amended

and un-amended soils. In response to organic several bacterial taxa became significantly more abundant in amended soils and were associated with amendment quality. Alpha and beta diversity of soil bacteria were compared between agricultural soil and prairie strip soil at two sites. Prairie strips examined were less than five years old and did not significantly differ from agricultural soils at the two sites. Bacterial richness, Shannon's diversity, and Simpson's diversity were significantly greater in prairie soils than agricultural soils at one site and only in response to rainfall. The abundance of manure-associated bacteria in runoff water was compared between plots receiving manure without prairie strips and plots receiving manure. Prairie strip appended plots had lower abundances of manure associated taxa and lower detection of antibiotic resistance genes than plots without prairie strips.

Conservation practices designed to reduce nutrient loss, such as the use of composts or the installation of prairie strips, are essential practices that can improve agricultural sustainability, yet have impacts on bacterial dynamics that are not well understood. Characterizing bacterial communities in soils under various conservation practices will inform our understanding of how these practices impact the soil bacterial community.

CHAPTER 1. GENERAL INTRODUCTION

1.1 Introduction

Is soil the foundation of life? Many people believe so, as it provides many services for humans, from providing a stable foundation for building our homes to supporting the agriculture that feeds our growing population. Supporting the production of the four 'Fs': Feed, Fuel, Fiber, and Food is one roles of soil; in addition to providing climate regulating services and supporting healthy ecosystems. Agricultural crop production covers nearly one third of the world's land surface (Ramankutty et al., 2008). Ensuring that this production is sustainable is vital to ensuring that we can continue to provide the four 'Fs' through agriculture. Minimizing the negative impacts of that agriculture can have on the environment and biodiversity is a critical challenge facing us today. Some of these impacts include degraded soil and water quality, loss of biodiversity, and greenhouse gas emissions (Doran, 2002; Gomiero et al., 2011; Jones et al., 2018; Rabalais et al., 2001). Fortunately, soils may provide the pathway to address multiple problems associated with agriculture, from storing carbon in the ground to offset climate change to reducing the threat of antimicrobial resistance and supporting healthy plant communities (Chaparro et al., 2012; Lehman et al., 2015; Pérez-Valera et al., 2019; Welbaum et al., 2004; Wiesmeier et al., 2019).

Soils represent an incredibly diverse and heterogeneous habitat, fostering some of the most complex biodiversity in nature. Diverse soil microbes drive functions critical to efficient agricultural practices, such as nutrient cycling and plant protection. One gram of soil may contain up to 1.5×10^{10} bacteria in combination with millions of fungi and other microorganisms and fauna (Torsvik et al., 1990). Understanding soil bacteria's functional role is challenging because less than 1% of soil bacteria may be cultured in the laboratory setting, limiting the

ability to investigate individual bacterial genomes (Torsvik and Øvreås, 2002). Recent advances in bacterial sequencing and agricultural conservation present exciting opportunities to explore the link between bacterial communities and sustainable agricultural management decisions. Though it is understood that bacteria represent a crucial component of soil's functional potential, there remain gaps in scientific understanding of how bacterial communities and their functions respond to management. Bacteria and archaea are especially important to agricultural soils, where they are more dominant than fungi (Pereira C. Silva et al., 2012). The relationship between sustainable agriculture, environmental health, and centrality of microbial communities in soils highlights the importance of understanding bacterial community dynamics.

Bacteria can facilitate climate change mitigation through the decomposition of carbon-rich materials and developing soil organic matter. For example, tillage can introduce oxygen into the soil facilitating a burst in microbial growth followed by the associated release of carbon dioxide, contributing to the concentration of carbon dioxide in the atmosphere and promoting global warming. In addition to climate change, soil bacteria can facilitate organic amendments such as manure, green manures, and composts (Saleh, 2013; Talgre et al., 2012). Within these organic amendments, bacteria can help cycle necessary carbon and nitrogen for plant growth and availability of soil organic matter. Offsetting harmful agrochemicals is a primary goal of sustainable agriculture. Soil bacteria play an important role in liberating plant-available nutrients from these organic amendments and minimize the need for chemical fertilizers. Additionally, soil bacteria may help support conservation practices such as native plantings used as filter strips (Kardol and Wardle, 2010). When agricultural soil is inoculated with rhizobial bacteria from a prairie microbiome, prairies' legume establishment is improved compared to control soils (Grman et al., 2020). By understanding and potentially manipulating the bacteria in the soil

microbiome, agriculture may be chaperoned into a more sustainable future. To do this, more investigations into the way soil bacteria can promote sustainable agriculture are needed.

1.2 Advances in Methods for Characterizing Soil Microbiomes

The characterization and quantification of diversity is a metric that has been used to link soil microbiomes to soil health. The diversity and abundance of soil microbes representing specific functional groups contribute to the soil's ability to access a range of nutrients (Ferris and Tuomisto, 2015). Therefore, diversity can contribute to functional stability and resilience, an important consideration when attempting to improve a system's sustainability, particularly agricultural systems (Shade et al., 2012). Plant communities impart a significant influence on soil microbes, recruiting desired species to colonize their rhizospheres by releasing compounds that are recognized by bacteria or fungi. The best-known example of this is the relationship between soybeans and rhizobacteria that end up colonizing root nodules allowing the plant to access atmospheric nitrogen (Udvardi and Kahn, 1993). In addition to plants recruiting soil microbes, the soil's physical properties directly contribute to the microbiome's composition. The soil characteristics of pH, moisture content, texture, and nutrient content influence microbial species (Jackson, 2006). The heterogeneous nature of soils contributes to the spatial diversity of soil microbes (O’Brien et al., 2016). Changes in pH, moisture, and oxygen content can change within a soil aggregate, creating spatial changes in microbial composition at scales smaller than 1mm (Upton et al., 2019). Together these factors contribute to the incredible diversity of soils making it more diverse than any other habitat (Vigdis and Lise, 2002). This presents an incredible challenge in cataloging the diversity of soil microbiomes, a challenge that has been made easier by recent advances in sequencing and metagenomics.

Previously, determining what microbes were present in a soil sample was done by plating techniques. This approach included plating a soil and buffer solution on agar plates of different

media and observing growth. Colonies were then identified by physiological features or by sequencing isolated colonies. While useful in identifying some soil microbes, this method is limited because it is estimated that > 90% of soil microbes are unculturable (Pham and Kim, 2012). The unique conditions of soil environments are challenging to replicate in the lab and limits our ability to culture the full microbial community. Culture-independent techniques have allowed us to characterize the soil microbiome in a new way and have led to better understanding of the soil microbiome. Improvements in the sequencing of conserved phylogenetic markers in all soil bacteria have led to an explosion of understanding what soil organisms are present (Sievers et al., 2011). Bacteria contain several highly conserved genetic elements that allow us to compare sequences between species to determine evolutionary relationships. In bacteria, the most commonly used gene is the 16S rRNA small subunit (SSU) gene. By extracting the DNA from an environmental sample and amplifying the 16s rRNA SSU gene, we can obtain sequences that describe the taxa present in a soil sample without culturing. We can subsequently compare the abundance of bacterial species in varying soils or management conditions. This technique allows us to characterize the diversity in soils through quantifying the membership of bacterial communities. Leveraging this technology can facilitate our understanding of soil bacterial community dynamics in agroecosystems and contribute to our understanding of how management practices impact the bacterial community's composition. Understanding how management changes soil microbial diversity can help us to understand its impacts on soil stability and health.

1.3 Rationale for Chapter 2: Comparison of Soil Microbial Community Responses to Alfalfa and Compost Organic Amendments

Multiple Understanding dynamics of soil bacteria are key to enhancing agroecosystem sustainability. Organic fertilizers such as manure, compost, or green manures serve as

alternatives to synthetic fertilizers with many documented benefits to soil health. The benefits of organic fertilizers over synthetic is that: improved soil structure, increases SOM, increased microbial biomass and activity, and can reduce nutrient losses (Cambardella et al., 2015). Agricultural production in the Midwest significantly contributes to nitrate loading to nearby waterways, distally contributing to the size of the hypoxic zone in the Gulf of Mexico and local degradation of water quality (Rabalais et al., 2001; Robertson and Vitousek, 2009; Syswerda et al., 2012). One challenge for the adoption of organic amendments is not understanding how it changes nutrient availability for plants. In contrast to synthetic fertilizer, nitrogen is made available from biological forms when using organic fertilizers. Nitrogen (N) is made available through decomposition of plant residues or compost and microbially mediated mineralization of the organic nitrogen. Soil organisms convert N containing compounds to plant-available N; however, this process can be influenced by the bacterial community's composition.

The first step in making organic N available to crops is depolymerization (Schimel and Bennett, 2004), a process which requires a variety of bacteria and their exoenzymes (Nguyen et al., 2019; Noll et al., 2019). The timing of mineralization of organic N is hard to predict. Farmers facing agronomic decisions often cannot afford any unnecessary uncertainty in their management. The potentially asynchronous mineralization of organic N and N needs from plants contributes to the limited adoption of these amendments (Crews and Peoples, 2005; Kaleeem Abbasi et al., 2015). To address this challenge, characterizing the phylogenetic response to organic amendments in the soil will contribute to our understanding of the microbiome during mineralization. Further, characterizing these phylogenic dynamics over time will improve our knowledge of the microbiome's temporal changes in response to amendment. This information can then be leveraged to evaluate the soil microbiome for the needed bacterial species that will

be able to facilitate the most efficient organic N mineralization. In addition to reducing agrochemical use, the sustainability of agriculture may further be improved by preserving and improving agroecosystems' biodiversity. Recent studies have provided support for soil biodiversity conferring resilience to disturbance induced by agricultural practices.

1.4 Rationale for Chapter 3: Comparison of Bacterial Community Diversity Under Prairie Conservation Strips and Agricultural Production

Several factors may contribute to differences in bacterial diversity between prairie strip soil and agricultural soils. The rhizosphere is a zone of high microbial activity, providing organic material in the form of sloughed root cells, organic compounds and simple sugars to the soil (Meier et al., 2017). This zone of increased energy and nutrient cycling density fosters greater microbial diversity, and resource competition around roots may lead shifts in species composition (Upton et al., 2019). The presence of prairie plants has an impact on soil structure in comparison to crop soils, a comparison of crop and prairie soils found an increase in small and medium sized aggregates compared to agricultural soils (Brittenham et al., 2017). The impact of prairie therefore has implications for the micro-habitats of soil aggregates, hot spots of microbial activity, especially in deep rooted prairie systems combined with aggregates at the deeper soil profiles (Kuzyakov and Blagodatskaya, 2015). These differences may affect microbial community dynamics and therefore bacterial diversity. Differences in aggregate fractions between prairie and crop soils have been shown to harbor different bacterial species composition and abundance, depending on the size of the aggregate fraction being analyzed, particularly, highest diversity is observed in microaggregates of the soil (Bach et al., 2018; Upton et al., 2019). Further, a related study has shown differences in the functional potential of aggregate size by showing differences in cellulase genes and a significantly higher abundance of cellulase genes in the medium aggregate fraction was observed (Choi et al., 2018).

Heterogeneous nature of the soil and relatively small habitat occupied by bacteria means that most bacteria may encounter relatively few other cells. A study done by photographing thin slices of soil and evaluating the proximity of bacterial cells showed a cell may encounter up to 120 other bacteria in the soil at cell densities of 10^9 cells g^{-1} of soil, while densities of 10^{10} cells g^{-1} of soil may encounter 1,000 other cells (Raynaud and Nunan, 2014). As previous research has shown, the presence of prairie plants may have a range of impacts on factors that influence the microbial community, showing their potential for altering microbial dynamics in comparison to crop soils, which may contribute to differences in bacterial diversity.

An exciting prospect for inducing changes in bacterial diversity is integrating the benefits of prairies with the necessity of agricultural production. Known as STRIPS (Science based Trial of Row crops Integrated with Prairie Strips), narrow strips of native grasses and forbs are strategically planted in row crop fields to replace 5 to 10 % of the total area (Schulte et al., 2017). Early research shows a multi-faceted benefit for this practice, with increases in diversity and richness of multiple species from avian to mammal and now investigations into the microbial impacts (Grudens-Schuck et al., 2017; Kordbacheh et al., 2020; Schulte et al., 2017, 2016). Greater diversity of bacteria fostered by prairie plants, compared to crop soils, offers multiple ways in which prairie strips may attenuate persistence and transfer of antibiotic resistant bacteria and genes.

Interspersing strips of prairie in row crops will give a portion of the agroecosystem a much more extensive and diverse rhizosphere for a longer portion of the growing season in comparison to conventional row crops. Diversity of plant species in general often fosters a greater compositional diversity below ground (Prober et al., 2015). Indeed, metagenomic

analysis of crop and prairie soils showed a difference of 835,590,292 microbial DNA sequences, indicating a higher abundance of bacteria in the prairie versus the crop (Howe et al., 2014).

1.5 Rational for Chapter 4: Impact of Prairie Strips on Transport of Poultry Manure-associated Bacteria and Antibiotic Resistance Genes Following Simulated Rainfall

Finally, agroecosystems can incorporate conservation practices to mitigate impacts on human health. Traditionally conservation practices have been used to reduce nutrient loss, provide perennial habitat, limit erosion, and reduce air pollution (Jaynes and Isenhart, 2014; Ucar and Hall, 2001; Zhou et al., 2014). However, there is a rising threat that is not generally associated with row crop agriculture: antibiotic resistance. The over-use of antibiotics in animal agriculture has led to an increase in antibiotic-resistant organisms in proximity to animal production facilities. While antimicrobial resistance's (AMR) threat might be thought of as only affecting those working in animal production, the abundance of both antibiotic-resistant organisms and genes in manure represents a potential vector for AMR to move to the broader environment. When AMR laden manures are applied to agricultural fields, there is the potential that AMRs may enter waterways and move through environments where they may pose a threat to human health. Recent changes in agricultural guidelines regarding antibiotic use have sought to reduce its use for growth promotion in an attempt to address this (Food and Drug Administration, 2015). However, this may be too little too late; several studies have indicated that AMR may persist in agricultural facilities past the cessation of use (Ghosh and LaPara, 2007). Therefore, management of AMR loss may be needed into the foreseeable future, even with reduced antibiotic use.

The use of conservation practices to attenuate AMR transport is being explored to address this issue. The use of filter strips has been shown to reduce the presence of antibiotic-resistant bacteria. In a study at the University of Nebraska, narrow-grass hedges were shown to

reduce antimicrobials and antibiotic-resistant genes in agricultural runoff (Soni et al., 2015). These authors applied swine slurry with bacitracin to cropland appended with switchgrass hedges. The efficacy of hedges in reducing AMR was assessed through rainfall simulation and runoff water collection. The switchgrass hedges significantly reduced tylosin, erm(B), and 16S rRNA gene copies in runoff water (Soni et al., 2015). Further evidence for filter strips attenuating the loss of manure associated bacteria was shown in another simulated rainfall experiment using poultry manure. Grass filter strips of 4.5 m and 9.0 m width were evaluated for their efficiency in removing sediment, fecal coliforms, and fecal streptococci from runoff water. Filter strips 4.5 m wide removed 95% of sediment, while 9 m wide strips removed 98% of sediment. The strips showed efficiency in trapping fecal coliforms at 75% and 91% for 4.5 m and 9.0 m filter strips. Efficient trapping of fecal streptococci was also observed, at 68% and 74% for 4.5 m and 9.0 m wide filter strips respectively. However, the strips did not reduce the fecal contaminant load below minimum water quality standards (McMurry et al., 1998). What if these filter strips could be leveraged to support native flora and fauna and reduce the threat of AMR, Prairie strips may be one such solution. Developed at Iowa State University, this conservation practice utilizes plantings of prairie species on a portion of an agricultural field to reduce soil and nutrient loss while promoting habitat restoration for one of the most degraded ecosystems in the Midwest. While the previous studies mentioned evaluated single-species hedges, there has yet to be an evaluation of the impact that a multi-species system, such as prairie strips, can play on attenuating the transport manure associated bacteria and genes.

1.6 Organization of Dissertation

This work aims to elucidate the impact of agricultural management on the bacterial community; also, I evaluate the potential for conservation practices to attenuate the transport of bacteria and antibiotic resistance genes. The goal is to contribute to our understanding of the

bacterial community in agricultural systems in order to promote sustainable farming practices. The specific objectives are three-fold. First, in chapter 2, my objective is to evaluate how bacterial communities change as they decompose organic amendments of varying quality. Using a controlled laboratory incubation, I measure the bacterial community composition and inorganic nitrogen release over response over 97 days. Secondly, in chapter 3, I describe the differences in bacterial diversity of soil under agricultural production and prairie strips. By comparing soils from standard farming practices and promising conservation practice, I describe agricultural management's effect on soil bacterial diversity. Finally, in chapter 4, I evaluate whether prairie strips can reduce the transport of antibiotic resistance genes and bacteria from poultry manure fertilized fields fertilized. By comparing runoff water from plots with and without prairie strips, we can determine if this new agricultural conservation practice can effectively reduce the threat of antibiotic pharmaceuticals in the environment. Altogether these efforts advance our understanding of soil bacterial communities in agroecosystems. Further, we specifically address the characteristics of soil bacteria during decomposition of organic amendments, the diversity of bacteria under prairie and agricultural production, and the ability of prairie strips to reduce the threat of antibiotic resistance.

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CHAPTER 2. COMPARISON OF SOIL MICROBIAL COMMUNITY RESPONSES TO ALFALFA AND COMPOST ORGANIC AMENDMENTS

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2.1 Abstract

Addressing the growing demand for food from a burgeoning population requires agricultural methods that sustainably support increases in crop production while maintaining environmental health. Agricultural practices that include the use of compost, in lieu of mineral fertilizers, have been shown to reduce environmental impacts and improve soil health. To better understand nutrient dynamics in soils amended with organic fertilizers, we compared the soil microbial community response to amendments with differing carbon and nitrogen content. Composted horse manure was chosen to represent an organic high carbon amendment, and alfalfa hay was chosen to represent a high nitrogen amendment. Amended soils were incubated for 97 days and destructively sampled on seven progressively longer incubation intervals. DNA was extracted for 16s rRNA gene characterization. Additionally, inorganic nitrogen and other soil health characteristics were measured. Our results showed significant shifts in soil microbial communities to both time and amendment. Nutrient release was tightly associated with amendment composition, with alfalfa showing the greatest release of plant available N. We observed complex interactions between soils and amendments, with specific bacteria enriched

following amendment with varying C:N ratios. After amendment, we observed two groups of microbial response, early and late response groups. Within these groups, amendment-specific bacterial membership was observed to increase in abundance. Overall, we found that these bacteria mainly originated from the soil and not amendments. Further, these bacteria were from similar phyla, suggesting phylogenetic responses to amendments. This study highlights linkages between soil bacterial membership and their links to C and N cycling with organic amendments.

2.2 Introduction

Using organic nitrogen (N) sources, rather than synthetic fertilizers, has shown promise as one tool to make modern agriculture more sustainable and regenerative (Kramer et al., 2006; Stopes et al., 2006). These sources include compost, manure, and green manures are often used as amendments to supply N to the soil (Gaskell and Smith, 2007).. Several investigations into organic N use in agricultural production systems have shown evidence that supports the role of organic N in sustainable systems. A comparison of water quality between organic and conventional maize production in Iowa showed significant differences in cumulative nitrate losses to drainage water, with two times as much nitrate-N lost over three years in conventional plots compared to organic plots (Cambardella et al., 2015). Conventional plots in commercial apple production were also observed to leach more than five times the annual nitrate of organic plots in Washington orchards (Kramer et al., 2006).

Despite the increased use of organic N sources, we lack understanding of the synchrony between N released from amendment and the availability of N for plant uptake. When the timing of N mineralization does not match crop needs, significant losses of N may occur. Therefore, for efficient crop production, application of organic N amendments must be carefully timed with consideration of factors contributing to mineralization, including stoichiometry of the amendment, soil physical properties, chemical properties, and environmental factors such as

temperature and moisture. These factors each contribute in different ways to the overall timing of decomposition and depolymerization of organic amendments. One factor that remains largely uncharacterized is the microbial response in soils to organic amendments and N availability. Understanding the complexities and compositions of communities responding to amendment will inform our understanding of the biological factors associated with organic N mineralization.

N from organic amendments is bound in large complex molecules such as proteins or poly-amino acids and must be depolymerized into smaller and lighter bio-available forms before mineralization can proceed (Schimel and Bennett, 2004). Generally, complex interactions between soil bacteria and organic N composition of amendments influence N mineralization and nutrient availability in these agricultural systems. Further, there are diverse microbes associated with extracellular enzyme production, which is essential for depolymerization and contributes to variations in mineralization in soils. Therefore, investigations into the specific bacteria responding to various amendments intended to supply organic N are needed to identify the interactions of soil microbial members during depolymerization and mineralization. Identification of these bacteria will contribute to our understanding of microbial community dynamics and result in improved management for more efficient organic N use.

Given the importance of organic N sources in sustainable agriculture – but our lack of understanding of bacterial communities' role in their decomposition – we used a 97-day laboratory incubation to monitor bacterial communities and their role in net N mineralization of three organic amendments. The amendments chosen in this study are alfalfa, composted cattle manure, and the combination and each reflect amendments commonly used for organic grain production systems. We evaluate whether there are distinct groups of bacteria responding to the amendments in incubated microcosms and whether predictable patterns are observed by

amendment type and if these patterns are consistent over time. The outcome of this study helps improve our understanding of the biological players involved in the release of nutrients and will help to improve agricultural production while still optimizing environmental benefits from organic N amendments.

2.3 Methods

2.3.1 Incubation Experimental Design and Logistics

Three organic N amendments and a control (no amendment) were chosen based on current use in organic agriculture as well as by their predicted effects on soil N cycling: (1) alfalfa: an amendment of alfalfa residue with low C: N ratio, simulating plow down of alfalfa hay as a N source before maize production; (2) compost: stable composted horse manure with a high C: N ratio used as an amendment replicating the use of composted manure on many organic farms; (3) mix: an amendment consisting of a mixture of alfalfa residue and compost was constructed to represent a neutral C: N ratio; and (4) reference: a control treatment receiving no amendment. Alfalfa hay samples were collected following hay harvest and processed by passing fresh hay through a grinder and then through a 2 mm mesh screened cyclone mill. Dry alfalfa was then stored in an air-tight vessel prior to use as amendment. A single bulk sample of compost was collected on September 23rd, 2015 from a large windrow of composted horse manure and saw dust bedding, from the Iowa State University (ISU) Compost Facility, Ames, Iowa. Compost was dried, processed, and stored in the same manner as the alfalfa amendment. Soil originated from the USDA-ARS Organic Water Quality research site, situated near Boone, Iowa, on the ISU Agronomy Research Farm. Surface soil (0-15 cm) for microcosms was taken from 2nd year alfalfa plots that were in a four-year corn-soybean-oat/alfalfa/alfalfa rotation under organic management (Cambardella et al., 2015). The site was located on the Clarion-Nicollet-

Webster soil association with fine-loamy texture soils. A total of 25 kg of soil was taken and processed through a 2 mm sieve, allowed to air-dry and stored in air-tight vessels before use.

Each microcosm consisted of 50 g of air dried, 2-mm sieved soil plus amendment, applied at a rate of 134.55 kg/ha of total N (as calculated by chemical analysis of each amendment, Table 1). To achieve this, we mixed 50 g of soil with either 0.882 g of alfalfa, 1.883 g of compost, or a combination of 0.411 g of alfalfa and 0.942 g of compost to achieve the three different C:N ratios. Soil samples were then wetted to 60% water-filled pore capacity and placed into the incubator. Amended and control soil samples were incubated for 97 days under aerobic conditions at 30.0 ^{o}C in 3.79 L glass jars. For each treatment, twelve 3.79 L glass jars containing six microcosms were assembled. These six microcosms were destructively sampled during the incubation. During the incubation, samples were aerated every 24 hours by removing the lid and kept moist via addition of deionized water to the bottom of the jar. Samples were subsequently analyzed on day 7, 14, 21, 35, 45, and 97 days yielding a total of 288 incubated samples for the four treatments, with n = 12 replicates for each treatment at each incubation time. Samples representing Day 0 conditions were constructed by extracting DNA from dry soils mixed with amendments in the same ratios as incubated samples, with n = 12 replicates for each amendment treatment, yielding a total of 48 amendment samples.

2.3.2 DNA Extraction and Sample Processing

To identify the bacterial community composition of amendment and soil samples, amplicon sequencing of the 16S rRNA gene was performed on extracted DNA. Microcosm soils were homogenized during destructive sampling and a sub-sample of soil was frozen immediately using dry ice for preservation at -80 ^{o}C until extraction could be performed. DNA extraction was performed using the HTP 96 well power soil kit from Qiagen using 0.25 g of soil. Following extraction, 16S rRNA genes were sequenced using the V4 region of the 16S SSU rRNA on an

Illumina MiSeq at Argonne National Laboratory following the protocol described by the Earth Microbiome project (Caporaso et al., 2012). The mothur (version 1.41.0) pipeline was used for sequence processing of the 150 bp paired end reads and identification of operational taxonomic units (OTUs) based on 97% genomic similarity of amplicons (Schloss et al., 2009). Taxonomic assignment of OTUs was completed by alignment to the most similar representative gene in the Silva 16S ribosomal database (version 123) (Pruesse et al., 2007) as described by (Wang et al., 2007).

2.3.3 Microbial Biomass Carbon and Nitrate Nitrogen

Standard methods outlined by (Delate et al., 2013) were used to measure pH, organic C, microbial biomass C (MBC), total N and inorganic N with slight alterations made to microbial biomass methods to accommodate limited sample mass, as described below.

MBC was quantified using moist microcosm soil subsampled during destruction of microcosms on sampling days. MBC was calculated and measured using standard soil fumigation-extraction methods modified for a 20 g sample. The 50 ml beakers with 20 g of soil were placed into a fumigation chamber and fumigated with chloroform overnight and extracted after 24 hours with 0.5 M K_2SO_4. Dissolved organic C in the filtrate was determined using flow injection technology using a Torch TOC Combustion analyzer (Teledyne Tekmar, Mason, Ohio), and MBC was calculated using the correction factor (k=0.33) (Vance et al., 1987). Inorganic N was determined by extraction with 2.0 M KCl from homogenized moist microcosm soil. Concentrations of NO_3 and NH_4 were quantified in the filtered extracts using a flow injection analyzer (Lachat Instruments, Milwaukee, WI).

2.3.4 Ancillary Soil and Amendment Measurements

Organic C and N in microcosm soils was determined using dry combustion analysis of 2 g of air-dry, soil ground with mortar and pestle. Dry homogenized soil was combusted using

Thermo Scientific FLASH Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA). The pH of soils was measured in a 2:1 soil-to-water slurry using a dual electrode pH meter (model, company, city). Soil water content was determined gravimetrically with overnight drying at 105°C.

2.3.5 Data handling and statistical analyses

Dissimilarities in the composition of sample bacterial communities were calculated based on the weighted UniFrac distances between samples and visualized by NMDS ordination. NMDS ordinations were performed using the "ordination" function from the Phyloseq package in R (R version 3.6.0, Phyloseq version 1.28.0) and were visualized with the "plot_ordination" function. To test if the composition of OTUs was different in samples based on treatment or day, the *adonis()* function from the vegan package (version 2.5-6) in R was used. The function *adonis()* uses a non-parametric multivariate analysis of variance (NPMANOVA) method to test the null hypothesis that there are no differences in microbial communities. To characterize the impact that the measured environmental variables had on the dissimilarities of communities between microcosms, we performed distance-based redundancy analysis (dbRDA) of the variables of nitrate, C:N ratio, pH, and total organic C (TOC) in addition to the experimental variables of treatment and day using the weighted-UniFrac distance matrix. dbRDA displays ordinations along with explanatory variables to evaluate the impact they have on observed dissimilarities. Additional variables were not included as they were not found to be significantly contributing to differences in community composition as shown by the *ordiR2step*() function from the R package Vegan (Oksanen et al., 2019). To account for uneven number of reads between microcosm communities, data was rarefied to an even depth of 6,000 reads per sample using the function *rarefy_even_depth()* in phyloseq.

All environmental variables were individually fit to a linear model using the function *aov()* from the "stats" package in R to compare means between treatments within each day. ANOVA was performed on the results from the linear model using the *aov()* function. To determine significance between treatments, we implemented multiple comparisons by means of Tukey range test using the function *HSD.test()* from the "agricolae" package in R. To test whether each treatment was significantly different from the reference, we implemented a paired t-test with the *stat_compare_means()* function from the R package "ggpubr".

To determine how bacterial membership from soil samples within the treatments cluster based on time, we performed hierarchical cluster analysis on the binary Bray-Curtis dissimilarity of each treatment. To identify amendment enriched taxa, we implemented "DESeq2" R package (1.24.0) to calculate the log2-fold change in taxon abundance between amended microcosms and reference microcosms, with an adjusted p-value threshold of 0.01 (Love et al., 2014). This package uses differential abundance analysis based on the negative binomial distribution with Wald's test with nonrarefied data.

Representative sequences for OTUs were used to generate a phylogenetic tree using Fasttree with default settings and plotted using the R package GGtree (Price et al., 2009; Yu et al., 2017). The representative sequence was the sequence within each OTU which is the minimum distance based on nucleotide similarity to the other OTU sequences.

2.4 Results

2.4.1 Chemical and Biological Characterization of Source Materials

Microbial communities from our source materials, prior to incubation, were significantly different from each other (NPMANOVA, $P < 0.001$). Generally, the most abundant phyla from each amendment and soil comprise the majority of the observed microbiome (Appendix Figure 2-1), with at least 75% of the total bacterial community represented in the five most abundant

phyla. The starting soil was comprised of Proteobacteria, Actinobacteria, Acidobacteria, unclassified bacteria (sharing no homology to a known reference gene), and Verrucomicrobia at 22%, 20%, 17%, 13%, and 10%, respectively. The five most abundant phyla in the alfalfa amendment were unclassified bacteria, Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes with relative abundances of 47%, 40%, 7%, 5%, and 1%, respectively. Composition of the compost amendment included the phyla Actinobacteria, unclassified bacteria, Firmicutes, Proteobacteria, Chloroflexi with abundances of 33%, 26%, 25%, 7%, and 4%, respectively.

The chemical characteristics of the amendments and starting soil also varied in inorganic N concentration and C:N ratio. Inorganic N concentration of the starting soil was 3.3 mg/kg, while the concentration in the alfalfa and compost amendments was 25.6 and 13.9 mg/kg, respectively (Table 1). In regard to C:N ratio, the starting soil was measured at 12.6, while alfalfa and compost inputs C:N ratios were 20.3 and 29.0, respectively (Table 1).

2.4.2 Biochemical Responses after Adding Organic Amendments

Following the incorporation of amendments with soil, inorganic N increased in both the alfalfa-amended and mix-amended microcosms from day 7 to 97 of the incubation. Similarly, this trend of increasing inorganic N also occurred in reference soils (Appendix Figure 2-2). In contrast, inorganic N decreased in the compost amended microcosms from day 7 to 49 before increasing slightly by day 97. Alfalfa amended soils had significantly higher inorganic N concentrations on days 14 to 97 than the reference treatment, and by day 97 had 2-fold greater inorganic N compared to reference soils. Mix-amended soils initially had lower concentrations of inorganic N compared to reference but increased to levels comparable to reference soils by day 97. Compost amendments resulted in a significantly lower concentration of inorganic N on all days of the incubation compared to the reference soil and had inorganic N concentrations less than one mg/kg on days 21, 35, and 45.

Microbial biomass C was increased in all amended soils relative to unamended, control (Appendix Figure 2-3). Alfalfa amendments had the highest observed MBC, with a two to three-fold higher MBC than reference and achieving the most MBC within the experiment, 780 mg C kg^{-1} on day 14. Reference soils also nearly doubled in microbial biomass on day 21 before declining to baseline concentrations on day 97. By day 35, amended microcosms had similar MBC and maintained this relationship throughout the experiment, while remaining significantly higher than reference MBC levels. Within day mean comparisons between treatments for all measured variables are summarized in Appendix Table 1.

2.4.3 Microbial Community Dynamics after Adding Organic Amendments

Amendment type and time were the two factors that most significantly contributed to differences in observed bacterial community structure (NPMANOVA, $P < 0.001$, $R2 = 0.34$ and NPMANOVA, $P < 0.001$, $R2 = 0.16$, respectively, Table 2). We observed clustering patterns of reference soils without amendment, soils with compost, alfalfa, and a mixture of alfalfa and compost (Appendix Figure 2-4).

To better understand the dynamics of these microbial communities, we evaluated whether the observed bacteria most likely originated from amendments or soils. Specifically, we identified amendment specific OTUs that were not detected in original soils or incubated reference microcosms. In total, 67, 307, and 386 amendment specific OTUs were identified from alfalfa, compost, and mix amendments, respectively. Compared to the total number of observed OTUs in amended microcosms, these amendment specific OTUs comprised a very small fraction of the total microbial community, 0.46% in alfalfa, 0.74% in mix, and 1.14% in compost.

To understand the influence of inorganic N, organic C, organic N, C:N ratio, and microbial biomass C on the microbial community, in addition to treatment and time, distance-based redundancy analysis was performed (Figure 2-1). Sampling day, amendment type, nitrate

concentration, C:N ratio, pH and organic C were identified as significantly contributing to 50.35% of the variation observed, while other environmental variables explain only 0.21% of the variation and were not considered significant contributors (Appendix Table 2).

To better understand the impacts of time on the bacterial communities, hierarchical clustering based on binary Bray-Curtis community dissimilarity within each treatment was used to identify groups with similar responses over time. For all amendments, we observed two distinct temporal groupings of microbial communities. These groups were characterized as either associated with "early" or "late" stages of the incubation and corresponded to the days 7, 14, and 21 and days 35, 49, and 97, respectively (Appendix Figures 2-5:2-8).

Within early and late incubation times, we identified specific OTUs that had significantly increased in abundance (greater than 4 log2(fold change) in abundance) in amended treatments relative to reference soils at the same sampling day (Appendix Table 3). We evaluated if there were phylogenetic relationships between observed enriched OTUs sequences in early and late groups (Figure 2-2). We observed trends in the phylogeny associated with OTUs identified in early and late groups of amended microcosms and classified these trends into ten clades of bacteria with consistent responses to either amendment type or response time.

Clade 1 was dominated by Bacteroidetes-associated OTUs that exhibited early response to amendment and were mainly associated with alfalfa amendment. Four OTUs related to Bacteroidetes Flavobacterium were enriched in either early alfalfa groups or early mix alfalfa-compost groups with an additional OTU on the same branch of responding bacteria from the genus Dyadobacter.

In clade 2, OTUs showed strong association with the early response group in all amended microcosms and are most likely associated with Planctomycetes. Most of these OTUs are

unclassified at the phyla level, but the sole OTU from this clade with genus assigned was from the genus Rhodopirellula. All OTUs in this clade were enriched specifically during later response times, with the exception of one OTU which was enriched throughout the experiment and is from the phyla Planctomycetes and the family Planctomycetaceae.

In clade 3, Verrucomicrobia-associated OTUs dominated and were observed in the early alfalfa response group almost exclusively. Clade 3 contains four OTUs, two of which could be classified at the genera level and are related to Verrucomicrobium and Roseimicrobium.

Clade 4 consists of three OTUs from the phyla Chloroflexi and an unclassified OTU at the phyla level. Clade 4 is associated with early compost samples, with one OTU related to the genus Sphaerobacter enriched throughout all compost amended microcosms. Further, this Sphaerobacter-associated OTU exhibited the highest increase in relative abundance compared to reference of all OTUs during the early response period of all amended samples.

Clades 5 and 6 were associated with alfalfa and compost samples, respectively. Clade 5 contains eight OTUs from the phyla Firmicutes, and generally this clade is associated with alfalfa amended samples in both early and late response groups. In contrast, clade 6 OTUs are associated with the phyla Actinobacteria and were found in enriched in compost-amended soils.

Clades 7, 8, 9, and 10 contain OTUs from the phyla Proteobacteria, which make up the majority of observed enriched OTUs. Clade 7 OTUs are from the order Myxococcales and are associated with OTUs enriched in the late response compost groups. Other Proteobacteria-associated OTUs in this clade are broadly enriched throughout incubations in both compost and alfalfa amended soils. Clade 8 contains five OTUs, with two OTUs most similar to the genus Hailea. Clade 8 OTUs are associated with mainly compost amended soils. Clade 9, in contrast, is comprised mainly of OTUs associated with alfalfa amended soils and are similar to

representatives from the genus Pseudomonas. Clade 10 contains three OTUs associated with early response groups in both alfalfa and compost amended soils and are associated with the genus Cellvibrio.

We evaluated the detection of enriched OTUs in the originating amendments and soils for the microcosms to understand the potential origin of these bacteria. Overall, only a small fraction of amendment-specific bacteria were enriched (Figure 2-2, green and brown branches), with none enriched in the late response group. This observation suggests that amendment specific bacteria do not persist past early response groups and are unlikely to colonize soils over the long term. One exception was OTU 00022 (Figure 2-2, Clade 4), which was not identified in compost or alfalfa yet shares sequence similarity to compost specific OTUs. This OTU was enriched in all compost and mix response groups and is most similar to bacteria from the genus Chloroflexi *sphaerobacter* and originated from the soil.

2.5 Discussion

The overall objective of the study was to identify whether distinct groups of bacteria respond to organic amendments in soils. We observed in two amendments of varying C:N ratios that there appeared to be two distinct, but consistent responses based on the time, which identified as an early (up to day 21) and late (day 37 up to day 97) response. In each of these time periods, we observed distinct groups of bacteria becoming enriched, but there were differences observed depending on the amendment and soil nutrient availability. Overall, these results are consistent with strong interactions between microbial communities and amendment C:N ratio during these response periods.

For each amendment, the early and late periods were associated with contrasting availability of nutrients. While both amendments initially were similar in total N content, they differed in the ratio of C:N. Alfalfa amendments had low C:N while compost amendments had

high C:N. Thus, in alfalfa-amended samples, the early response period is associated with high mineralization of inorganic N, while compost-amended samples early period is associated with immobilization of inorganic N.

We observed specific bacteria enriched in these conditions for each amendment. Generally, these enriched bacteria were taxonomically diverse, as observed through their broad phylogenetic associations. Overall, we find that the large majority of enriched bacteria in response to amendments most likely originate from soil communities rather than the amendment bacterial communities. Below, we synthesize our identification of distinct groups of bacteria with previous reports of their roles in soils.

2.5.1 Key OTUs Enriched in Response to Alfalfa Amendments

OTUs associated with Bacteroidetes, and specifically *Flavobacterium* and *Dyadobacter*, were observed as enriched in early alfalfa groups. Initially, we expect alfalfa amendments to have a high cellulose content compared to manure compost (Chen et al., 1989). Previous studies have identified *Flavobacterium* as capable of degrading cellulose and polysaccharides (Lednická et al., 2000), consistent with our observations of their enrichment in early alfalfa samples. Bacteria associated with *Dyadobacter* were also enriched in the early response to groups to alfalfa amendments, and these bacteria have been identified as plant-associated endophytes (Haichar et al., 2008; Reisberg et al., 2012). In our study, these OTUs are not likely to be endophytes, however, as they were not detected in the amendment-specific samples (e.g., ground alfalfa plant material alone). Strains of *Dyadobacter* have also been characterized as associated with nitrate reduction (Liu et al., 2006), though this trait is not common among species in this genus. Thus, it is possible that the high levels of nitrate during early alfalfa amendment responses may have resulted in *Dyadobacter*-associated OTU enrichment.

We also observed enrichment of bacteria related to the phyla *Verrucomicrobia*, specifically the genera *Verrucomicrobium* and *Roseimicrobium,* in early alfalfa decomposition. Several studies have identified *Verrucomicrobia sp.* from soils (Bergmann et al., 2011; Schlesner et al., 2006), including one that found *V. spinosum* enriched soil amended with incubated wheat straw after 168 days (Bastian et al., 2009). Our observed enrichment of *Verrucomicrobium* in early alfalfa groups contrasts with previous studies which found *Verrucomicrobium* to have a negative relationship with soil nutrient availability; these studies found that *Verrucomicrobia* decreased in relative abundance following N addition (Navarrete et al., 2015; Ramirez et al., 2012). We observed enrichment of *Verrucomicrobium* with elevated levels of nitrate compared to reference soils, suggesting that at least some members of this phyla may respond to high nutrient conditions and potentially diverse functional capabilities within this phylum.

In both early and late alfalfa-amendment response groups, we identified OTUs within the phyla Firmicutes and sharing similarity to the genera *Paenibacillus, Cohnella, Brevibacillus, Sporosarcina,* and *Tumebacillus.* The presence of *Firmicutes sp.* enrichment after alfalfa amendment agrees with past studies that found these organisms to be stimulated following wheat and alfalfa addition to soils (Pascault et al., 2010) and with further observations of copiotrophic activities as they grow in association with higher levels of inorganic N (Pascault et al., 2013). OTUs within the genus *Pseudomonas* were observed to be consistently enriched in early incubation alfalfa-amendment samples, as well as in late responding alfalfa-amendment and early mix response groups. The enrichment of *Pseudomonas* species may be linked to the N availability in soil. Previous studies have shown *Pseudomonas* species exhibiting N limitation in soils amended with high C:N ratio amendments, such as straw (Jensen and Nybroe, 1999). We did not observe the enrichment of *Pseudomonas* species in compost samples, likely due to N

limitations as a result of the high C:N ratio of our compost amendment (29:1) created conditions for the immobilization of inorganic N from the soil. In contrast, the mineralization of organic N in alfalfa amended soil results in higher levels of inorganic N, potentially favoring the growth of *Pseudomonas*.

2.5.2 Key OTUs Enriched in Response to Compost Amendments

In contrast to alfalfa, composts from the early and late incubation periods were enriched with OTUs associated with the phyla *Actinobacteria.* Specifically, one OTU was observed to be enriched in all early and late-incubation compost and alfalfa-compost mix response groups and is most similar to *Thermobifida fusca*, a previously characterized cellulolytic species (Lykidis et al., 2007). Enrichment of this OTU was greatest in the early mix and compost response groups and declined though still enriched in later groups. These results suggest that it may play a role in the degradation of the cellulose from the compost amendments and its decreasing availability to bacterial communities.

OTUs associated with *Proteobacteria* were also enriched in compost groups, specifically OTUs sharing similarity to Deltaproteobacteria and specifically from the order Myxococcales. Myxococcales are gliding bacteria that consume insoluble organic compounds and have previously been isolated from compost (Mohr et al., 2016). Their enrichment in compost-amended microcosms may be stimulated by the presence of recalcitrant compounds following the composting process or in response to low nutrient conditions stimulating sporulation, which may have been induced by the immobilization of inorganic N observed in mix and compost soil microcosms (Huntley et al., 2011). Consistent with our observations, Myxococcales have been found to be positively correlated with the C:N ratio of soil, significantly increasing in relative abundance between C:N ratios of 11.5 to 15.0 in a study of German grassland soils (Herzog et al., 2015).

In compost early response groups, we observed the enrichment of bacteria related to the phyla *Chloroflexi*. Bacteria associated with this group were also some of the few OTUs detected during the incubation that originated from the compost. These genes share similarity to *Sphaerobacter thermophilus.* Previously, *Sphaerobacter* species were reported as enriched following the application of bio-organic fertilizer in apple orchards (Wang et al., 2016). Early compost groups were also enriched with bacteria associated with *Cellvibrio japonicas* and *Cellvibrio fulvus*, which are known cellulose degrading saprophytic bacteria (Gardner, 2016; Lednická et al., 2000). We did not observe enrichment of these species in the mixed amendment groups, which also contained compost. This result may suggest that the mixed groups which did not exhibit significant immobilization during early response may not provide conditions favorable to *Cellvibrio*, and that these bacteria may prefer low the N conditions stimulated by compost amendment or not compete well in nutrient rich environments.

2.5.3 Key OTUs Enriched in Response to Mixture of Alfalfa and Compost Amendments

Within late response groups of both alfalfa and compost amendments, we observed unique clades of enriched bacteria, specifically related to the phyla *Planctomycetes.* Generally, *Planctomycetes* are considered slow growing bacteria (Lage and Bondoso, 2012; Whitman et al., 2016). Their enrichment in late groups is thus consistent with a slow growth rate. Additionally, this phylum has been found to respond to exopolysaccharide (EPS) production by other community members (Wang et al., 2015), and it is possible that late responders may be responding to metabolites produced by other community members. One specific OTU associated with *Planctomycetes* was enriched both in early and late response groups and across all amendment treatments, suggesting that these bacteria may also have a ubiquitous response to compounds available during the incubation and not specific to an amendment.

2.5.4 Conclusion

We find that amendment bacterial communities do not appear to persist in the soils, and this result has implications for understanding best practices for agricultural management. The main response to amendments appears to be the enrichment of native soil bacteria, suggesting that the most successful strategies for improving nutrient management in organic soils may be associated with the timing and quality of amendments rather than the specific amendment treatment. Overall, our observations suggest that the large majority of amendment-added bacteria may not persist, making it unlikely that they directly provide long-term benefits. Based on our observations, benefits most likely result from the interactions of both the amendment (both nutrients and microbiome) and native soil communities.

Overall, we observe strong phylogenetic responses to early and late response groups between organic amendments to soils. These results are consistent with previous studies finding the functional ability to degrade available compounds is often phylogenetically conserved (Morrissey et al., 2016). However, at a more granular scale, we observe patterns of genera level associations within each amendment response groups, evidence to specific microbial membership and its response to amendment quality. This result supports our hypothesis that there are specific communities that may be necessary for optimizing nutrient cycling in organic amendments, depending on the characteristics of the amendment.

This study emphasizes the need and opportunity for characterizing the microbial activity and composition for organic amendments and management practices with the goal of identifying enriched and responding bacteria. Future studies that include various amendments at the same C:N ratio would help to elucidate the role that amendment type plays in lieu of C:N ratio. The unique response of OTUs to specific amendments suggests specialization for decomposing the specific substrate and highlights the importance of a diverse microbial community for

decomposition and emphasizes the role of complex inputs in supporting a diverse microbial community. Further, our study indicates that there are predictable patterns of microbial response to organic amendments of soils, and a further investigation into the functional benefits of these responses will be useful for understanding the nutrient availability in soils.

2.5.6 Data availability

Sequences are available under the BioProject accession number PRJNA663308. R code used to generate statistics and figures is available at https://github.com/jflater/Incubation

2.5.7 Acknowledgements

We would like to thank Jody Ohmacht and Derek Carney of the USDA ARS NLAE in Ames, Iowa for their significant contributions to sample processing and analysis. In addition, we would like to extend thanks to Jin Choi who was helpful for this analysis.

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2.7 Figures and Tables

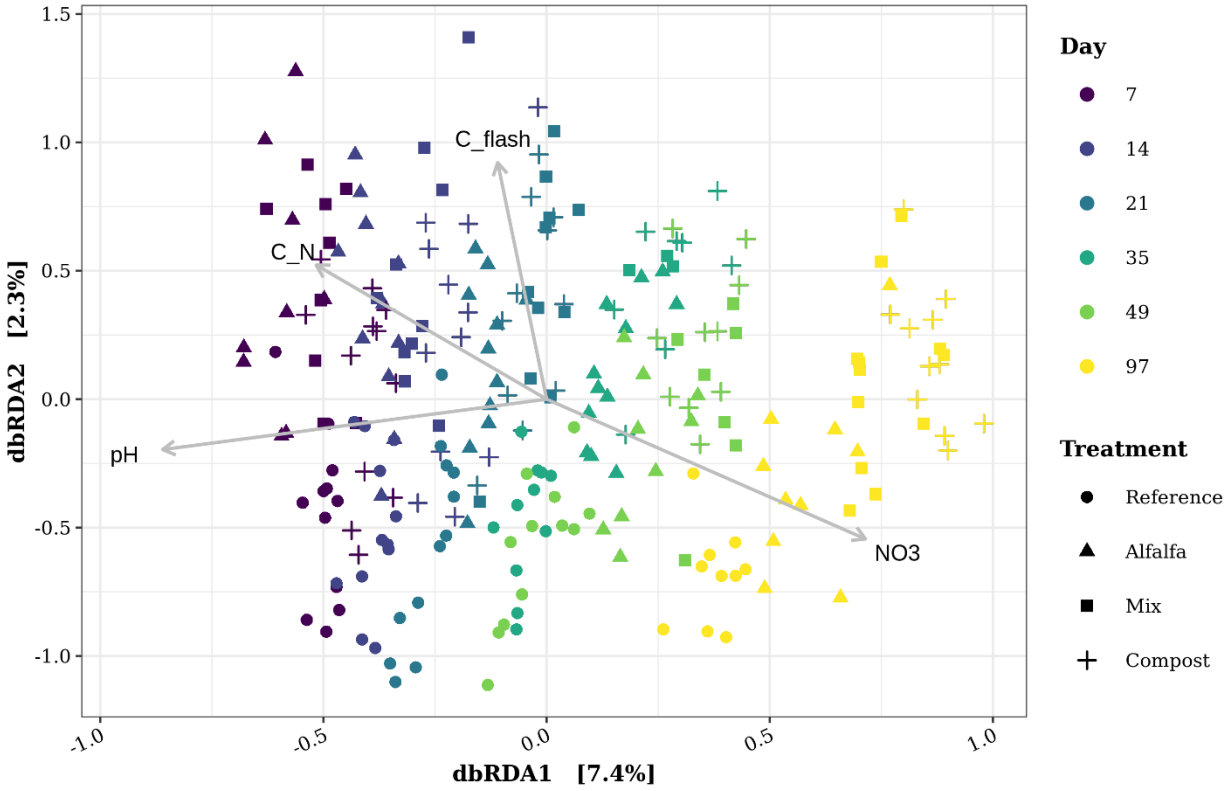
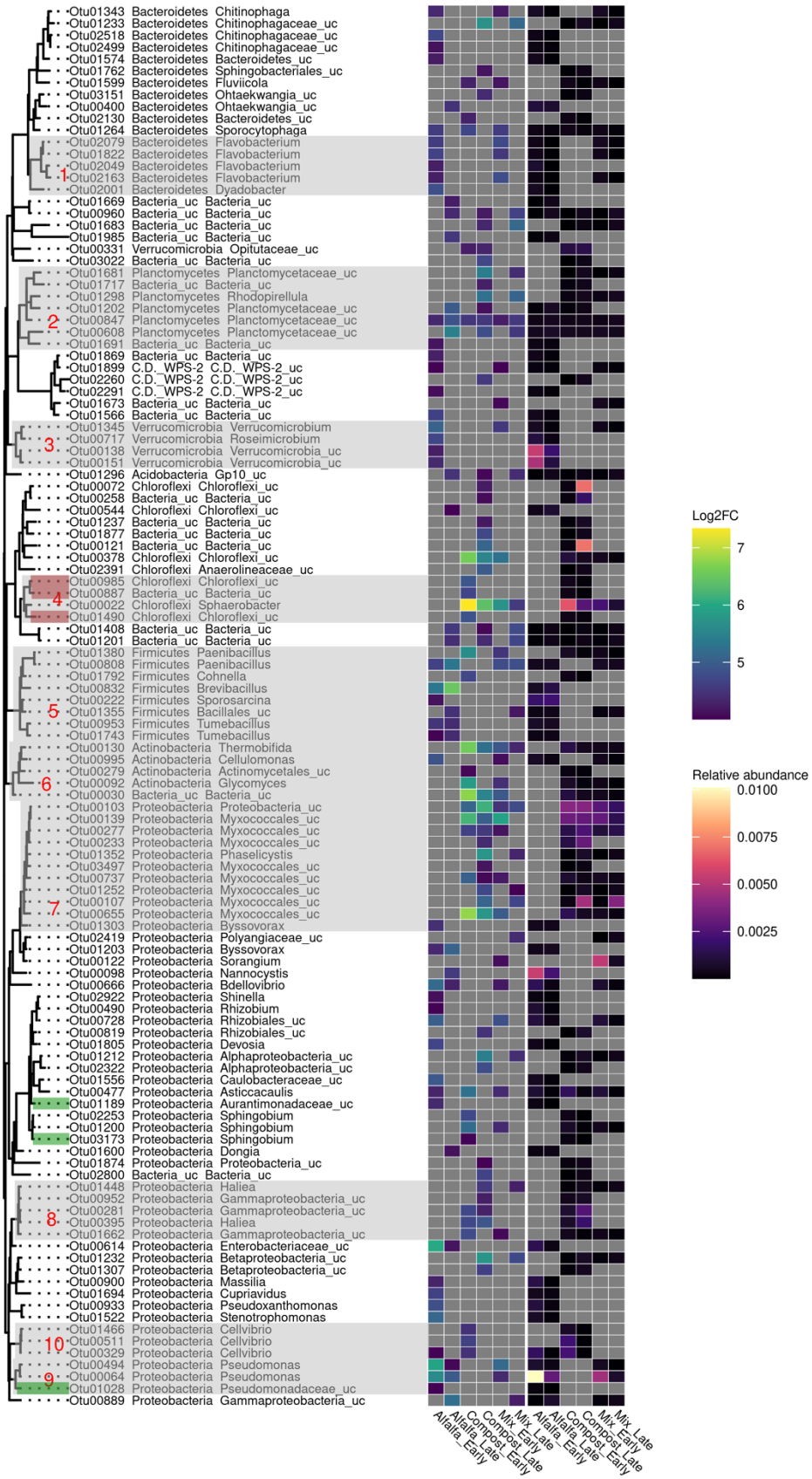


Figure 2-1: Distance-based redundancy analysis of rarefied (6,000 reads per sample) bacterial OTUs from incubated microcosms. Arrows represents variables that had significant effects on the community variation. (Bonferroni adjusted ANOVA Pr (>F) 0.001).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Otu01762 Bacteroidetes Sphingobacteriales_uc
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Otu02130 Bacteroidetes Bacteroidetes_uc
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Otu02049 Bacteroidetes Flavobacterium
Otu02163 Bacteroidetes Flavobacterium
Otu02001 Bacteroidetes Dyadobacter
1
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Otu01691 Bacteria_uc Bacteria_uc
2
Otu01869 Bacteria_uc Bacteria_uc
Otu01899 C.D._WPS-2 C.D._WPS-2_uc
Otu02260 C.D._WPS-2 C.D._WPS-2_uc
Otu02291 C.D._WPS-2 C.D._WPS-2_uc
Otu01673 Bacteria_uc Bacteria_uc
Otu01566 Bacteria_uc Bacteria_uc
Otu01345 Verrucomicrobia Verrucomicrobium
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9
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Otu00889 Proteobacteria Gammaproteobacteria_uc
Alfalfa_Early
Alfalfa_Late
Compost_Early
Compost_Late
Mix_Early
Mix_Late
Alfalfa_Early
Alfalfa_Late
Compost_Early
Compost_Late
Mix_Early
Mix_Late
Log2FC
7
6
5
Relative abundance
0.0100
0.0075
0.0050
0.0025

Figure 2-2: Phylogenetic tree of OTUs enriched by the addition of alfalfa, compost, or a mixture of alfalfa and compost. Associated heatmap (left) displays the log2 fold change of enriched OTUs in response to amendments in the early (days 7-21) and late (days 35-97) time points of the incubation; the relative abundance of OTUs in the early and late time points. Clades of enriched OTUs with specific amendment association are highlighted with grey boxes and labeled with red numbers. Branches highlighted with green indicate OTUs that originated from the alfalfa amendment while brown indicates OTUs originating from the compost amendment. Abbreviations in tip labels: "uc" is equivalent to "unclassified" and "C.D." is equivalent to "candidate_division".

Table 2-1: C and N content of soil and amendments used to construct microcosms for incubation.

	Total N %	Organic C %	NH_3-N mg/kg	NO_3-N mg/kg	C:N ratio
Soil	0.3	3.2	2.2	1.2	12.6
Alfalfa amendment	2.1	41.7	20.3	5.3	20.3
Compost amendment	1.2	33.8	12.6	1.4	29.0

Table 2-2: Nonparametric multivariate analysis of variance on the weighted UniFrac distances of taxa observed in incubated microcosms. Significant effects from both treatment (amendment type) and incubation or residence time (day) were found ($P<0.001$). (Df = degrees of freedom).

	Df	R^2	Pr (>F)
Treatment	3	0.16	0.001
Incubation time	5	0.34	0.001
Treatment x Incubation time	15	0.06	0.001
Residual	223	0.44	NA
Total	246	1	NA

2.8 Appendix

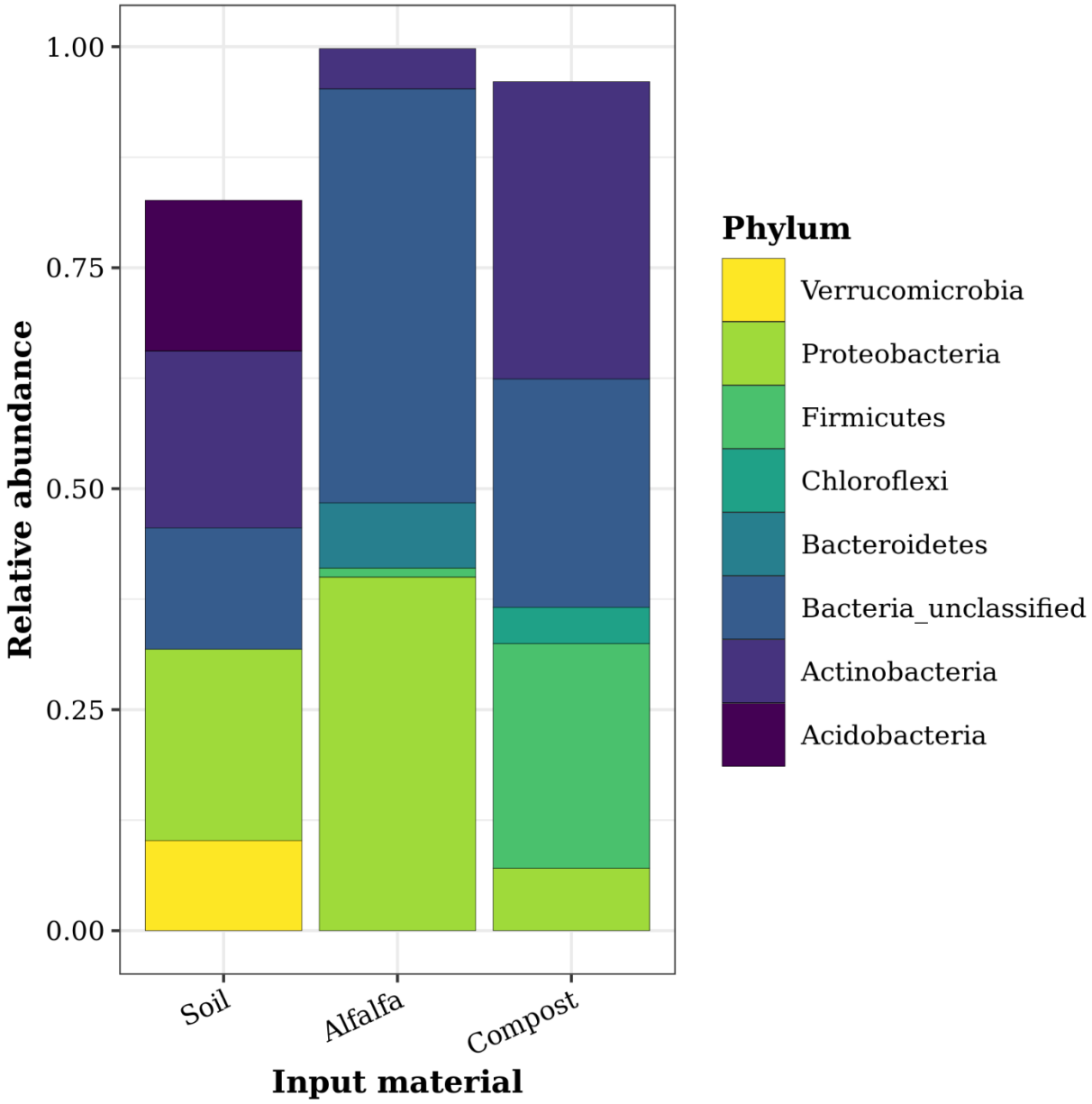

Figure A2-1: The relative abundance of the top five phyla from each of the substrates used to construct microcosms.

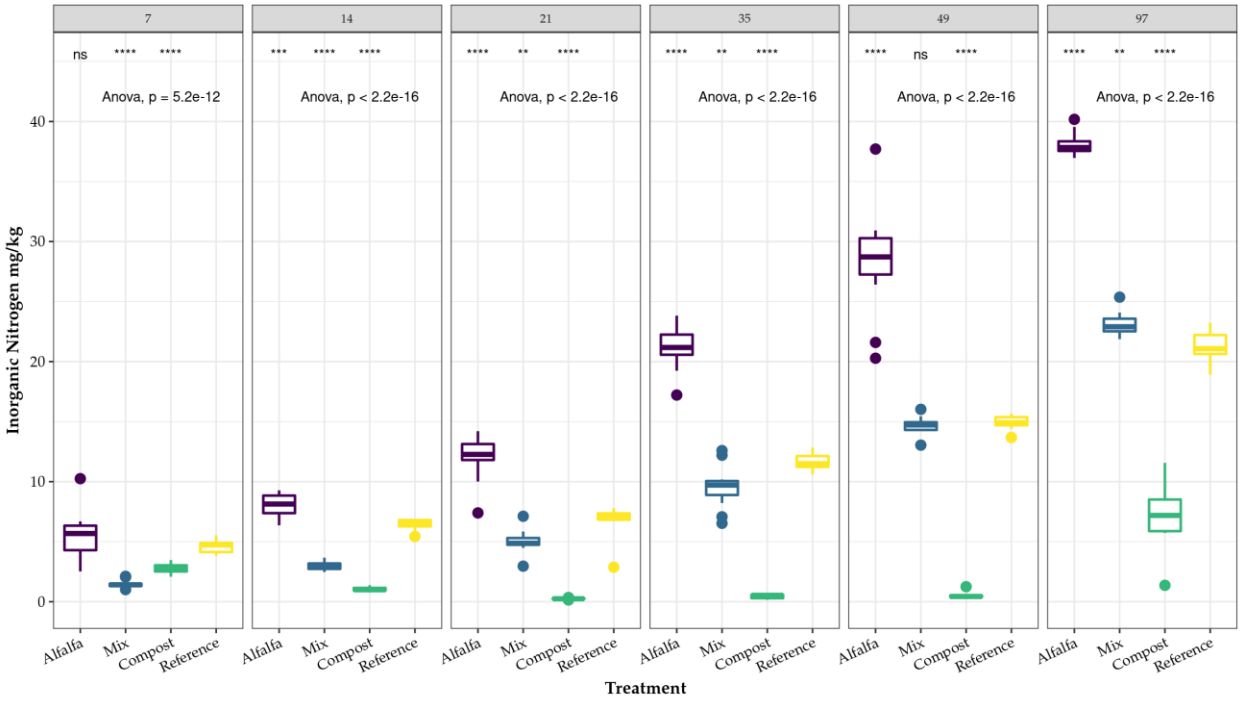

Figure A2-2: Boxplot showing mean inorganic N concentrations in soil (reference) or soil amended with alfalfa, compost or an alfalfa-compost mix. The panels represent the length of incubation from left to right (days are shown at the top). Each amended group mean was compared to the reference group from the same day to estimate significance (T-test, ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001 is shown, “Anova” = ANOVA global p-value).

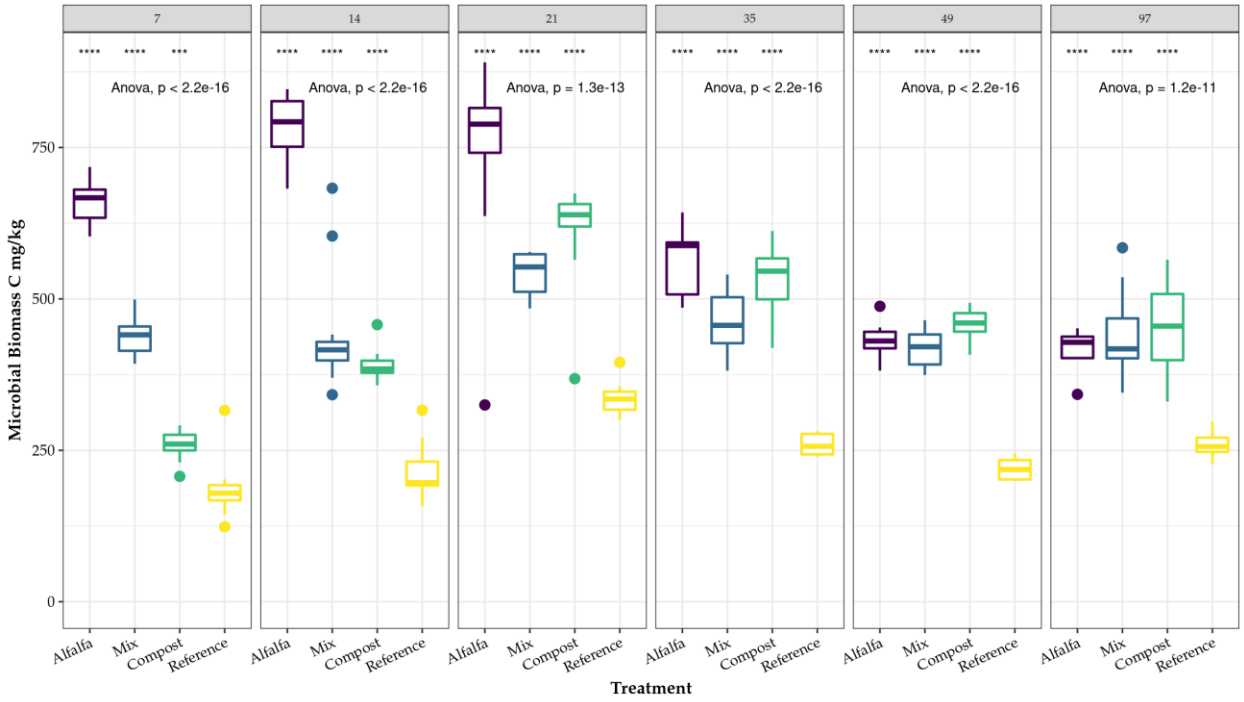

Figure A2-3: Boxplots showing the levels of microbial biomass C in incubated microcosms. The panels represent the sampling day of the incubation from left to right. Each amended treatment mean was compared to the reference mean from the same day using ANOVA followed by a t-test (T-test, ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001 is shown, "Anova" = ANOVA global p-value).

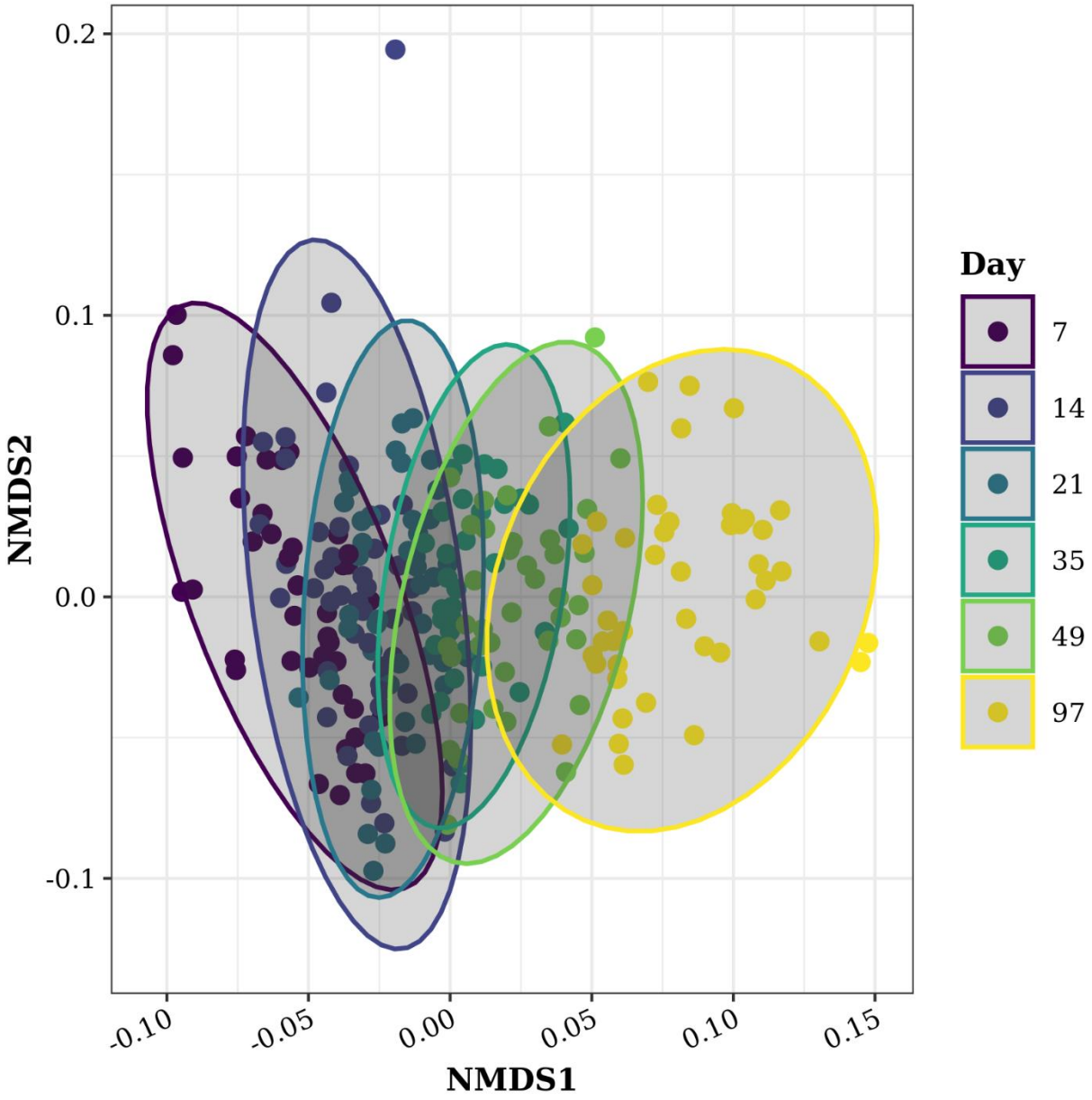

Figure A2-4: Non-metric multidimensional scaling (NMDS) of rarefied (6,000 reads per sample) bacterial taxa from incubated microcosms. Ordinations show dissimilarity from the weighted UniFrac distance matrix. Centroid lines show distinct communities among sampling days (NPMANOVA R2 = 0.34, Pr (>F) = 0.001, STRESS = 0.1260212).

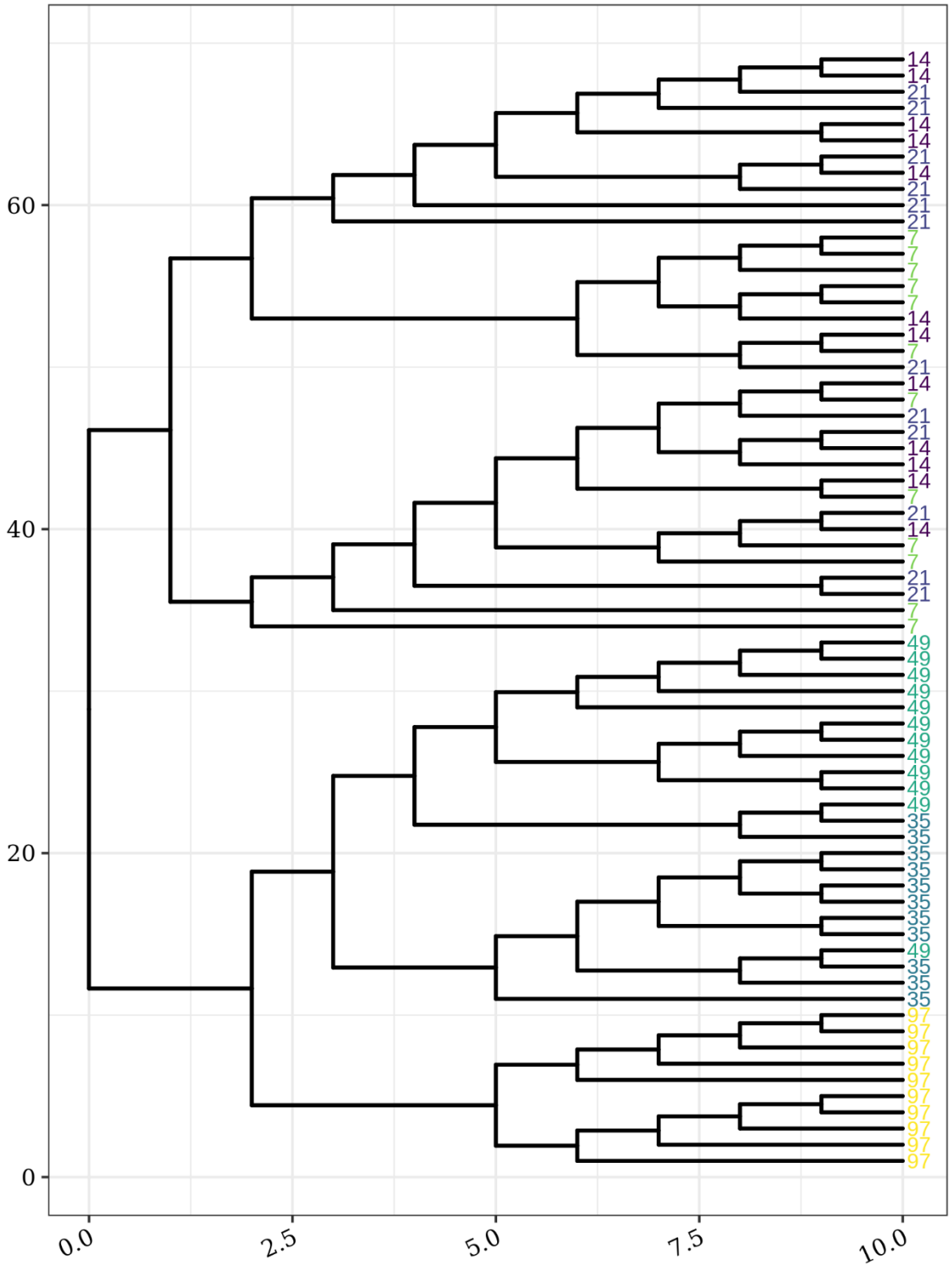

Figure A2-5: Hierarchical clustering of bacterial communities, based on binary Bray-Curtis dissimilarity within reference soils. Labels indicate day of sampling.

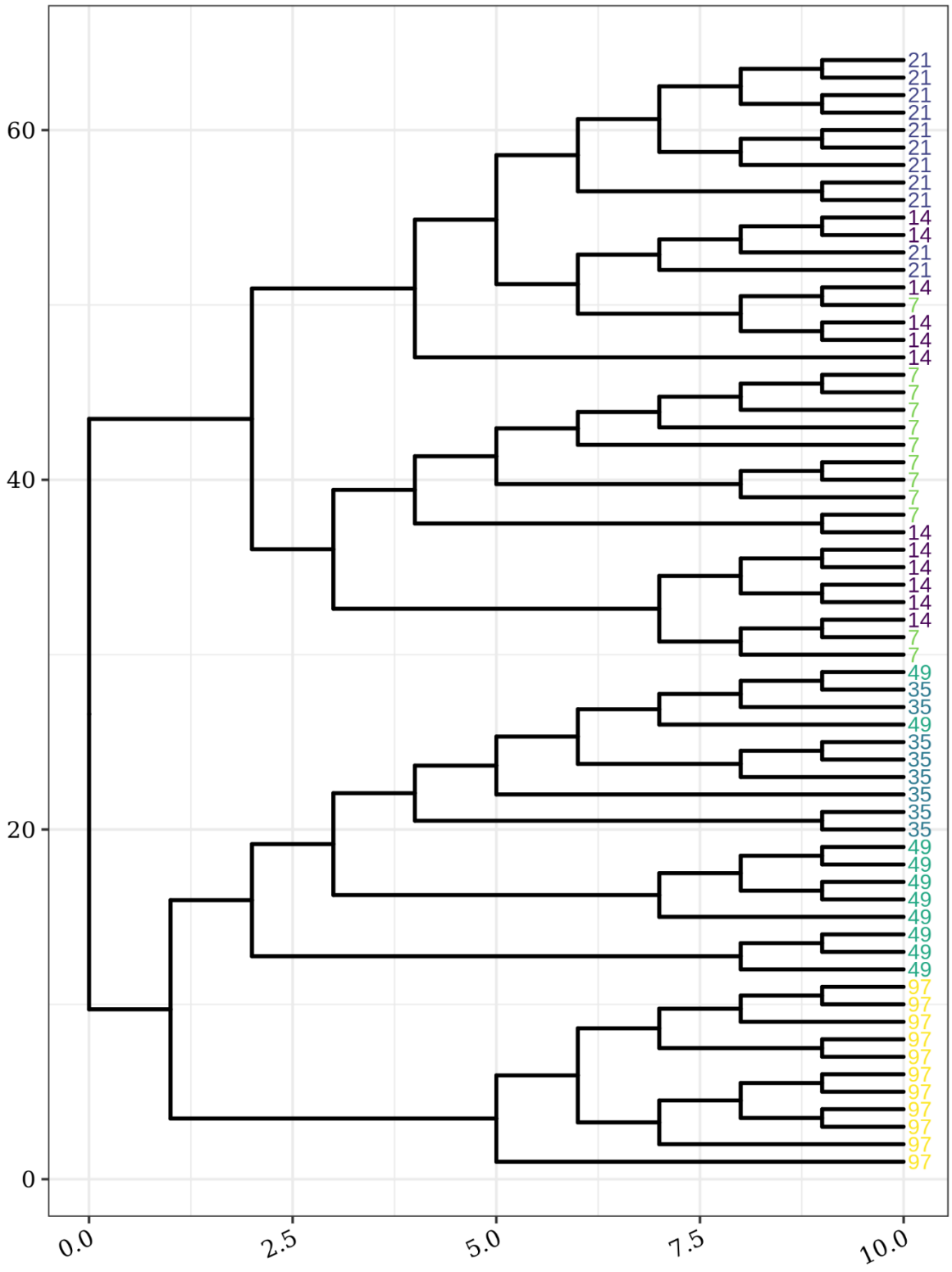

Figure A2-6: Hierarchical clustering of bacterial communities, based on binary Bray-Curtis dissimilarity within compost soils. Labels indicate day of sampling.

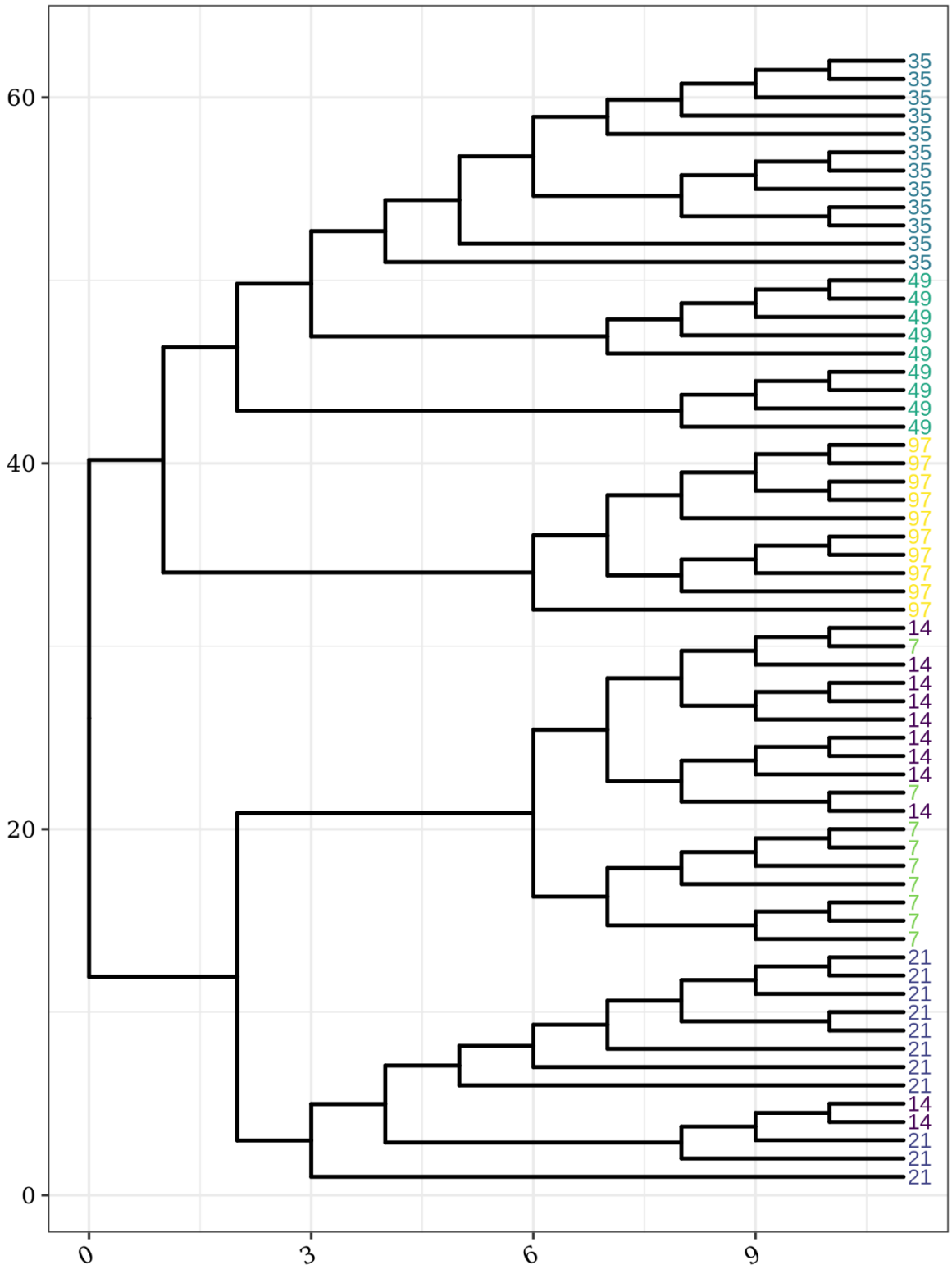

Figure A2-7: Hierarchical clustering of bacterial communities, based on binary Bray-Curtis dissimilarity within alfalfa soils. Labels indicate day of sampling.

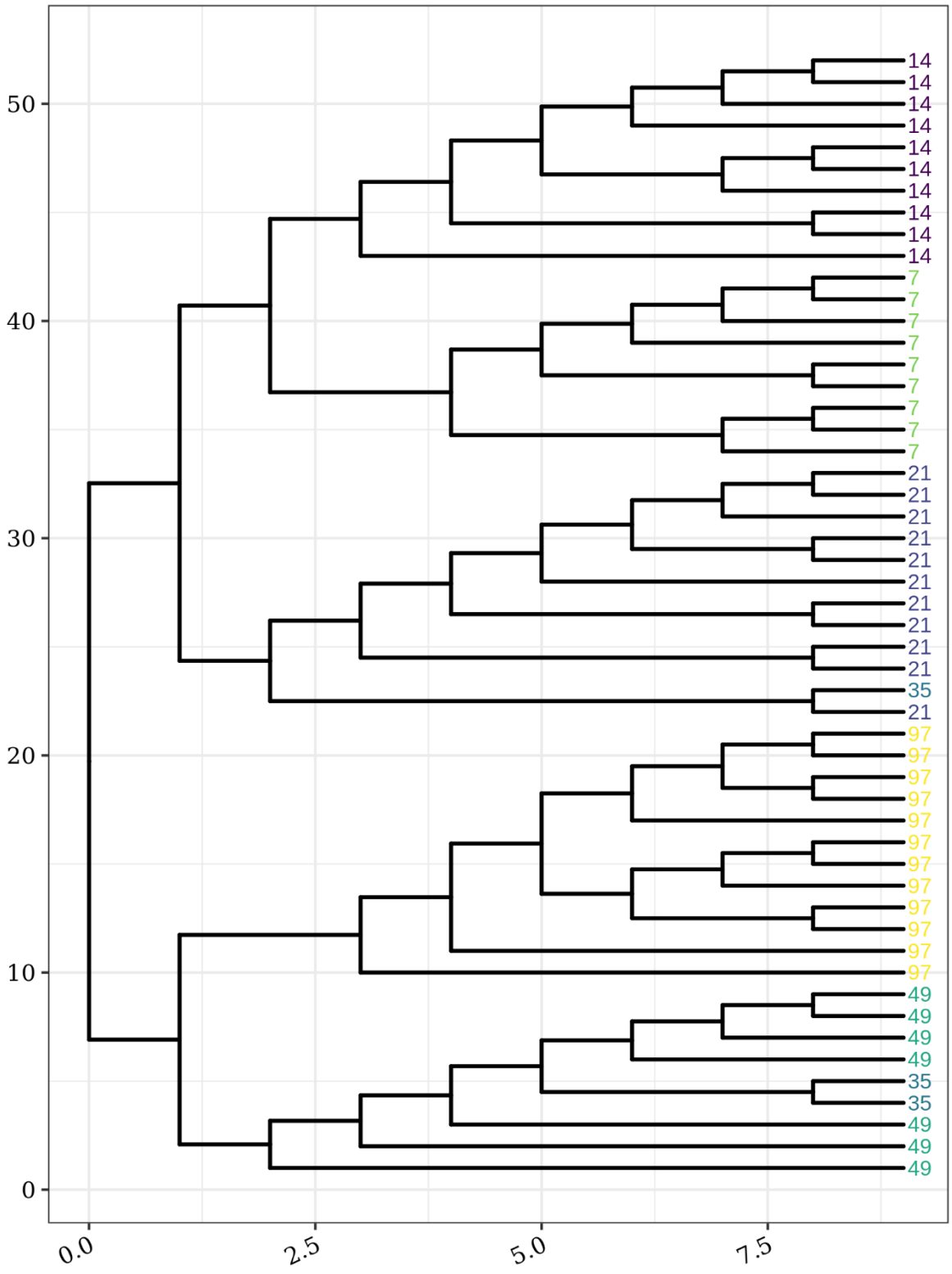

Figure A2-8: Hierarchical clustering of bacterial communities, based on binary Bray-Curtis dissimilarity within mix soils. Labels indicate day of sampling.

Table A2-1: Within day comparisons of soil characteristics of each treatment from linear model and analysis of variance, significance indicated by letter with $P < 0.05$. Values are mean (standard deviation).

Treatment	Day	Organic C	Total N	C:N	Inorganic N	NO3	NH3	MBC	pH	Gravimetric Water Content
Compost	7	3.72 (0.21) a	0.26 (0.01) b	14.44 (0.65) a	2.77 (0.40) b	2.39 (0.42) b	0.38 (0.11) b	259.23 (24.15) c	6.93 (0.08) c	0.37 (0.01) b
Mix	7	3.59 (0.14) a	0.27 (0.01) a	13.24 (0.51) b	1.47 (0.32) c	0.79 (0.27) c	0.68 (0.11) b	438.53 (30.27) b	6.96 (0.06) c	0.38 (0.01) ab
Alfalfa	7	3.3 (0.18) b	0.27 (0.02) a	12.12 (0.21) c	5.55 (1.95) a	4.02 (1.40) a	1.53 (0.86) a	660.82 (36.52) a	7.11 (0.06) b	0.39 (0.02) a
Reference	7	2.81 (0.12) c	0.23 (0.01) c	12.21 (0.26) c	4.59 (0.54) a	4.33 (0.46) a	0.26 (0.25) b	185.16 (46.73) d	7.22 (0.10) a	0.39 (0.01) a
Compost	14	3.93 (0.28) a	0.27 (0.02) a	14.34 (0.60) a	1.03 (0.17) d	0.18 (0.09) d	0.86 (0.16) bc	389.81 (25.94) b	6.88 (0.08) bc	0.36 (0.01) b
Mix	14	3.42 (0.17) b	0.26 (0.01) b	13.25 (0.26) b	2.96 (0.37) c	1.95 (0.32) c	1.01 (0.14) ab	442.17 (99.22) b	6.82 (0.08) c	0.37 (0.00) b
Alfalfa	14	3.21 (0.12) c	0.27 (0.01) ab	11.85 (0.22) c	8.03 (0.96) a	6.8 (1.02) a	1.24 (0.24) a	780.13 (56.26) a	6.94 (0.06) b	0.38 (0.01) a
Reference	14	2.89 (0.08) d	0.24 (0.01) c	12.03 (0.19) c	6.45 (0.44) b	5.71 (0.38) b	0.74 (0.30) c	214 (45.51) c	7.04 (0.06) a	0.38 (0.01) a
Compost	21	4.01 (0.87) a	0.29 (0.07) a	14.06 (0.49) a	0.26 (0.05) d	0.04 (0.03) d	0.22 (0.05) c	615.18 (83.72) b	6.6 (0.04) b	0.37 (0.01) ab
Mix	21	3.35 (0.17) b	0.26 (0.01) ab	12.98 (0.44) b	5.01 (0.96) c	4.65 (0.94) c	0.36 (0.07) b	542.4 (35.42) b	6.51 (0.03) c	0.36 (0.05) b

Table A2-1 continued

Treatment	Day	Organic C	Total N	C:N	Inorganic N	NO3	NH3	MBC	pH	Gravimetric Water Content
Alfalfa	21	3.1 (0.10) bc	0.26 (0.01) ab	11.81 (0.27) c	12.09 (1.87) a	11.63 (1.89) a	0.45 (0.04) a	742.68 (146.07) a	6.58 (0.04) b	0.39 (0.01) a
Reference	21	2.83 (0.12) c	0.23 (0.02) b	12.2 (0.40) c	6.8 (1.27) b	6.66 (1.23) b	0.14 (0.06) d	335.52 (26.36) c	6.66 (0.05) a	0.39 (0.00) a
Compost	35	3.68 (0.15) a	0.27 (0.01) a	13.46 (0.25) a	0.45 (0.17) d	0.13 (0.10) d	0.32 (0.17) a	530.14 (56.66) a	6.58 (0.09) b	0.36 (0.01) a
Mix	35	3.33 (0.13) b	0.27 (0.01) a	12.32 (0.24) b	9.55 (1.77) c	9.28 (1.76) c	0.27 (0.11) ab	461.81 (52.04) b	6.35 (0.02) c	0.34 (0.06) a
Alfalfa	35	3.02 (0.09) c	0.27 (0.01) a	11.3 (0.24) d	21.12 (1.73) a	20.83 (1.74) a	0.29 (0.06) a	560.07 (51.43) a	6.59 (0.06) b	0.37 (0.02) a
Reference	35	2.79 (0.07) d	0.23 (0.01) b	11.92 (0.36) c	11.62 (0.63) b	11.45 (0.62) b	0.17 (0.07) b	259.11 (16.50) c	6.7 (0.05) a	0.36 (0.01) a
Compost	49	3.54 (0.21) a	0.26 (0.01) a	13.65 (0.50) a	0.52 (0.25) c	0.2 (0.21) c	0.31 (0.05) a	458.52 (26.00) a	6.66 (0.13) b	0.37 (0.01) b
Mix	49	3.18 (0.09) b	0.26 (0.01) a	12.04 (0.19) b	14.67 (0.74) b	14.42 (0.71) b	0.26 (0.08) ab	417.92 (30.02) b	6.48 (0.05) c	0.37 (0.01) b
Alfalfa	49	2.98 (0.12) c	0.26 (0.01) a	11.34 (0.23) c	28.36 (4.49) a	28.14 (4.48) a	0.22 (0.05) b	431.19 (27.67) ab	6.72 (0.07) ab	0.38 (0.01) a
Reference	49	2.76 (0.12) d	0.24 (0.01) b	11.7 (0.27) b	14.93 (0.57) b	14.72 (0.59) b	0.22 (0.08) b	220.19 (17.03) c	6.79 (0.07) a	0.39 (0.01) a
Compost	97	3.32 (0.18) a	0.28 (0.01) a	12.05 (0.31) a	7.07 (2.43) d	6.61 (2.40) c	0.46 (0.58) a	455.58 (75.43) a	6.64 (0.11) a	0.39 (0.02) ab

Table A2-1 continued

Treatment	Day	Organic C	Total N	C:N	Inorganic N	NO3	NH3	MBC	pH	Gravimetric Water Content
Mix	97	3.1 (0.09) b	0.28 (0.01) a	11.12 (0.30) b	23.13 (0.96) b	22.29 (0.93) b	0.85 (0.42) a	438.41 (66.55) a	6.63 (0.11) a	0.36 (0.01) b
Alfalfa	97	2.94 (0.07) c	0.28 (0.01) a	10.47 (0.19) c	38.1 (0.95) a	38.1 (0.95) a	0 (0.00) b	418.85 (29.80) a	6.62 (0.10) a	0.38 (0.05) ab
Reference	97	2.73 (0.07) d	0.24 (0.01) b	11.26 (0.23) b	21.31 (1.23) c	21.31 (1.23) b	0 (0.00) b	258.92 (19.54) b	6.72 (0.07) a	0.41 (0.01) a

Table A2-2: Variance partitioning of the variables contributing to differences in community structure of the incubated microcosms.

Variable	Explains (%)	Pr(>F)
Day	26.77	0.011 *
Treatment	14.7	0.011 *
pH	3.54	0.011 *
Nitrate	1.24	0.011 *
C:N	0.69	0.022 *
Organic C	0.27	0.374
Others	0.45	
Unexplained	52.35	
Total	100	

Table A2-3: OTUs and phylogeny of enriched bacteria compared to reference (greater than 4 log2(fold-change)), NA signifies that OTU was not enriched in the response group compared to the same reference response group.

	Phylum	Genus	Alfalfa Early	Alfalfa Late	Compost Early	Compost Late	Mix Early	Mix Late
Otu00064	Proteobacteria	Pseudomonas	5.61	4.95	NA	NA	4.36	NA
Otu00138	Verrucomicrobia	Verrucomicrobia_unclassified	4.24	NA	NA	NA	NA	NA
Otu00151	Verrucomicrobia	Verrucomicrobia_unclassified	4.36	NA	NA	NA	NA	NA
Otu00222	Firmicutes	Sporosarcina	4.14	NA	NA	NA	NA	NA
Otu00329	Proteobacteria	Cellvibrio	4.02	NA	4.45	NA	NA	NA
Otu00477	Proteobacteria	Asticcacaulis	4.31	NA	5.25	NA	4.37	NA
Otu00490	Proteobacteria	Rhizobium	4.01	NA	NA	NA	NA	NA
Otu00494	Proteobacteria	Pseudomonas	5.94	4.05	NA	NA	5.16	NA
Otu00614	Proteobacteria	Enterobacteriaceae_unclassified	6.02	4.15	NA	NA	NA	NA
Otu00666	Proteobacteria	Bdellovibrio	5.17	4.28	NA	NA	4.18	NA
Otu00717	Verrucomicrobia	Roseimicrobium	4.70	NA	NA	NA	NA	NA
Otu00728	Proteobacteria	Rhizobiales_unclassified	5.01	NA	NA	NA	4.82	NA
Otu00808	Firmicutes	Paenibacillus	4.64	5.32	NA	NA	4.78	4.80
Otu00832	Firmicutes	Brevibacillus	5.31	6.50	NA	NA	NA	NA
Otu00847	Planctomycetes	Planctomycetaceae_unclassified	4.34	4.86	4.52	4.53	4.24	4.54
Otu00900	Proteobacteria	Massilia	4.30	NA	NA	NA	NA	NA
Otu00933	Proteobacteria	Pseudoxanthomonas	4.82	NA	NA	NA	NA	NA
Otu00953	Firmicutes	Tumebacillus	4.49	4.38	NA	NA	NA	NA
Otu00995	Actinobacteria	Cellulomonas	4.87	NA	NA	NA	4.09	NA
Otu01028	Proteobacteria	Pseudomonadaceae_unclassified	4.01	NA	NA	NA	NA	NA
Otu01189	Proteobacteria	Aurantimonadaceae_unclassified	4.36	NA	NA	NA	NA	NA
Otu01203	Proteobacteria	Byssovorax	4.35	5.06	NA	NA	NA	NA
Otu01264	Bacteroidetes	Sporocytophaga	4.59	NA	4.70	NA	4.59	NA
Otu01303	Proteobacteria	Byssovorax	4.44	NA	NA	NA	NA	NA
Otu01343	Bacteroidetes	Chitinophaga	4.35	NA	NA	NA	4.20	NA
Otu01345	Verrucomicrobia	Verrucomicrobium	5.06	NA	NA	NA	4.53	NA
Otu01522	Proteobacteria	Stenotrophomonas	5.10	NA	NA	NA	NA	NA
Otu01556	Proteobacteria	Caulobacteraceae_unclassified	4.93	NA	NA	NA	NA	NA
Otu01566	Bacteria_unclassified	Bacteria_unclassified	4.63	NA	NA	NA	NA	NA

Table A2-3 continued

	Phylum	Genus	Alfalfa	Alfalfa	Compost	Compost	Mix	Mix
Otu01574	Bacteroidetes	Bacteroidetes_unclassified	Early	Late	Early	Late	Early	Late
Otu01691	Bacteria_unclassified	Bacteria_unclassified	4.16	NA	NA	NA	NA	NA
Otu01694	Proteobacteria	Cupriavidus	4.63	NA	NA	NA	NA	NA
Otu01743	Firmicutes	Tumebacillus	4.02	4.38	NA	NA	NA	NA
Otu01805	Proteobacteria	Devosia	4.67	NA	NA	NA	NA	NA
Otu01822	Bacteroidetes	Flavobacterium	4.64	NA	NA	NA	4.52	NA
Otu01869	Bacteria_unclassified	Bacteria_unclassified	4.26	NA	NA	NA	NA	NA
Otu01899	candidate_division_WPS-2	candidate_division_WPS-2_unclassified	4.01	NA	NA	NA	4.01	NA
Otu02001	Bacteroidetes	Dyadobacter	4.81	NA	NA	NA	NA	NA
Otu02049	Bacteroidetes	Flavobacterium	4.25	NA	NA	NA	NA	NA
Otu02079	Bacteroidetes	Flavobacterium	4.58	NA	NA	NA	4.75	NA
Otu02163	Bacteroidetes	Flavobacterium	4.25	NA	NA	NA	4.80	NA
Otu02291	candidate_division_WPS-2	candidate_division_WPS-2_unclassified	4.08	NA	NA	NA	NA	NA
Otu02499	Bacteroidetes	Chitinophagaceae_unclassified	4.12	NA	NA	NA	NA	NA
Otu02518	Bacteroidetes	Chitinophagaceae_unclassified	4.72	NA	NA	NA	NA	NA
Otu02922	Proteobacteria	Shinella	4.10	NA	NA	NA	NA	NA
Otu00098	Proteobacteria	Nannocystis	NA	4.56	NA	NA	NA	NA
Otu00400	Bacteroidetes	Ohtaekwangia_unclassified	NA	4.46	NA	NA	NA	NA
Otu00544	Chloroflexi	Chloroflexi_unclassified	NA	4.02	NA	NA	NA	NA
Otu00608	Planctomycetes	Planctomycetaceae_unclassified	NA	5.46	NA	4.79	NA	4.53
Otu00889	Proteobacteria	Gammaproteobacteria_unclassified	NA	5.21	NA	NA	NA	4.23
Otu00960	Bacteria_unclassified	Bacteria_unclassified	NA	4.35	NA	4.20	NA	4.65
Otu01201	Bacteria_unclassified	Bacteria_unclassified	NA	4.40	NA	4.38	NA	4.70
Otu01202	Planctomycetes	Planctomycetaceae_unclassified	NA	4.95	NA	4.11	NA	NA
Otu01296	Acidobacteria	Gp10_unclassified	NA	4.47	NA	4.05	NA	4.40
Otu01355	Firmicutes	Bacillales_unclassified	NA	4.45	NA	NA	NA	4.17
Otu01408	Bacteria_unclassified	Bacteria_unclassified	NA	4.54	NA	4.06	NA	4.76
Otu01600	Proteobacteria	Dongia	NA	4.12	NA	NA	NA	NA
Otu01669	Bacteria_unclassified	Bacteria_unclassified	NA	4.23	NA	NA	NA	NA
Otu01985	Bacteria_unclassified	Bacteria_unclassified	NA	4.51	NA	NA	NA	NA
Otu00022	Chloroflexi	Sphaerobacter	NA	NA	7.34	6.39	5.70	4.56
Otu00030	Bacteria_unclassified	Bacteria_unclassified	NA	NA	6.75	5.51	4.93	NA
Otu00092	Actinobacteria	Glycomyces	NA	NA	5.83	NA	4.43	NA
Otu00103	Proteobacteria	Proteobacteria_unclassified	NA	NA	5.10	6.14	4.49	4.77

Table A2-3 continued

	Phylum	Genus	Alfalfa	Alfalfa	Compost	Compost	Mix	Mix
Otu00130	Actinobacteria	Thermobifida	NA	NA	6.57	5.23	4.89	4.34
Otu00139	Proteobacteria	Myxococcales_unclassified	NA	NA	6.19	5.01	5.93	NA
Otu00277	Proteobacteria	Myxococcales_unclassified	NA	NA	4.64	4.53	4.16	NA
Otu00279	Actinobacteria	Actinomycetales_unclassified	NA	NA	4.06	NA	NA	NA
Otu00281	Proteobacteria	Gammaproteobacteria_unclassified	NA	NA	4.72	4.27	NA	NA
Otu00331	Verrucomicrobia	Opitutaceae_unclassified	NA	NA	4.24	4.30	NA	NA
Otu00378	Chloroflexi	Chloroflexi_unclassified	NA	NA	6.60	5.52	5.24	NA
Otu00395	Proteobacteria	Haliea	NA	NA	4.72	4.90	NA	NA
Otu00511	Proteobacteria	Cellvibrio	NA	NA	4.63	NA	NA	NA
Otu00655	Proteobacteria	Myxococcales_unclassified	NA	NA	6.79	5.74	4.97	NA
Otu00737	Proteobacteria	Myxococcales_unclassified	NA	NA	5.04	4.05	4.21	NA
Otu00887	Bacteria_unclassified	Bacteria_unclassified	NA	NA	4.68	NA	NA	NA
Otu00985	Chloroflexi	Chloroflexi_unclassified	NA	NA	4.91	NA	NA	NA
Otu01200	Proteobacteria	Sphingobium	NA	NA	5.17	NA	4.36	NA
Otu01380	Firmicutes	Paenibacillus	NA	NA	5.64	NA	4.49	NA
Otu01466	Proteobacteria	Cellvibrio	NA	NA	4.44	NA	NA	NA
Otu01490	Chloroflexi	Chloroflexi_unclassified	NA	NA	4.86	NA	NA	NA
Otu01599	Bacteroidetes	Fluviicola	NA	NA	4.27	NA	4.15	NA
Otu01662	Proteobacteria	Gammaproteobacteria_unclassified	NA	NA	4.78	NA	4.06	NA
Otu01792	Firmicutes	Cohnella	NA	NA	4.70	NA	NA	NA
Otu02130	Bacteroidetes	Bacteroidetes_unclassified	NA	NA	4.27	NA	NA	NA
Otu02253	Proteobacteria	Sphingobium	NA	NA	4.74	NA	NA	NA
Otu03173	Proteobacteria	Sphingobium	NA	NA	4.02	NA	NA	NA
Otu00072	Chloroflexi	Chloroflexi_unclassified	NA	NA	NA	4.22	NA	NA
Otu00107	Proteobacteria	Myxococcales_unclassified	NA	NA	NA	5.07	NA	4.68
Otu00121	Bacteria_unclassified	Bacteria_unclassified	NA	NA	NA	5.09	NA	NA
Otu00233	Proteobacteria	Myxococcales_unclassified	NA	NA	NA	4.37	NA	NA
Otu00258	Bacteria_unclassified	Bacteria_unclassified	NA	NA	NA	4.08	NA	NA
Otu00819	Proteobacteria	Rhizobiales_unclassified	NA	NA	NA	4.46	NA	NA
Otu00952	Proteobacteria	Gammaproteobacteria_unclassified	NA	NA	NA	4.25	NA	NA
Otu01212	Proteobacteria	Alphaproteobacteria_unclassified	NA	NA	NA	5.43	NA	4.42
Otu01232	Proteobacteria	Betaproteobacteria_unclassified	NA	NA	NA	5.73	NA	4.74
Otu01233	Bacteroidetes	Chitinophagaceae_unclassified	NA	NA	NA	5.70	NA	5.15
Otu01237	Bacteria_unclassified	Bacteria_unclassified	NA	NA	NA	4.19	NA	NA

Table A2-3 continued

	Phylum	Genus	Alfalfa	Alfalfa	Compost	Compost	Mix	Mix
Otu01252	Proteobacteria	Myxococcales_unclassified	NA	NA	NA	4.72	NA	4.00
Otu01298	Planctomycetes	Rhodopirellula	NA	NA	NA	5.26	NA	5.08
Otu01307	Proteobacteria	Betaproteobacteria_unclassified	NA	NA	NA	4.62	NA	NA
Otu01352	Proteobacteria	Phaselicystis	NA	NA	NA	5.76	NA	4.30
Otu01448	Proteobacteria	Haliea	NA	NA	NA	4.53	NA	4.26
Otu01681	Planctomycetes	Planctomycetaceae_unclassified	NA	NA	NA	5.39	NA	4.40
Otu01683	Bacteria_unclassified	Bacteria_unclassified	NA	NA	NA	4.33	NA	5.15
Otu01717	Bacteria_unclassified	Bacteria_unclassified	NA	NA	NA	4.25	NA	NA
Otu01762	Bacteroidetes	Sphingobacteriales_unclassified	NA	NA	NA	4.15	NA	NA
Otu01874	Proteobacteria	Proteobacteria_unclassified	NA	NA	NA	4.02	NA	NA
Otu01877	Bacteria_unclassified	Bacteria_unclassified	NA	NA	NA	4.72	NA	NA
Otu02260	candidate_division_WPS-2	candidate_division_WPS-2_unclassified	NA	NA	NA	4.55	NA	NA
Otu02322	Proteobacteria	Alphaproteobacteria_unclassified	NA	NA	NA	4.80	NA	NA
Otu02391	Chloroflexi	Anaerolineaceae_unclassified	NA	NA	NA	4.96	NA	NA
Otu02800	Bacteria_unclassified	Bacteria_unclassified	NA	NA	NA	4.66	NA	NA
Otu03022	Bacteria_unclassified	Bacteria_unclassified	NA	NA	NA	4.71	NA	NA
Otu03151	Bacteroidetes	Ohtaekwangia_unclassified	NA	NA	NA	4.37	NA	NA
Otu03497	Proteobacteria	Myxococcales_unclassified	NA	NA	NA	4.14	NA	NA
Otu00122	Proteobacteria	Sorangium	NA	NA	NA	NA	4.09	NA
Otu01673	Bacteria_unclassified	Bacteria_unclassified	NA	NA	NA	NA	4.05	NA
Otu02419	Proteobacteria	Polyangiaceae_unclassified	NA	NA	NA	NA	NA	4.37

CHAPTER 3. COMPARISON OF BACTERIAL COMMUNITY DIVERSITY UNDER PRAIRIE STRIPS AND AGRICULTURAL PRODUCTION

Modified from a manuscript to be submitted to Agriculture, Ecosystems & Environment

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3.1 Abstract

The microbial communities of agricultural soils are receiving increasing attention, in part, thanks to the role soil microbes play in ecosystem services. Despite the importance of the soil bacterial community, there remain gaps in our understanding of how bacterial diversity is affected by agricultural management and how this relates to ecosystem services. Studies suggest that the management of above-ground plant communities can influence the below-ground bacterial community. In agroecosystems, edge and in-filed changes in plant communities maximize the conservation of resources. These practices include the establishment of perineal grass species as filter or buffer strips primarily target reducing erosion and nutrient loss. In the Midwest's tallgrass prairie region, the establishment of native prairie species is under study for its potential as a conservation practice. Prairie stirps can reduce soil loss, improve nutrient cycling, and increase above-ground diversity. However, we know little of the changes in the bacterial community of soils under conservation prairie strips. Multiple measures of alpha diversity characterize diversity in a bacterial community of one soil sample. First, the observed number of species is a simple measure of the number of species observed in a community.

We asked how the establishment of strips may alter these three measures of soil bacterial alpha diversity between prairie and crop soil over space and time in response to rainfall simulation. Further, we wished to describe the differences in diversity between two different soil

types and prairie strip ages. Here we investigated differences in bacterial diversity between the soils of row crops and prairie conservation strips. Prairie soils were compared to ag soils at two Iowa state research farms, at two depths and multiple days. The installations represent typical Iowa cropping systems in the Central and Southwest portion of the state. Prior to rainfall, we found no significant differences between crop and prairie soils. Following rainfall, we found that there was a significant increase in diversity in prairie soils at site one compared to croplands two days after rainfall.

Conversely, site two samples did not exhibit differences in diversity between crop and prairie at any sampling point nor in response to rainfall. These results are relevant because it suggests that young prairie strips are not significantly different from adjacent crop soils, and that observed ecosystem benefits are not likely to be driven by bacterial diversity changes. Nevertheless, more investigations are needed to determine how age and soil type impact alpha diversity.

3.2 Introduction

In Iowa, agricultural management has changed soils. This region was once occupied by the tallgrass prairie and its heavily rooted soil ecosystem (Smith, 1998). Prairies and their soils are one of the most diverse habitats on the planet. Under prairie for thousands of years, soil bacteria grew and evolved in close relationship with the plant communities above them, primarily through the extensive rhizosphere. This was a relatively stable regime, occasionally impacted by the grazing of herbivores, such as bison, or burning by Native Americans (Allen and Palmer, 2011). Following the turn of the 19th century, the prairie had begun to be converted to agricultural production in Iowa. The ensuing years have resulted in significant ecological changes, mainly in habitat loss and agricultural expansion. Soils that once supported diverse grasslands are now used to generate food, fiber, feed, and fuel in the Midwest's agricultural

states. Conservation and restoration of natural areas and systems such as prairies are receiving increased attention, in some cases for the first time, due to their importance for maintaining diverse and functional ecosystems (Asbjornsen et al., 2014). These efforts have resulted in some agricultural land being restored to a more natural and native state.

One conservation practice that has been promising in this region is the incorporation of native strips of prairie vegetation (Liebman and Schulte, 2015). This practice is based on adding strips of prairie vegetation in within an agricultural field, along grassed waterways, or at the edge of field. These plantings may occupy up to 25% of a tract and are between 9.1 m and 36.6 m wide. The goal of this conservation practice is to reduce erosion, improve water quality, and provide habitat for native species. These strips of prairie vegetation have resulted in improved ecosystems. For example, water quality is improved by the addition of prairie strips. Reduction of total N in surface water by 3.3 times as well as reduced nitrate in groundwater by 3.6 times has been observed. Compared to fully cropped fields, phosphorus in surface water was reduced by 4.3 times and sediment by 1900%. Incorporation of native plants in agricultural settings has also benefited the surrounding biodiversity. Compared to fully cropped fields, prairie strips have doubled the total observed taxa of insects and birds. Prairie strips are also associated with nearly 8 times more plant species richness (Schulte et al., 2017). These impacts provided by prairie strip installations make it an exciting conservation practice that is expanding across the prairie states.

Another area where prairie strips are most likely to have an impact is in the underlying soil microbial communities. Land use changes in the agricultural landscape have been observed to have significant impacts on the underlying microbial communities in the soil. In agroecosystems, these changes on soil-bacteria interactions can impact crop production, plant protection, nutrient cycling, and water quality (Benítez and McSpadden Gardener, 2009;

Expósito et al., 2017; Singh and Gupta, 2018). Soil bacteria are incredibly diverse, with estimates of one gram of soil containing up to 1.5×10^{10} bacteria (Torsvik et al., 1990). It is an open area of research to identify the relationships between this soil bacterial diversity and the functions it provides to agricultural soils and changes under various land management practices. Generally, microbial biodiversity has been observed to influence ecosystem services, but with inconsistent patterns. For example, it has been observed that a reduction in the number of bacterial species can negatively impact ecosystem functions (Delgado-Baquerizo et al., 2016). In contrast, greater diversity of bacterial species has been associated with a increased ecosystem stability (Konopka, 2009).

Agricultural management has been shown to influence soil microbiome diversity. In the Brazilian Amazon, conversion of rainforest to pasture increased the total unique observations of species (e.g., alpha diversity), but the pasture had lower species turnover (e.g., beta diversity) than the rainforest. These results indicate that the rainforest had a more heterogeneous community across samples and a less rich community within a sample (Rodrigues et al., 2013). In the conversion of grasslands in the U.K. to arable lands, no significant differences in total observed species were observed (French et al., 2017). Comparison of bacterial richness between fertilized grasslands with low plant diversity, plant species rich unfertilized grasslands, conservation grasslands, rapeseed fields, and poppyseed fields showed no variance between land use types. However, there were observations of increases in pathogenic organisms in fertilized fields (French et al., 2017). In the grasslands of tropical Laos, it was observed that after three years, diversity increased after transitioning to arable conditions (Lienhard et al., 2014). Overall, these studies indicate that land use change may have varied impact on bacterial species richness.

Generally, the impacts of restoration practices in agroecosystems on the soil microbiome are not well understood, though it has been observed that crop and prairie soils have significantly different microbial communities (Mackelprang et al., 2018). Additionally, it is estimated that it may take up to 28 years before agricultural soils with restored prairie vegetation to closely resemble remnants' bacterial community (Barber et al., 2017). As a management strategy, prairie strips are a unique conservation practice that provides benefits that are disproportionately greater than the area they occupy (Schulte et al., 2017). The diversity of plant species has been shown to alter soil bacterial community composition, but not soil bacterial diversity as indicated by the richness of species detected (Grüter et al., 2006; Prober et al., 2015). Thus, given the age of prairie strips currently installed and the associated evidence for decadal changes in bacterial diversity, differences in diversity may be limited.

To test these two hypotheses, we performed rainfall simulations and characterized the soil bacterial communities from two sites over time. Here we used three measures of alpha diversity to characterize the bacterial communities in agricultural soils and adjacent prairie strips. We tested two hypotheses: (1) that prairie conservation strip soil will increase alpha diversity compared to agricultural soil, and (2) alpha diversity measures will exhibit different responses in agricultural soil and prairie soil following rainfall. We found that there were no significant differences in alpha diversity between prairie and crop soils prior to rainfall.

3.3 Methods

3.3.1 Study Sites

Two field sites were selected with prairie strips installed at the edge of crop fields. Both field sites are on Iowa State University (ISU) research farms. The WOR research site is located southeast of the ISU campus in Ames, Iowa. It was established in Spring 2015 and sampled in 2017. The ARM research site is located near Lewis, Iowa in Southwest Iowa. It was established

in Fall 2014 and sampled in 2018. The WOR site is situated on soils with 3.9 slope percent and a major soil series is Clarion Loam. In 2017, it was under a no-till corn-soybean rotation typical of the area. The ARM site is situated on soils with slope of 6.6 percent and on major soil series of Marshal Silty Clay Loam. In 2018, it was under a no-till corn-soybean rotation typical in the area. All samples were collected in the fall following harvest of soybeans.

3.3.2 Soil Sampling

At WOR, three sampling plots were constructed along the border between the strip and the crop. Sampling plots were 3m x 1m with the long axis perpendicular to the strip/crop interface. The prairie occupied 1m of plot length while the crop occupied 2m of the plot. As part of a larger experiment (see Chapter 4), a rainfall simulation was performed on three replicated blocks between October 25th and 31st of 2017. Each plot was independently subject to simulated rainfall at a rate of 65 mm per hour until surface runoff was observed, with an additional 30 minutes of rainfall following. Soil samples were collected at six timepoints beginning with baseline samples taken before rainfall simulation and time zero samples taken approximately 30 minutes post rainfall. Subsequent samples were taken on days 2, 14, 21, and 42 post rainfall. At each sampling event, soil samples were taken along the long axis of each plot resulting in six soil transects evenly spaced through the plot. Soil cores were taken at a depth of 15 cm and split into two depths: upper 7.5 cm (D1) and lower 7.5 cm (D2) of the soil core. Along each transect, 9 samples were collected with 4 from the crop portion and 4 from the prairie portion of each plot with one sample from the prairie crop interface. At ARM, a similar rainfall simulation was performed with minor changes to the experimental design. Sampling plot dimensions were similar, except for increasing from 1 m to 1.5 m of plot length allowing for an even split between crop and prairie. Additionally, the rainfall rate was adjusted to 76 mm per hour and soil sample depths changed to 0-5 cm and 5-15 cm depths. Simulated rainfall on replicated blocks occurred

between October 24th and 26th 2018. Soil samples at baseline, time zero, day two, and day 14 were the same as WOR but the final sample occurred 153 days post rainfall. In total, 324 (3 plots x 6 timepoints x 9 soil cores x 2 depths) soil samples were taken from WOR and 270 (3 plots x 5 timepoints x 9 soil cores x 2 depths) from ARM for DNA extraction and bacterial community analysis (Figure 3-1).

3.3.3 DNA Extraction and 16S Sequencing

Soil DNA was extracted from 0.25 grams of soil from each sample with a Qiagen environmental DNA extraction kit. Extracted DNA was normalized to a concentration of 10 ng ml^{-1} in samples with concentrations greater than 10 ng ml-1. Samples below 10 ng ml-1 were not diluted and were deposited for amplicon sequencing. Amplification targeted the V4 region of the 16s rRNA gene. 10 µM each of 16S rRNA v4 region primers was used to perform amplification. The forward primer, 515F, used was GTGYCAGCMGCCGCGGTAA, and the reverse primer, 806R, used was GGACTACNVGGGTWTCTAAT. The target amplicon size was 390 bp. The specific protocol is described at https://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/16s. Sequencing of bacterial amplicons was performed on Illumina MiSeq with MiSeq Reagent Kit V2 (Illumina, San Diego, CA, USA) at United States Department of Agriculture, National Animal Disease Center (Ames, IA, USA), and sequencing libraries were comprised of 150 bp paired-end reads. Sequencing data will be deposited in the NCBI Short Read Archive (SRA).

3.3.4 Bioinformatic Analysis

The DADA2 pipeline was used to process the sequencing libraries using the R statistical language. Default parameters were used from the DADA2 pipeline and are described in the DADA2 pipeline tutorial (version 1.14) (Callahan et al., 2016). 16S rRNA sequences were filtered based on sequence library quality scores, taxa in each sample were assigned as amplicon

sequence variants (ASVs) and describe the taxa present in each sample. Taxa were assigned to each ASV through alignment with the Silva 16S rRNA gene database, v124 (Pruesse et al., 2007). Samples with less than 1,000 reads per sample were removed from the analysis, resulting in 317 bacterial communities from WOR and 269 from ARM. Alpha diversity and statistical analyses were done using R, complete code used for analysis and figure generation available at jflater.github.io/StripsDiversityWebsite/. Species richness, Shannon index, and Simpson's index were generated using the *estimate_richness()* function from the Phyloseq package (version 1.32.0) (McMurdie and Holmes, 2013). Beta diversity was estimated using the Bray-Curtis dissimilarity matrix and the function *ordinate()* from Phyloseq. To test significance between comparisons of alpha diversity, Wilcoxon's test was used to account for the varying number of samples between grouping comparisons and plots were generated using the ggpubr package in R. To test for significance in Beta diversity, the nonparametric analysis of variance function *adonis()* was applied to the Bray-Curtis distance matrix, this function is from the Phyloseq package in R.

3.4 Results

In total, we identified 45,241 unique taxa (minimum and maximum sample depth of 1,401 and 73,565 reads, respectively). These taxa were used to estimate the soil bacterial diversity of prairie strips and adjacent crops at both studied sites. To characterize diversity, we estimated both alpha (within sample) and beta (between samples) diversity. Within alpha diversity, three indices were used to estimate within sample diversity. Specifically, we estimated richness (total observed species), Shannon, and Simpson alpha diversity. The Shannon index assumes all species are represented in a sample and increases as both the richness and evenness of a community increase. The Simpson index gives more weight to dominant species and thus takes into account the number of species present and the relative abundance of each species. The

Simpson index is indicative of the likelihood that two sequences randomly chosen belong to the same classification. The value ranges from 0 to 1 and increases as communities become less diverse. Between the two sites, the alpha diversity of ARM soil microbial communities was generally greater than WOR communities in these samples (Table 2). This difference in observed alpha diversity between sites was the largest within the treatments in this study. The observation that ARM was associated with higher alpha diversity was consistent with samples from both depths, regardless of alpha diversity estimate. Prior to any simulated rain event, we observed no significant differences in alpha diversity between crop and prairie strip soil at either the ARM or WOR site (Figure 3-2). At WOR, D1 samples were associated with increased and more variable richness than D2 samples, and increased Shannon's diversity and Simpson's diversity than D2 samples (Figure 3-2). These results suggest that though richness might be increased in D1 soils, D2 soil communities are less even and not as dominated by few species as D1 soils. Overall, the ARM site exhibited greater similarity in alpha diversity measures from D1 and D2 soils than the WOR site (Figure 3-2).

At both WOR and ARM, we simulated a rainfall event to evaluate the impact of an environmental disturbance on the microbiome of crop and strip soils. Generally, we observed that rainfall had a significant impact on alpha diversity, but the magnitude and direction of this impact was dependent on the site. After rain simulation, we found that WOR prairie soils had several significant changes in diversity over time (Figure 3-3). Overall, the alpha diversity in WOR D1 soils fluctuated in its response to rainfall, with a general increase in all three alpha diversity indices after the rainfall event up to day 2 and from day 14 to day 21. This pattern was generally also observed in WOR D2 soils but with less magnitude. The greatest difference in WOR soil communities after rainfall was observed on day 2 in D1 soils. At this time, alpha

diversity of the prairie strip was significantly greater than crop soils. The significant difference in alpha diversity in strip and crop soils was not observed to be long lasting, evidenced by crop soil communities exhibiting similar alpha diversities to prairie strip soils 21 days after rainfall, except for crop soils having significantly higher Simpson diversity on day 42.

At the ARM site, we observed a very different response to rainfall relative to WOR soils (Figure 3-4). After the rainfall simulation, alpha diversity estimates were to decrease across richness, Shannon, and Simpson estimates. Prairie strips and crop soils were observed to share similar alpha diversity after the simulated rainfall. The magnitude of the observed change of alpha diversity at ARM was also lower than WOR. Like WOR soils, D1 and D2 soils of ARM shared similar profiles of alpha diversity over time, with the largest changes observed in D1 soils. We observed that there were more significant shifts from sample day to sample day within prairie strips and crop soils at both sites, though with the most observable differences at the prairie strip soils at the WOR site (Figure 3-5, 3-6 and Figure 3-7, 3-8). At WOR, we observed that species richness had more significant changes in both prairie and crop than either the Simpson or Shannon indices and that these changes were most significant in D1 relative to D2 soils. At the ARM site, we observed less changes over time, with the largest change occurring right after the simulated rain event. Overall, alpha diversity estimates consistently demonstrated differences of within sample diversity between sites and in response to rain events.

We next evaluated if the microbial composition between sites and soil types within sites were different. Consistent with observations of alpha diversity, we observed that the specific membership microbial communities associated with WOR and ARM were significantly different. These results are evident in the Bray-Curtis dissimilarities estimated between samples community composition (Figure 3-9, 3-10, 3-11). Within WOR soils, we observed significant

differences in microbial community composition between prairie strips and adjacent crop D1 and D2 soils (Table 1) prior to the rainfall simulation. These soil bacterial communities were observed to be different after rainfall but depended on the day of sampling and also the depth. For WOR D1 soils, microbial communities were observed to be significantly different between prairie strips and crop soils on Day 2 and Day 21 (Table 1). For the D2 soils, there were significant differences in community composition after rainfall and up to day 2. After Day 2, communities were observed to not be significantly different. Unlike the WOR soils, ARM prairie strip and crop soils were observed to be similar prior to the rainfall simulation and were only observed to be different on Day 2, Day 14, and Day 153 after rainfall. There were no significant differences in community dissimilarity of the D2 soil communities from crop and prairie over time (Table 1).

3.5 Discussion

The installation of prairie strips adjacent to crop soils has been found to provide benefits associated with the increased diversity of prairie strips. Here, we studied the impacts of prairie strip installations on below ground microbial diversity at two different sites and two different depths. Additionally, we examined the impacts of rainfall on this diversity. Generally, we found that the largest differences in diversity were observed in our two different sites. These results were observed in both within sample and between sample measurements of diversity. These results are consistent with differences in the locations and soils of these sites. The WOR site is located on the Des Moines lobe (loamy Wisconsin glacial till) with Clarion Loam soils and prairie strips were 2.5 years of age at the time of sampling. The ARM site is located on the Southern Iowa Drift Plain (loess with glacial till outcrops) with Marshal Silty Clay Loam soils and prairie strips were 4 years of age at the time of sampling.

We also observed differences between prairie strips and adjacent crop soils at both sites, but these differences were not consistently observed. Within each site, the alpha diversity between prairie strips and crop soils were generally not observed to be different. The microbial community turnover was measured by beta-diversity and contrasted between prairie strips and crop soils. These results suggest that the composition and not number of specific taxa in these soils is import in distinguishing the microbial communities of prairie strips and soils. The significance of the contrasting microbial communities was observed in our experiment in response to simulated rainfall events, where we observed both sites and soils to have contrasting responses. The largest observed difference occurred two days post simulated rainfall, where prairie communities in D1 soils were significantly more diverse and had higher turnover than crop communities at the WOR site. Prairie strip and crop soils at ARM were not associated with as many significant differences, though were different after rainfall. Generally, we observed that the shallower D1 soils rather than the deeper D2 soils had more dynamic responses, with more variability and change. Overall, these results highlight that soil characteristics and site history have a larger influence on diversity than differences in soil management (prairie vs. crop). Despite that, we observe that prairie strips can have a significantly different response to rainfall than agricultural soils.

Our observations are consistent with previous research supporting that bacteria are limited in their capacity to independently move through the environment and generally move with water flow (Abu-Ashour et al., 1994; Wong and Griffin, 1976). As a result, their dynamics in soils without environmental changes may be relatively consistent. The installation of prairie strips introduces a significant change to the soil. New vegetation is planted on what has often been under agricultural production for a relatively long period. Our results that crop and prairie

soils are largely similar within a site is consistent with prairie strip associated soil bacteria developing from long-term crop associated communities. Similarly, with the introduction of prairie strips, new species, nutrients, or soils may change and alter the existing soil community. Our results suggest that these changes are depending on the existing soil and environmental perturbations. We observed that rain events could significantly alter communities, which is consistent with the importance of soil moisture in previous studies (Bickel and Or, 2020; De Menezes et al., 2015; Griffin et al., 2020). We speculate that a major contribution to the observed differences between prairie strips and soils is related to the age of the installed prairie strips, the amount of time for interactions with strips, and below ground communities. A previous study of grasslands from 1 to 28 years old found that younger and older prairies had significantly different bacterial communities comparatively (Barber et al., 2017). This supports the idea that older prairie strips may have a different impact on soils than younger ones. It has also been found that restored prairies moved toward convergence with remnants, suggesting soil communities respond to prairie installations. Another study comparing crop and native prairie alpha diversity between sites under long term cultivation to native prairie showed no significant differences between crop and prairie, highlighting the stability of alpha diversity in the bacterial community (Mackelprang et al., 2018). These results are consistent with our observations that alpha diversity is more consistent between prairie strips and crops than beta diversity and the importance of community composition in evaluating their differences.

Based on our results, future experiments should be carefully designed to also consider site specific differences. Future research could be directed to study specifically the impacts of prairie strip ages. An opportunity for future characterization of the impacts of prairie strips and adjacent crop soils on microbial communities is their changes, both through a season and through

years of installation. In this study, we capture up to 153 days of a comparison of two sites limiting observation of any longer-term benefits of prairie strips. This amount of time is a small snapshot of soil communities, and we are not fully capturing the potential differences between two systems across growing seasons. Another direction for future research is to observe the benefits to the agroecosystem or water quality of these differences in communities.

In conclusion, our analysis of soil diversity under differing management, specifically agricultural and conservation management, suggest that young prairie strips are similar to crop soils yet can have a distinct response to rainfall events. These results are in agreement with past studies indicating decadal time scales for permanent changes in prairie communities when converting from crop soil communities. Further, our analysis was restricted to the fall season, possibly suggesting that monitoring microbial communities throughout the growing season may reveal more differences in bacterial diversity, particularly due to differences in the growing season of prairie plants compared to agricultural crops. Our results help contribute to scientific understanding of how management decisions impact diversity, specifically the installation of prairie conservation strips on altering soil microbial communities. While more research is needed, our observations suggest that there is an impact of prairie strips on diversity. A direction for future research is quantifying the benefits to the agroecosystem or water quality of these differences in communities.

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3.7 Figures and Tables

Figure 3-1: Schematic of soil samples collected at WOR and ARM, with alterations made to plot width at ARM: cropland and strips were each 1.5 m in length. Additional transects of soil samples were collected adjacent and parallel to the first sampling.

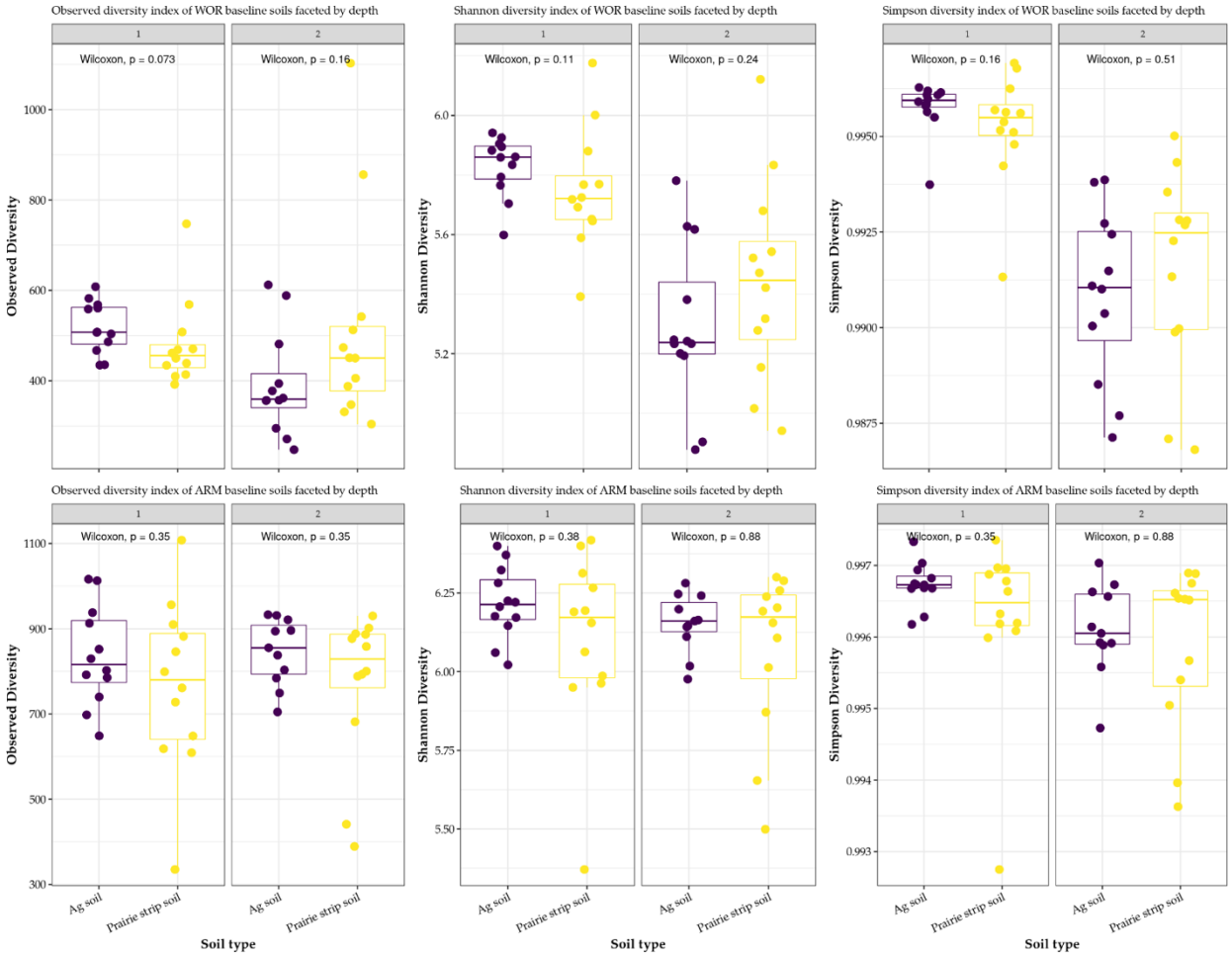

Figure 3-2: Alpha diversity means of baseline soil communities. Top row: WOR. Bottom row: ARM. From left to right: Observed, Shannon Index, and Simpson Index. Comparison of Agricultural soil (purple) and Prairie soil (yellow). Soil core depth 1 on left panel and soil core depth 2 on right panel of each plot.

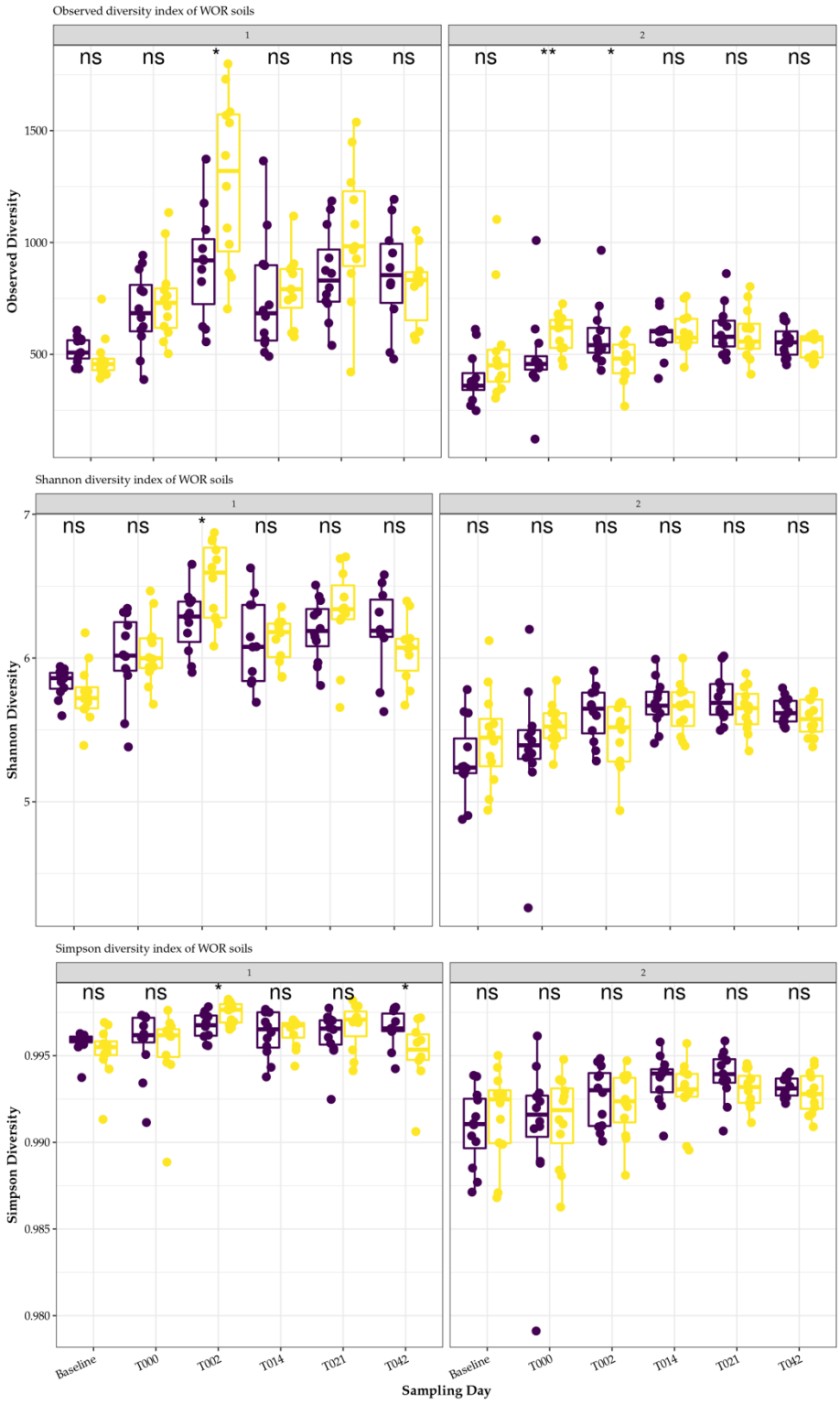

Figure 3-3: Alpha diversity of WOR soil communities over time. Pairwise comparisons made between ag soil (purple) and prairie soil (yellow). Top to bottom panel is observed diversity, Shannon Index, and Simpson Index. Left to right is depth 1 and depth 2. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

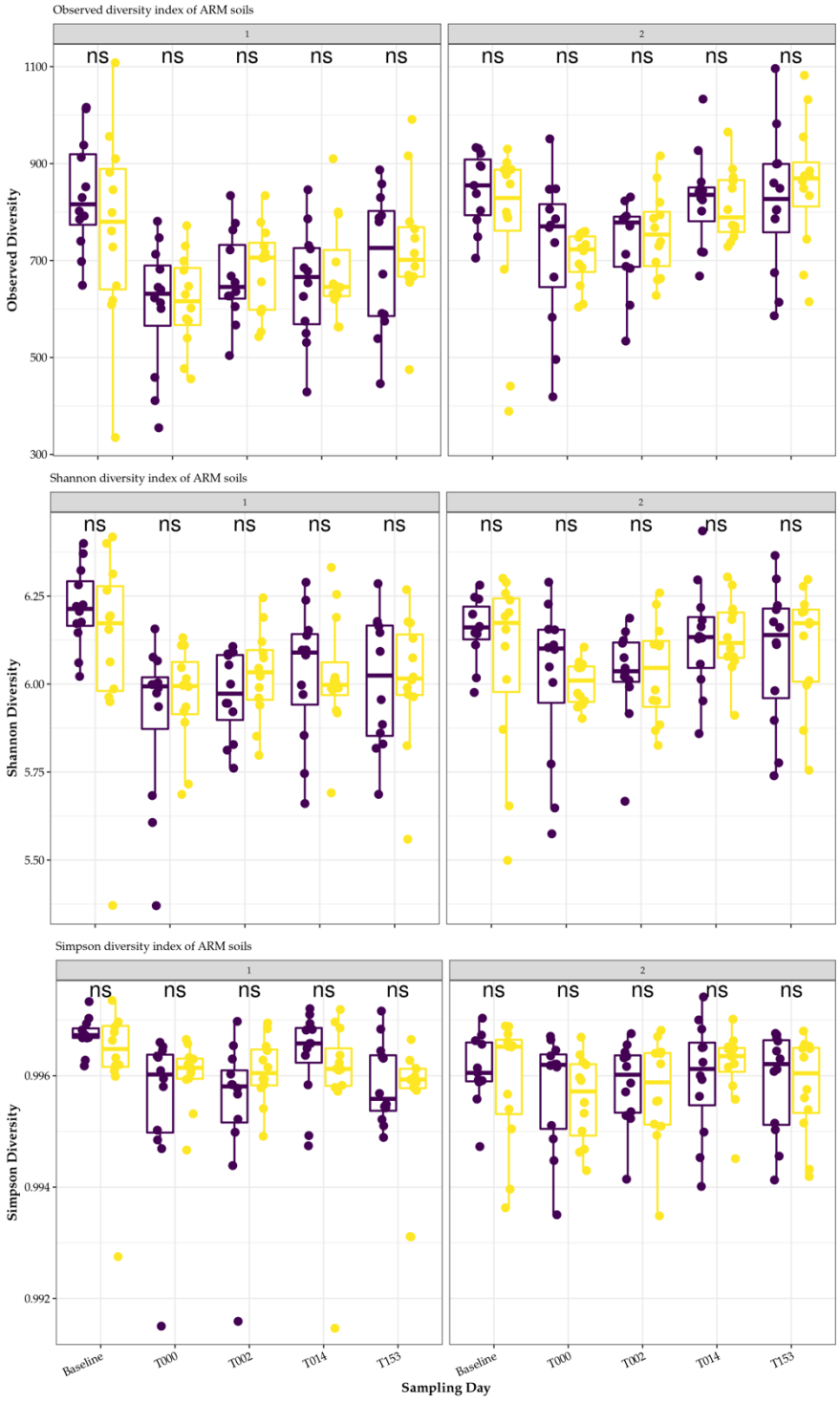

Figure 3-4: Alpha diversity of ARM soil communities over time. Pairwise comparisons made between ag soil (purple) and prairie soil (yellow). Top to bottom panel is observed diversity, Shannon Index, and Simpson Index. Left to right is depth 1 and depth 2. ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

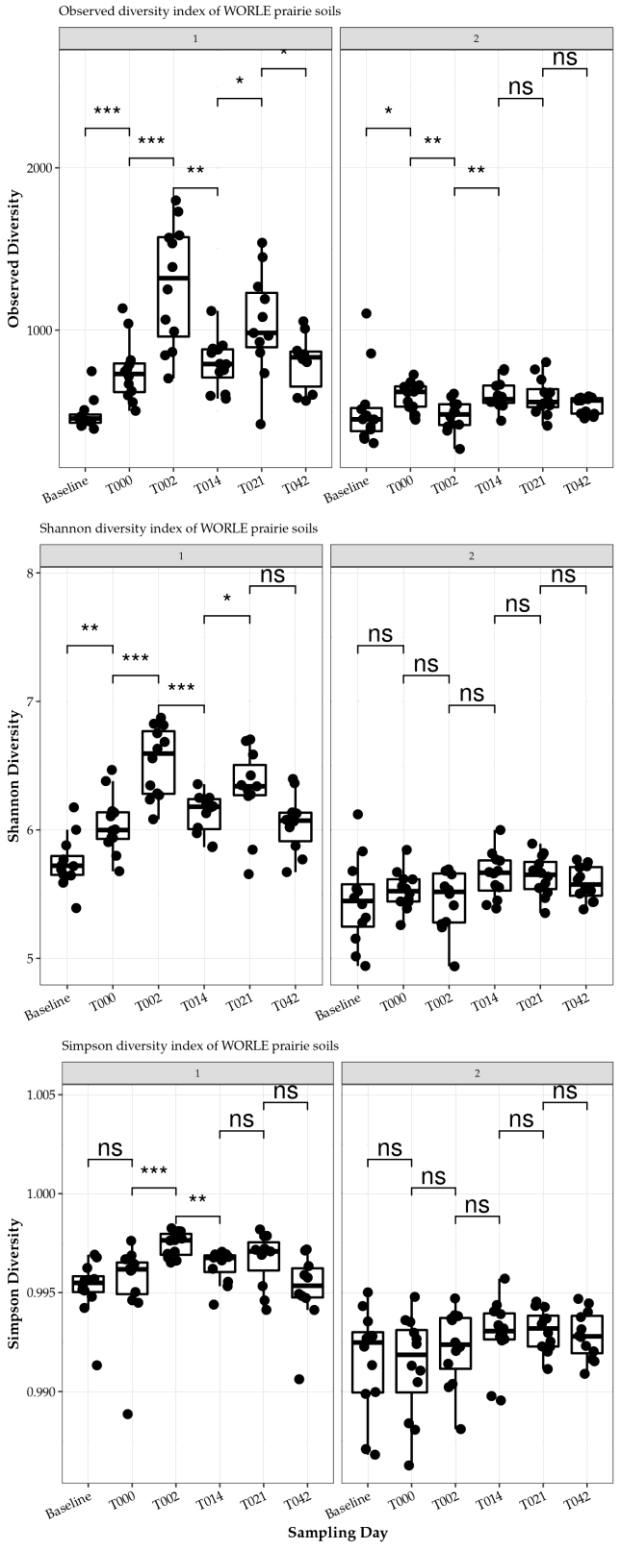

Figure 3-5: Alpha diversity in WOR prairie soils. Top to bottom: Observed, Shannon Index, Simpson Index. From left to right: depth 1 and depth 2. Pairwise comparisons ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

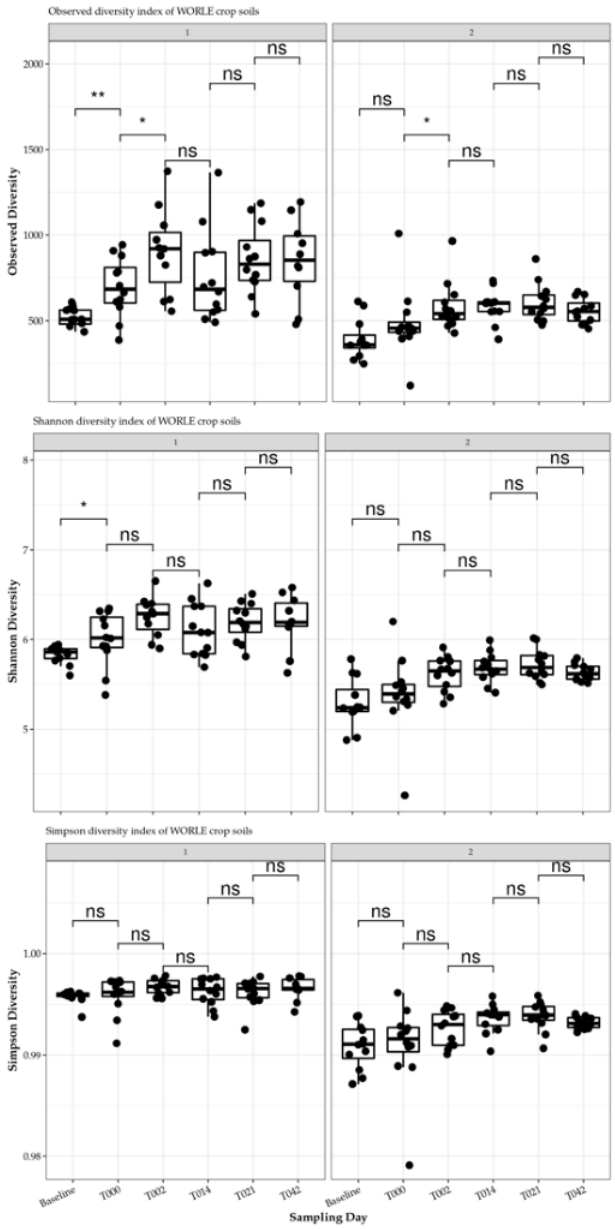

Figure 3-6: Alpha diversity in WOR crop soils. Top to bottom: Observed, Shannon Index, Simpson Index. From left to right: depth 1 and depth 2. Pairwise comparisons ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

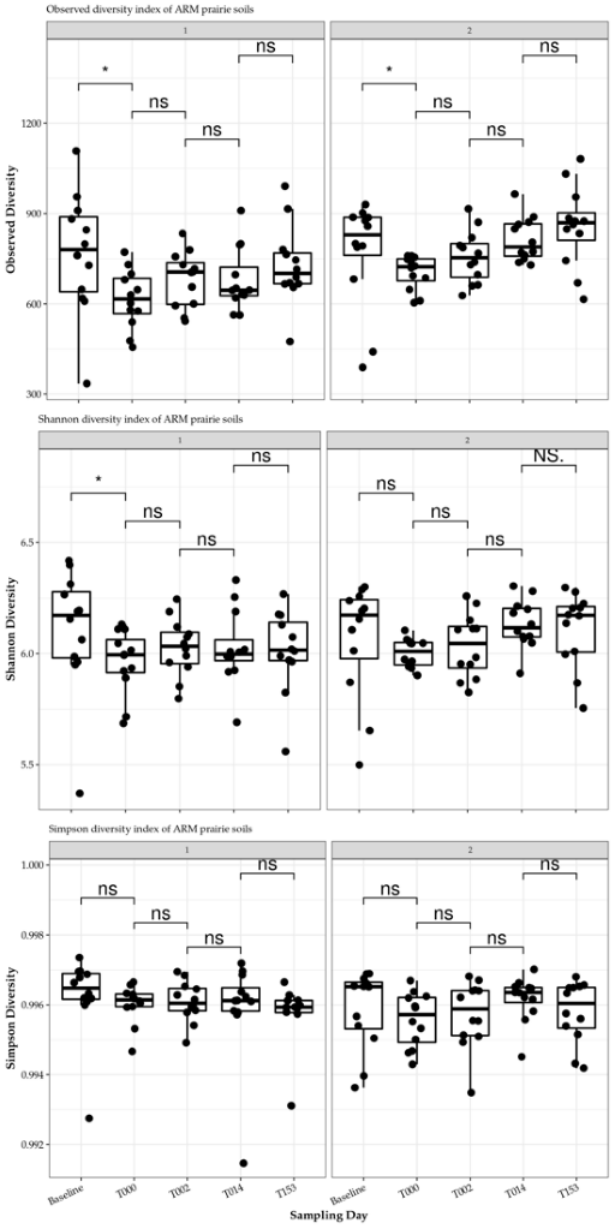

Figure 3-7: Alpha diversity in ARM prairie soils. Top to bottom: Observed, Shannon Index, Simpson Index. From left to right: depth 1 and depth 2. Pairwise comparisons ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

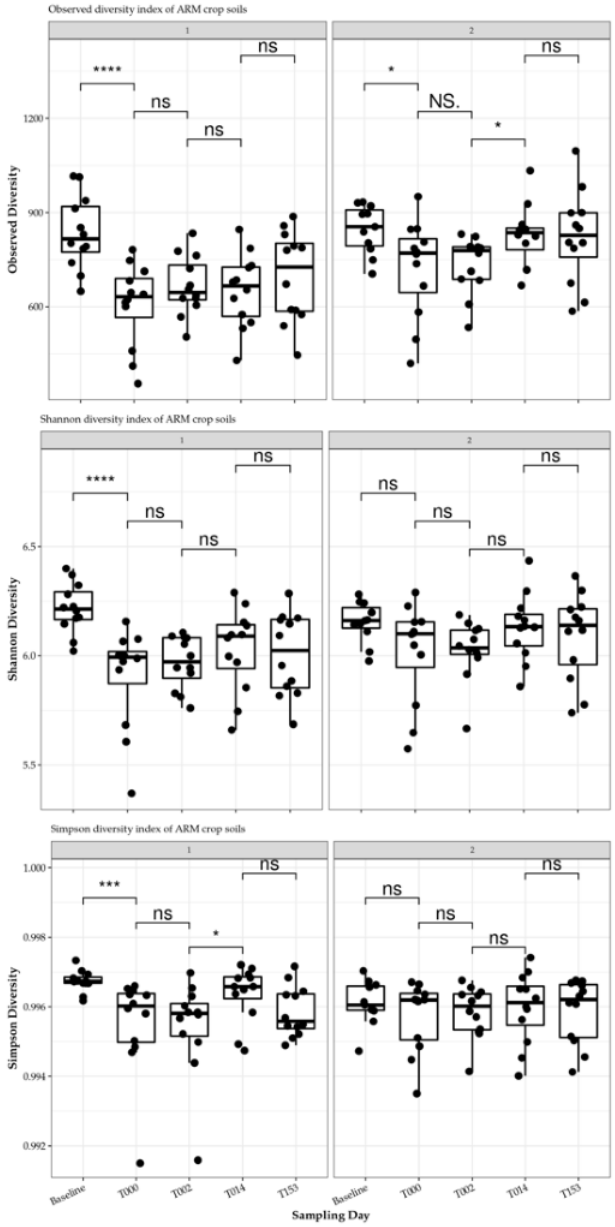

Figure 3-8: Alpha diversity in ARM crop soils. Top to bottom: Observed, Shannon Index, Simpson Index. From left to right: depth 1 and depth 2. Pairwise comparisons ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

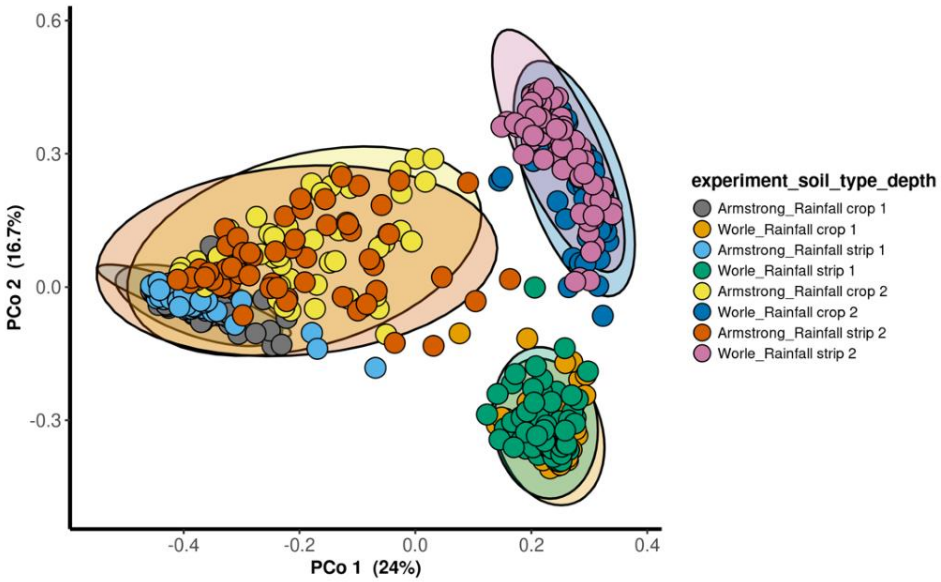

Figure 3-9: PCoA ordination of the Bray-Curtis dissimilarity matrix showing clustering of soil communities from both sites. Grouped by site, soil type, and depth.

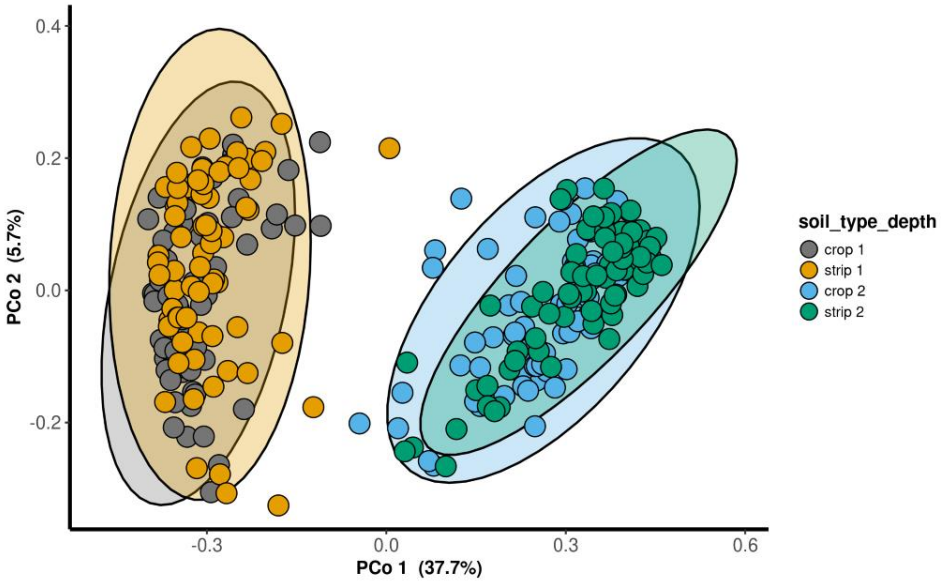

Figure 3-10: PCoA ordination of the Bray-Curtis dissimilarity matrix showing clustering of soil communities from WOR site. Grouped by site, soil type, and depth.

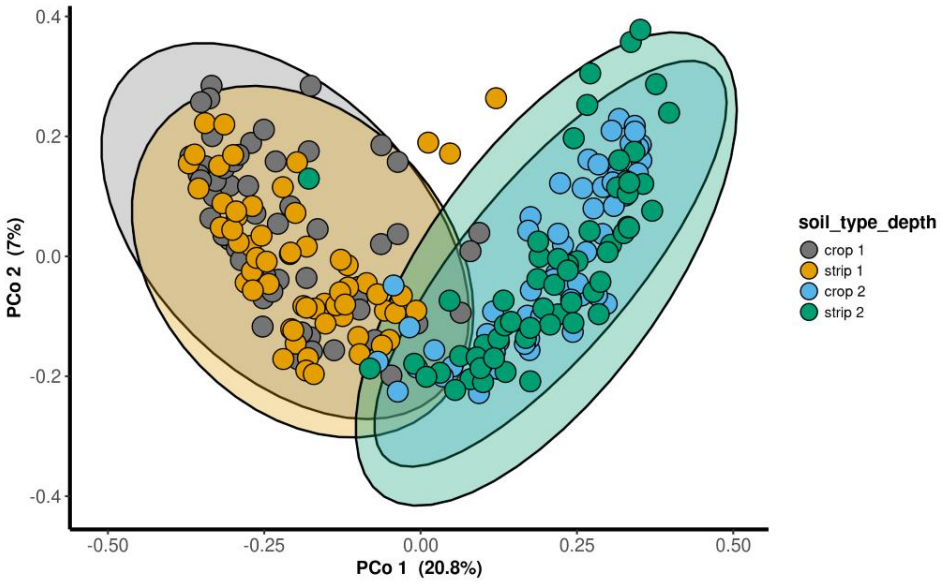

Figure 3-11: PCoA ordination of the Bray-Curtis dissimilarity matrix showing clustering of soil communities from the ARM site. Grouped by site, soil type, and depth.

Table 3-1: NPMANOVA significance in Bray-Curtis dissimilarity. Comparison is between crop and strip soil for each site, depth, and day. NS = not significant and NA = not applicable (i.e. no samples on that day at that site).

	Baseline	Day 0	Day 2	Day 14	Day 21	Day 42	Day 153
WORD1	Pr(>F): 0.025	NS	Pr(>F): 0.007	NS	Pr(>F): 0.010	NS	NA
WORD2	Pr(>F): 0.021	Pr(>F): 0.002	Pr(>F): 0.042	NS	NS	NS	NA
ARMD1	NS	NS	Pr(>F): 0.012	Pr(>F): 0.048	NA	NA	Pr(>F): 0.001
ARMD2	NS	NS	NS	NS	NA	NA	NS

Table 3-2: Mean of reads, and the three diversity indices for each group of site, soil type, day, and depth.

Site	Soil type	Day	depth	Mean Sample Reads	Mean Richness	Mean Shannon Index	Mean Simpson Index	Number Of Samples
ARM	crop	Baseline	1	17785.33	835.67	6.22	0.99674	12
ARM	crop	Baseline	2	20731.00	846.27	6.15	0.99611	11
ARM	crop	T000	1	11276.33	605.83	5.90	0.99551	12
ARM	crop	T000	2	15281.92	723.33	6.02	0.99574	12
ARM	crop	T002	1	13346.75	665.42	5.97	0.99543	12
ARM	crop	T002	2	16613.92	733.92	6.03	0.99585	12
ARM	crop	T014	1	12548.75	651.25	6.03	0.99637	12
ARM	crop	T014	2	19634.42	826.25	6.13	0.99597	12
ARM	crop	T153	1	13302.42	695.67	6.01	0.99586	12

Table 3-2 continued

Site	Soil type	Day	depth	Mean Sample Reads	Mean Richness	Mean Shannon Index	Mean Simpson Index	Number Of Samples
ARM	crop	T153	2	17940.33	821.33	6.09	0.99588	12
ARM	strip	Baseline	1	16196.50	766.67	6.11	0.99626	12
ARM	strip	Baseline	2	17975.00	769.58	6.07	0.99587	12
ARM	strip	T000	1	10800.08	615.75	5.96	0.99602	12
ARM	strip	T000	2	14722.08	703.50	6.00	0.99557	12
ARM	strip	T002	1	13750.67	681.17	6.03	0.99609	12
ARM	strip	T002	2	17472.50	756.08	6.04	0.99572	12
ARM	strip	T014	1	13906.92	679.33	6.03	0.99590	12
ARM	strip	T014	2	19542.83	813.83	6.13	0.99618	12
ARM	strip	T153	1	14298.42	727.83	6.01	0.99556	12
ARM	strip	T153	2	20459.50	856.67	6.11	0.99580	12
WOR	crop	Baseline	1	11686.42	518.42	5.83	0.99577	12
WOR	crop	Baseline	2	10670.00	391.67	5.29	0.99085	12
WOR	crop	T000	1	16518.92	694.92	6.00	0.99581	12
WOR	crop	T000	2	13158.83	485.42	5.38	0.99084	12
WOR	crop	T002	1	24851.64	902.00	6.25	0.99671	11
WOR	crop	T002	2	18223.75	584.25	5.61	0.99263	12
WOR	crop	T014	1	19167.75	753.58	6.10	0.99626	12
WOR	crop	T014	2	16671.17	583.50	5.68	0.99360	12
WOR	crop	T021	1	21227.25	857.83	6.19	0.99618	12
WOR	crop	T021	2	16993.58	606.08	5.72	0.99383	12
WOR	crop	T042	1	21809.80	850.70	6.19	0.99655	10
WOR	crop	T042	2	15658.08	556.08	5.63	0.99319	12
WOR	strip	Baseline	1	10232.25	480.42	5.75	0.99524	12
WOR	strip	Baseline	2	15861.00	513.75	5.44	0.99155	12
WOR	strip	T000	1	19116.17	745.75	6.04	0.99546	12
WOR	strip	T000	2	19686.92	594.42	5.53	0.99129	12
WOR	strip	T002	1	42213.08	1277.25	6.53	0.99744	12
WOR	strip	T002	2	13805.33	470.33	5.45	0.99222	12
WOR	strip	T014	1	20210.50	792.08	6.13	0.99635	12
WOR	strip	T014	2	18789.67	600.75	5.64	0.99290	12
WOR	strip	T021	1	31502.09	1038.27	6.31	0.99666	11
WOR	strip	T021	2	17219.67	588.33	5.64	0.99309	12
WOR	strip	T042	1	23892.90	800.40	6.05	0.99515	10
WOR	strip	T042	2	15749.25	536.67	5.58	0.99284	12

CHAPTER 4. IMPACT OF PRAIRIE STRIPS ON TRANSPORT OF POULTRY MANURE-ASSOCIATED BACTERIA AND ANTIBIOTIC RESISTANCE GENES

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4.1 Abstract

The use of antibiotics in animal agriculture has led to an increase in antibiotic resistance genes and resistant bacteria near areas of animal production and in animal manure. While animal manure is a valuable nutrient resource for agricultural production, it is also a potential vector for the transport of antibiotic resistance genes (ARGs) and bacteria (ARBs) and subsequently antibiotic resistance to the environment. To manage water quality in agricultural settings, prairie strips have been proposed as an effective conservation practice and have shown several benefits, including reducing nutrient loss, increasing ecosystem health, and improving biodiversity. We hypothesize that prairie strips as a field treatment can also attenuate the transport of ARGs and ARBs in surface runoff following rainfall events in manured agricultural fields. We performed two rainfall simulation experiments comparing the impacts of prairie strip installations on ARBs and ARGs associated with poultry or swine manure in soils and water. Soil and surface runoff were collected before and following manure application, as well as during a rain simulation event, resulting in 1,380 soil samples and 108 water samples from the two experiments. The bacterial community and over 300 ARGs were characterized in these samples by 16s sequencing and microfluidic qPCR.

Our results identify distinct microbial communities unique to crop soils, prairie strip soils, and manure. These manure-associated bacteria were identified in soil receiving manure but

were not found to be transported to adjacent prairie strip soils by surface runoff. Further, we observed reductions in ARG and ARB concentrations in runoff water from plots with prairie strips compared to plots without prairie strips, despite significant concentrations of ARGs in the manure. For example, the ARGs *tet(M)* and *tet(T)* were detected in the runoff water in 33% of plots with prairie strips compared to 66% of plots with no prairie strips. We conclude that prairie strips attenuate the transport of ARGs and ARBs to the wider environment in agroecosystems. These findings add to the benefits associated with prairie strips used as a conservation practice.

4.2 Introduction

Antibiotics are crucial for fighting infectious diseases caused by bacteria. Antibiotics are used frequently in animal production for medical purposes and formerly for growth promotion. In 2009, the annual use of antibiotics was quantified from a producer survey and reported that 533,973 kg of chlortetracycline, 165,803 kg of tylosin, and 154,973 kg of oxytetracycline was found in the feed of swine facilities (Bush et al., 2012). In 2015, antibiotics used in animal agriculture make up an estimated 70% of all antibiotic used in the United States (Food and Drug Administration, 2015). In 2015, the introduction of the veterinary feed directive (VFD) limited the use of antibiotics, those important for human health, in animal agriculture for the sole purpose of promoting growth. However, antibiotics are still allowed for medical purposes in animal agriculture, with veterinary approval (Food and Drug Administration, 2015). The use of antibiotics for animal production have raised concerns about its role in the spread of antibiotics into the environment (Cloud-Hansen et al., 2010; Han et al., 2018). Land application of poultry manure leads to transport of fecal bacteria to surface waters in concentrations that exceed Federal standards (Soupir et al., 2006). Fecal bacterial attachment to soil particles and associated sediment loss during rainfall highlight the potential for sediment associated transport of fecal bacteria (Soupir et al., 2010). Previous studies have shown that repeated applications of manure

from antibiotic treated animals to farm fields has resulted in an increase in the number of antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARBs) present in soil (Heuer et al., 2011).

Poultry animal production in the US used 892,882 kg of medically important antibiotics in 2018, representing 15% of medically important antibiotic use in animal agriculture (FDA, 2018). Poultry production results in significant manure production, and the use of poultry manure to fertilize soils serves to dispose of manure as waste and provide nutrients to soils. However, this manure can act as a reservoir of antibiotic resistance genes (ARGs) and its use in fields represents a potential vector for ARG transport to the wider environment. Poultry manure has been previously found to harbor ARGs, such as Aminoglycoside, Beta lactamase, Macrolide-Lincosamide-Streptogramin B, multidrug, Sulfonamide, Tetracycline, and Vancomycin (Zhang et al., 2017). Following land application, several studies have identified increases in soil ARGs from poultry manure (Han et al., 2018). Fewer studies have examined the distribution of poultry manure associated ARGs in surface water as a result of agricultural amendment, but some have showed that ARGs and bacteria from poultry manure are readily transported to surface and ground waters, highlighting the threat of pathogens and ARGs entering the environment (Hubbard et al., 2020; Soupir et al., 2006). The presence of mobile genetic elements in manure and potential horizontal gene transfer, suggests that reduction of pathogenic and fecal bacterial transport may not sufficiently reduce the threat of ARG spread to soil organisms (Klümper et al., 2015). Manure associated ARGs persist in soil for extended periods following manure application, studies show a range of 120-130 days following application, suggesting that transport from soil to water may persist for extended periods following manure application (Han et al., 2018; Zhang et al., 2017).

Soil characteristic can impact the spread of ARGs in the environment. For example, native soil bacterial communities can impact the persistence of ARGs. Irradiation of soils to kill native communities resulted in significantly higher tetracycline resistance genes when compared to non-irradiated soils, implying an effect of native bacteria on ARGs (Pérez-Valera et al., 2019). In addition to native bacterial communities, physical properties of the soil may influence the amount of ARGs that could be found. A previous study found that ARG presence showed a positive correlation with the concentrations of Ca, Na, and P in the soil, which suggest that soil chemical properties may affect ARG abundance, suggesting that conservation practices that influence soil characteristics may subsequently impact ARG presence (Cadena et al., 2018). Moisture conditions in the soil can influence where ARBs and ARGs are deposited in the soil, saturated soils may result in rapid transport of bacteria to the vadose zone (McMurry et al., 1998). Further, soil macropores in well-structured soils are likely paths for bacterial movement into the soil profile and groundwater (Unc and Goss, 2003).

The observation that soil management can impact the spread of ARGs/ARBs presents a potential opportunity to use land management practices to reduce its spread. Specifically, land management at the edge of crop fields may be an opportunity to influence the fate and spread of ARBs. For example, grass buffers were shown to attenuate manure associated ARG transport when installed in crop fields in Nebraska. The presence of narrow switchgrass (Panicum virgatum L.) buffers effectively reduced the abundance of 16s rRNA and *erm*(b) genes in runoff water following application of swine manure (Soni et al., 2015).

Prairie strips are another conservation practice and have been observed to provide benefits to biodiversity, sediment loss, and water quality when installed at the edge of a field. These narrow strips of native grasses and forbs are strategically planted in row crop fields to

replace 5 to 25 percent of the total area (Schulte et al., 2017). Early research shows a multi-faceted benefit for this practice, with increases in diversity and richness of multiple species from avian to mammal (Iqbal et al., 2015; Liebman and Schulte, 2015; Schulte et al., 2017; Zhou et al., 2014). These results suggest that these strips would also influence underlying bacterial diversity and potentially ARBs.

In this study, we characterize the impacts of prairie strips on the transport of poultry manure-associated bacteria and antibiotic resistance genes. This study has two primary objectives, the first is to understand the distribution of ARGs and bacteria in poultry manure and its movement from manured-crop soil into prairie strip soils following simulated rainfall. The second is to understand the role of prairie strips, when interspersed in row-crops, to attenuate the transfer of poultry manure associated bacteria and antibiotic resistance genes through surface runoff water. To evaluate the potential spread of poultry associated bacteria and ARGs following manure application, we performed a rainfall simulation on agricultural plots bordered by prairie strips following manure application. We hypothesize that poultry manure will introduce manure associated ARGs and bacteria into the soil, and the presence of prairie strips will decrease abundance of antibiotic resistance genes in soil and runoff water when compared to plots without prairie strips.

4.3 Methods

4.3.1 Study Sites

A field site was selected with prairie strips installed as an in-field conservation practice. This WOR research site is on an Iowa State University (ISU) research farm and is located southeast of the ISU campus in Ames, Iowa. WOR prairie strips were established in Spring 2015 and sampled in 2017 for this study. The site is situated on soils with a slope of 3.9 percent and a

major soil series of Clarion Loam. In 2017, WOR was under a no-till corn-soybean rotation typical of the area. The experiment was performed following harvest of soybeans.

4.3.2 Manure application

Poultry manure was surface broadcast at a rate of 3.5 tons acre $^{-1}$ before simulated rainfall was applied. Manure was lightly incorporated into the surface soil using a gravel rake.

4.3.3 Samples of Soil and Water

Six plots were constructed along the border between strip and crop sections at WOR and divided into strip + crop treatments, three receiving manure and the other three no manure. Three additional sampling plots were constructed in the adjacent cropland, approximately 10 m from the prairie strip, and assigned the treatment of crop + manure. Sampling plots were 3m x 1m with the long axis perpendicular to the strip/crop interface. The nine plots were grouped into three replicated blocks with the treatments of crop + manure (WCM), crop + manure + prairie strip (WCSM), and crop + strip without manure (WCS). The prairie occupied 1 m of the plot length, while the crop occupied 2 m of the plot in the WCS and WCSM treatments. Rainfall simulation was performed on the three replicated blocks between October 25th and 31st of 2018. Each plot was independently subject to simulated rainfall at a rate of 65 mm per hour until surface runoff was observed, with an additional 30 minutes of rainfall following (Kovar et al., 2011). Soil samples were collected at six timepoints beginning with baseline samples taken before rainfall simulation. We refer to samples taken approximately 30 minutes post rainfall as “time 0” samples. Subsequent soil samples were taken on days 2, 14, 21, and 42 post rainfall. At each sampling event, soil samples were taken along the long axis of each plot resulting in six soil transects evenly spaced through the plot. Soil cores were taken to a depth of 15 cm and split into two depths, upper 7.5 cm and lower 7.5 cm of the soil core. Along each soil transect, a total of 9 samples were collected, with 4 from the crop portion, 4 from the prairie portion, and one at the

prairie-crop interface portion of each crop for WCSM and WCS plots. In WCM plots, 5 samples were collected. Runoff water samples were collected when runoff achieved steady flow and were taken every five minutes for 30 minutes. In total, 552 soil samples (6 plots x 9 transect samples x 6 timepoints x 2 depth and 3 plots x 5 transect samples x 6 timepoints x 2 depths), 54 water samples, and 3 poultry manure samples were taken for this study. For HTqPCR analysis, soil sample DNA was combined according to in plot location and vegetation type. For example, soil DNA from WCM treatment was combined to reduce the number of samples from 5 to 2. For plots in the WCSM and WCS treatment, samples within the cropland and samples within the prairie strip were combined and reduced from 4 to 2, the sample from the prairie-crop interface were kept independent. See supplemental data for sample layout and combination scheme used for HTqPCR and sample data table (Figure 4-12, Table 4-2).

4.3.4 DNA extraction and 16s rRNA sequencing

Soil DNA was extracted from 0.25 grams of soil and manure samples with a Qiagen environmental DNA extraction kit. Water DNA was extracted from runoff water samples after filtering through 0.22 µm filter with Qiagen environmental DNA extraction kit. DNA was normalized to a concentration of 10 ng ml^{-1} in samples with concentrations greater than 10 ng ml^{-1} samples below 10 ng ml^{-1} were left at their initial concentration, following normalization DNA was submitted for amplicon sequencing. Amplification targeted the V4 region of the 16s rRNA gene. 10 µM each of 16S rRNA v4 region primers was used to perform amplification. The forward primer, 515F, used was GTGYCAGCMGCCGCGGTAA, and the reverse primer, 806R, used was GGACTACNVGGGTWTCTAAT. The target amplicon size was 390 bp. The specific protocol is described at https://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/16s. Sequencing of bacterial amplicons was performed on Illumina Miseq with Miseq Reagent Kit V2 (Illumina, San Diego, CA, USA) at United States Department of Agriculture, National Animal

Disease Center (Ames, IA, USA), and sequencing libraries were comprised of 150 bp paired-end reads. Sequencing data will be deposited in the NCBI Short Read Archive (SRA).

4.3.5 Detection of ARGs

DNA from poultry manure and soils without manure was initially screened against a suite of 384 ARGs using high-throughput qPCR as previously described (Stedtfeld et al., 2018). Based on detection, 32 ARG probes were selected because of their presence in poultry manure and absence in soils. A total of 32 probes against 114 soil, 54 water, and 3 manure sample reactions were performed for detection of ARGs using high throughput qPCR. Standards for 32 probes were also included for absolute quantification of these targets. Fluidigm Biomark HT-qPCR assays were run on a total of 3 96.96 Fluidigm Dynamic Array Integrated Fluidic Circuits (IFCs)(Fluidigm) according to the manufacturer's Evagreen protocol without pre-amplification. Standard curves were generated for every probe set using 3 technical replicates of 10-fold dilutions of synthetic oligonucleotide standards ranging from 10^{-6} to 10^{-1} ng/ul. Standards were synthesized using gBlock Gene Fragments (Integrated DNA Technologies). All plates were loaded using a HX IFC Controller (Fluidigm) and placed in a BioMark HD (Fluidigm) for thermal cycling at 95°C for 1 min, 30 cycles at 96°C for 5 sec and 60°C for 20 sec followed by melt curve analysis for 60-95°C at a ramp of 1°C/3s. Data were exported using the Real-Time PCR Analysis software, version 4.12 (Fluidigm) with the default peak sensitivity set to 7, peak ratio threshold of 0.7, melt temperature (Tm) ranges individually set based on peaks observed in standards, quality threshold of 0.65 and linear baseline correction. The data were then processed and analyzed using RStudio, version 1.2.5001. Samples that were above the upper Ct cutoff value of 28 were removed from analysis. Copy numbers were calculated using the standard curve for each gene.

4.3.6 Bioinformatic analysis

Sequencing libraries were processed using DADA2 and the R statistical language. DADA2 was performed with default parameters as described in the DADA2 Pipeline Tutorial (version 1.14). This analysis filters 16S rRNA sequences for quality based on sequencing library quality scores and assigns sequences to amplicon sequence variants (ASVs) to describe the taxa identified in each sample. ASVs were assigned taxonomy by alignment with the Silva 16S rRNA gene database, v124. The output of this software is abundances of each observed ASV and its most closely related taxa in the database. Manure associated bacteria were assigned by comparing taxa identified in manure and soil not receiving manure to define taxa with unique association to manure (i.e., not present in non-manured soils and not common between the two). To test significance of a treatment effect on ARG abundance, a non-parametric Kruskal-Wallis test was used to account for the varying sample sizes between grouping comparisons. When significant treatment effects were detected between more than two groups, pairwise comparisons were evaluated using Wilcoxon rank-sum test to determine which groups were significantly different. Figures were generated in the ggpubr package in R. All code is available at github.com/jflater/biomark.

4.4 Results

Several antibiotic compounds and degradation products were found in the poultry manure. The chlortetracycline concentration was 3958.48 ng/g of manure sampled. The concentration of the sulfonamide sulfachlorpyrazidine was 261.39 ng/g of manure. The concentration of the ionophore monensin was 15.52 ng/g of manure. Based on personal communication, the only antibiotic used during poultry production of the source manure was sulfonamide.

To determine if prairie strips attenuate the transport of manure associated bacteria and ARGs. To do this, we first characterized manure-associated bacteria and ARGs associated with the poultry manure used in this experiment. The identification of bacteria and ARGs that were specifically from manure was important to distinguish these genetic elements from those that are naturally present in soils or may be enriched due to rainfall and not manure amendment.
In total, we identified 46,655 unique taxa from manure and soil communities (minimum and maximum sample depth of 1,132 and 84,339 reads respectively). From manure communities we identified 1,073 unique taxa and from soil communities we identified 7,247 taxa unique to crop soils, 8,755 taxa unique to strip soils and 7,681 taxa common between strip and crop soils. To identify manure associated bacteria, we compared the bacterial community of manure with the bacterial community of soils prior to manure treatment. The bacterial community of the poultry manure shared 46 ASVs with the soil communities that did not receive manure. We then identified manure associated taxa as those ASVs not detected in soils without manure amendment or the 46 ASVs common between them. In total, 1,020 ASVs were identified as unique to manure (Table 4-1).

The majority of ARGs identified in manure were similar to previously described genes conferring resistance to tetracyclines, sulfonamides, and erythromycins and associated with mobile genetic elements (MGEs) such as transposases (Figure 4-2). In the same manner used to identify to manure-associated ASVs, we identified manure-associated ARGs that were specific to manure samples. We classified ARGs and MGEs specific to manure by comparing manure ARGs and MGEs to those present in soil that did not receive manure. We found 16 ARGs unique to manure: *"tetbP", "aphA3", "tetT", "strB", "tetW", "erm(C)", "tetX", "int1-a-marko", "tnpA1", "tnpA2", "tetH", "aadD", "lnuA", "tnpA5", "tetM", "cmr"*. Together with manure-associated taxa,

these results indicate a manure specific profile of taxa and ARGs that can be used to target manure associated antibiotic resistance genes following manure application.

To understand the movement of manure-associated bacteria and ARGs in soils with and without prairie strip installation, we performed a rainfall simulation on manured and non-manured plots. We first evaluated the presence and quantity of manure-associated bacteria and diverse ARGs in response to manure amendment and the presence of prairie strips in runoff water. Manure associated bacteria were transported in the runoff water from manured plots, both with and without prairie strips, following the rainfall simulation. In manured plots without prairie strips (WCM), the average relative abundance of manure associated bacteria ranged between 14% and 48 % of the total observed taxa in runoff water across the 30-minute collection period (Figure 4-3). The manured prairie strip plots (WCSM) had relative abundances of manure associated bacteria that ranged from 0.008% to 65% across the collection period. We observed trends that were plot specific. Specifically, one of three plots, plot 4 in the WCSM treatment had much higher detection (65%) of manure-associated bacteria in runoff compared to the other two plots in the treatment (0.03 and 0.008%) (Table 2). With the exception of plot 4, we found that WCSM plots had a smaller percentage of manure associated bacteria than WCM plots. Further evidence of these plot-specific trends was observed in the estimation of total 16S rRNA gene copies (qPCR-based) in each plot. Plot 4 impact on bacterial abundance is shown with data from HTqPCR amplification of the 16S gene. When comparing all plots, there was not a significant difference in 16S rRNA gene counts between manured no strip and strip runoff WCM and WCSM for any of the 6 water sampling time points (Figure 4-4). However, if plot 4 is excluded from analysis, in WCSM runoff is then observed to have significantly lower bacterial loads as estimated by 16S rRNA gene than the WCM runoff, with the exception of water sample 6, which

there was no significant difference in 16S rRNA gene copies between WCM and WCS runoff (Figure 4-5). These results highlight the effect of prairie strips in reducing the abundance of manure associated bacteria in runoff water.

These plot specific trends between manured strip and no strip runoff were also observed in our detection ARGs. Only one plot, plot 4, in the WCSM treatment consistently had detection of manure associated ARGs. Besides plot 4, there were no ARGs detected in plots 5 and 6 of the WCSM treatment runoff water. These results suggest that in addition to manure-associated bacteria, prairie strips are also observed to decrease ARGs in manure applied runoff, though these results were observed to be plot-specific at this site.

The most abundant manure associated ARGs that we detected in runoff were associated with tetracycline genes *'tetbP', 'tetT', 'tetW', 'tetX', 'tetH', 'tetM'*. These ARGs were mainly detected in WCM plots and a single WCSM (plot 4), and their abundances varied depending on the time of sampling. Among tetracycline genes, '*tetM*' was the most abundant and detected in the most runoff samples. There were significant treatment effects detected in water samples 15, 25, and 30 minutes after runoff was achieved, ($P < 0.05$), in each case, WCSM had significantly greater 'tetM' copies than WCS (Figure 4-6). However, there was strong plot effect observed, removing plot 4 from WCSM results in no detection of 'tetM' in WCSM plots. 'tetT' was detected as the second most abundant ARG in runoff water samples. Once again, lack of amplification in multiple samples limited our ability to make determinations of significance between treatments. Only water sample 25 minutes into runoff showed significant differences between WCS and WCSM, with WCSM having significantly higher gene copies at this time point (Figure 4-7). Once again, all WCSM samples with copies of 'tetT' were from plot 4. 'tetX' copies were not significantly different between WCS and WCSM. Samples 10, 20, and 25

minutes into runoff had no amplification in WCS, and all amplification in WCSM occurred in samples from plot 4 (Figure 4-8). The remaining *'tet'* genes exhibited limited amplification and sample size of groups was too small to make statistical inferences. Like previously described samples, all amplification of the remaining *'tet'* genes in WCSM samples occurred in plot 4 (Figure 4-9, 1-10, 4-11). These results indicate the potential for prairie strips to reduce ARG transport when corrected for plot effect. However, the limited detection in multiple samples resulted in group size for treatment and sample number that prevent statistical determinations.

4.5 Discussion

Given the above-ground diversity differences associated with prairie strips relative to row crops, we expected that below ground microbial diversity in prairie strips to also be different. We were specifically interested in the impacts prairie strips may have on the movement of poultry manure associated bacteria and ARGs after rainfall. First, to understand the transport of poultry manure bacteria and ARGs, we characterized a specific manure associated profile of bacteria and ARGs associated with the poultry manure used in this study. These results allowed us to track the movement of these genetic elements independent of existing soil bacteria or those enriched as a result of rainfall and not manure application. The poultry manure profile we identified was dominated by bacteria from the phyla Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria (Figure 4-1). These bacteria are consistent with manure-associated bacteria identified in previous studies. Swine manure associated bacteria were also found to be dominated by Firmicutes and Bacteroidetes (Looft et al., 2012; Rieke et al., 2018), and these results suggest that bacteria from the phyla Firmicutes and Bacteroidetes are highly associated with manure. For addressing the impacts of poultry manure addition in the soils, the ability to classify numerous bacteria and ARGs as manure-associated was helpful in interpreting the consistency of the manure impact over our experiment.

In addition to identifying manure-associated bacteria, we were also able to identify the origin of specific bacteria and ARGs as from soils (prior to manure amendment). Consequently, we compared runoff water from plots with and without prairie strip installations and their composition of manure- and soil-associated bacteria and genes. We conducted experiments on 3 paired strip and no strip plots and observed results suggestive of plot variation. On average, we found that cumulatively prairie strips did not significantly reduce the abundance of ARGs associated with manure in runoff water. However, in a plot-specific analysis, we identified one plot (plot 4) as having high abundance in runoff of both manure-associated bacteria and ARGs. The two other plots had significantly less manure-associated bacteria and no manure associated ARGs detected. It was previously unknown if prairie strips effectively reduced manure associated bacteria in runoff water, despite evidence indicating significant sediment loss reductions following prairie strip installations (Helmers et al., 2012). Taken together, these results suggest that there can be high variability of the fate of bacteria and ARGs from manure in prairie strip installations even within the same soil type. We observe that prairie strips can have a mixed effect on manure-associated bacteria and that they can both reduce the observed bacteria and ARGs or have similar bacteria and ARGs as plots where strips were not installed. In runoff water where manure associated bacteria and ARGs are detected, these bacteria and genes are observed to have similar abundances over time. These results suggest that these bacteria are most likely being transported directly with water flow. Our observation that these genes and bacteria are also not widely identified in soils further supports their transport with water flow. These results are important when considering that water flow from our fields will connect to other waterways. Our observation that indicator antibiotic resistance genes move mainly through water

indicates that it is a potential and important pathway for future study regarding the spread of antibiotic resistance.

Tetracycline resistance genes were the most widely detected manure associated ARGs in our samples. Tetracycline resistance is of concern because of its importance to human medicine where it is used to treat bacterial infections, particularly respiratory infections (Smilack, 1999). The significant concentrations of tetracycline associated resistance genes is concerning as to the best of our knowledge, these poultry were administered ionophores only. This result, while unexpected, is consistent with other studies that have shown that antibiotic resistant genes may be present following discontinuation of antibiotic use (Ghosh and LaPara, 2007). It may be a legacy effect from past antibiotic use, or possibly unrelated to antibiotic usage at all (Cadena et al., 2018; Dcosta et al., 2011). The presence of ARGs cannot alone be used as an indicator of anthropogenic impacts on bacterial communities. In a study of native prairie soils, several *tet* genes were found, suggesting that ARGs from this class are present in pristine soils (Cadena et al., 2018). However, *tetM* may be an indicator as it is less common in native or pristine environments but was detected in relatively high abundance in several animal manures. European investigations in to ARG and agriculture have identified *tetM* as an indicator of ARG movement in agricultural settings (Storteboom et al., 2010). Our observation of *tetM* as the most abundant in poultry manure, manure associated, and present in the most runoff water samples adds support to using *tetM* as an indicator of ARG movement (Berendonk et al., 2015). Overall, our results are consistent with previous observations of ARGs in poultry manure, where ARGs associated with resistance to Tetracycline, Sulfonamide, and Aminoglycoside were found to be abundant (Awasthi et al., 2019; Han et al., 2018).

A limitation of this study is that we only studied the impact of manure application on surface water flows. It has previously been observed that macropores in grassland soils, which are similar to prairie strips, form a varied soil matrix due to high abundances of earthworms and their burrows in combination with undisturbed soil structure (Zachmann et al., 1987). These macropores offer an avenue for water movement that bypasses surface flow and instead move into the vadose zone. Further, the undisturbed nature of prairie strips allows macropores to remain open for large periods of time, creating channels into the soil profile. Bacteria can be transported through these channels, and rainfall on these soils has resulted in rapid bacterial transport facilitated by the presence of macropores (Unc and Goss, 2003). Previously, it has been shown that bacterial transport through undisturbed soils is highly affected by macropore presence (Jamieson et al., 2002). Thus, it is likely that by characterizing runoff water and not subsurface water, we are potentially missing an important component of the water budget (and flow of antibiotic resistant indicators) due to the presence of deep-rooted prairie plants and macropores. Subsequent investigations of prairie strips would benefit from examining subsurface water for concentrations of manure associated bacteria and ARGs.

This work contributes to an existing body of knowledge indicating that strips of vegetation can reduce sediment and manure associated bacteria loss in agroecosystems. This finding is important in that it adds evidence to the use of these strips as management practice that can improve the sustainability of manure use, facilitating the application of this valuable resource. We did observe the movement of antibiotic resistant indicators in water flows after manure application, and the use of edge of field mitigation technologies to mitigate these risks is promising to prevent further environmental harm and water quality damage. Finally, our results

indicate that there is high plot variability in strip installations, and likely the need for considering this variability in future, especially field-based, experiments.

4.6 References

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4.7 Figures and Tables

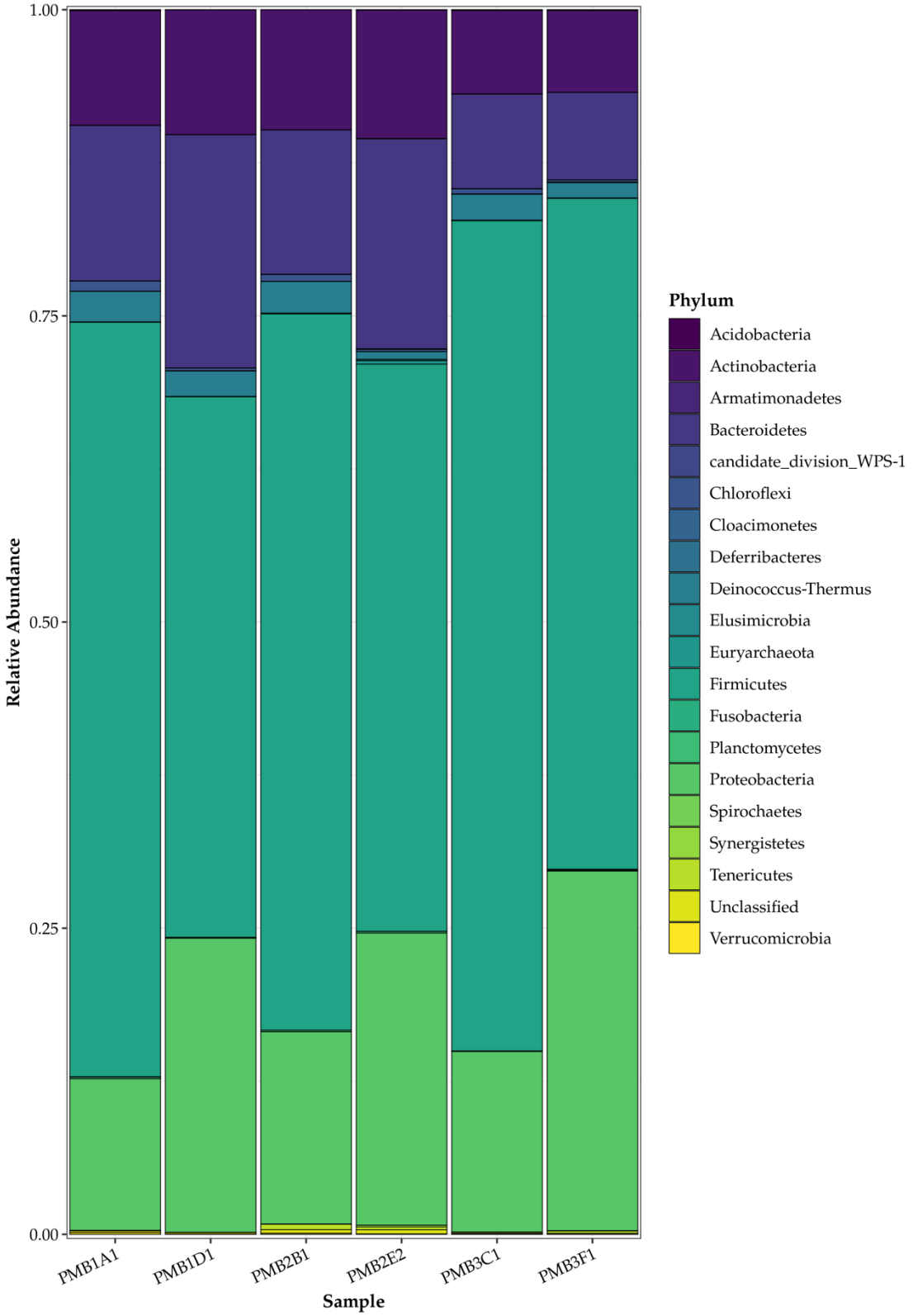

Figure 4-1: The relative abundance of phyla in the six poultry manure samples. Two samples were collected from manure used on each simulation day resulting in six manure samples.

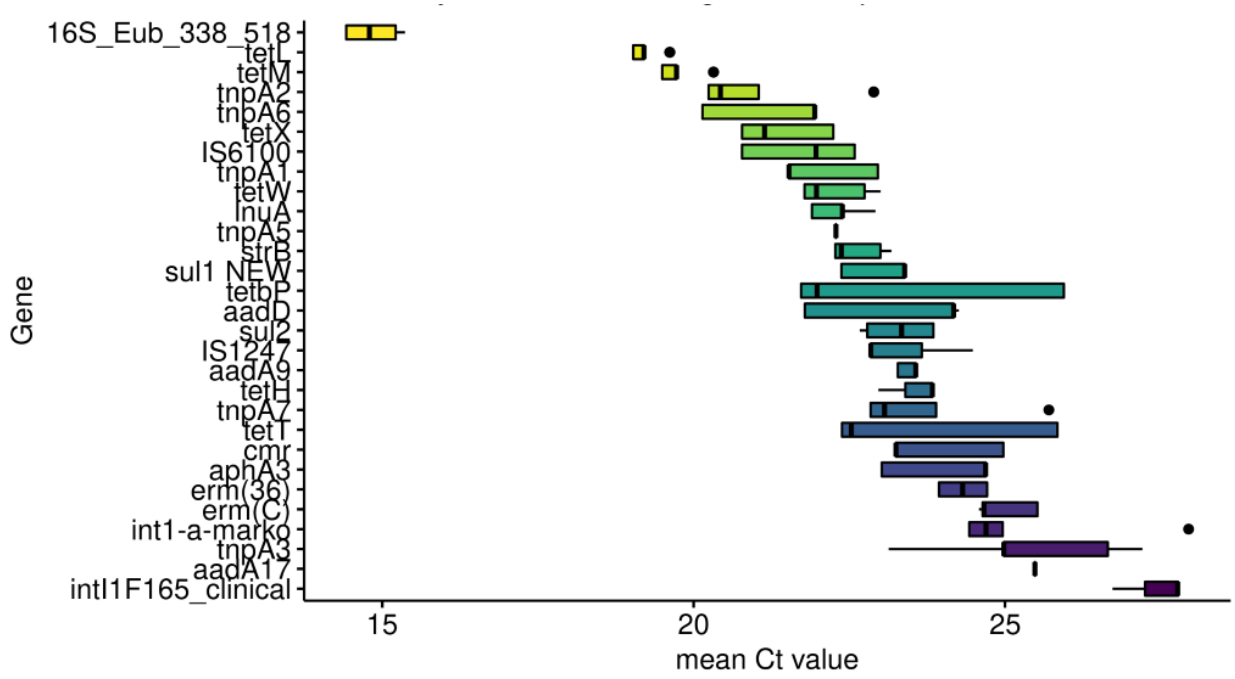

Figure 4-2: Cycle time for ARGs specific to manure.

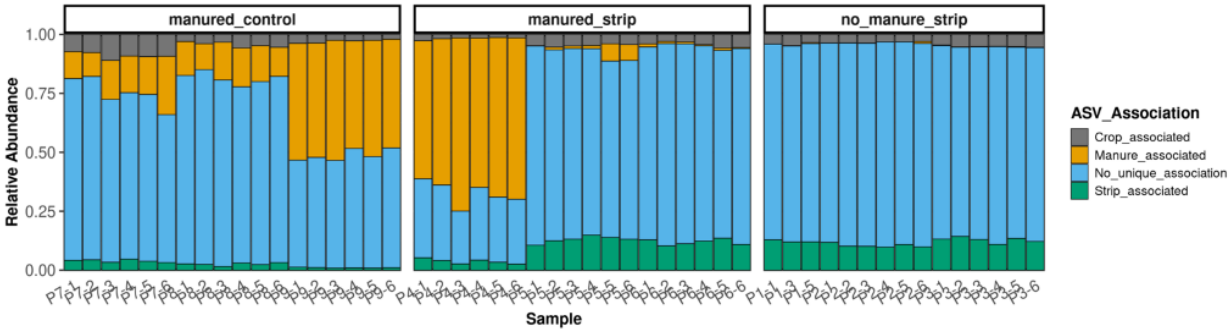

Figure 4-3: Relative abundance of ASVs by association in runoff water samples. Label scheme: P7-1 = Plot 7 water sample 1. Six water samples collected at 5, 10, 15, 20, 25 , and 30 minutes after the initiation of runoff event.

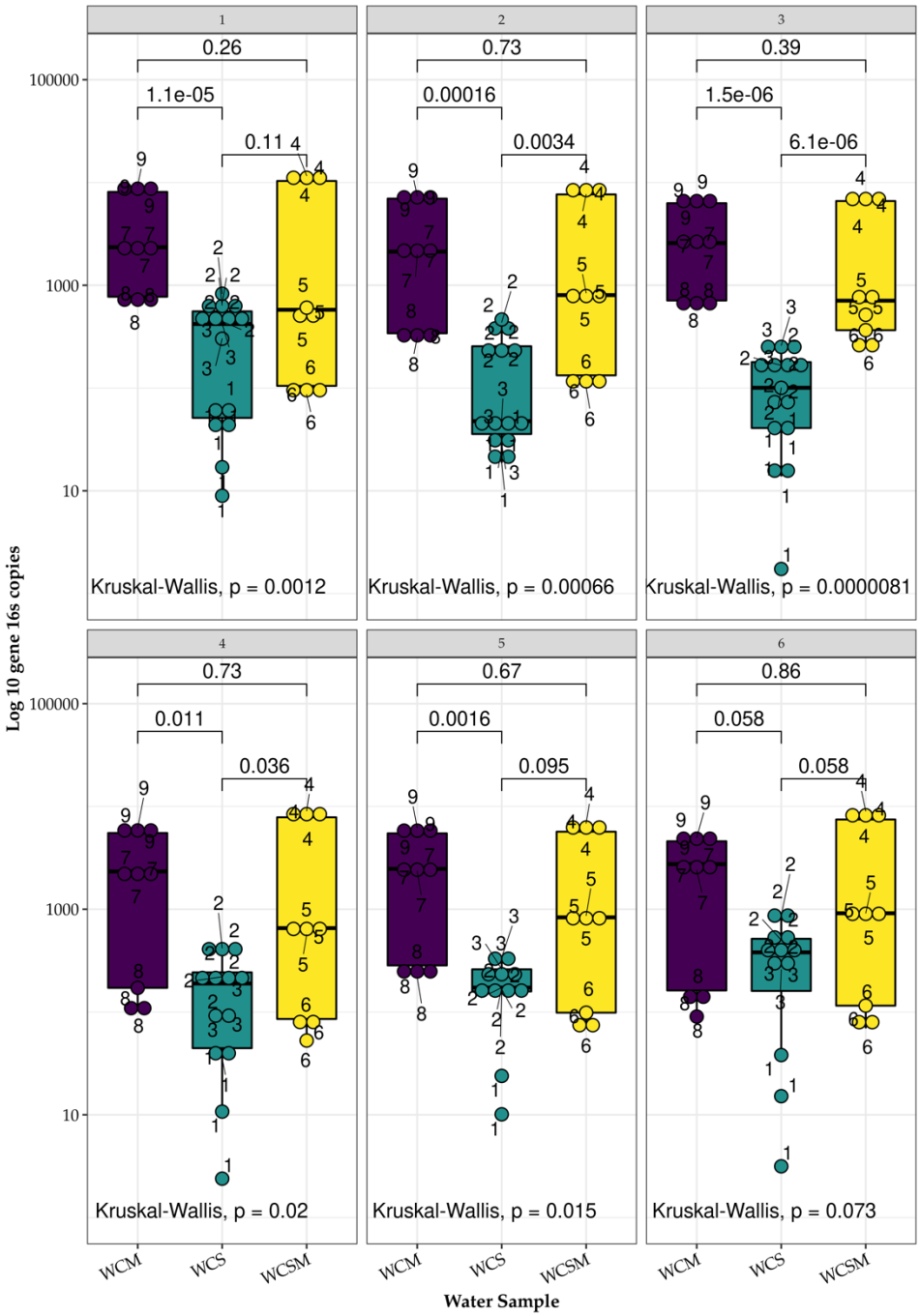

Figure 4-4: Log 10 gene copies of the 16S gene in runoff water samples. Panels are 1-5 and represent runoff 5, 10, 15, 20, 25, and 30 minutes into runoff respectively. Dots on boxplots are labeled with the plots from each treatment.

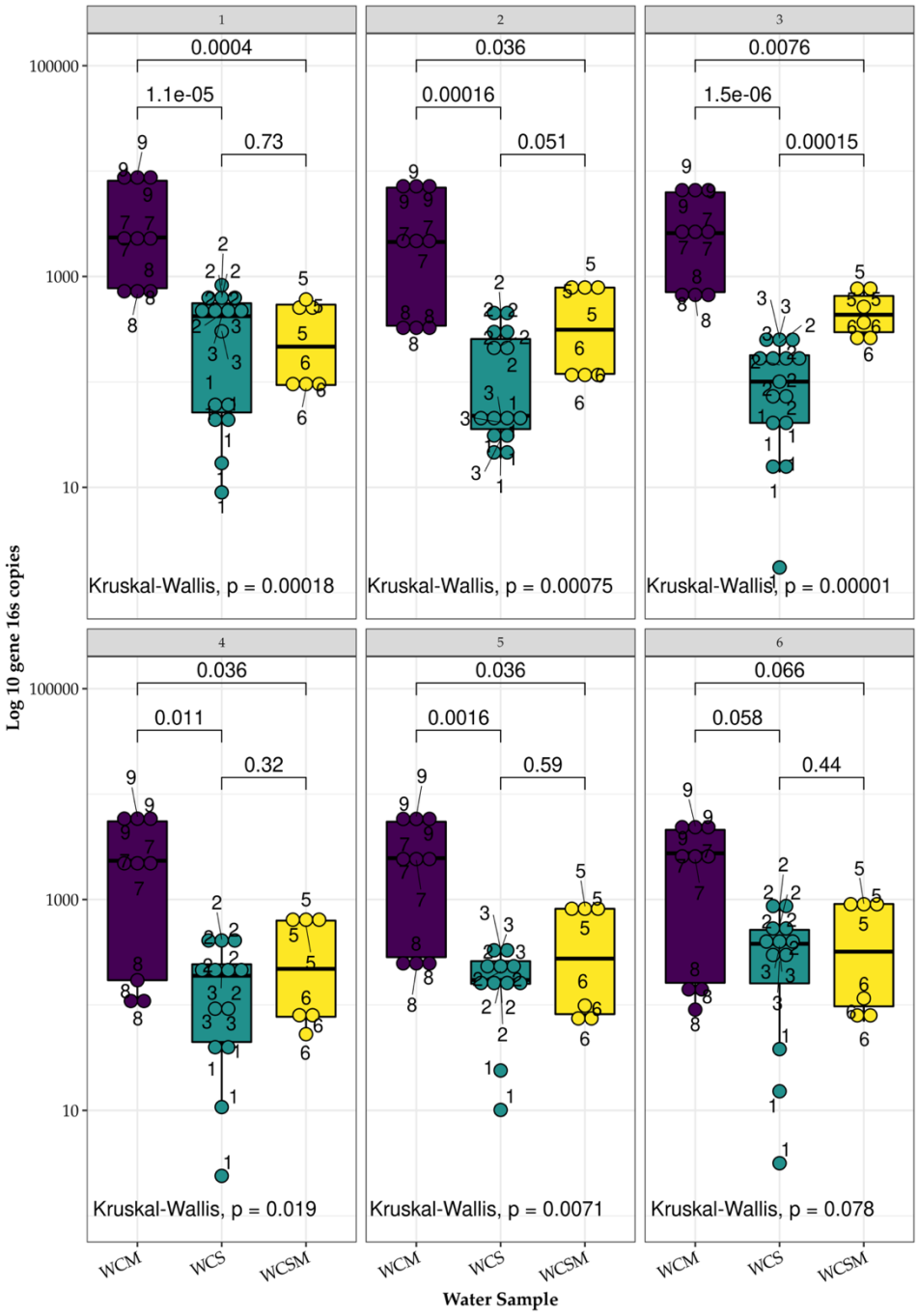

Figure 4-5: Log 10 gene copies of the 16S gene in runoff water samples. Panels are 1-5 and represent runoff 5, 10, 15, 20, 25, and 30 minutes into runoff respectively. Dots on boxplots are labeled with the plots from each treatment. Plot 4 has been removed to show the impact of that plot on copy numbers.

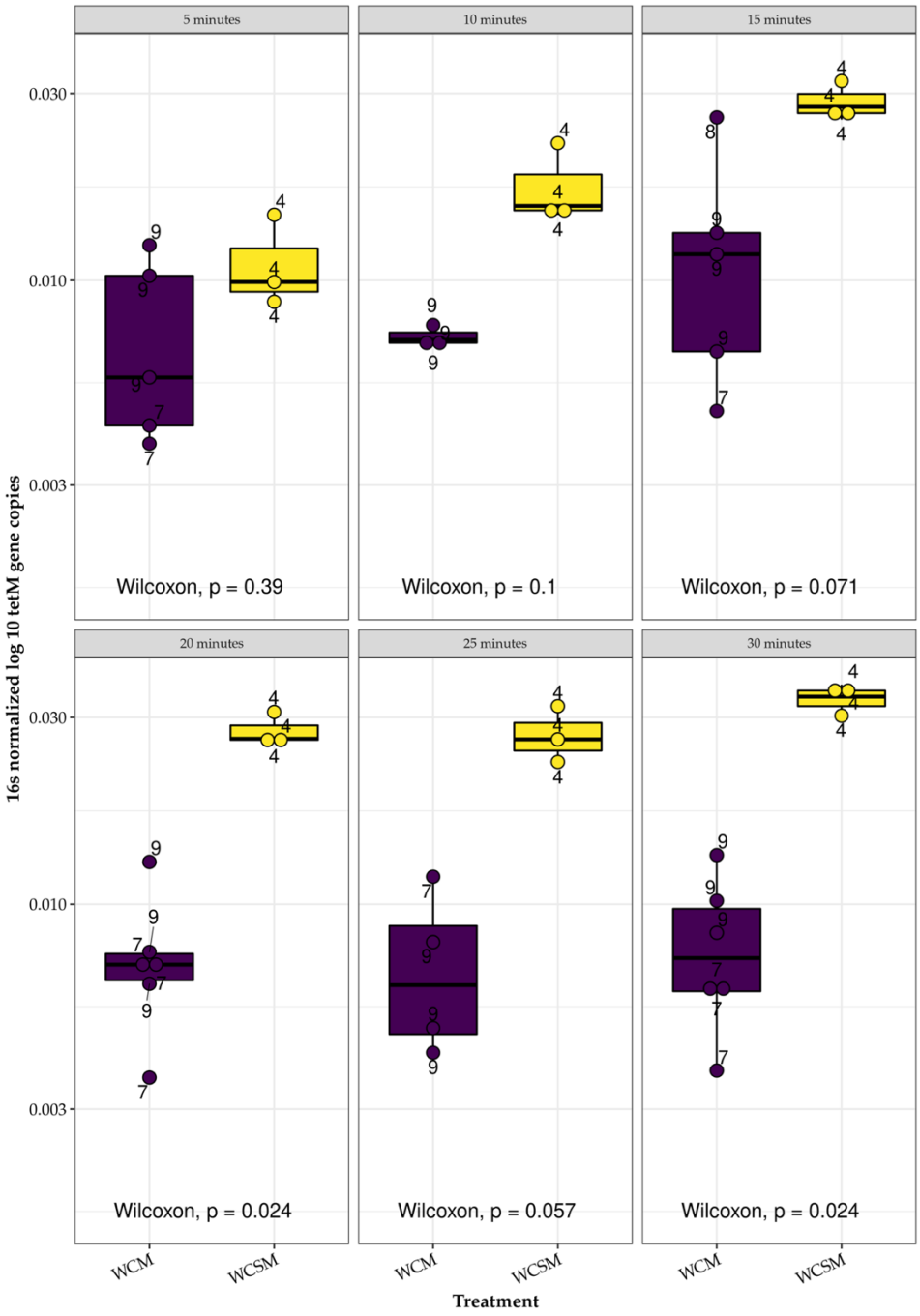

Figure 4-6: 6 Log 10 gene copies of *tet*M normalized by 16S copies. Panels represent time into runoff and dots are labeled with plot numbers.

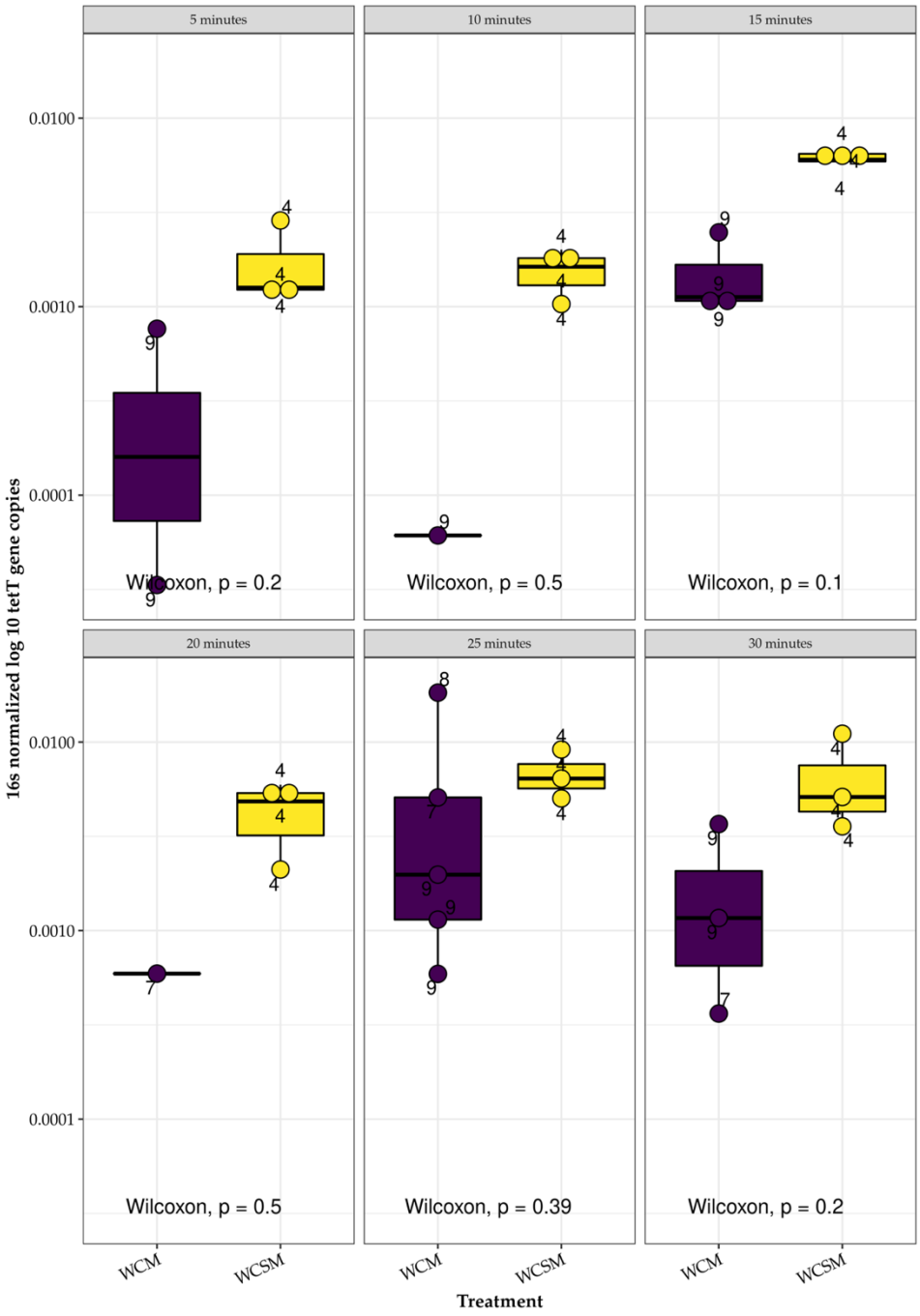

Figure 4-7: Log 10 gene copies of *tet*T normalized by 16S copies. Panels represent time into runoff and dots are labeled with plot numbers.

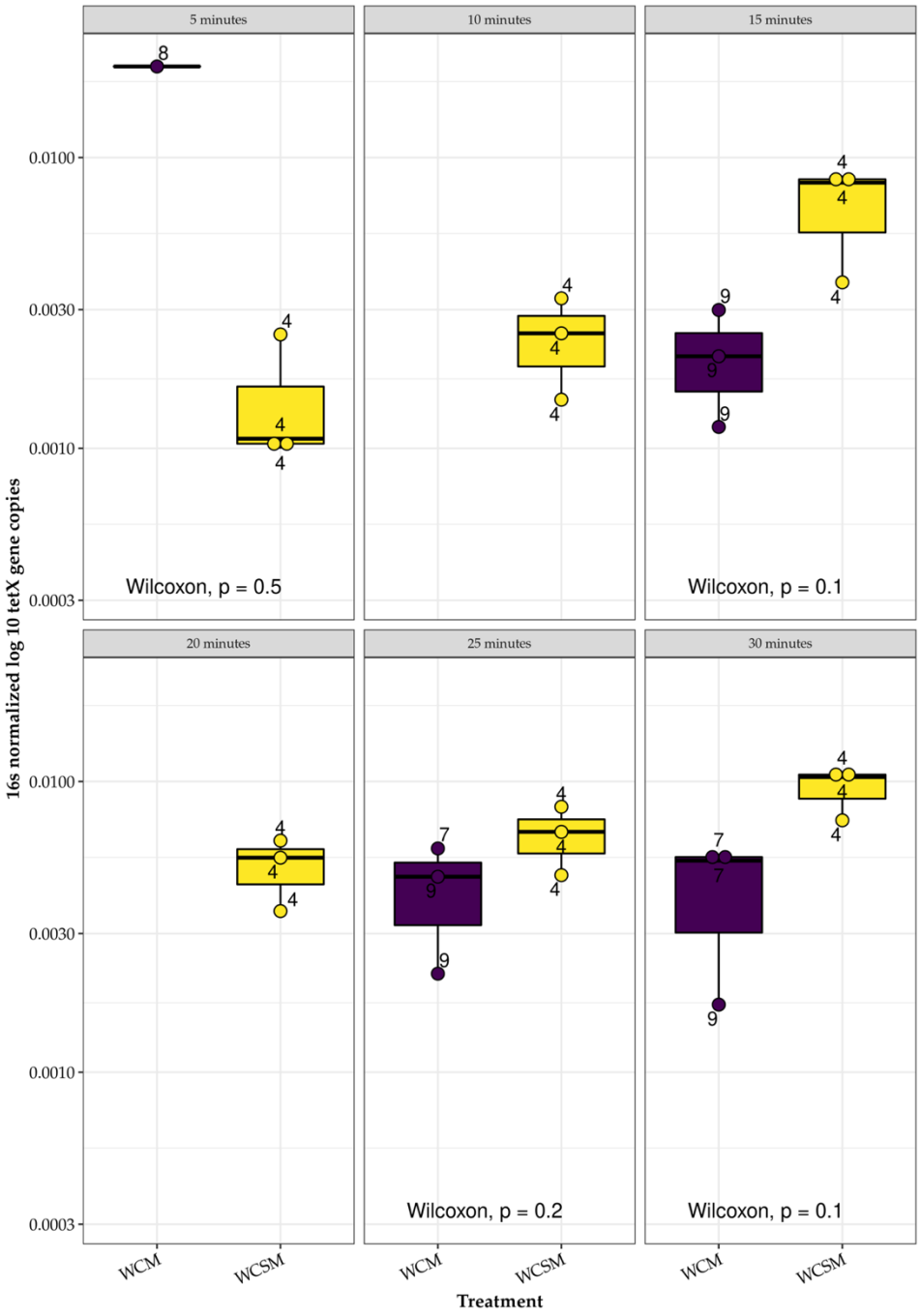

Figure 4-8: Log 10 gene copies of *tet*X normalized by 16S copies. Panels represent time into runoff and dots are labeled with plot numbers.

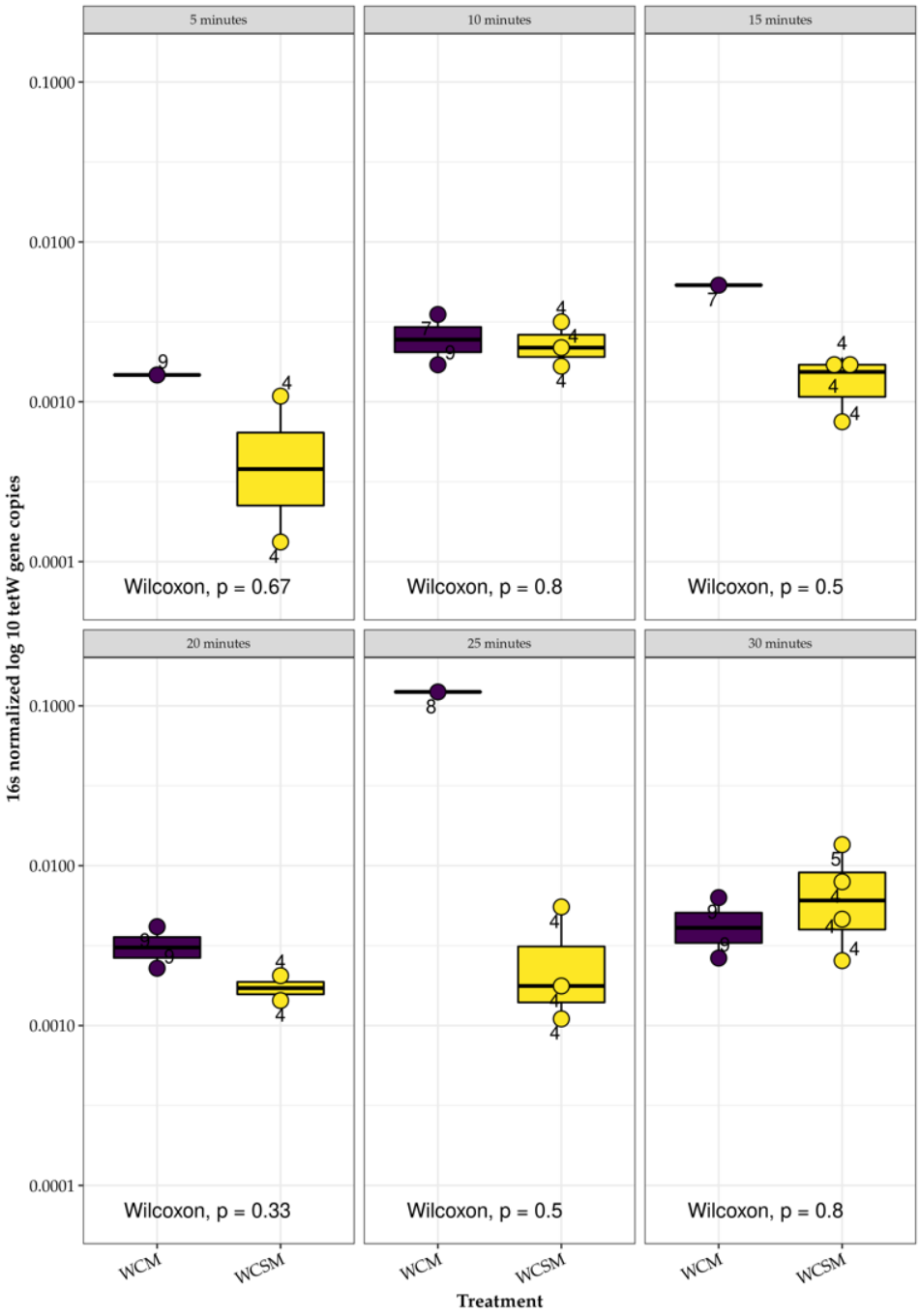

Figure 4-9: Log 10 gene copies of *tet*W normalized by 16S copies. Panels represent time into runoff and dots are labeled with plot numbers.

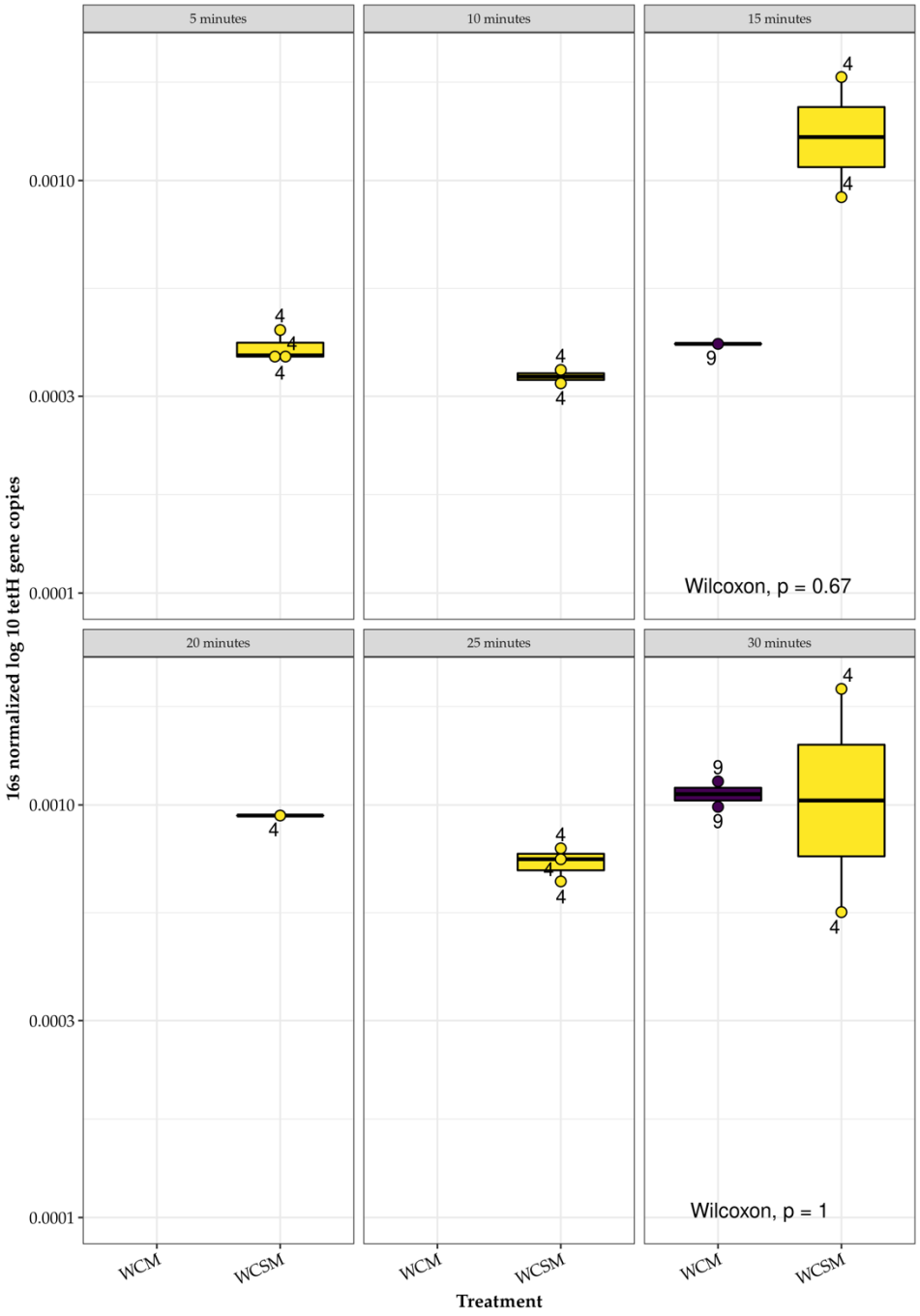

Figure 4-10: Log 10 gene copies of *tet*H normalized by 16S copies. Panels represent time into runoff and dots are labeled with plot numbers.

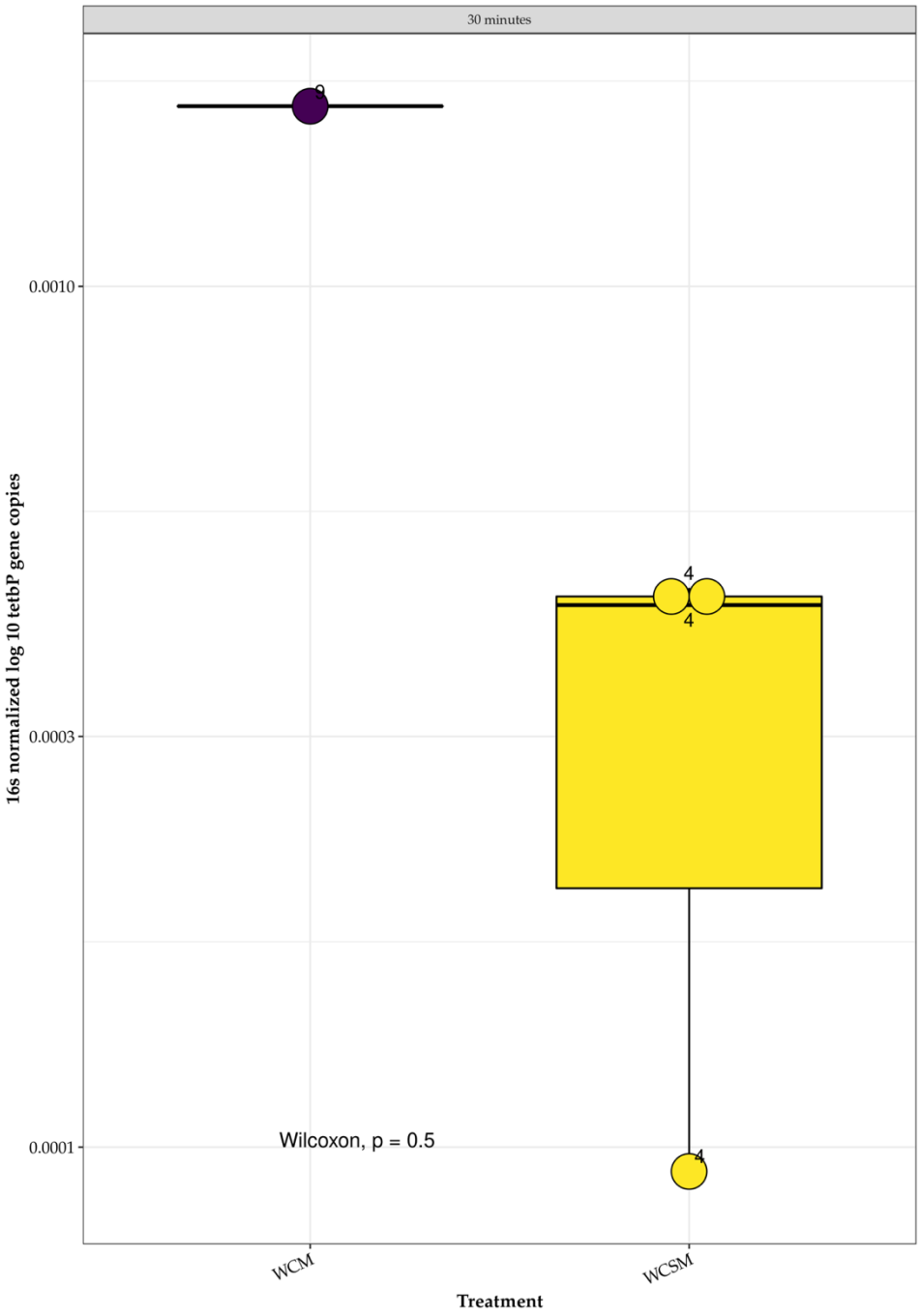

Figure 4-11: Log 10 gene copies of *tet*bP normalized by 16S copies. Panels represent time into runoff and dots are labeled with plot numbers.

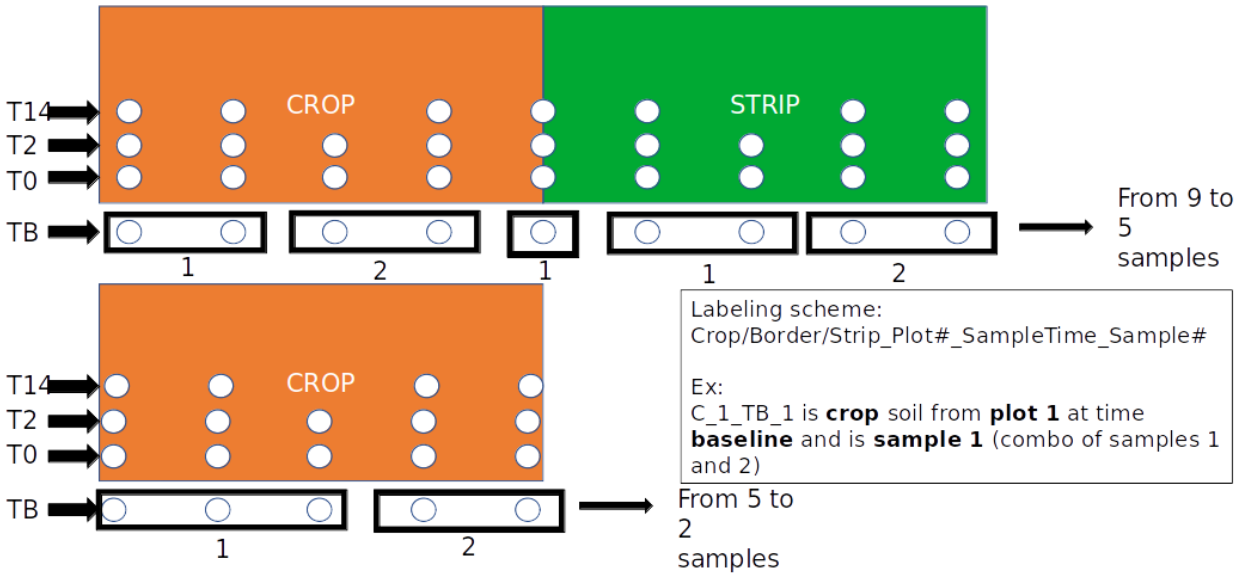

Figure 4-12: Schematic of the combination scheme used to combine extracted DNA for WOR soil samples that was used for biomark analysis.

Table 4-1: Mean sample reads for each combination of treatment and sample day. Samples indicated number of samples used for analysis following quality control.

Matrix	Treatment	Sample day	Mean reads per sample	Samples
manure	NA	T042	26522.67	6
soil	WCM	Baseline	19112.83	29
soil	WCM	T000	17201.69	29
soil	WCM	T002	16045.83	30
soil	WCM	T014	19445.48	29
soil	WCM	T021	19814.26	27
soil	WCM	T042	16587.41	29
soil	WCSM	Baseline	17628.16	51
soil	WCSM	T000	14907.81	54
soil	WCSM	T002	17535.43	53
soil	WCSM	T014	24184.00	52
soil	WCSM	T021	20667.96	53
soil	WCSM	T042	16483.02	51
soil	WCS	Baseline	11904.07	54
soil	WCS	T000	17218.94	54
soil	WCS	T002	24715.92	53
soil	WCS	T014	18450.72	53
soil	WCS	T021	21019.15	53
soil	WCS	T042	17898.76	50
water	WCM	T042	24354.21	19
water	WCSM	T042	24632.44	18
water	WCS	T042	17343.60	15

Table 4-2: Sample names and metadata used for biomark analysis. See figure 4-12 for label scheme.

Sample_Name	soil_type	plot	treatment	sample_day	sample_type
WM_B1	na	na	na	T00	manure
WM_B2	na	na	na	T00	manure
WM_B3	na	na	na	T00	manure
B_1_TB_1	border	1	WCS	TB	soil
C_1_TB_1	crop	1	WCS	TB	soil
C_1_TB_2	crop	1	WCS	TB	soil
S_1_TB_1	strip	1	WCS	TB	soil
S_1_TB_2	strip	1	WCS	TB	soil
B_2_TB_1	border	2	WCS	TB	soil
C_2_TB_1	crop	2	WCS	TB	soil
C_2_TB_2	crop	2	WCS	TB	soil
S_2_TB_1	strip	2	WCS	TB	soil
S_2_TB_2	strip	2	WCS	TB	soil
B_3_TB_1	border	3	WCS	TB	soil
C_3_TB_1	crop	3	WCS	TB	soil
C_3_TB_2	crop	3	WCS	TB	soil
S_3_TB_1	strip	3	WCS	TB	soil
S_3_TB_2	strip	3	WCS	TB	soil
B_4_TB_1	border	4	WCSM	TB	soil
C_4_TB_1	crop	4	WCSM	TB	soil
C_4_TB_2	crop	4	WCSM	TB	soil
S_4_TB_1	strip	4	WCS	TB	soil
S_4_TB_2	strip	4	WCS	TB	soil
B_5_TB_1	border	5	WCSM	TB	soil
C_5_TB_1	crop	5	WCSM	TB	soil
C_5_TB_2	crop	5	WCSM	TB	soil
S_5_TB_1	strip	5	WCS	TB	soil
S_5_TB_2	strip	5	WCS	TB	soil
B_6_TB_1	border	6	WCSM	TB	soil
C_6_TB_1	crop	6	WCSM	TB	soil
C_6_TB_2	crop	6	WCSM	TB	soil
S_6_TB_1	strip	6	WCSM	TB	soil
S_6_TB_2	strip	6	WCSM	TB	soil
C_7_TB_1	crop	7	WCM	TB	soil
C_7_TB_2	crop	7	WCM	TB	soil
C_8_TB_1	crop	8	WCM	TB	soil
C_8_TB_2	crop	8	WCM	TB	soil
C_9_TB_1	crop	9	WCM	TB	soil
C_9_TB_2	crop	9	WCM	TB	soil
B_1_T00_1	border	1	WCS	T00	soil
C_1_T00_1	crop	1	WCS	T00	soil
C_1_T00_2	crop	1	WCS	T00	soil
S_1_T00_1	strip	1	WCS	T00	soil
S_1_T00_2	strip	1	WCS	T00	soil
B_2_T00_1	border	2	WCS	T00	soil
C_2_T00_1	crop	2	WCS	T00	soil
C_2_T00_2	crop	2	WCS	T00	soil
S_2_T00_1	strip	2	WCS	T00	soil
S_2_T00_2	strip	2	WCS	T00	soil

Table 4-2 continued

Sample_Name	soil_type	plot	treatment	sample_day	sample_type
B_3_T00_1	border	3	WCS	T00	soil
C_3_T00_1	crop	3	WCS	T00	soil
C_3_T00_2	crop	3	WCS	T00	soil
S_3_T00_1	strip	3	WCS	T00	soil
S_3_T00_2	strip	3	WCS	T00	soil
B_4_T00_1	border	4	WCSM	T00	soil
C_4_T00_1	crop	4	WCSM	T00	soil
C_4_T00_2	crop	4	WCSM	T00	soil
S_4_T00_1	strip	4	WCSM	T00	soil
S_4_T00_2	strip	4	WCSM	T00	soil
B_5_T00_1	border	5	WCSM	T00	soil
C_5_T00_1	crop	5	WCSM	T00	soil
C_5_T00_2	crop	5	WCSM	T00	soil
S_5_T00_1	strip	5	WCSM	T00	soil
S_5_T00_2	strip	5	WCSM	T00	soil
B_6_T00_1	border	6	WCSM	T00	soil
C_6_T00_1	crop	6	WCSM	T00	soil
C_6_T00_2	crop	6	WCSM	T00	soil
S_6_T00_1	strip	6	WCSM	T00	soil
S_6_T00_2	strip	6	WCSM	T00	soil
C_7_T00_1	crop	7	WCM	T00	soil
C_7_T00_2	crop	7	WCM	T00	soil
C_8_T00_1	crop	8	WCM	T00	soil
C_8_T00_2	crop	8	WCM	T00	soil
C_9_T00_1	crop	9	WCM	T00	soil
C_9_T00_2	crop	9	WCM	T00	soil
B_1_T02_1	border	1	WCS	T02	soil
C_1_T02_1	crop	1	WCS	T02	soil
C_1_T02_2	crop	1	WCS	T02	soil
S_1_T02_1	strip	1	WCS	T02	soil
S_1_T02_2	strip	1	WCS	T02	soil
B_2_T02_1	border	2	WCS	T02	soil
C_2_T02_1	crop	2	WCS	T02	soil
C_2_T02_2	crop	2	WCS	T02	soil
S_2_T02_1	strip	2	WCS	T02	soil
S_2_T02_2	strip	2	WCS	T02	soil
B_3_T02_1	border	3	WCS	T02	soil
C_3_T02_1	crop	3	WCS	T02	soil
C_3_T02_2	crop	3	WCS	T02	soil
S_3_T02_1	strip	3	WCS	T02	soil
S_3_T02_2	strip	3	WCS	T02	soil
B_4_T02_1	border	4	WCSM	T02	soil
C_4_T02_1	crop	4	WCSM	T02	soil
C_4_T02_2	crop	4	WCSM	T02	soil
S_4_T02_1	strip	4	WCSM	T02	soil
S_4_T02_2	strip	4	WCSM	T02	soil
B_5_T02_1	border	5	WCSM	T02	soil
C_5_T02_1	crop	5	WCSM	T02	soil
C_5_T02_2	crop	5	WCSM	T02	soil
S_5_T02_1	strip	5	WCSM	T02	soil

Table 4-2 continued

Sample_Name	soil_type	plot	treatment	sample_day	sample_type
S_5_T02_2	strip	5	WCSM	T02	soil
B_6_T02_1	border	6	WCSM	T02	soil
C_6_T02_1	crop	6	WCSM	T02	soil
C_6_T02_2	crop	6	WCSM	T02	soil
S_6_T02_1	strip	6	WCSM	T02	soil
S_6_T02_2	strip	6	WCSM	T02	soil
C_7_T02_1	crop	7	WCM	T02	soil
C_7_T02_2	crop	7	WCM	T02	soil
C_8_T02_1	crop	8	WCM	T02	soil
C_8_T02_2	crop	8	WCM	T02	soil
C_9_T02_1	crop	9	WCM	T02	soil
C_9_T02_2	crop	9	WCM	T02	soil
B_1_T14_1	border	1	WCS	T14	soil
C_1_T14_1	crop	1	WCS	T14	soil
C_1_T14_2	crop	1	WCS	T14	soil
S_1_T14_1	strip	1	WCS	T14	soil
S_1_T14_2	strip	1	WCS	T14	soil
C_2_T14_1	crop	2	WCS	T14	soil
P1_1	na	1	WCS	T00	water
P1_2	na	1	WCS	T00	water
P1_3	na	1	WCS	T00	water
P1_4	na	1	WCS	T00	water
P1_5	na	1	WCS	T00	water
P1_6	na	1	WCS	T00	water
P2_1	na	2	WCS	T00	water
P2_2	na	2	WCS	T00	water
P2_3	na	2	WCS	T00	water
P2_4	na	2	WCS	T00	water
P2_5	na	2	WCS	T00	water
P2_6	na	2	WCS	T00	water
P3_1	na	3	WCS	T00	water
P3_2	na	3	WCS	T00	water
P3_3	na	3	WCS	T00	water
P3_4	na	3	WCS	T00	water
P3_5	na	3	WCS	T00	water
P3_6	na	3	WCS	T00	water
P4_1	na	4	WCSM	T00	water
P4_2	na	4	WCSM	T00	water
P4_3	na	4	WCSM	T00	water
P4_4	na	4	WCSM	T00	water
P4_5	na	4	WCSM	T00	water
P4_6	na	4	WCSM	T00	water
P5_1	na	5	WCSM	T00	water
P5_2	na	5	WCSM	T00	water
P5_3	na	5	WCSM	T00	water
P5_4	na	5	WCSM	T00	water
P5_5	na	5	WCSM	T00	water
P5_6	na	5	WCSM	T00	water
P6_1	na	6	WCSM	T00	water
P6_2	na	6	WCSM	T00	water

Table 4-2 continued

Sample_Name	soil_type	plot	treatment	sample_day	sample_type
P6_3	na	6	WCSM	T00	water
P6_4	na	6	WCSM	T00	water
P6_5	na	6	WCSM	T00	water
P6_6	na	6	WCSM	T00	water
P7_1	na	7	WCM	T00	water
P7_2	na	7	WCM	T00	water
P7_3	na	7	WCM	T00	water
P7_4	na	7	WCM	T00	water
P7_5	na	7	WCM	T00	water
P7_6	na	7	WCM	T00	water
P8_1	na	8	WCM	T00	water
P8_2	na	8	WCM	T00	water
P8_3	na	8	WCM	T00	water
P8_4	na	8	WCM	T00	water
P8_5	na	8	WCM	T00	water
P8_6	na	8	WCM	T00	water
P9_1	na	9	WCM	T00	water
P9_2	na	9	WCM	T00	water
P9_3	na	9	WCM	T00	water
P9_4	na	9	WCM	T00	water
P9_5	na	9	WCM	T00	water
P9_6	na	9	WCM	T00	water

CHAPTER 5. GENERAL CONCLUSIONS

Managing agriculture for sustainable production of food, fiber, feed, and fuel is needed to support a growing population while maintaining and improving ecosystems. Agricultural management that leverages microbial communities is an approach that has shown promise for improving agricultural sustainability. Microbial communities are important in agricultural management because they mediate ecosystem functions, such as nutrient cycling and decomposition. Thus, management decisions, with and understanding of how they can improve the sustainability of agriculture, are needed. Some examples of such decisions include the use of compost and manures in lieu of mineral fertilizers and the use of edge of field practices to reduce soil and nutrient loss. These practices impact soil microbial communities, but their specific benefits are under characterized. In this dissertation, three studies were presented to improve our understanding of soil microbial communities under varying management practices. One study improves our understanding of bacterial response to organic fertilizers, and two studies contribute to our understanding of how prairie conservation strips impact soil bacterial diversity and how they impact manure-associated bacterial and gene transport following simulated rainfall.

In chapter two, we answer the question of which bacteria respond to organic amendments and when they respond. Our results were based on a performed experiment designed to identify the phylogenetic and temporal response of bacteria to organic amendments with equivalent nitrogen contents combined with high, medium, and low C:N ratios. To simulate variable carbon and nitrogen availability, we used mixtures of amendments from manured compost and alfalfa. We simulated decomposition of these amendments in laboratory soil microcosms, which were amended and incubated for 97 days. For the first 21 days, we

characterized the soil microbial communities and nutrient availability in the soil every week. To assess longer term impacts, we also sampled on day 35, 49, and 97. This study directly addresses an open question of what happens during decomposition of organic amendment and specifically links microbial community membership to the availability of nutrients over time. Our results highlight how soil microbial communities consistently shift during the course of the incubation, regardless of the amendment type. This result suggests that there are groups of bacteria that have a rapid response to nutrient availability, and a separate group of bacteria that has a secondary and later response. Comparing the two amendments, we found that alfalfa microcosms had the greatest mineralization rate while compost microcosms exhibited immobilization. Consistent with these results, we also observed distinct communities responding to the availability of nutrients from both amendments in early and late response groups. Finally, we observed that there were phylogenetic patterns of response, where more closely related bacteria were observed to be associated with either early or late responses to nutrient availability, or associated with alfalfa or compost in the amendment. Overall, this work emphasized the linkages between soil microbial membership and nutrient availability. The methods used in this study involved identification and analysis of the bacterial communities via amplification of the 16s rRNA gene. This method allowed for highly resolved evaluation of soil community membership.

The research in chapter 2 also had a couple notable limitations. The use of the 16s rRNA gene limited the inference of the function of the membership in the soil based on community and cannot describe the functions of the microbes. Further, we used soil microcosms to control often highly variable climate and environmental conditions of soil studies. Future studies would be improved by establishing linkages between microbiomes and soil health or nutrient responses in field conditions. As a result, in chapter 3, we use similar methods to assess the impacts of soil

microbial communities in the field. In chapter 4, we expand the characterization of soil community membership to functions related to the risks of antibiotic resistance from manure applications.

In chapter three, our main question was how soil diversity is impacted by the installation of prairie conservation strips. This is directly related to the impacts of soil microbiomes and their relationship to management because changes in management related to fertilization and vegetation type have direct effects on microbial communities. This effort showcased a field experiment designed to characterize the differences in bacterial diversity between prairie and agricultural soil communities. The experiment utilized simulated rainfall and 16s rRNA gene sequencing to identify differences in diversity in soils and to characterize diversity changes in response to simulated rainfall. Two sites with prairie strips installed adjacent to row crop production were utilized for the experiment. Bacterial communities were characterized from soil samples prior to and at multiple points following simulated rainfall. Our results in this study are important because they highlight several factors that may contribute to bacterial diversity differences, or lack thereof. We observed that site and depth of soil contributed to community structure more than vegetation type. In regard to beta diversity, we found that site and soil depth significantly impacted community dissimilarity between soil communities. This result highlights the role of site and soil characteristics in shaping soil bacterial communities, which had a greater impact than vegetation type in our analysis. Comparing changes in bacterial diversity following simulated rainfall revealed differing response based on site, further supporting the role of site characteristics shaping bacterial diversity. When comparing alpha diversity response between soil depths, we observed greater variation in shallow depths compared to the deeper soil profiles. These results highlight the more dynamic environment of the upper soil profile, where there are

greater changes in environmental and nutrient conditions that may stimulate a response from a greater range of soil bacteria. These results suggest that soil type or site was a dominant factor in determining differences in diversity, perhaps due to the young age of the prairie stirp installations and long site histories associated with agricultural production. These results are important because they indicate that young prairie strips have not significantly impacted soil bacterial diversity but may promote higher diversity following rainfall depending on site.

The work in chapter 3 was limited by several factors. The analysis of only two sites limited our ability to distinguish the impact of prairie strips in shaping diversity over site history. Contributing to this limitation is the narrow range of age in strips analyzed, both less than five years old. Finally, we analyzed bacterial communities independent of soil characteristics, limiting our ability to attribute the impact of soil characteristics such as pH, nutrient content, and soil texture on bacterial diversity. Future studies would benefit from including older prairie strips to establish how strip age impacts bacterial diversity. In addition, future work would be enhanced by including analysis of soil characteristics to help determine the contribution of soils to variation in bacterial diversity. Finally, future studies would be improved by investigating deeper into the soil profile where deep-rooted prairie plants could influence bacterial diversity.

In chapter four, we expanded on our investigations into the impacts of prairie strips on both soil bacterial communities and the soil resistome, or the profile of antibiotic resistant genes. Poultry manure was applied to crop soils adjacent to prairie strips and rainfall was simulated to evaluate the impact of prairie strips on the transport of manure associated bacteria and resistance genes. This is related to agricultural management and soil microbiomes because poultry manure introduces bacteria and ARGs to the environment and strips of vegetation have been shown to effectively reduce ARG transport. Our analysis revealed several antibiotic resistance genes in

poultry manure. Further, these genes were specific to poultry manure, enabling us to track them in soil and water. This result highlights the ability to detect genes of interest specific to manure, validating the approach of the experiment. Overall, we were not readily able to detect resistance genes in soil communities. This result could be because genes move rapidly through soils with the water or are below our detection limits. We suspect that genes move rapidly through the soil as we found that manure associated bacteria were not abundant in prairie strip soils. In runoff water, we were able to detect manure associated bacteria and antibiotic resistance genes. In 2 of the 3 plots that received manure and had installed prairie strips, we observed significantly reduced abundances of manure associated bacteria when compared to runoff water from plots without prairie strips. In regard to antibiotic resistance genes, tetracycline resistance genes were the most abundant and detected in plots receiving manure without prairie stirps. In plots with prairie strips, we observed amplification *of tet* ARGs in only 1 of 3 plots. These results suggest that prairie strips can effectively reduce the transport of manure associated bacteria and ARGs, but this is site-specific within our results. The observance of inconsistent results from one plot warrant further investigations to truly elucidate the impact of prairie conservation strips on the fate and transport of manure associated bacteria and resistance genes. Further, these results highlight the difficulty of working with complex environmental ecosystems.

The investigation in chapter 4 had several limitations. One limitation was the number of replicates used for detecting ARGs in runoff waters. Inconsistent detection in many, but not all, samples prevented statistical inference. Further, it was unclear if a gene was not detected if it was absent or below detection limits. It is also possible that inhibitors from soil interfered with detection of genes. Another opportunity for this study is to obtain information on soil type, especially porosity, pH, and bulk density, that may inform our understanding of the site-specific

differences observed. Finally, detection of ARGs in the environment indicates a risk to human health, however, the presence of a gene does not mean it is active and therefore the bacteria may contain the gene yet not be phenotypically resistant to an antibiotic. Future efforts would benefit from increased sampling to enhance statistical power. Sampling sub-surface waters would also enhance future studies and potentially capture the effect of soil properties, such as the presence of macropores, on the transport of ARGs.

The findings from the studies described here enhance our understanding of bacterial community response to amendment and contribute to knowledge of bacterial dynamics under prairie strips in response to manure and simulated rainfall. The soil bacterial community is necessary for stable ecosystem functioning and agricultural practices have significant impacts of bacterial communities. Characterizing bacterial response to inputs is one of the first steps in understanding bacterial communities of agroecosystems and the knowledge gained from these studies may contribute to future efforts to manage soil bacterial communities for more sustainable agricultural production. In chapter 2, we identified specific bacterial phyla responding to amendments over time. This characterization will contribute to our understanding of the bacteria present during decomposition of complex organic amendments and highlights the diverse soil community response. These results will contribute to developing a bacterial profile associated with efficient decomposition of organic amendments and inform our understanding of the complex bacterial response. By beginning to characterize the bacteria present in organic amendment decomposition, we hope to contribute to future studies that may utilize this knowledge to facilitate the adoption of organic amendments in lieu of mineral fertilizers and reduce the environmental impact of mineral fertilizer use on the environment. Several studies have identified increases in bacterial diversity following conversion of grasslands to agricultural

production and generally attribute this to increased nutrient availability as a result of fertilization practices. Our investigations into the effect of prairie strip installations in chapter 3 did not reveal significant differences in alpha diversity between prairie strip soils and agricultural soils. Studies suggest that it may take decades for soil communities in restored prairies to resemble those of remnants. The young age of the prairie strips we investigated may therefore explain the lack of observed differences in our studies. Further supporting this is the observation that both site and soil depth drove greater dissimilarities between prairie and crop than the different management. However, we did identify differences in diversity as a response to simulated rainfall at one site, suggesting that prairie strips may have more dormant species that may be stimulated by moisture changes, but this response was site specific. This could be, perhaps, due to differences in soil type. Future work should incorporate analysis of soil characteristics and assessment of community variation induced by them. The threat of antibiotic resistance and the association of resistance with agricultural settings makes them an ideal target for conservation practices that may attenuate the transport of ARGs. Previous work in Nebraska identified the efficacy of narrow hedges of switchgrass in reducing the transport of ARGs from manure. We evaluated if prairie strips showed similar reductions in ARG transport in chapter 4. What we found was that several ARGs were detectable in runoff from manured plots but observed limited detection in samples from plots with prairie strips. We had significant plot effects, limiting our ability to conclusively determine the role of prairie stirps in reducing ARG transport.

In conclusion, this dissertation reveals unique bacterial responses to agricultural management decisions. The results indicate a complex relationship between the soil and bacterial communities that are influenced by plant communities, nutrient inputs, and environmental events. Given the importance of bacteria for many soil functions, further investigations into soil

bacterial communities are warranted. In conclusion, we found that prairie strips do not have significantly altered alpha diversity for their age when compared to adjacent crop soils. However, site specific increases in alpha diversity were observed in plots with prairie strips receiving simulated rainfall, suggesting that site history may contribute to the observed differences. Future studies to evaluate the role of bacterial communities should incorporate rigorous soil analysis to elucidate the role of soil characteristics in bacterial diversity of prairie strips.