

RESPONSE OF SOIL BACTERIAL COMMUNITIES TO CROPPING SYSTEMS, TEMPORAL CHANGES,
AND ENVIRONMENTAL CONDITIONS IN THE NORTHERN GREAT PLAINS

by

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TABLE OF CONTENTS

1. INTRODUCTION.....	1
Soil Health and Soil Microbial Communities	1
Challenges Faced by Soil Bacterial Communities in Semiarid Agriculture	4
Soil Bacterial Communities in Cropping Systems.....	8
Impact of Organic Cropping Systems on Soil Bacterial Communities	8
Impact of Diversified Crop Rotations on Soil Bacterial Communities	10
Impact of Cover Crop Mixtures on Soil Bacterial Communities	11
Impact of Cover Crop Termination Methods on Soil Bacterial Communities	12
Research Objectives.....	14
References	17
2. TEMPORAL SOIL BACTERIAL COMMUNITY RESPONSES TO CROPPING SYSTEMS AND CROP IDENTITY IN DRYLAND AGROECOSYSTEMS OF THE NORTHERN GREAT PLAINS	31
Introduction.....	31
Materials and Methods.....	36
Site Description	36
Study Design.....	37
Soil Collection	39
Laboratory Methods.....	39
Bioinformatics	40
Statistical Procedures	41
Results	43
Alpha Diversity	43
Overall Community Composition: Abundance	46
Beta Diversity	49
Discussion	52
Differences Between Cropping Systems.....	53
No Impact of Crop Rotation Phase	54
Differences Between Sampling Months	56
Conclusion.....	57
References	58

TABLE OF CONTENTS CONTINUED

3. SOIL BACTERIAL COMMUNITY RESPONSE TO COVER CROPS, TERMINATION, AND TEMPERATURE AND MOISTURE CONDITIONS IN A DRYLAND CROPPING SYSTEM.....	66
Introduction.....	66
Materials and Methods.....	70
Site and Experimental Design.....	70
Termination of Cover Crops.....	73
Winter Wheat.....	73
Temperature and Moisture Conditions.....	74
Soil Sampling.....	74
Laboratory Methods.....	75
Bioinformatics.....	76
Statistical Procedures.....	77
Results.....	79
Alpha Diversity.....	79
Beta Diversity.....	83
Community Composition: Relative Abundance.....	85
Discussion.....	89
Interaction Between the Presence and Composition of Cover Crops and Predicted Climate Conditions.....	90
Effects of Temperature and Moisture Conditions.....	92
Cover Crop Termination and Soil Bacterial Communities.....	93
Conclusion.....	94
References.....	96
4. SUMMARY OF FINDINGS AND FUTURE RESEARCH.....	106
Summary of Findings.....	107
Future Directions.....	109
References.....	111
REFERENCES CITED.....	113
APPENDICES.....	134
APPENDIX A: Supplementary Information for Chapter Two.....	135
APPENDIX B: Supplementary Information for Chapter Three.....	140

LIST OF TABLES

Table	Page
2.1 Statistics from Type III Analysis of Variance for mixed effect linear models of bacterial richness, Shannon's Diversity, and Shannon's Evenness	45
2.2 Pairwise comparisons from Tukey's post-hoc analysis of alpha diversity models	46
2.3 PERMANOVA of the effects of cropping system, crop, month, and the interaction between cropping system and crop on soil bacterial communities	49
3.1 Statistics from Type III Analysis of Variance for mixed effect linear models of bacterial richness, Shannon's Diversity, and Shannon's Evenness	80
3.2 Pairwise comparisons from Tukey's post-hoc analysis of alpha diversity models	81

LIST OF FIGURES

Figure	Page
2.1. Alpha Diversity of Soil Bacterial Communities.....	44
2.2. Heatmap of Abundant Sequence Variants	47
2.3. Random Forest: Log Relative Abundance of Predictor Taxa	48
2.4. Soil Bacterial Community Composition in Cropping Systems.....	50
2.5. Soil Bacterial Community Composition in Crop Phases of Cropping Systems.....	52
3.1. Experimental Design	72
3.2. Alpha Diversity of Soil Bacterial Communities.....	82
3.3. 2018 Soil Bacterial Community Composition	84
3.4. 2019 Soil Bacterial Community Composition	85
3.5. Relative Abundance of Common Phyla	86
3.6. Heatmap of Abundant Sequence Variants	87
3.7. Random Forest: Log Relative Abundance of Predictor Taxa in 2018	89

ABSTRACT

Soil bacterial communities are essential components of the soil ecosystem that support crop production. However, agriculture in semiarid drylands and their associated soil bacterial communities face increasingly warmer and drier conditions due to climate change. Two complementary studies were conducted to assess the response of soil bacterial communities to cropping systems, temporal changes, and soil temperature and moisture conditions in semiarid, dryland agricultural systems of the Northern Great Plains.

The first study focused on soil bacterial community response to crop phase in contrasting cropping systems (chemical inputs and no-till, USDA-certified organic tilled, and USDA-certified organic sheep grazed) over a growing season. Organic grazed management supported more diverse bacterial communities than chemical no-till, though diversity in all systems decreased over the growing season. Organic grazed bacterial communities were distinct from those in the organic tilled and chemical no-till systems. An interaction between cropping system and crop phase affected community dissimilarity, indicating that overarching management systems and environmental conditions are influential on soil bacterial communities.

The second study evaluated soil bacterial communities in a winter wheat - cover crop or fallow rotation. Observations were conducted in the summer fallow and two cover crop mixtures differing by species composition and phenologies, terminated by three different methods (chemical, grazing, or haying), and subjected to either induced warmer/drier or ambient soil conditions. Only the presence and composition of cover crops affected bacterial community dissimilarity, where mid-season soil bacterial communities were distinct from early season and fallow communities. Bacterial communities responded to an interaction between the presence and composition of cover crops and environmental conditions, but not termination. No treatment effects were observed in bacterial communities in 2019, which could be attributed to above average rainfall.

The results of these studies suggest cover crop mixtures including species tolerant to warmer and drier conditions can foster diverse soil bacterial communities compared to fallow soils. Overall, these studies contribute to a better understanding of how soil bacterial communities respond to soil health building practices in the Northern Great Plains. Cropping systems can foster unique soil bacterial communities, but these effects may be moderated by environmental and temporal conditions.

CHAPTER ONE

INTRODUCTION

Agricultural systems support nearly 8 billion people but focusing on crop production comes with environmental consequences. Intensified, input-heavy agriculture as the dominant approach to production has resulted in significant soil degradation and topsoil loss, disruptions to global biogeochemical cycling, and widespread biodiversity loss (Foley et al. 2005; Landis 2017; Vanwalleghem et al. 2017; Doran and Zeiss 2000). These negative impacts have spurred an interest in understanding the mechanisms impacting both soil quality and soil health, a required step to developing sustainable agricultural systems. Our understanding of soil has changed from a medium for plant growth to that of an extremely complex, living ecosystem characterized by a high degree of interconnectivity among its biotic components (Lal 2016; Lehmann et al. 2020). Recognizing the ecological interactions of organisms in agricultural systems is important because soils, plant communities, and soil microbial communities affect nutrient cycling, plant health, and soil health (Wagg et al. 2014).

Soil Health and Soil Microbial Communities

Soils are an indispensable resource and complex ecosystem, yet the importance of belowground communities to agricultural systems has gained recognition only within the past few decades. With nearly 40% of all agricultural land considered degraded (Doran and Zeiss 2000), the pressing issues of soil degradation and loss of productivity on agricultural lands have

brought a focal shift towards restoring and maintaining soil health. Soil health is the ability of soils to function as a living ecosystem while supporting life aboveground and sustaining the socio-ecological functions of the land (Lehmann et al. 2020; Janzen et al. 2021). This is an idea that is separate from, but has grown out of the concept of, soil quality, or the extent to which a soil can function and support agricultural systems (Lehmann et al. 2020). Doran and Zeiss (2000) presented soil as an ecosystem vital to environmental quality and human systems and Lal (2016) defined soil as “a living biological entity that affects plant health.” Overall soil health is assessed through soil properties, including soil organic carbon concentration, soil organic matter, soil aggregate size, pore space, water infiltration, water holding capacity, and aeration, but is also based on soil biotic communities (Maharjan et al. 2020).

Soil organic carbon underlies several physicochemical and biological soil properties essential to many ecosystem services humans depend on, such as agricultural production (Lal 2016; Maharjan et al. 2020; Brussaard 2013). Soil organic carbon is a product of the short-term carbon cycle, which encompasses net primary production and the decomposition of soil organic matter (Horwath 2007). As a major component of soil organic matter, soil organic carbon improves physical soil properties by increasing aggregation and water storage capacity while decreasing bulk density and compactibility, indirectly enhancing crop production (Blanco-Canqui et al. 2013b). A considerable amount of soil organic carbon is root-derived (Schmidt et al. 2011) and an important source of nutrients for the soil microbial communities (Lal 2016) that form the basis of the soil food web.

Two of the more notable biological soil properties are the immense biodiversity and complex networks arising from soil biota communities at several scales, ranging from micrometers to landscapes (Wakelin 2018; Brussaard et al. 2007). The heterogeneous nature of soils creates a multitude of habitats suitable for multitrophic soil food webs (Bardgett and van der Putten 2014; Digel et al. 2014) where interactions among micro-, meso-, and macrofauna influence physical soil structure, soil geochemistry, and nutrient cycling (Wilpiseski et al. 2019; Becker et al. 2001). Beyond physicochemical indicators such as bulk density, pH, water content, soil carbon and soil nitrogen that can inform a soil's ability to function (Seaton et al. 2020), soil microbial communities can be used as indicators of soil health because they are an essential component of the soil ecosystem underpinning numerous ecosystem functions (Lal 2016). For example, soil bacteria and soil fungi cycle nutrients directly through the processes of decomposition and mineralization (Geisen and Bonkowski 2018) and indirectly as a source of food for other organisms such as bacterivore and fungivore nematodes (Ferris and Tuomisto 2015).

The soil microbiome is the array of bacteria, fungi, archaea, protozoa, viruses, and their collective genomes which interact with one another and with macroorganisms in the soil matrix. These multitrophic communities influence physical soil structure, drive nutrient cycling by decomposing organic matter, mineralize nitrogen and can help promote growth and suppress disease in plants (Miller and Jastrow 2000; Brussaard, de Ruiter, and Brown 2007; Martínez-García et al. 2018). For example, soil bacteria can produce hormones that directly influence plant growth or modify the production of plant growth hormones (Patten and Glick

2002; Mohite 2013), and mutualistic soil biota aid in water and nutrient uptake by plants, thereby increasing aboveground productivity (Bender and van der Heijden 2015; van der Heijden et al. 2016). Understanding how the interactions between aboveground and belowground communities influence crop production is essential to enhance the sustainability of farming, especially as intensive agricultural management is known to reduce micro- and macro-biological diversity with associated negative consequences to ecosystem functions (Tsiafouli et al. 2015; Dudley and Alexander 2017).

Challenges Faced by Soil Bacterial Communities in Semiarid Agriculture

The interactions between aboveground and belowground communities influence crop production and are essential to agricultural systems, especially in semiarid regions where soils are at risk for degradation and aridification (Garcia-Franco et al. 2018). Over a third of the world's population depends on semiarid dryland agriculture, highlighting the need for continued research on soil microbial community behavior in these systems and implementation of soil health-building practices (Delgado-Baquerizo et al. 2017). Drylands are defined as water scarce areas that, while adapted to variable and dry conditions, are vulnerable to land degradation and unsustainable land use (IUCN). The importance of understanding the relationships between dryland agricultural systems and soil microbial communities is magnified as semiarid regions become warmer, potentially drier, and more widespread. Currently, semiarid ecosystems span almost 45% of the planet's terrestrial surface but, according to climate change projections, their range may increase to nearly 80% by the end of the 21st

century (Delgado-Baquerizo et al. 2017). Climate change poses a challenge for soil microbiota and agriculture alike (Classen et al. 2015; Kumar et al. 2020), especially in semiarid ecosystems where annual precipitation is already low.

Meta analyses often overlook semiarid systems when examining the impacts of cropping systems on soil bacterial communities (Housman et al. 2021), but mean annual precipitation is a main driver of bacterial community structure (Tan et al. 2020). Soil bacteria in semiarid ecosystems often do not have as significant a response to soil health building practices as communities in areas that receive more rainfall, as bacterial utilization of substrates is affected by environmental conditions (Housman et al. 2021). In water limited systems, precipitation events are often accompanied by a spike in bacterial activity as the influx of moisture reactivates carbon cycling in the microbial community (Barnard et al. 2015). These events may not be consistent enough to build up soil, as the responses to extreme wetting are short-lived (Cruz-Martínez et al. 2012). In more humid climates, soil bacteria can more consistently utilize resources and take advantage of inputs from cover crops and other regenerative practices (Taskin et al. 2021). Water stress is still detrimental, but the benefits from cover crops can accumulate over the years. In irrigated Mediterranean climates, no-till and cover cropping encouraged a shift towards slow-growing and stress tolerant organisms, especially deeper in the soil (Schmidt et al. 2018). These practices are expected to increase soil health in rainfed and humid systems but are more challenging to implement in drier environments (Mitchell et al. 2017).

Soil bacterial and soil fungal community response to drought and soil warming can be moderated by nutrient availability, and land use. Soil bacterial communities in drylands are particularly sensitive to changes in aridity and can decrease in abundance and diversity as a result (Maestre et al. 2015). Across the semiarid sections of the Northern Great Plains, the region where this study was conducted, temperatures and precipitation are both projected to increase by the end of the century (Wienhold et al. 2018). On the scale of where our field sites are located, precipitation is expected to increase before the growing season and decrease during the growing season. However, the higher evapotranspiration rates predicted are likely to reduce effective soil moisture (Whitlock et al. 2017). Decreased soil moisture tends to destabilize or apply selective pressure to the soil microbial community (Naylor and Coleman-Derr 2018; de Vries et al. 2018) because without mobility of their own, they are limited by the spatial and temporal availability of resources. This is problematic if drought-sensitive keystone taxa are affected because they are heavily involved in soil ecosystem functioning (Berry and Widder 2014; Banerjee et al. 2018). Keystone taxa are species integral to a community to the extent that their removal has far-reaching, negative impacts (Berry and Widder 2014). They are often connected to several other taxa in the community as part of a co-occurrence network, or the relationships and associations among members of a community (Barberán et al. 2012; Banerjee et al. 2018). Disturbances such as drought may have a cascading effect on bacterial co-occurrence networks because their high connectivity makes them less stable (de Vries et al. 2018). This has consequences for the stability of a larger ecological network and its ability to resist environmental disturbances. A loss of stability in semiarid agricultural soil microbial

communities may impact the ecosystem functions they help regulate and the associated services these managed systems provide, including crop production (Rossato et al. 2017; Daryanto et al. 2016).

Increasing soil organic carbon is a possible solution to ameliorate the impact of climate change on soil microbiota (Whitmore et al. 2015), as this may bolster soil bacterial community resistance to drought (Moreno et al. 2019) and benefit agricultural production. Soil organic carbon can improve soil aggregation and stability, which increases macropore space, water filtration, and water storage (Blanco-Canqui et al. 2013b). However, soil texture class affects the extent to which increases in soil organic carbon can change soil water storage. Water retention ability is more responsive to increases in soil organic carbon in coarse-textured soils than in fine-textured soils (Rawls et al. 2003). It has been estimated that for medium-textured soils, a 1% increase in soil organic carbon concentration can result in soil aggregate size increasing by 1.5mm and plant available water increasing by 12.5mm in the top 20cm of a soil with a 1.25 Mg m^{-3} bulk density (Blanco-Canqui et al. 2013b). One way to increase soil organic carbon is through ecological intensification, or the process of integrating ecosystem services management into agricultural systems (Bommarco et al. 2013). This can be accomplished in water-limited systems such as the Northern Great Plains, where diversified no-till cropping systems have already become more commonplace (Hansen et al. 2016). The remainder of this review will explore how soil bacterial communities may respond to ecological intensification and soil health building practices in different cropping systems.

Soil Bacterial Communities in Cropping Systems

Several soil health building practices are also components of regenerative agriculture, a popular sustainability concept that focuses on rebuilding soil and increasing diversity in agricultural systems through ecological intensification, with an emphasis on minimizing off-farm inputs (Pearson 2007) and moving away from tillage and fallow (LaCanne and Lundgren 2018). The goal is to improve agricultural lands and counteract practices that have contributed to their degradation (Rhodes 2017). Regenerative agriculture encompasses practices that prioritize soil building over crop production, under the paradigm that soil, ecosystem, and human health are interconnected (Lal 2020). Elements of cropping systems often categorized under the umbrella of regenerative agriculture include organic farming, diversified crop rotations, cover crops, and integrated crop-livestock management.

Impact of Organic Cropping Systems on Soil Bacterial Communities

Organic farming is one approach to improve agricultural sustainability through increasing reliance on ecological processes rather than synthetic inputs (Adesope et al. 2012). Compared to chemically managed systems, organic systems place a greater emphasis on soil regeneration and aboveground biodiversity. Previous literature has examined the differential impact of organic and conventional management systems on soil microbiota, which can lead to distinct belowground communities (Li et al. 2012; Hartmann et al. 2015; Harkes et al. 2019). For example, soil microbiota are reported to increase in phylogenetic richness and community variability in organic systems (Lupatini et al. 2017), but this may be linked to the greater

bioavailability of nutrient inputs in a more neutral soil pH (Lauber et al. 2009; Zhalnina et al. 2015). Nutrient input from different fertilizer regimes may be the driving force of these observations (Hartmann et al. 2015; Semenov et al. 2020). Across the Northern Great Plains, organic cropping systems are characterized by more complex crop rotations and diverse associated biodiversity than those observed in conventional wheat-summer fallow rotations (Adhikari and Menalled 2020).

Despite these benefits, excessive reliance on tillage in organic systems can be detrimental to soil health. Tillage is used to cultivate fields, remove crop and weed residues, and incorporate manure inputs. Intensive conventional tillage is known to facilitate soil erosion and a loss of nutrients (Jat et al. 2019; Schneekloth et al. 2020), particularly in dry regions (Clay et al. 2014). Moreover, this practice can dramatically reduce bacterial (Dörr de Quadros et al. 2012; García-Orenes et al. 2013; Ishaq et al. 2020) and fungal (Drijber et al. 2000; Castillo et al. 2006) diversity in soil, potentially impairing the functionality of these systems. Alternatives to conventional tillage are being sought for organic systems that maintain crop productivity but prevent soil or biodiversity loss. For example, conservation or reduced tillage lessens disruption to soil organic carbon pools and no tillage leaves cover crop residue on the soil surface. This can result in increased soil carbon, soil nitrogen, and microbial biomass at the surface of the soil (Nivelle et al. 2016; Helgason et al. 2010; Sapkota et al. 2012) but, in conventional systems, has been criticized for its heavy reliance on herbicides and the associated selection of herbicide-resistant weed biotypes (Menalled et al. 2016).

Impact of Diversified Crop Rotations on Soil Bacterial Communities

One strategy for ecological intensification includes replacing a fallow period following the commercial crop harvest with cover crops (Wittwer et al. 2017). Winter wheat - summer fallow rotations are commonly used in the semiarid sections of the Northern Great Plains as a method of soil moisture conservation (Kumar et al. 2020). These highly simplified agricultural landscapes that dominate conventional agriculture of the region are characterized by low plant diversity and a high dependence on synthetic inputs (Adhikari et al. 2019) which negatively impact soil biota and result in less diverse belowground communities (Chaudhry et al. 2012; Ishaq et al. 2017). However, a winter wheat - summer fallow rotation has only 10-40% water storage efficiency (Nielsen and Vigil 2010) and does not contribute as many benefits to the soil as more diverse cropping systems (Rosenzweig et al. 2018). Fields left bare for a growing season are susceptible to erosion, reductions in soil organic matter, salinization, and decreases in soil health (Nielsen and Calderón 2011; Sharratt et al. 2018; Carr et al. 2020). Replacing summer fallow with cover crops has the potential to increase soil organic matter and sequester soil organic carbon, even in semiarid regions (Blanco-Canqui et al. 2013a).

Diversified crop rotations that include for-profit or beneficial (*i.e.*, green manure or bioremediation) plant species reduce or replace fallow periods, which can benefit soil ecosystems and increase soil biodiversity (Steenwerth and Belina 2008; Maarastawi et al. 2018; Peralta et al. 2018). While precipitation is a limiting factor in drylands, increasing the number of crops in rotation may be feasible though financial risks of potential lower yields must be assessed (Smith et al. 2017). Carbon and nitrogen from microbial biomass can increase by

roughly 20% and 27%, respectively, in diversified crop rotations compared to a simple monoculture (McDaniel et al. 2014). Functional and metabolic diversity in heterotrophic soil bacterial communities also increase under more diverse crop rotations (D'Acunto et al. 2018). It is unclear whether the soil physicochemical changes, increases in crop detritus, host-specific promotion, or inclusion of various functional groups associated with diversifying crop rotations have the greatest impact on soil microbial communities (Venter et al. 2016). While there is not a specific sequence of crops that will achieve these benefits, cover crop mixtures are often included in these rotations towards the same end.

Impact of Cover Crop Mixtures on Soil Bacterial Communities

Cover crops can recruit specific microorganisms via root exudates and rhizodeposits (Ishaq et al. 2017; Cardinale et al. 2015), and while there is not a comprehensive understanding of what microbial communities are recruited by specific plant species, there is some evidence that certain plants act more selectively than others (Aguilera et al. 2017; Massenssini et al. 2015; Trognitz et al. 2016). Plants produce organic compounds and phytochemicals during their growth that vary by species and life stages (Hansen et al. 2018; Tiemann et al. 2015), resulting in the ability for cover crops such as brassicas and legumes to foster unique communities in their rhizospheres (Thapa et al. 2021a). Differences in litter quality between root inputs, root turnover, and crop residue can alter soil microbial community structure. For example, while high quality plant litter has a lower carbon to nitrogen ratio that supports microbiota with fast growth rates and the capacity to utilize temporally limited nutrients, low quality plant litter, with a high carbon to nitrogen ratio, is better suited for fungal communities (Castellano et al.

2015; Wortman et al. 2013; Frasier et al. 2016). These inputs of biomass and root exudates shape soil microbial activity and diversity, sometimes even after cover crops are terminated for the season (Wortman et al. 2013; Calderón et al. 2016). Cover crops need to be regularly included in a crop rotation to maintain their positive effects on soil health because post-termination benefits do not last as long in semiarid systems (Blanco-Canqui et al. 2013a). However, limited soil moisture places constraints on dryland cover cropping because it can further decrease the amount of soil moisture available for subsequent commercial crops (Unger and Vigil 1998; Thapa et al. 2021b). Lower evapotranspiration rates in the Northern Great Plains compared to further south may increase the success of adding cover crops to a rotation, as would choosing species that optimize the trade-off between soil benefits and soil moisture loss (Blanco-Canqui et al. 2015; Thapa et al. 2021b).

Impact of Cover Crop Termination Methods on Soil Bacterial Communities

Cover crops grown in semiarid regions such as the Northern Great Plains are often terminated between late spring and early summer to preserve soil moisture (Carr et al. 2012; Nielsen et al. 2016; Lehnhoff et al. 2017). Contrasting termination methods may have different effects on soil microbial communities (Liang et al. 2014; Kim et al. 2020; Castellano-Hinojosa and Strauss 2020). Glyphosate is widely used for cover crop termination but its effects on soil microbiota is less understood because a consistent reaction has not been observed (Khan et al. 2006; Mijangos et al. 2009; Castillo et al. 2012; Meena et al. 2020). Factors such as location, cropping system, tillage, and land use history are more significant drivers of soil microbial community diversity than glyphosate use, as application at recommended rates do not appear

to affect most bacterial taxa (Schlatter et al. 2017; Kepler et al. 2020). It has been suggested that glyphosate may change community composition by decreasing plant defenses and creating a favorable environment for pathogenic microbiota or suppressing taxa beneficial for plant growth (Van Bruggen et al. 2018), though this is controversial (Kepler et al. 2020). Alternate methods of cover crop termination and weed management include haying and integrated crop-livestock management.

Soil bacterial diversity may increase in response to haying, possibly through mechanisms such as disturbance-induced changes to root biomass, root exudates, and soil moisture (Foster et al. 2010). A combination of warming and aboveground biomass removal can lead to a decrease in soil carbon inputs from plant litter and an increase in the abundance of microbial genes responsible for nutrient cycling, though responses are not consistent (Xue et al. 2016). Available studies for the effect of haying on microbial communities are scarce. More thoroughly studied is the integration of crop and livestock practices as an approach to reduce tillage intensity in organic cropping systems (Miller et al. 2015, McKenzie et al. 2016; Larson et al. 2021) while providing forage (Blanco-Canqui et al. 2015) and increasing soil nitrogen, soil carbon, and microbial biomass (Ishaq 2017). The reintegration of livestock management with crop production has gained traction since the mid-2000s (Kumar et al. 2019). As discussed in Russelle et al. (2007), studies on crop-livestock management have been conducted in several regions of the United States (Allen et al. 2007; Franzluebbers 2007; Sulc and Tracy 2007), and the practice can be successfully adapted to the semiarid Northern Great Plains (Sulc and Franzluebbers 2014; Kumar et al. 2019). Associated resistance to soil water infiltration and a

loss of plant cover would need to be mitigated to avoid increases in soil temperature and decreases in soil moisture (Blanco-Canqui et al. 2013a), factors that can negatively impact soil microbial communities (de Vries et al. 2018; Naylor and Coleman-Derr 2018; Bérard et al. 2011).

The ecological interactions within integrated crop-livestock systems demand investigation beyond their effects on crop production. Grazing can influence microbial diversity and community structure directly through nutrient deposition and soil compaction (Zhou et al. 2010) or indirectly by encouraging root production and enhancing rhizodeposition (Hamilton III et al. 2008; Zhou et al. 2010; Yang et al. 2019). Other indirect effects include changes to plant community composition (Yang et al. 2019) or soil nutrient availability. Grazing can remove carbon from the system, suppressing the copiotrophic dryland taxa Actinobacteria and supporting the oligotrophic Acidobacteria and Chloriflexi (Eldridge et al. 2017). Interactions with physicochemical soil characteristics such as pH and soil moisture may also explain grazing-induced changes in soil bacterial community structure (Yang et al. 2019).

Research Objectives

The overall goal of this research was to assess how soil bacterial communities respond to contrasting cropping systems of the Northern Great Plains. Our objectives were to evaluate soil bacterial community response to 1) cropping systems, crop phase, and temporal changes, and 2) cover crop composition, termination methods, and altered temperature and moisture

conditions. We conducted two studies examining the effects of cropping systems and environmental conditions on soil bacterial diversity and community composition.

Chapter two focuses on the first objective, which utilized an experiment conducted at the Montana State University Fort Ellis Teaching and Research Center that began in 2012. The cropping systems used in this study were conventional off-farm inputs with no tillage, USDA certified organic with tillage for weed control and cover crop termination, and USDA certified organic with targeted sheep grazing and reduced tillage. Samples were taken in June and August 2017 from the first three phases (safflower undersown with sweet clover, sweet clover, and winter wheat) of a five-year crop rotation. The purpose of this experiment was to assess the effects of system-level differences and crop phase across a single growing season on soil bacterial community alpha (within-group) and beta (between-group) diversity. We hypothesized that the USDA certified organic systems would support higher soil bacterial diversity than the conventional system, but that tillage and grazing with reduced tillage would select for dissimilar soil bacterial communities. We also hypothesized that soil bacterial diversity would be highest during the cover crop phases.

For the second objective and third chapter of this thesis, we took part in the final two years of a field experiment at the Montana State University Northern Agricultural Research Center in Havre, MT that also began in 2012. The study was conducted from 2018 to 2019 in two fields under a biphasic crop rotation: a winter wheat phase and a cover crop or fallow phase. We compared the winter wheat - summer fallow rotation with winter wheat in rotation with cover crop mixtures of different species richness and phenologies. The fallow, early season

mixture (five species), and mid-season mixture (seven species) phases were terminated by glyphosate application, haying, or cattle grazing. This study also tested the effects of decreased soil moisture and increased soil temperature on soil bacterial communities to mimic climate change projections for the region. We hypothesized that the mid-season and early season treatments would have more diverse soil bacterial communities than fallow, that ambient conditions would support higher soil bacterial diversity than warmer and drier conditions, and that there would be a gradient in soil microbial community diversity from warmer and drier fallow plots terminated by glyphosate (lowest) to ambient mid-season plots terminated by grazing (highest).

The final chapter of this thesis presents a summary of these findings in the overall context of how soil bacterial communities respond in dryland agricultural systems common to the semiarid sections of the Northern Great Plains. It also gives recommendations for future studies.

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CHAPTER TWO

TEMPORAL SOIL BACTERIAL COMMUNITY RESPONSES TO CROPPING SYSTEMS AND CROP
IDENTITY IN DRYLAND AGROECOSYSTEMS OF THE NORTHERN GREAT PLAINSIntroduction

Modern industrial agriculture results in overly simplified landscapes where soil mediated regulatory ecosystem functions have been hampered or replaced with intensive off-farm inputs aimed principally at securing high yields. The overreliance on synthetic chemical and mechanical inputs, however, comes with a high potential for soil degradation and soil loss (Landis 2017; Vanwalleghem et al. 2017). Worldwide, agricultural erosion has diminished soil capacity to function as a resource and a living ecosystem on 40% of all agricultural land, underscoring the need to restore and maintain soil health. (Doran and Zeiss 2000). In particular, tillage is used in many conventional and organic agricultural systems to cultivate fields, remove crop and weed residues, and incorporate manure inputs. Intensive conventional tillage is known to facilitate soil erosion and a loss of nutrients (Jat et al. 2019; Schneekloth et al. 2020), particularly in dry regions (Clay et al. 2014). Moreover, this practice can dramatically reduce bacterial (Dörr de Quadros et al. 2012; García-Orenes et al. 2016; Ishaq et al. 2020a) and fungal (Drijber et al. 2000; Castillo et al. 2006;) diversity in soil, potentially impairing the functionality of these systems. Alternatives to conventional tillage are being sought for organic systems which maintain crop productivity but sustain soil or biodiversity loss.

The soil microbiome - the array of bacteria, fungi, archaea, protozoa, viruses, and their collective genomes which interact with each other and with macroorganisms, is an essential component of the soil ecosystem underpinning numerous ecosystem functions (Lal 2016). Soil microorganisms influence soil physical structure, drive nutrient cycling through decomposition of organic matter and mineralization of nitrogen, and suppress disease in plants (Miller and Jastrow 2000; Brussaard et al. 2007; Martínez-García et al. 2018). Soil bacteria can produce hormones that directly influence plant growth or modify the production of plant growth hormones (Patten and Glick 2002; Mohite 2013), and mutualistic soil biota aid in plant water and nutrient uptake, increasing aboveground productivity (Bender and van der Heijden 2015; van der Heijden et al. 2016). Understanding how these interactions between aboveground and belowground communities condition productivity is essential to enhance the sustainability of farming, especially as intense agricultural management is known to reduce micro- and macro-biological diversity with associated negative consequences to ecosystem functions.

Organic farming is one approach to improve agricultural sustainability through increasing reliance on ecological processes rather than synthetic inputs. Compared to chemically managed systems, organic systems place a greater emphasis on soil regeneration and aboveground biodiversity. Previous literature has examined the differential impact of organic and conventional management systems on soil microbiota, which can lead to distinct belowground communities (Li et al. 2012; Hartmann et al. 2015; Harkes et al. 2019). For example, soil microbiota is reported to increase in phylogenetic richness and community variability in organic systems (Lupatini et al. 2017), but this may be linked to the greater

bioavailability of nutrient inputs in a more neutral soil pH (Lauber et al. 2009; Zhalina et al. 2015). Nutrient input may be the driving force of these observations (Hartmann et al. 2015; Semenov et al. 2020). There is also a knowledge gap of how soil microbial communities vary among contrasting organic systems.

In the semiarid regions of the Northern Great Plains, fallow periods where fields are left bare for a growing season to aid in the preservation of soil moisture do not contribute as many benefits to the soil as more diverse cropping systems (Rosenzweig et al. 2018). Also, the highly simplified landscapes that dominate conventional agriculture of the region are characterized by low plant diversity and a high dependence on synthetic inputs (Adhikari et al. 2019) which negatively impact soil biota and result in less diverse belowground communities (Chaudhry et al. 2012; Ishaq et al. 2017). In contrast, organic cropping systems are characterized by more complex crop rotations and diverse associated biodiversity, including weed communities, than those observed in conventional wheat-summer fallow rotations across the region (Adhikari and Menalled 2020).

Diversified crop rotations that include for-profit or for-health (i.e., green manure or bioremediation) plant species reduce or replace fallow periods, benefiting soil ecosystems and increasing soil biodiversity (Maarastawi et al. 2018; Peralta et al. 2018). Carbon and nitrogen from microbial biomass can increase by roughly 20% and 27%, respectively, in diversified crop rotations compared to a simple monoculture (McDaniel et al. 2014). Functional and metabolic diversity in heterotrophic soil bacterial communities also increase under more diverse crop rotations (D'Acunto et al. 2018). It is unclear whether the soil physicochemical changes,

increases in crop detritus, host-specific promotion, or inclusion of various functional groups associated with diversifying crop rotations have the greatest impact on soil microbial communities (Venter et al. 2016). While there is not a specific sequence of crops that will achieve these benefits, cover crop mixtures are often included in these rotations towards the same end.

Cover crops can recruit specific microorganisms via plant inputs (Ishaq et al. 2017), and while there is not a comprehensive understanding of what microbial communities are recruited by specific plant species, there is some evidence that certain plants act more selectively than others (Aguilera et al. 2017; Massenssini et al. 2015; Trognitz et al. 2016). It is also known that cover crops can alter soil microbial community dynamics because microbiota with fast growth rates and the capacity to utilize temporally limited nutrients can take advantage of organic carbon from cover crop roots, root turnover, and crop residue (Wortman et al. 2013) and may favor bacterial communities while those that become low quality residue favor fungi (Frasier et al. 2016). These inputs of biomass and root exudates shape soil microbial activity and diversity, even after cover crops are terminated for the season (Wortman et al. 2013; Calderón et al. 2016).

In semi-arid agroecosystems, cover crops must be terminated early enough in the growing season to preserve soil moisture for the commercial crop. Cropping systems employ various methods of cover crop termination that have their own effects on soil microbial communities. For example, conservation or reduced tillage lessens disruption to soil organic carbon pools and no tillage leaves cover crop residue on the soil surface. This can result in

increased soil carbon, soil nitrogen, and microbial biomass at the surface of the soil (Nivelle et al. 2016; Helgason et al. 2010; Sapkota et al. 2015) but has been criticized for its heavy reliance on herbicides and the associated selection of herbicide-resistant weed biotypes (Menalled et al. 2016). In recent years, consumer demands and market opportunities have driven an expansion of organic agricultural systems which use tillage to terminate cover crops and incorporate residue into the soils. However, tillage disturbs the soil ecosystem by accelerating soil organic matter oxidation and labile carbon cycling (McLauchlan 2006), factors that have driven a growing interest to reduce soil disturbance practices in organic systems (Carr 2017). The integration of crop and livestock practices has been explored as an approach to reduce tillage intensity in organic cropping systems (Miller et al. 2015, McKenzie et al. 2016) while increasing soil nitrogen, soil carbon, and microbial biomass (Ishaq et al. 2017). However, little is known about specific effects of grazing on soil microbial communities.

Semiarid, dryland agriculture makes up a significant portion of global crop production and understanding how soil communities interact with crops under different farming practices is crucial for global agricultural security (Delgado-Baquerizo et al, 2017). In this study, we assessed soil bacterial community responses to contrasting cropping systems in semiarid regions of the Northern Great Plains. To do so, we took advantage of a field experiment that began in 2012 at the Montana State University Fort Ellis Research and Teaching Center in Bozeman, MT to test a five-year crop rotation under three different management systems: chemical inputs with no-tillage, a USDA-certified organic system with tillage, and a USDA-certified organic system with targeted sheep grazing for cover crop and weed termination

(Ishaq et al. 2020a). We hypothesized that 1) organically managed systems support higher soil bacterial diversity than chemically managed systems, 2) the two organic systems result in dissimilar soil bacterial communities, and 3) diversity is highest in the cover crop phases. We did this by comparing bacterial communities from winter wheat, safflower/sweet clover, and sweet clover under different management systems in fields where the crop rotation has been established for several years.

Methods

Site Description

A field experiment to evaluate approaches to minimize soil disturbances in organic cropping systems was established in 2012 at the Montana State University Fort Ellis Research and Teaching Center in Bozeman, MT (45.653 N, -110.972 W). The underlying soil is a Blackmore silt loam (fine-silty, mixed superactive, frigid Typic Argiustolls) with 0-4% slopes, composed of about 22% clay, 10% sand, and 68% silt down to 25cm (NRCS 1999; UC Davis 2019). Fort Ellis usually receives 465mm of precipitation annually, with monthly mean air temperatures between -5.7 to 18.9°C. Prior to 2004, the study site was planted with perennial grasses (*Bromus inermis* L., *Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey, and *Poa compressa* L.). Between 2004 and 2009, the experimental site followed either continuous spring wheat (*Triticum aestivum* L.), spring wheat-fallow, or winter wheat-fallow crop rotation. From 2009 to 2012, the study site followed either a continuous alfalfa (*Medicago sativa* L.) or a three-year crop rotation consisting of spring wheat in the first year followed by pea (*Pisum*

sativum L.), and hay barley (*Hordeum vulgare* L.) in the second and third years, respectively. In the spring of 2012, the entire experimental site was planted with glyphosate tolerant rapeseed (*Brassica napus* L.) and treated with herbicide. The rapeseed was tilled to a depth of 15 cm in July 2012 and planted in September 2012 following the experimental design described below. Additional site information can be found in Miller et al. (2015) and Barsotti et al. (2013).

Study Design

This study followed a split-plot design with three replications where cropping systems were the main plot level and crop phases the split-plots (Figure S2.1). The cropping systems included a chemical no-till system where synthetic off-farm inputs were utilized to manage weeds, pathogens, and soil nutrient levels (hereafter, chemical no-till). This system, often referred to as conventional in the context of industrial-age farming, was considered the basis of comparison for other treatments. The other cropping system treatments included a USDA-certified organic system with tillage used for cover crop termination and weed management (hereafter, organic tilled), and a USDA-certified organic system that employed reduced tillage and targeted sheep grazing (*Ovis aries* L.) for cover crop termination and weed management with the overall goal of reducing tillage intensity (hereafter, organic grazed) (Ishaq et al. 2020a). The organic tilled treatment represents current organic practices which are effective yet not ecologically sustainable, and the organic grazed treatment provided an experimental approach to reduce tillage intensity. Each cropping system was randomly assigned to a 75 x 90m plot with three entire field replications and further divided into five 90 x 13m split-plots separated by a 1m fallow track, and randomly assigned to one phase of a 5-year crop rotation: (Year 1)

safflower (*Carthamus tinctorius* L.) under-sown with yellow sweet clover (*Melilotus officinalis* (L.) Lam), (Year 2) yellow sweet clover, (Year 3) winter wheat (*Triticum aestivum* L.), (Year 4) lentils (*Lens culinaris* Medik), (Year 5) winter wheat. Austrian winter pea (*Pisum sativum* subsp. *arvense*.) was planted in fall 2012 for the first year (2013) of the experiment because the biennial nature of yellow sweet clover would have required a previous year of seeding. In 2017, sweet clover was terminated in early July (with allowance for regrowth) and winter wheat was harvested in the last week of July. Safflower, a late spring crop, was performing negligible soil chemistry by the time samples were collected in August and was harvested in September.

A no-till double-disk seeder was used on all cropping systems to minimize soil disturbance. Chemical inputs in the conventional system mimicked standard practices in the Northern Great Plains and included 2,4-D, bromoxynil, dicamba, fluroxypyr, glyphosate, MCPA, pinoxaden, and urea to manage weeds and nutrient availability (Adhikari and Menalled 2020). Crops from both organic treatments were USDA certified by 2015, after completing the transition to organic that began in 2012. A chisel plow, tandem disk, and field cultivator were utilized in the organic tilled treatment as needed to terminate cover crops, prepare the seedbed, and incorporate cover crop residue into the soil (Ishaq et al. 2020a). Targeted sheep grazing at a stocking density of 50 sheep/ha for 30 days terminated cover crops and managed weeds in the organic grazed treatment (Menalled et al. 2020). The agronomic management details are provided in more detail elsewhere (see Adhikari and Menalled 2020).

Soil Collection

Soil samples for microbial DNA extraction and sequencing were collected from the safflower/sweet clover, sweet clover, and year 3 winter wheat split-plots, from each of the three cropping systems, in each of the three cropping system replicates, in June and in August of 2017 (i.e., 3 systems x 3 replications x 3 rotations x 2 time points). Due to logistic problems, two safflower/clover split-plots (one chemical no-till and the other organic grazed) in the second field replication were not sampled in June, but all were sampled in mid-August following residue removal in the respective treatments for a total of 52 soil samples. Each split-plot was divided into quartiles to account for spatial variation in the soil environment and allow for more representative microbial community samples. One bulk soil sample from each of these quartiles was taken with a 2cm diameter core sampler to a depth of 15cm after clearing the ground of debris and crop residue. The four cores were manually homogenized to one sample per subplot and kept at -20°C until analysis. Extremely dry soil conditions in August 2017 necessitated the use of a pickaxe to break the ground before sampling. The soil sampler was sterilized with 70% isopropyl alcohol and air dried between samples to prevent cross contamination of microbial communities.

Laboratory Methods

Metagenomic soil DNA was extracted from 0.25g of the 20g - 30g homogenized samples using a Qiagen DNeasy PowerSoil kit (Qiagen, Hilden, Germany). Amplicon libraries of the V4 region of the bacterial 16S rRNA gene were prepared following procedures outlined by Kozich et al. (2013), using dual indexed primers (515F and 806R) to target soil bacteria active at the

time of sampling. This method amplifies both bacteria and archaea, but the resulting libraries are primarily bacterial as the latter are not amplified well. Amplicons were normalized using SequelPrep plate normalization kit (Invitrogen) and the resulting product was pooled to equimolar concentrations. These pooled amplicons were cleaned with AmpureXP magnetic beads at a concentration of 0.8 (vol/vol) beads to pool ratio. Paired-end sequencing was performed at Michigan State University's RTSF Genomics Core using an Illumina MiSeq with a maximum read length of 250 base pairs on MiSeq v2 reagent cartridge (Illumina Inc.). Illumina Real Time Analysis v1.18.54 was used to perform base calling, and the resulting output was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.19.1. Raw sequences and metadata are available from NCBI under BioProject Accession PRJNA672991.

Bioinformatics

The DADA2 pipeline run in the software environment R 3.6.1 was used to filter paired end reads, remove chimeric sequences, and conduct taxonomic assessment (Callahan et al. 2016; R Development Core Team 2020). Sequences were trimmed by 10 bases at the start and end positions during filtering, with no ambiguous bases allowed and a maximum expected error of two. The error rates were learned on 2×10^6 randomly selected dereplicated reads and then used to identify Amplicon Sequence Variants (SVs), which are analogous to individuals in that sequences have been grouped down to single-nucleotide polymorphism/single base differences by assessing overall error rate and probability of base error versus polymorphism occurrence.

Then, using DADA2, two-parent chimeras (bimeras) were removed. The Silva NR version 138 database was used to assign taxonomy to sequence variants (Yilmaz et al. 2014). Data were

first rarefied to the size of the smallest sample library (38,776 sequenced reads), which allowed all 52 samples to pass this quality control step. One sample with only 220 sequence variants made it through as a result. This low outlier was from an organic tilled safflower/clover plot sampled in August 2017. Data were rarefied to the next lowest number of reads per sample (65,165 sequenced reads). The outlier was subsequently removed along with 42 absolute sequence variants and the 6,932 sequenced reads unique to them.

Statistical Procedures

All data analysis was performed in R 3.6.1 - 4.0.2 (R Development Core Team 2020). The phyloseq and vegan packages were used for statistical analysis (McMurdie and Holmes 2013; Okansen et al. 2019), while ggplot2 was used for data visualization (Wickham 2016). Alpha or within-community diversity was assessed via observed sequence variants (bacterial community richness), Shannon's Diversity, and Shannon's Evenness. Linear regression with mixed effect models explored the relationships between treatments and alpha diversity, using the lme4 package (Bates et al. 2020). The model for bacterial richness and diversity included cropping system and sampling month as fixed effects and plot as a random effect, while the model for evenness used only month as a fixed effect and plot as a random effect. Crop phase (i.e., plant species identity) was tested as a predictor for richness, diversity, and evenness, but was not a significant predictor for any of them. Field replication was also considered as a random effect because of variation at the main plot level, but split-plot explained more variation in the model for bacterial richness, Shannon's Diversity, and Shannon's Evenness. Nesting split-plot in plot also did not increase the conditional R^2 of these models enough to be considered an

improvement. Soil data on percent carbon, percent nitrogen, C:N ratio, and pH were available along with crop and weed biomass but could not be used to model alpha diversity because all except C:N ratio and weed biomass (Figures S2.2 and S2.3) varied by cropping system, split-plot, or were highly correlated with one another ($r=0.90$). There were no interactions between treatments in any of the models for alpha diversity. Type III One-Way Analysis of Variance determined which variables significantly affected alpha diversity while Tukey's post-hoc comparisons performed with the emmeans packages evaluated the differences in means among treatment levels (Lenth et al. 2020).

Changes in relative bacterial abundance from June to August among cropping systems were represented with heatmaps that displayed the fifty most abundant sequence variants labeled by genera. Permutational random forest analysis was used with the rfpermute package to determine the taxa which were significantly discriminatory between treatment states, highlighting the ones that were unique or a defining feature of the three cropping systems (Archer 2021). For each comparison, 500 trees were made with 100 permutations each. Relative abundance of important taxa was then visualized by sampling month within each cropping system. Unspecified genera in both the heatmaps and random forest analysis were identified at the family level.

Bray-Curtis dissimilarities (based on species' presence/absence and relative abundance) were calculated to assess between-community diversity and visualized using nonmetric multidimensional scaling ordination. Mean dissimilarities of soil bacterial communities grouped by treatment were compared using analysis of variance and Tukey's post hoc tests. A

permutational analysis of variance (PERMANOVA) compared differences among bacterial communities using the adonis function with 9,999 permutations (Anderson 2017). Data were stratified by split-plot to account for repeated measures. Homogeneity of dispersions - an assumption for using a PERMANOVA - was evaluated using the betadisper function.

Results

Alpha Diversity

There were 11,193 unique sequence variants in the 51 soil samples collected in 2017. Bacterial richness decreased in every crop across all three cropping systems between June and August but varied the most in the chemical no-till cropping system and the least in the organic systems (Figure 2.1a). Safflower/clover and winter wheat in the chemical no-till systems had bacterial communities with a wider range of Shannon's diversity compared to those under organic management (Figure 2.1b). Bacterial evenness changed the least in organic tilled winter wheat, organic tilled safflower/clover, and chemical no-till safflower/clover (Figure 2.1c).

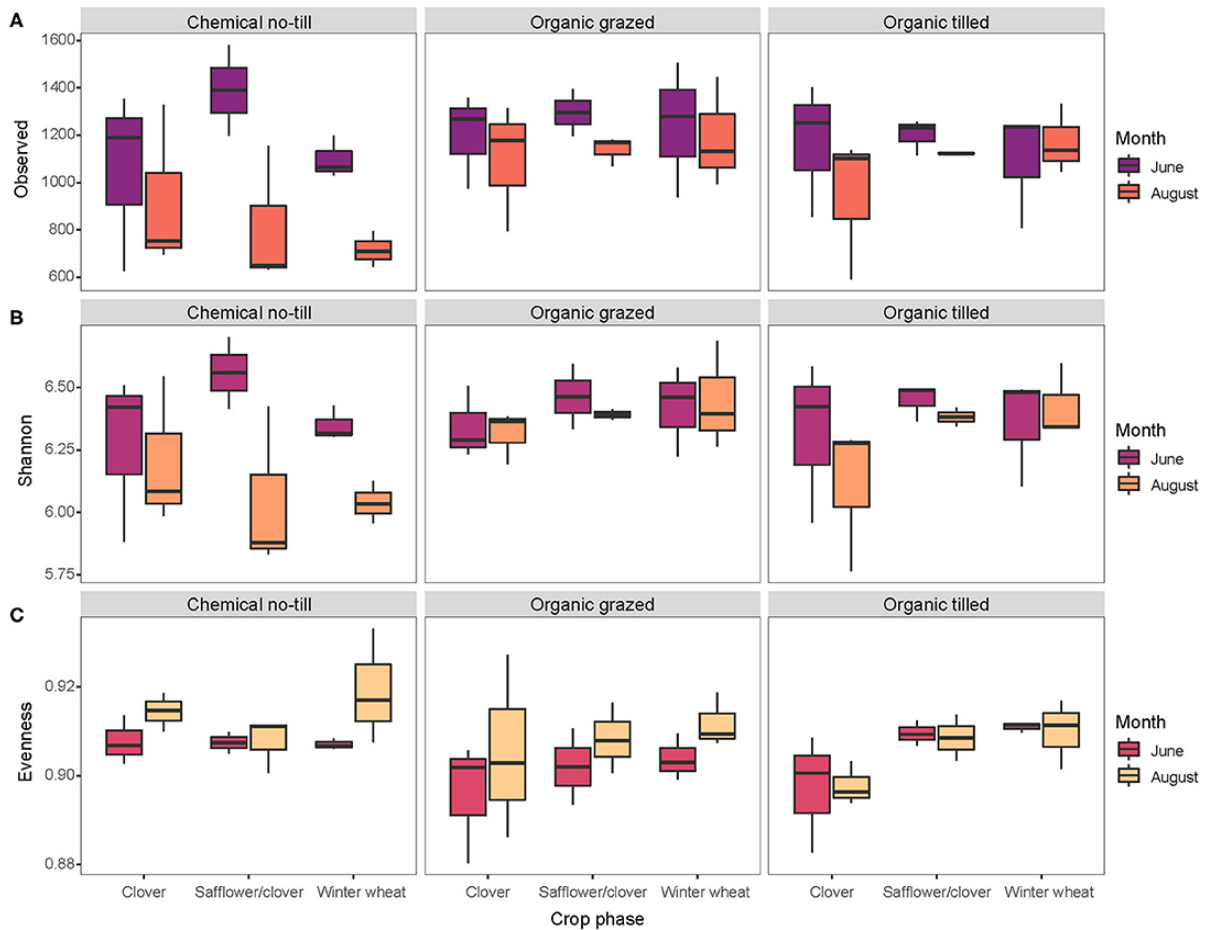


Figure 2.1. Alpha diversity metrics for soil bacterial communities associated with three crops in three contrasting dryland cropping systems in June and August.

All measures of alpha diversity changed with respect to sampling month (Table 2.1).

Bacterial richness varied as a function of cropping system and month (p -value = 0.049, equivocal, 0.008, respectively, Table 2.1). Richness was higher in the organic grazed system than in the chemical no-till system, an estimated difference in means of 212 observed sequence variants when averaged across months (p -value = 0.041, Table 2.2). Bacterial richness was significantly higher in June than in August with an estimated difference in means of 171 observed sequence variants when averaged across cropping systems (p -value = 0.008, Table

2.2). Bacterial richness did not differ between the grazed and tilled organic systems. Cropping system as a predictor of Shannon's diversity was trending towards significance (p -value = 0.09, Table 2.1).

Table 2.1. Statistics from Type III Analysis of Variance for mixed effect linear models of bacterial richness, Shannon's Diversity, and Shannon's Evenness.

	Numerator Df	Denominator Df	F statistic	p-value
<i>Richness</i>				
Cropping System	2	24.14	3.43	0.049
Month	1	25.70	8.41	0.008
<i>Diversity</i>				
Cropping System	2	23.38	2.62	0.094
Month	1	25.08	4.87	0.037
<i>Evenness</i>				
Month	1	24.61	7.57	0.011

Chemical no-till system was trending towards less diversity than the organic grazed system, with an estimated difference in mean Shannon's diversity measure of 0.169 when averaged across months (p -value = 0.08, Table 2.2). Soil bacterial diversity and evenness also changed significantly by month (p -value = 0.037 and p -value = 0.011, respectively, Table 2.1). Shannon's diversity was higher in June than in August, with an estimated difference in means of 0.123 (p -value = 0.011, Table 2.2). Mean Shannon's evenness, however, increased by an estimate of 0.005 by the end of the summer (p -value = 0.011, Table 2.2).

Table 2.2. Pairwise comparisons from Tukey's post-hoc analysis of alpha diversity models.

	Contrast	Estimate	t ratio	p-value
<i>Richness</i>				
Cropping System	Organic grazed – Chemical no-till	212.7	2.59	0.041
	Organic tilled – Chemical no-till	131.5	1.60	0.266
	Organic grazed – Organic tilled	81.2	0.99	0.592
Month	June – August	171	2.89	0.008
<i>Diversity</i>				
Cropping System	Organic grazed – Chemical no-till	0.169	2.26	0.082
	Organic tilled – Chemical no-till	0.107	1.43	0.344
	Organic grazed – Organic tilled	0.062	0.83	0.690
Month	June – August	0.123	2.20	0.037
<i>Evenness</i>				
Month	June – August	-0.005	-2.74	0.011

Overall Community Composition: Abundance

Taxa from the phyla Actinobacteria, Proteobacteria, Verrucomicrobia, and Firmicutes consistently had the highest relative abundance across all three cropping systems (Figure S2.2), though there were some shifts in community composition. Sequence variants from *Bradyrhizobium* (Proteobacteria) and *Candidatus Udaeobacter* (Verrucomicrobia) were more abundant in June while those from *Candidatus Nitrososphaera* (Thaumarchaeota, Archaea) and *Blastococcus* (Actinobacteria) were more abundant in August (Figure 2.2). *Pseudarthrobacter* (Actinobacteria) remained highly abundant throughout the summer in almost every treatment plot (Figure 2.2). Some changes in bacterial abundance occurred within particular crop phases or cropping systems. For example, one sample in an organic tilled clover plot had a high abundance of *Candidatus Udaeobacter* in June but not in August, while organic tilled plots had higher abundance of Bacillaceae in August than in June. Other sequence variants were

abundant throughout the summer, such as Nitrososphaeraceae, Xanthobacteraceae (Proteobacteria), *Sphingomonas* (Proteobacteria), and several taxa not identified at the genus level (Figure 2.2).

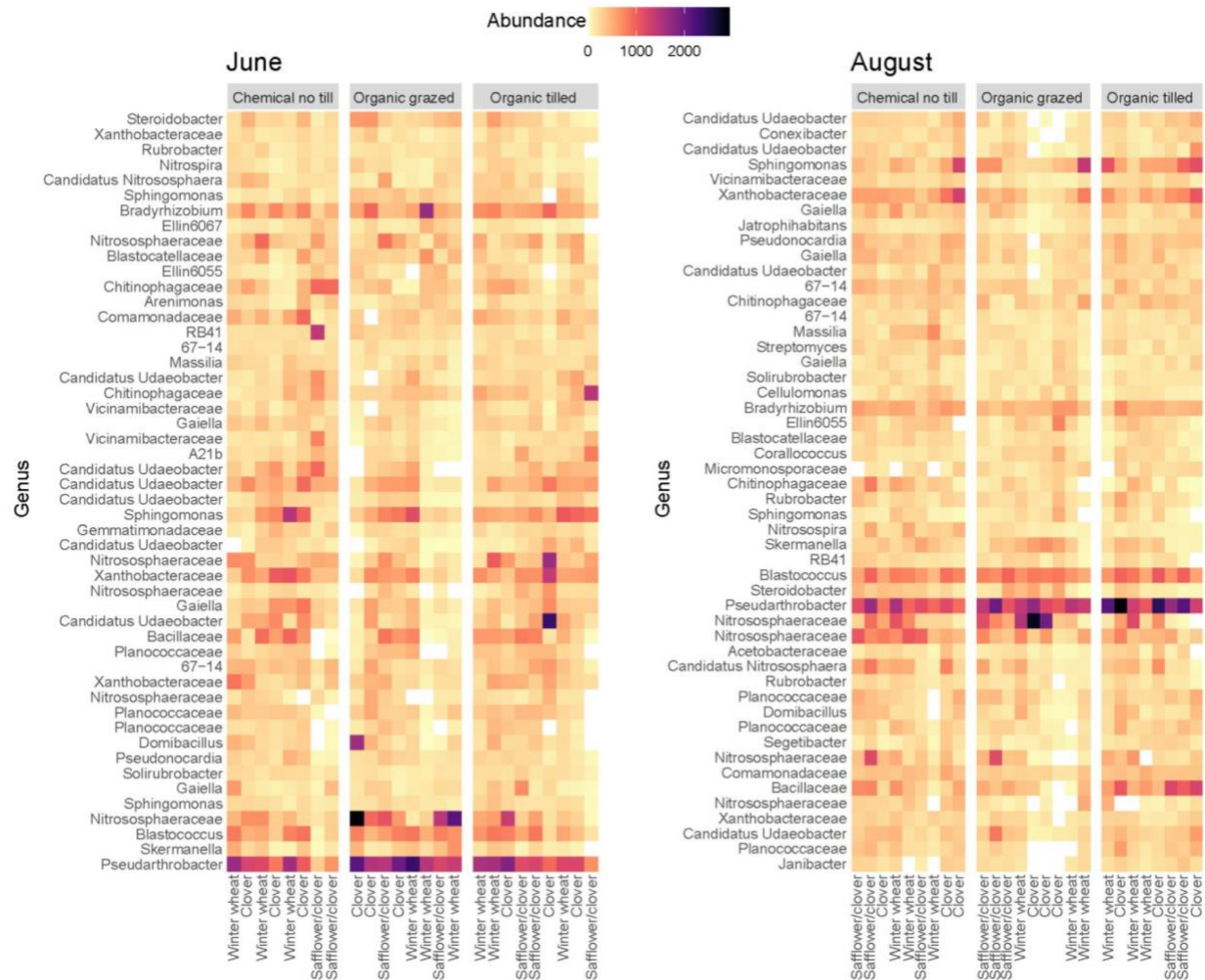


Figure 2.2. Abundance of the most abundant sequence variants identified at the genus level in three contrasting dryland cropping systems. Sequence variants from unspecified genera are identified at the family level.

A random forest analysis identified the top 50 sequence variants, by calculated importance ($p < 0.05$), that responded to cropping systems in each month (Figure 2.3). The

relative abundances of these taxa faceted by month indicated which predictor taxa were important to different cropping systems in June and August (Figure 2.3). *Pseudarthrobacter* was the most abundant, followed by Nitrososphaeraceae and *Candidatus Udaeobacter*. Log relative abundance of *Pseudarthrobacter*, increased in all three cropping systems from June to August. Nitrososphaeraceae increased in the chemical no-till and organic tilled systems and decreased in the organic grazed system while *Candidatus Udaeobacter* did the opposite (Figure 2.3). The random forest analysis had an out-of-bag error estimate of 19.61% as a prediction error for bootstrapped samples that did not contain elements of the original dataset.

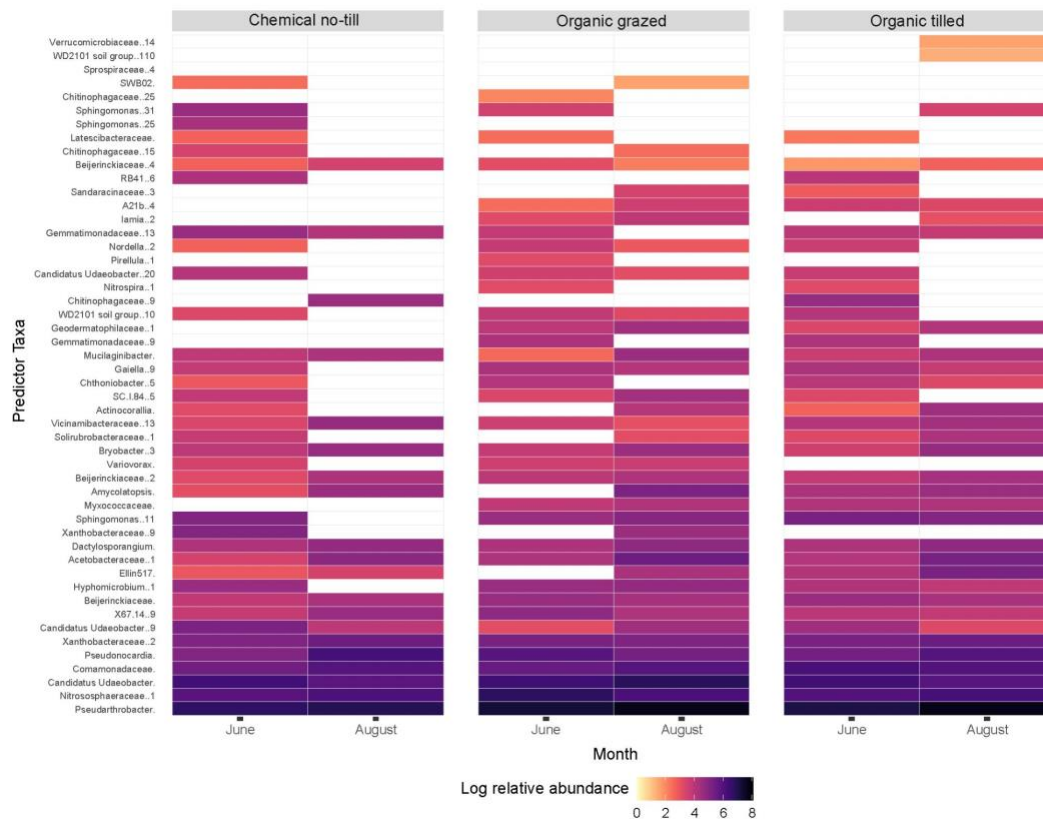


Figure 2.3. Log relative abundance of the top 50 predictor sequence variants in all cropping systems by month represented by a random forest analysis. White spaces indicate absent or very low abundance taxa. Sequence variants from unspecified genera are identified by family.

Beta (Between-Community) Diversity

The PERMANOVA indicated that cropping system, crop, month, and the interaction between cropping systems and crop identity impacted bacterial communities (Table 2.3). Of the three treatments, cropping system explained the most variation among communities (10.1%) and the interaction between cropping system and crops explained the second (8.3%). The organic grazed system had different soil bacterial communities from the organic tilled and chemical no-till systems (Figure 2.4). Organic grazed communities and chemical no-till communities had a mean dissimilarity of 55.06%, followed by 54.94% between organic grazed and organic tilled communities. Chemical no-till and organic tilled systems had less dissimilar communities in comparison with a mean dissimilarity of 52.26%. Dissimilarity decreased in the chemical no-till system, increased in the organic grazed system, and changed little in the organic tilled system as the summer progressed (Figure 2.4). Communities in organic grazed systems had the highest within-group mean dissimilarity of 53.25% on a weighted Bray-Curtis scale. Communities in the organic tilled and chemical no-till systems had within-group dissimilarities of 50.65% and 51.04% respectively.

Table 2.3. PERMANOVA of the effects of cropping system, crop, month, and the interaction between cropping system and crop on soil bacterial communities.

Variable	Df	F Model	R ²	p-value
Cropping system	2	2.85	0.101	0.0001
Crop	2	1.288	0.045	0.0001
Month	1	2.434	0.043	0.0001
Cropping system: Crop	4	1.176	0.083	0.0003
Residuals	41		0.727	
Total	50		1	

Soil microbial communities become more dissimilar throughout the summer, and this was driven more by cropping system than the crop phase (i.e., plant species identity) (Figure 2.4). June communities were 53.95% dissimilar from August communities on average. An analysis of dissimilarities between just the two organic systems was performed to determine if the chemical no-till system masked differences between organic grazed and organic tilled microbial communities. However, between-group and within-group mean dissimilarities for bacterial communities did not significantly change for cropping system, crop phase, or month.

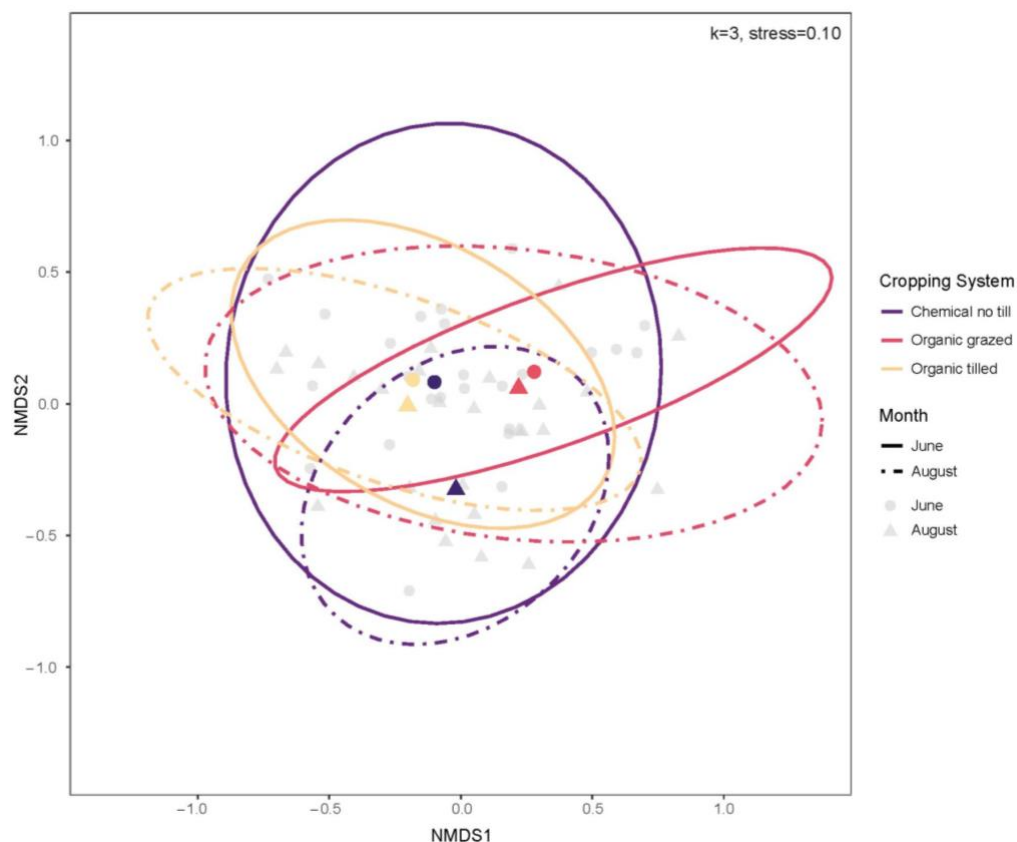


Figure 2.4. Ordination of soil bacterial communities by cropping system for June and August using nonmetric multidimensional scaling on weighted Bray-Curtis dissimilarities. Centroids and multivariate t-distribution ellipses for bacterial communities in the three cropping systems are designated by color for June (circles, solid line) and August (triangles, dashed line). For the NMDS calculation, $k=3$, and stress = 0.10.

Within crop phases, safflower/clover split-plots and clover split-plots had soil microbial communities with the highest mean dissimilarity at 54.65%, followed by clover and winter wheat split-plots at 53.60%. Bacterial communities in safflower/clover and winter wheat split-plots were 51.73% dissimilar, on average. Winter wheat bacterial communities were less dissimilar across cropping systems and had the lowest within-group mean dissimilarity of 51.09%. Organic grazed clover split-plots had dissimilar soil bacterial communities from the clover split-plots of the organic tilled and chemical no-till systems, and communities under organic tilled safflower/clover split plots were more dissimilar from the organic grazed and chemical no-till safflower/clover (Figure 2.5). Clover communities had the highest mean within-group dissimilarity in all cropping systems at 56.05%. Bray-Curtis dissimilarities did not differ among crop phases in the cropping systems, but there was an interaction between cropping system and month (p -value = 0.033). However, there were no significant post hoc comparisons to indicate which systems changed in beta diversity as a function of month.

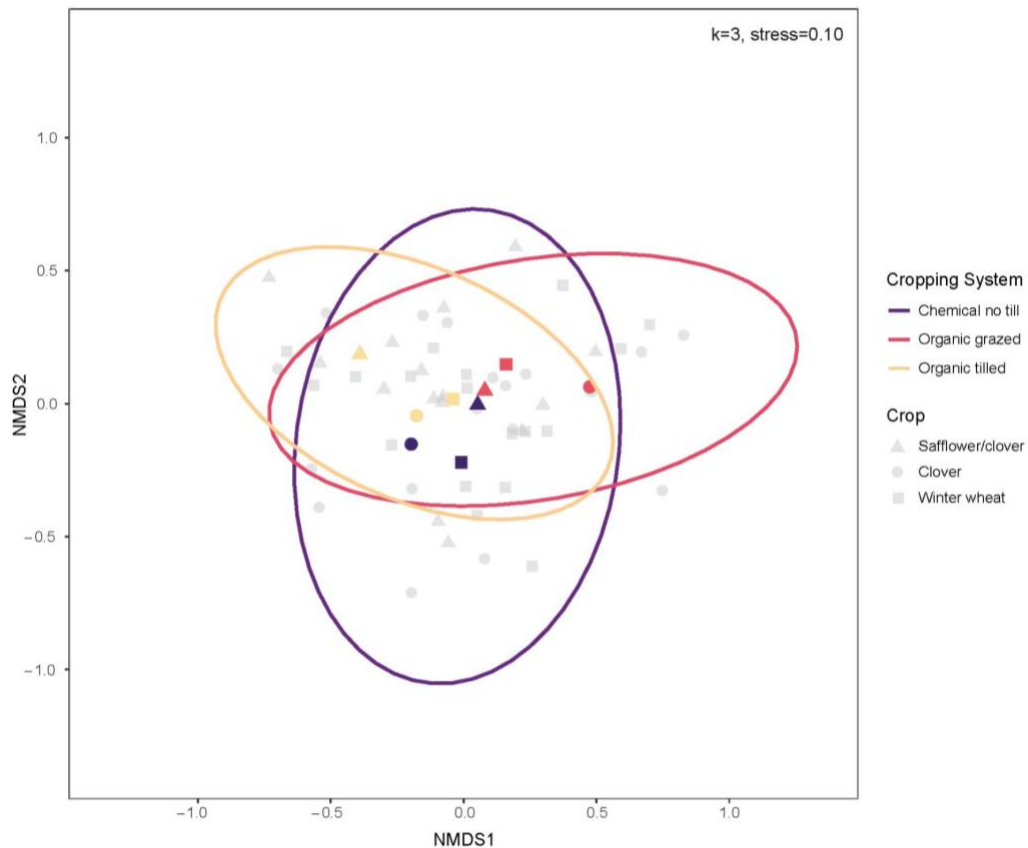


Figure 2.5. Ordination of soil bacterial communities by crop for three contrasting dryland cropping systems using nonmetric multidimensional scaling on weighted Bray-Curtis dissimilarities. The centroids and multivariate t-distribution ellipses for bacterial communities in the three cropping systems are designated by color for cropping system and shape for soil growing clover (circles), safflower/clover (triangles), or winter wheat (squares). For the NMDS calculation, $k=3$, and stress = 0.10.

Discussion

The sustainable productivity of arable land is dependent on preventing or remediating soil degradation, and alternatives to common agricultural practices need to be thoroughly studied to allow researchers to make accurate recommendations for producers. With that goal in mind, this study was part of a field experiment comparing the effects of cropping systems on crop production, soil bacterial communities, and other ecosystem dynamics. In this study, we

confirmed that organically grazed systems support higher soil bacterial richness and Shannon's diversity than chemically managed systems but did not find evidence suggesting that soil bacterial diversity differed between organic systems or was higher in cover crops compared to winter wheat when accounting for cropping systems and sampling month.

Differences Between Cropping Systems

Management practices used by chemical and organic systems can result in divergent microbial communities, principally because of tillage, fungicides, fertilizers, herbicides, and divergent plant communities, factors which are known to affect macro- and microbial diversity (Smith et al. 2015; Nettles et al. 2016; Zuber and Villamil, 2016; Hartmann et al. 2015). In this study, the chemical no-till system resulted in the least diverse microbial communities, which were dissimilar to those communities under grazed but not tilled organic management. Both organic systems in this study utilized different levels of tillage for weed management and cover crop termination. Specifically, the organic grazed system did not receive any tillage for the first three years of the rotation while soil at the organic tilled system was mechanically disturbed on a regular basis during this study. Tillage can act as a selective pressure leading to a difference in soil microbial community composition under contrasting soil disturbance regimes (Navarro-Noya et al. 2013). Breaking apart soil aggregates exposes previously protected soil organic matter to oxidizing conditions (Six et al. 2000; Van Groenigen et al. 2010) favorable to fast growing copiotrophic bacteria (Srour et al. 2020). However, findings on the effects of tillage on soil microbial communities are not consistent. Studies have reported higher microbial richness

and diversity in no-till systems compared to tilled systems while others find no difference between the two (Navarro-Noya et al. 2013).

No impact of crop rotation phase

Soil microbial community alpha diversity was less affected by plant species in the crop rotation than by the overall cropping system and seasonality, a result previously demonstrated in this field experiment (Ishaq et al. 2020a). The lack of a response to crop phase (i.e., plant species identity) indicates other factors were more important in determining microbial community composition. It is presumed that chemical- versus organic-based inputs and soil disruptions from tillage (Ishaq et al. 2020a), as well as seasonal temperature and precipitation (Ishaq et al. 2020b), are stronger selective pressures of soil microbial communities than the effects that crop species have on quality of plant residue inputs (Schmatz et al. 2019) or microbial-recruitment by plants (Ishaq et al. 2017). A similar study of crop rotations and management strategies in the Central Great Plains found that conservation tillage and arable weed diversity affected soil microbial communities much more than cover crop diversity (Wortman et al. 2013).

This study identified that soil microbial communities were impacted by an interaction between cropping systems and crop identity. Within-group community dissimilarity was higher in the organic grazed system, regardless of crop. Soil bacteria from clover subplots had the highest within-group dissimilarity in all cropping systems, indicating that local environmental factors created more variation in the membership of bacterial communities. This may be due to the effect of local soil conditions on the soil microbial assembly (Brown et al. 2020), or to

genetic variation in plants and their relative interactions with soil microbiota (Brown et al. 2020; Pérez-Jaramillo et al. 2019).

Previous research on the impact of tillage and crop identity on soil microbial communities indicated that tillage selects for faster growing taxa while cover crops select for moderate-growth taxa with more biochemical capacities (Schmidt et al. 2018). Moreover, the crop species used in rotations can alter soil microbial communities both taxonomically and functionally. While clover is known to recruit nitrogen-fixing bacteria in root nodules, clover and grass crops increase soil respiration from the community as a whole and presumably digestion of complex carbohydrates (Martínez-García et al. 2018). The effects of safflower planted with clover on soil microbiota has not been thoroughly evaluated, but previous studies linked safflower fertility to high soil bacterial abundance and low fungal abundance (Lu et al. 2013). This may be due to the phosphate-solubilizing bacteria recruited to the safflower rhizosphere (Zhang et al. 2019) taking the place of mutualistic soil fungi that create bioavailable phosphorus, which form the basis of their nutritional symbiosis with plants in exchange for sugars.

In the present study, given the drought resistance of safflower, the diversity of two plant species in the safflower/clover year, and the bacterial recruitment capacity of clover, it was anticipated that the bacterial diversity in soil would differ among crop phases. It was expected that winter wheat would recruit the lowest bacterial diversity, clover the next highest, and safflower/clover would result in the highest bacterial diversity. Variation in bacterial diversity among crop phases was moderated by cropping system and did not differ based solely

on crop. Safflower/clover did not have a higher bacterial richness, but it did exhibit less variability in richness between plots and appeared to lose fewer taxa between June and August. Climate data at the experimental site show a trend of decreasing precipitation and increasing temperature over the course of the growing season (Adhikari and Menalled 2020), which can lead to a decrease in microbial community diversity (Naylor and Coleman-Derr 2018).

Differences between sampling months

Low bacterial richness observed in August was presumably related to low soil moisture due to severely dry late summer conditions, an effect which has been observed previously (Fuchslueger et al. 2014; Ishaq et al. 2020b; Naylor and Coleman-Derr 2018; de Vries et al. 2018). Soil bacteria are in closer contact with their surroundings and are limited by the availability of resources. In addition to the lack of moisture itself, microbial communities are altered by a reduction in plant-soil feedbacks which occur under drought conditions (Fuchslueger et al. 2014; de Vries et al. 2018). The large decrease in alpha diversity observed in the chemical no-till safflower/clover split-plots may have been compounded by crop senescence in these plots, heightening community vulnerability to dry soil conditions.

Dissimilarity in soil communities between June and August reflect a reduction in species richness and an increase in evenness associated with late summer dry soil conditions. In agreement with Ishaq et al. (2020b), we observed that soil bacterial communities become more disparate towards the end of the growing season when plant growth and moisture are no longer selecting bacterial growth and localized differences in fields may determine which bacteria can survive. If these spatially specific effects persist over time, it can lead to legacy

effects on the microbial community over several growing seasons, affecting system resiliency (Seipel et al. 2019).

Conclusion

Overall, soil microbial communities in bulk soil respond to overarching management systems but not necessarily crop species. Conditions within the growing season such as precipitation and soil moisture have a more pronounced effect on community richness and composition than crop phase. Looking to future research, this study generated additional hypotheses and considerations. The rhizosphere needs to be sampled rather than bulk soil to assess how microbiota respond to the specific phases of a crop rotation (i.e., plant species identity), as differences in bulk soil microbial communities among the crop rotation plots were not detectable. Additionally, studies need to incorporate multiple time points in the growing season and assess long-term changes in soil microbial communities caused by disturbances. This knowledge, in turn, will allow an improved understanding of how management systems and their associated ecological disturbances create circumstances from which it is more difficult for microbial communities, soil health, and plant productivity to recover.

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CHAPTER 3

SOIL BACTERIAL COMMUNITY RESPONSE TO COVER CROPS, TERMINATION, AND TEMPERATURE
AND MOISTURE CONDITIONS IN A DRYLAND CROPPING SYSTEMIntroduction

Soils are an indispensable resource and complex ecosystems home to microbial communities vital to agricultural systems because of their roles in nutrient cycling and plant growth. While semiarid climates have been previously underrepresented in research on agricultural soil microbiota (Kim et al. 2013), over a third of the world's people depend on semiarid dryland agriculture (Delgado-Baquerizo et al. 2017). The importance of understanding the relationships between dryland agricultural systems and soil microbial communities magnifies as semiarid regions are predicted to become warmer, as well as more widespread. Currently, semiarid ecosystems span 45.4% of the planet's terrestrial surface (Právělie et al. 2019) but, according to climate change projections, their range may increase to nearly 80% by the end of the 21st century (Delgado-Baquerizo et al. 2017).

Soil microbial communities are an important component of soil health, a relative measure of the ability of soils to function as a living ecosystem while supporting life aboveground and sustaining the socio-ecological functions of the land (Lehmann et al. 2020; Janzen et al. 2021). These belowground communities are involved in several key ecosystem functions interconnected with plant growth such as litter decomposition, nutrient cycling, and primary production (Philippot et al. 2013; Wagg et al. 2014; Delgado-Baquerizo et al. 2016).

Climate change poses a challenge for soil microbiota and agriculture alike (Classen et al. 2015; Kumar et al. 2020), especially in semiarid ecosystems where annual precipitation is already low. Across the semiarid sections of the Northern Great Plains, the region where this study was conducted, temperatures and precipitation are both projected to increase overall by the end of the century (Wienhold et al. 2018). However, the higher evapotranspiration rates predicted are likely to reduce effective soil moisture (Whitlock et al. 2017). In the area where this study was performed, precipitation is projected to increase before the growing season but decrease during the growing season (Whitlock et al. 2017). Maintaining biodiverse soils with ample soil organic carbon through farming management will be an important part of climate change adaptation.

Soil bacterial communities in drylands are particularly sensitive to changes in soil moisture and aridity, and they decrease in abundance and diversity as soils become warmer and drier (Maestre et al. 2015; Naylor and Coleman-Derr 2018; de Vries et al. 2018). Increasing soil organic carbon is a possible solution to ameliorate the impact of climate change on soil microbiota (Whitmore et al. 2015), as this may bolster soil bacterial community resistance to drought (Moreno et al. 2019) and benefit agricultural production. One way to increase soil organic carbon is by reducing fallow periods and increasing the plant diversity of crop rotations (Bommarco et al. 2013).

Winter wheat - summer fallow rotations are commonly used in the semiarid sections of the Northern Great Plains as a method of soil moisture conservation (Kumar et al. 2020). However, this practice is only 10-40% efficient at storing water (Nielsen and Vigil 2010) and

fallow fields are susceptible to erosion, reductions in soil organic matter, salinization, and decreases in soil health (Carr et al. 2020). Replacing summer fallow with cover crops has the potential to increase soil organic matter and sequester soil organic carbon, even in semiarid regions (Blanco-Canqui et al. 2013a). This can aid in water retention over longer periods because soil organic carbon can increase potential water storage (Blanco-Canqui et al. 2013b), which is beneficial for both microbial communities and crops (Steenworth and Belina 2008).

Cover crops grown in semiarid regions are often terminated between late spring and early summer to preserve soil moisture (Carr et al. 2012; Nielsen et al. 2016). Contrasting termination methods may have different effects on soil microbial communities based on their inputs into soil and amount of physical disturbance (Liang et al. 2014; Kim et al. 2020; Castellano-Hinojosa and Strauss 2020). Glyphosate is commonly used to terminate cover crops, but its effects on soil microbiota are not well understood, as climate, soil, cropping system, tillage, and land use history appear to be significant drivers of soil microbial community diversity (Schlatter et al. 2017; Kepler et al. 2020). It has been suggested that glyphosate may change soil microbial community composition by decreasing plant defenses and creating a favorable environment for pathogenic microbiota or by suppressing taxa beneficial for plant growth (Van Bruggen et al. 2018), though this is controversial (Kepler et al. 2020). Alternate methods of cover crop termination and weed management include haying and integrated crop-livestock management.

Soil bacterial diversity can increase under haying, possibly through disturbance-induced changes to root biomass, root exudates, and soil moisture (Foster et al. 2010), but studies are

scarce. In contrast, the reintegration of livestock management with crop production is more thoroughly studied and has gained traction since the mid-2000s (Kumar et al. 2019), though the impacts on soil microbial communities are not fully understood. However, the ecological interactions of integrated crop-livestock production demand investigation beyond yields and soil quality. Grazing can influence microbial diversity and community structure directly through nutrient deposition and soil compaction (Zhou et al. 2010) or indirectly by encouraging root production and enhancing rhizodeposition (Hamilton III et al. 2008; Zhou et al. 2010; Yang et al. 2019), by changing plant community composition (Yang et al. 2019), or by soil nutrient availability. For example, grazing can remove carbon from the system, suppressing the copiotrophic dryland bacterial phyla Actinobacteria and supporting the oligotrophic Acidobacteria and Chloriflexi (Eldridge et al. 2017). Interactions with physicochemical soil characteristics such as pH and soil moisture may also explain grazing-induced changes in soil bacterial community structure (Yang et al. 2019). In a dryland USDA-certified organic cropping system, sheep grazing with reduced tillage intensity has been shown to foster distinct soil bacterial communities in comparison with organic tilled and conventional no-till systems (Ouverson et al. 2021).

To our knowledge, no study has disentangled the relationships among cover crop diversity, agricultural management, and environmental factors mimicking predicted climate conditions on soil microbiota. As many sections of northern Montana are expected to experience higher temperatures and decreased summer precipitation (Whitlock et al. 2017), understanding these relationships is imperative for the future agricultural stability of the

region. This study assessed how soil bacterial communities respond to greater cover crop diversity terminated by contrasting methods under both ambient and induced warmer/drier conditions. Specifically, we tested the impacts of a winter wheat - cover crop rotation with cover crop mixtures varying in diversity (a chemical fallow control, an early season mixture of five species, and a mid-season mixture of seven species) terminated by three different methods (glyphosate application, cattle grazing, or haying). Open-top chambers and rain-out shelters were added to simulate climate change projections for the region by increasing temperature and decreasing precipitation compared to ambient conditions. We hypothesized that 1) the mid-season and early season treatments would have more diverse soil bacterial communities than fallow, 2) on average, ambient conditions would support higher soil bacterial diversity than warmer/drier conditions, and 3) there would be a gradient in soil microbial community diversity from warmer/drier fallow plots terminated by glyphosate (lowest) to ambient cover crop plots terminated by grazing (highest).

Materials and Methods

Site and Experimental Design

This study was conducted in 2018 and 2019 at the Montana State University Northern Agricultural Research Station, located southwest of Havre, Montana (48.49689°N, 109.8029°W). The study site has an annual low and high temperature of 0°C and 13.3°C, respectively, with an average annual precipitation of 305mm (Western Regional Climate Center, 2020). The soil underlying the study area is a mix of Joplin and Telstad clay loam (Fine-loamy, mixed,

superactive, frigid Aridic Argiustolls) with an average pH of 7.34, 1.27% organic matter, and 46.5 ppm of total soil nitrogen (UC Davis 2021). These deep soils (>150cm) have similar surface textures of loam, parent material of till, and water holding capacities of over 18 centimeters (NRCS 1999; Soil Survey Staff 2021).

Details of the field trials, including experimental design, crop management, treatments, and field sampling procedures, are described in Dupre et al. (2021). Briefly, the experiment utilized two replicated field trials established as a restricted-randomized strip plot design (Figure 1). The two field trials, one planted with winter wheat (*Triticum aestivum* L.) and the other with either different types of cover crops or fallow, were rotated each year. This study focused on a subset of the cover crop phase which included a summer fallow treatment, an early season cover crop mixture of 5 species planted in early spring, and a mid-season cover crop mixture of 7 species planted about two weeks later. The location of each treatment was randomized in the first year (2012-2013) of the experiment to 8 x 44m strip plots and maintained throughout the duration of the study. Termination methods were assigned perpendicular to the fallow and cover crop strip plots (herbicide, grazing, and haying and baling) and applied in a strip plot design. We established two 0.56 m² frames in each cover crop and fallow strip plots (8 x 13m), then randomly assigned them to two different temperature and moisture conditions (“ambient” and “warmer/drier,” Figure 3.1).

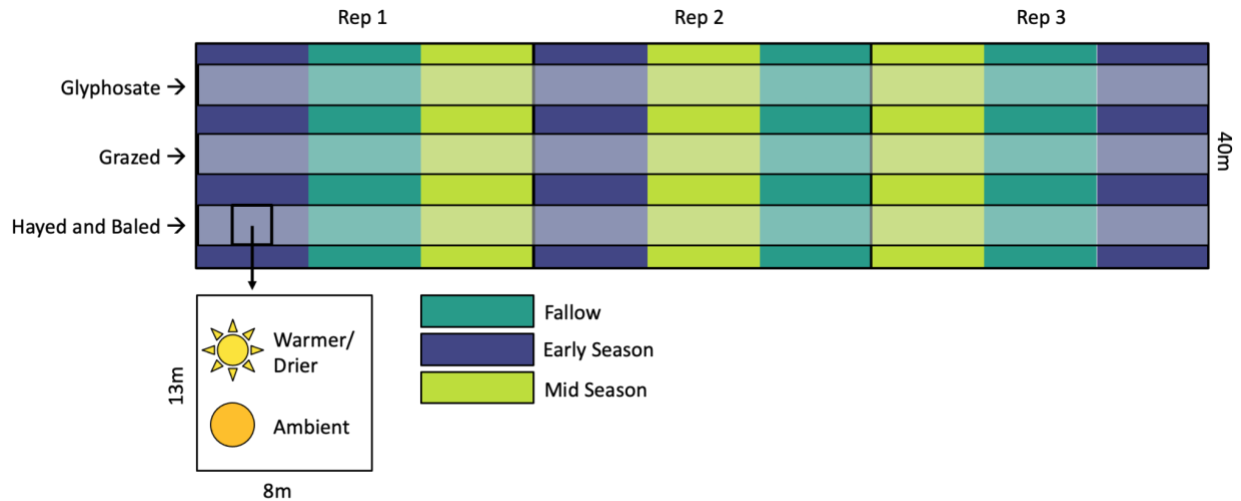


Figure 3.1. Experimental design showing one year of the winter wheat - cover crop rotation. Open-top chambers with rain-out shelters were located at each combination of the cover crop or fallow strip plots and termination treatments (9 per replication). Samples were taken from the 0.56m² frames placed within the open-top chamber and a marked area approximately 1m away from the structure.

Experimental units were in summer fallow, an early season cover crop mixture, or a mid-season cover crop mixture. Summer fallow began after the winter wheat harvest on July 12, 2017, and July 26, 2018, and ended on September 21, 2018, and September 13, 2019. All strip plots were sprayed with 2240g/ha of ai RTIII glyphosate before planting. Summer fallow plots were also treated with 1120g of ai RTIII glyphosate plus 340 g/ha of ai dicamba during the growing season to manage weed populations as needed.

Two cover crop mixtures were selected to include a variety of functional groups and life history traits. The early season mixture was dominated by C3 species while the mid-season mixture was a mix of C3 and C4 species (Table S3.1). The species included in the mid-season cover crop mixture varied slightly from 2018 to 2019 due to logistics. Cover crops planting dates varied each year depending on local weather conditions. The early season mixture was planted

on May 4, 2018, and April 29, 2019, while the mid-season mixture was planted on May 14, 2018, and May 9, 2019. Cover crops were seeded at a depth of 2.54cm at different seeding rates (Table S3.1) using a 3.7-meter Conservapak no-till disk planter and fertilized with 112 kg/ha N-P-K (20-20-20).

Termination of Cover Crops

Cover crops were terminated shortly before oat anthesis to preserve soil moisture for the next crop (O'Dea et al., 2013). Termination methods included herbicide (ai glyphosate applied at 2500 g/ha), haying, and grazing. Herbicide application and haying occurred on July 9, 2018, and July 8, 2019. Cattle grazing involved 8-10 yearling bulls fenced in the 12 x 360m termination strip across the fallow and cover crop mixtures from July 11 - 13, 2018, and July 9 - 11, 2019. Four weeks after initial termination, regrowth of cover crops and weeds was terminated with 2000 g/ha of ai RTIII glyphosate plus 340 g/ha of ai dicamba because haying and grazing did not sufficiently kill the cover crops.

Winter Wheat

Winter wheat (*Triticum aestivum* L., Judee variety) seeds were pretreated with the fungicides Vitavas (carboxin-2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin) and Enhance (captab-N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide). Seeds were planted on September 21, 2017, and September 13, 2018, at a depth of 2.5cm and seeding rate of 67.2 kg/ha using a 3.7 Conservapak no-till planter. Plots were fertilized with 112 kg/ha of N-P-K (100-40-20). 1120 g/ha of ai bromoxynil and 2-methyl-chlorophenoxyacetic acid (MPCA) were

applied to the winter wheat on May 14, 2018, and May 9, 2019, to control for broad-leaf weed populations.

Temperature and Moisture Conditions

Open-top chambers were combined with rain-out shelters during the growing season to mimic the warmer/drier climate projected for summers in Northern Montana. The open-top chambers, designed to increase the temperature by 1 to 2°C (Marion et al. 1997) had a basal diameter of 1.5m and a top diameter of 1m and were built out of low-opacity Sun-lite HP fiberglass material (1mm thick; Solar Components Corporation, Manchester, New Hampshire). Following Yahdjian and Sala (2002), rain-out shelters decreased precipitation by up to 55% and were constructed out of wooden frames that supported gutters made of corrugated clear polycarbonate (Sunturf). The rain-out shelters were placed at an aspect of 220° to 230° to account for the prevailing southwestern wind. GB-1 gypsum sensor blocks with a KS-D1 digital soil moisture tester (Delmhorst Instrument Company, Towaco, New Jersey) and iButtons (Maxim Integrated, San Jose) recorded soil moisture and temperature, respectively, 10cm belowground in the ambient and warmer/drier treatments.

Soil Sampling

Soil samples from the cover crop/fallow phase were collected on July 1 of both years before termination. Three 10cm depth x 1.6cm diameter soil cores were taken from a 0.56m² frame in the warmer/drier treatment and homogenized. The same procedure was repeated in the soil under ambient conditions. Instruments were cleaned with 70% isopropyl alcohol in

between plots to minimize cross contamination. 1g of the homogenized soil was fixed in 9mL of 70% isopropyl alcohol in the field and stored at ambient temperatures.

Laboratory Methods

DNA extraction, amplification, and sequencing library preparation was performed as described in Ishaq et al. (2017, 2020). In 2018, a PowerLyzer PowerSoil DNA isolation kit was used to extract metagenomic DNA and in 2019, DNA extraction was done with the Qiagen equivalent (Mo-Bio Laboratories Inc., Carlsbad, California; Qiagen, Hilden, Germany). In both years, a negative control of molecular grade water was added to each extraction batch and an additional cleaning step was performed after extraction to precipitate DNA with ethanol and sodium acetate (Ishaq et al. 2020). The V3-V4 region of the 16S rRNA gene was amplified through 25 cycles of PCR with barcoded and indexed primers using the Q5 High-Fidelity Mastermix (New England Biolabs, Inc.) with 25 μ L of mix, 6 μ L molecular-grade water, 21 μ L of forward and reverse barcoded primer mix at 1.09 μ M concentration, and 4 μ L sample DNA. The PCR protocol was as follows: 98 °C for 30 sec; 30 cycles of denaturation at 98 °C for 10 sec, annealing at 55 °C for 30 sec, elongation at 72 °C for 30 sec; then a final elongation at 72 °C for 2 min. Primers included the MiSeq adaptors (A for forward, B for reverse), the sample index/barcodes, the two-nucleotide linker, and primers 341F (5'-ACTCCTACGGGAGGCAGCAG-3') (Fadrosh et al. 2014) and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso et al., 2011). Amplicons were purified using Omega Mag-Bind beads (Omega Bio-Tek, Norcross, Georgia) and quantified with a Qubit 4 Fluorometer (Invitrogen, Thermofisher Scientific). High-throughput sequencing with an Illumina MiSeq (Illumina, San Diego, CA) at the University of Oregon

Genomics Core (Eugene, OR, USA) was performed using a 2 x 300-nucleotide V3 kit. PhiX at a 10% spike-in and molecular-grade sterilized water were used as positive and negative controls respectively.

Bioinformatics

The DADA2 pipeline run in the software environment R 4.0.2 was used to filter paired end reads, remove chimeric sequences, and conduct taxonomic assignment (Callahan et al. 2016; R Development Core Team 2020). The 2018 and 2019 samples were processed separately because they came from two different sequencing runs. Due to low quality in the reverse reads, only the forward reads were used for this analysis. Sequences were trimmed by 10 bases at the start and 30 at the end positions during filtering, with no ambiguous bases allowed, a maximum expected error of two, and anything matching the phiX controls were removed. On average, the 2018 samples retained 75.1% of sequences with 88,413 reads in 35,082 unique sequences and the 2019 samples retained 70.5% sequences with 58,540 reads in 20,041 unique sequences. Error rates were learned on 2×10^6 randomly selected dereplicated reads and then used to identify Amplicon Sequence Variants (SVs). These are analogous to individuals as sequences have been grouped down to single-nucleotide polymorphism/single base differences by assessing overall error rate and probability of base error versus polymorphism occurrence. Two-parent chimeras (bimeras) were removed, and the Silva NR version 138 database was used to assign taxonomy to sequence variants (SVs) (Yilmaz et al. 2014). Laboratory negative controls were used to remove identical SVs from experimental samples, using adapted code (Ishaq 2017). Data were rarified to the size of the smallest sample library above 20,000 reads per

sample, which was 33,572 reads per sample for 2018 and 21,423 reads per sample for 2019. This removed 10 samples and 284 absolute sequence variants from the 2018 dataset and 13 samples and 630 absolute sequence variants from the 2019 dataset.

Statistical Procedures

All data analysis was performed in R 4.0.3 - 4.1.0 (R Development Core Team 2021). The phyloseq and vegan packages were used for statistical analysis (McMurdie and Holmes 2013; Okansen et al. 2019), while ggplot2 was used for data visualization (Wickham 2016). Alpha or within-community diversity was assessed via observed sequence variants (bacterial community richness), Shannon's Diversity, and Shannon's Evenness. Linear regression with mixed effect models was used to explore the relationships between treatments and alpha diversity. Alpha diversity, beta diversity, and relative abundance were analyzed separately by year to account for environmental factors external to the experimental design. All regression analyses were performed using the lme4 package (Bates et al. 2015).

One sample in each year was a low outlier for Shannon's Diversity and Evenness which obscured the resolution of other taxa and skewed the distribution of regression model residuals. For these two outliers, bar plots and heatmaps revealed that the 2018 sample had a high abundance of phylum Actinobacteria (*Pseudarthrobacter*) and the 2019 sample had a high abundance of phylum Proteobacteria (*Salmonella*) (Figures S3.2 and S3.3). Presence (fallow or cover crop) and composition (early season or mid-season) of cover crops, termination method, manipulated temperature and moisture conditions (ambient or warmer/drier), and the interactions among them were used as fixed effects with replication as a random effect in

explanatory models of alpha diversity. Type III One-Way Analysis of Variance determined which variables affected measures of alpha diversity while Tukey's post-hoc comparisons evaluated differences in means among treatment levels. Correlations between environmental variables and bacterial richness, diversity, and evenness were explored using Pearson's correlation (Wei and Simko 2021).

Bray-Curtis dissimilarities (based on species' presence or absence and relative abundance) were calculated to assess between-community diversity with nonmetric multidimensional scaling ordination. Mean dissimilarities of soil bacterial communities grouped by treatment were compared using analysis of variance and Tukey's post hoc tests. The betadisper function in the vegan package was used to assess the assumption of homogenous dispersion for performing a permutational analysis of variance (PERMANOVA), which was met. A PERMANOVA (Anderson 2017) compared bacterial community dissimilarity among treatments, using the adonis function with 999 permutations. Data were stratified by Replication to account for repeated measures. The pairwise.adonis2 function was used to examine post-hoc contrasts between significant factors (Martinez Arbizu 2020).

Changes in relative bacterial abundance from 2018 to 2019 for the presence and composition of cover crops were represented with bar plots of the ten most abundant phyla in each treatment. Heatmaps displayed the fifty most abundant sequence variants labeled by family. Differential abundance analysis was performed using the DESeq2 package to determine which sequence variants differed by the presence and composition of cover crops (Love et al. 2014). The rfPermute package was used to conduct a random forest analysis to determine

which taxa were significantly discriminatory between treatment states, highlighting the ones that were unique or defining features (Archer 2021). Specifically, this procedure calculated which sequence variants could predict bacterial communities based on manipulated temperature and moisture conditions or the presence and composition of cover crops. For each comparison, 500 trees were made, with 100 permutations each. Out-of-bag estimates were checked as a form of model validation; these were the prediction errors for bootstrapped samples that did not contain elements of the original dataset.

Results

Alpha Diversity

Of the 108 total samples collected, 83 (excluding the two outliers) passed through quality control: 43 samples with 16,994 unique sequence variants from 2018 and 40 samples with 16,989 unique sequence variants from 2019. When the 2018 and 2019 samples were analyzed together, year was the single highly significant predictor of bacterial richness. However, as the decrease from 2018 to 2019 in mean bacterial richness (Figure S3.1) could not be explained by any treatments, the two years were analyzed separately. In 2018, bacterial richness was less variable in mid-season cover crops than in early season or fallow soils under both ambient and warmer/drier conditions (Figure 3.2a). Bacterial richness responded to the presence and composition of cover crops, manipulated temperature and moisture conditions, and their interaction (Table 3.1).

Table 3.1. Statistics from Type III Analysis of Variance for mixed effect linear models of bacterial richness, Shannon's Diversity, and Shannon's Evenness for the 2018 samples. Treatments did not have a significant effect on measures of bacterial alpha diversity in 2019.

	Num. Df	Den. Df	F statistic	p-value
<i>Richness 2018</i>				
Cover crop	2	24.555	5.608	0.010
Climate	1	25.378	5.344	0.029
Cover crop: Climate	2	25.034	3.564	0.043
<i>Diversity 2018</i>				
Cover crop	2	23.298	13.208	< 0.001
Climate	1	24.267	6.056	0.021
<i>Evenness 2018</i>				
Cover crop	2	40	3.625	0.036

There were on average 457 more sequence variants in ambient early season cover crop soils than in warmer/drier early season cover crop soils (Table 3.2). Bacterial richness was also higher in both the ambient and warmer/drier mid-season cover crops than in the warmer/drier early season cover crops, with an estimated difference of 475 and 465 sequence variants, respectively (Table 3.2).

Table 3.2. Pairwise comparisons from Tukey's post-hoc analysis of alpha diversity models for the 2018 samples.

	Contrast	Estimate	t ratio	p-value
<i>Richness</i>				
Cover crop: Climate	early ambient - early warmer.drier	457.11	3.141	0.043
	mid ambient - early warmer.drier	475.16	3.265	0.033
	early warmer.drier - mid warmer.drier	-465.23	-3.255	0.034
<i>Diversity</i>				
Cover crop	early - mid	-0.173	-3.443	0.006
	fallow - mid	-0.232	-4.959	< 0.001
Climate	ambient - warmer.drier	0.100	2.380	0.026
<i>Evenness</i>				
Cover crop	Fallow - mid	- 0.011	-2.679	0.029

The diversity of bacterial communities also varied in response to the presence and composition of cover crops and manipulated temperature and moisture conditions (Table 3.1). The mid-season cover crop soils had greater bacterial diversity than the early season and fallow soils, with an estimated difference in means of 0.173 and 0.232, respectively (Table 3.2). Shannon's Diversity was also greater in ambient conditions than in warmer/drier conditions, by a difference in means of 0.010 (Table 3.2). Bacterial evenness was only affected by the presence and composition of cover crops (Table 3.1). Mid-season cover crop soils had higher evenness than fallow soils, an estimated difference in means of 0.011 (Table 3.2). Bacterial evenness was less variable overall than bacterial richness and diversity (Figure 3.2c). However, no treatments were significant predictors of bacterial richness, diversity, or evenness in 2019 (Table 3.1).

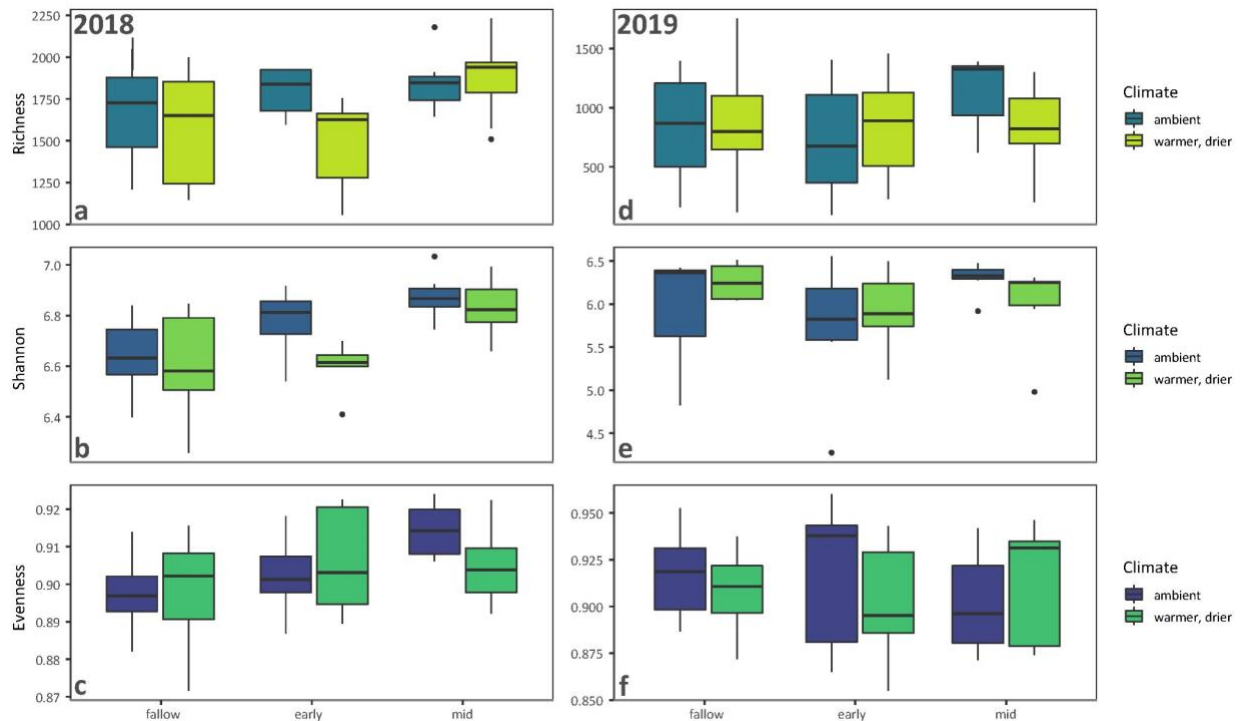


Figure 3.2. Alpha diversity metrics of soil bacterial communities early associated with fallow, early season cover crops, or mid-season cover crops in both ambient and warmer/drier conditions for each year.

Total aboveground plant biomass (g) collected from a 0.56m² frame placed in the ambient and warmer/drier treatments of each strip plot (see Dupre 2020, Chapter 2), average soil moisture the day of or prior to sampling, and the average soil temperature for one, three, and seven days prior or up to the day of sampling were not correlated with bacterial richness or evenness across both years (Figure S3.4). However, in 2018, bacterial diversity and plant biomass had a low positive correlation (0.34, *p-value* = 0.028) while plant biomass and soil moisture had a low negative correlation (-0.49, *p-value* = 0.002).

Beta Diversity

The permutational analysis of variance on the 2018 samples indicated that only the presence and composition of cover crops impacted bacterial community dissimilarity, accounting for 26.1% of the variation among communities (p -value = 0.001, Table S3.2). The mid-season cover crop mixture had distinct soil bacterial communities from the early season mixture and fallow soils (Figure 3.3), confirmed with PERMANOVA pairwise contrasts (p -values = 0.001). Mid-season and early season communities had the highest mean dissimilarity of 76.2%, followed by mid-season and fallow communities at 76.0%. Communities in the mid-season cover crops had the lowest within-group mean dissimilarity of 53.8% on a weighted Bray-Curtis scale while those in fallow soils had the highest mean within-group dissimilarity of 64.7%.

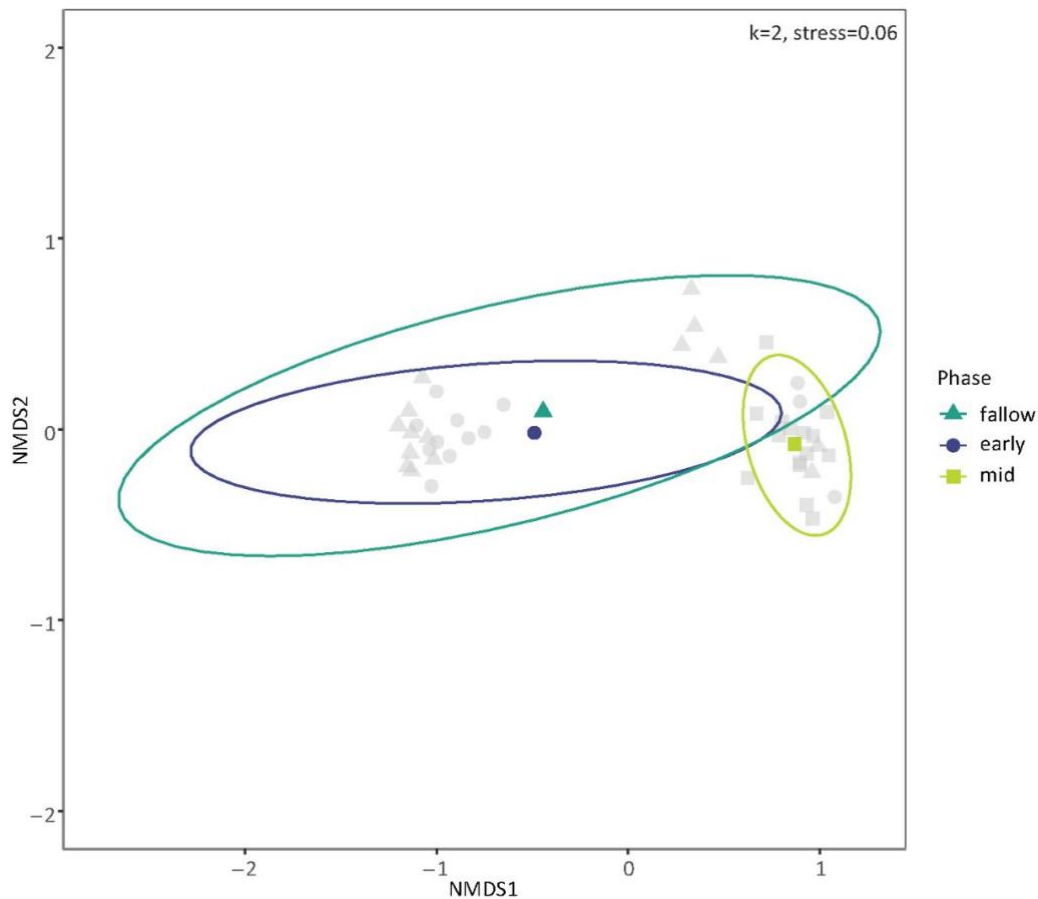


Figure 3.3. Ordination of soil bacterial communities in 2018 by the presence and composition of cover crops using nonmetric multidimensional scaling on weighted Bray-Curtis dissimilarities. The centroids and multivariate t-distribution ellipses for bacterial communities in the cover crop/fallow phase are designated by color and shape (circle for early season, triangle for fallow, and square for mid-season). For the NMDS calculation, $k=2$, and stress = 0.06.

The PERMANOVA on the 2019 samples indicated that none of the treatments or interactions impacted soil bacterial community dissimilarity. However, plant biomass was correlated with community dissimilarity (p -value= 0.037) and was included in the ordination as a vector. The average soil temperature for the seven days prior to sample collection was also included, though it was marginally significant (p -value = 0.052, Figure 3.4).

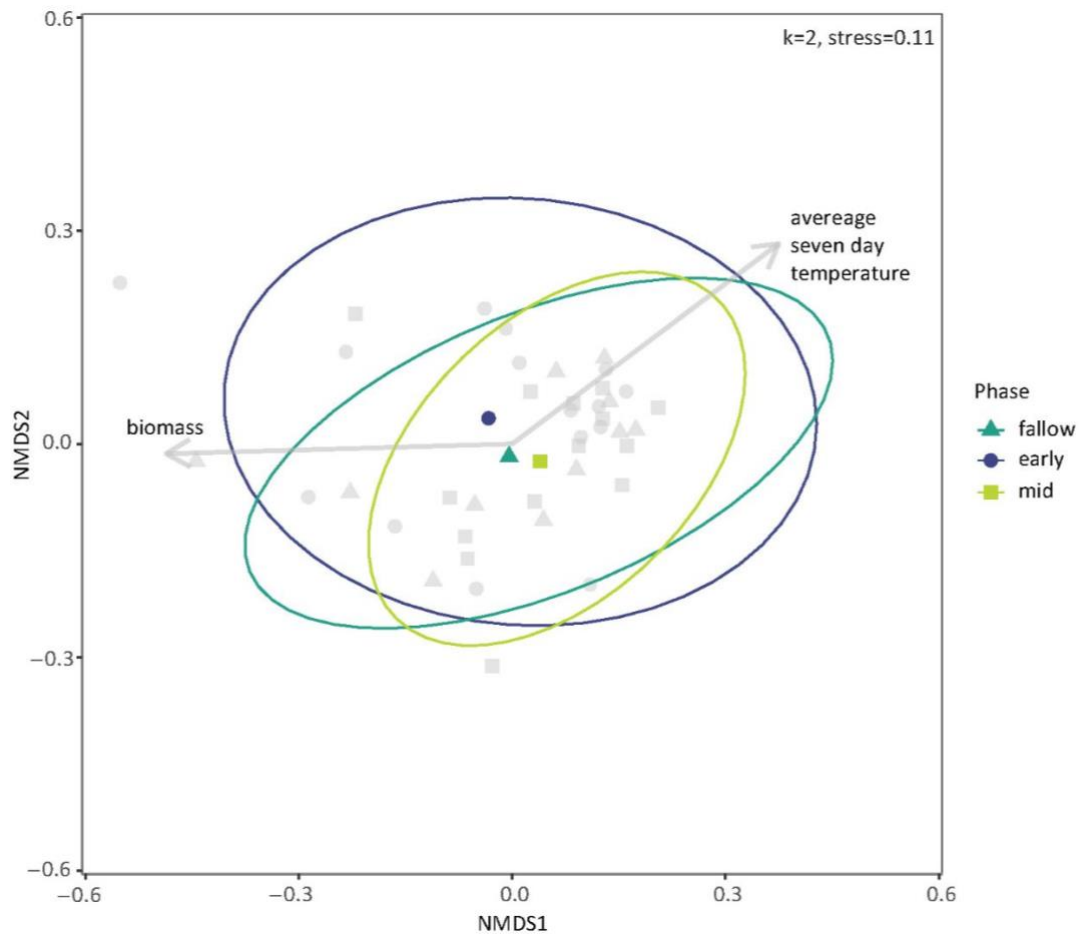


Figure 3.4. Ordination of soil bacterial communities in 2019 by presence and composition of cover crops using nonmetric multidimensional scaling on weighted Bray-Curtis dissimilarities. The centroids and multivariate t-distribution ellipses for bacterial communities are designated by color and shape (circle for early season, triangle for fallow, and squared for mid-season). Plant biomass and average soil temperature for the seven days preceding or including sampling were found to be potential vectors for the ordination. For the NMDS calculation, $k=2$, and stress = 0.11.

Community Composition: Relative Abundance

In 2018, sequence variants from the phyla Acidobacteria, Actinobacteria, and Proteobacteria had the highest relative abundance across the cover crop/fallow phase (Figure 3.5). Acidobacteria and Actinobacteria were dominant across the early season and fallow treatments but samples from the mid-season cover crop mixture tended to have less

Acidobacteria. Community composition shifted from 2018 to 2019, marked by an apparent increase in Actinobacteria and Chloroflexi, and a decrease in Acidobacteria and Gemmatimonadota (Figure 3.5). Between 2018 and 2019, the phylum with the lowest relative abundance of the ten most prevalent phyla shifted from Patescibacteria to Firmicutes (Figure 3.5).

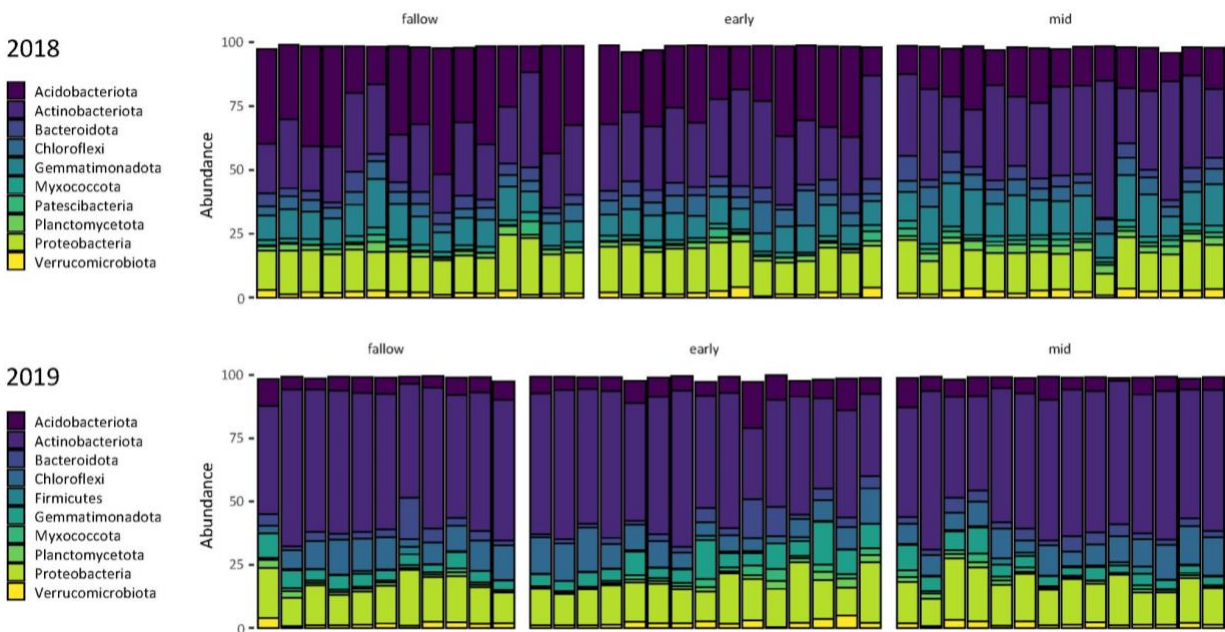


Figure 3.5. Soil bacterial communities at the phylum level of taxonomy by the presence and composition of cover crops for each year. The ten most abundant phyla are displayed.

Heatmaps show that sequence variants from Micrococcaceae were abundant in both years (Figure 3.6). Pyrinomonadaceae and Rubrobacteriaceae were more abundant in 2018, while bacteria from the family designator JG30-KF-CM45 were more abundant in 2019 (Figure 3.6). Based on the heatmap, the fifty most common taxa found in the 2018 samples were not as abundant in the mid-season cover crop soils as the early season and fallow soils.

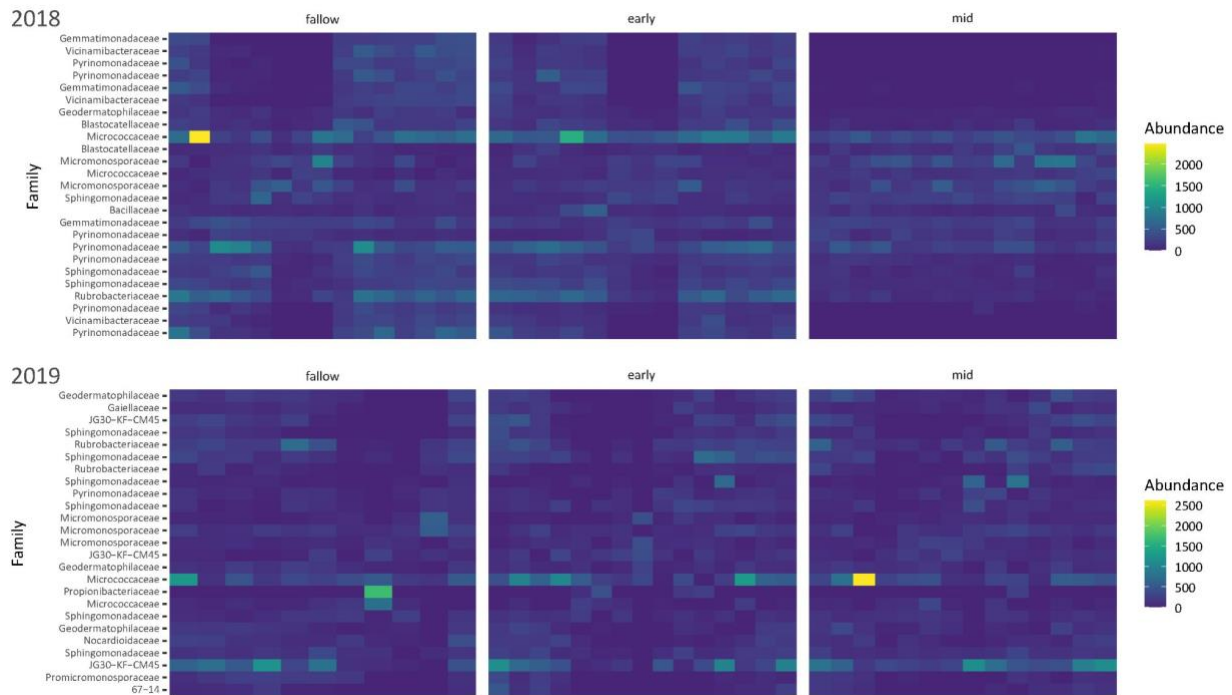


Figure 3.6. Heatmap of the most abundant sequence variants identified at the family level for each year. Taxa not present or at very low abundance in samples were set to display as the closest limit (0) for visual coherence.

DESeq2 was used on unrarified sample libraries to determine differential abundance of taxa among treatments (Weiss et al. 2017). Several bacterial genera relevant to soil health were differentially abundant between the 2018 mid-season and fallow soils as well as the mid-season and early season soils. Sequence variants from *Nitrospira* (family Nitrospiraceae) were more abundant in mid-season cover crop soils than in fallow soils (fold change 84.44 and 4.47) or early season cover crop soils (fold change 106.90). *Sphingomonas* (Sphingomonadaceae) were also more abundant in the mid-season cover crops than in fallow (fold change 216.77) or early season cover crops (fold change 207.94, 114.56). Nitrosomonadaceae and Nocardiodaceae were generally more abundant in the mid-season cover crops. However, *Actinoplanes* (Micromonosporaceae) were less abundant in mid-season soils than fallow (fold change -

68.11), but more abundant than in early season soils (fold change 79.34). No sequence variants were differentially expressed between the 2018 early season and fallow soils. In 2019, at most two sequence variants were differentially abundant by the presence and composition of cover crops.

The random forest analysis could not predict bacterial communities for ambient or warmer/drier soils in both years (out-of-bag error 62.8% and 50.0%, respectively). Bacterial communities based on the presence and composition of cover crops could be successfully predicted in 2018 but not 2019, with a large variation in error (20.9% and 57.5%, respectively). The resulting top 50 sequence variants were ordered by calculated importance ($p < 0.05$) and faceted by manipulated temperature and moisture conditions. *Pseudarthrobacter* was the most abundant in both ambient and warmer/drier soils, except for the ambient mid-season cover crops, which had the highest abundance of Micromonosporaceae (Figure 3.7).

Blastocatellaceae were more abundant in warmer/drier fallow than in ambient fallow conditions. *Sphingomonas* remained abundant in the mid-season cover crops under both ambient and warmer/drier conditions but decreased in the early season cover crops and fallow (Figure 3.7).

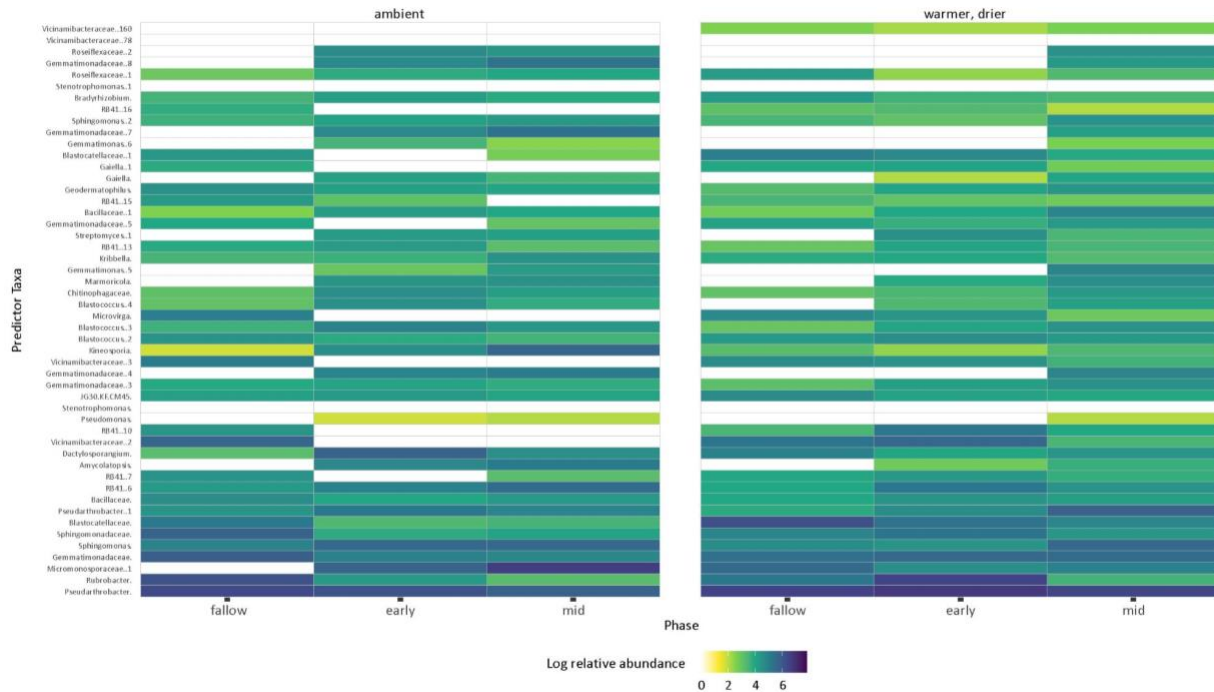


Figure 3.7. Random forest analysis of the 2018 samples showing the log relative abundance of the top 50 predictor sequence variants under ambient and warmer/drier conditions. White spaces indicate taxa that are absent or at very low abundance. Sequence variants from unspecified genera are identified at the family level.

Discussion

Cover crop mixtures have been suggested as a replacement for summer fallow to increase soil organic carbon, soil health, and soil bacterial diversity, though soil moisture use by cover crops represent a challenging issue, particularly under projected climate change conditions. This study examined the effects of the presence and composition of cover crops, cover crop termination methods, and predicted temperature and moisture conditions on soil bacterial communities. It is possible that the high rainfall observed in 2019 played a dominant role in homogenizing soil bacterial communities. However, the 2018 results allowed us to disentangle some of the management and environmental factors conditioning the abundance

and diversity of soil bacterial communities. In agreement with our first hypothesis, our results show that cover crop mixtures can support higher bacterial richness and diversity than fallow, though this was moderated by manipulated temperature and moisture conditions. Confirming our second hypothesis, we observed that ambient conditions can foster more diverse soil bacterial communities than warmer and drier conditions. We did not find evidence to support our third hypothesis of a gradient in diversity from chemically terminated fallow soils under warmer/drier conditions to grazed cover crops in ambient conditions because cover crop termination methods did not have an impact on soil bacterial communities.

Interaction Between the Presence and Composition of Cover Crops and Predicted Climate Conditions

The effects of cover crops on soil bacterial communities range from marginal (Hartmann et al. 2006) to a strong response by specific taxa (Martínez-García et al. 2018; Frasier et al. 2016; Finney et al. 2017). Within the rhizosphere, cover crops may cause a “priming effect” by affecting soil organic matter through root turnover (Nivelle et al. 2016). Other studies have found that conservation tillage and arable weeds (Wortman et al. 2013) or irrigation (Calderón et al. 2016) can be more impactful on soil microbial communities than cover crops. In the studied systems, the 2018 mid-season cover crops fostered a soil bacterial community distinct from early season cover crops and fallow, reinforcing the selectivity of some plant species.

In 2018, an interaction between the presence and composition of cover crops and temperature and moisture conditions affected bacterial richness while only the former accounted for community dissimilarity. A related study at the same field site found that an

interaction between these same variables affected plant biomass (Dupre 2020). Cover crop species in the early and mid-season mixtures responded differently to changes in temperature and moisture. Specifically, oat biomass was higher in the ambient early season mixture than in warmer/drier conditions, while radish and turnip biomass was higher in the warmer/drier mid-season mixture than in ambient conditions (Dupre 2020). Cereals exhibit greater stress under drier conditions compared to legumes (Daryanto et al. 2016), which may account for lower bacterial richness in the warmer/drier early season soils because microbial community resilience is linked to plant community resilience (de Vries et al. 2018). Additionally, brassicas select for unique microbial communities because of the compounds they produce (Rumberger and Marschner 2003) and have been shown to suppress plant pathogens while increasing soil bacterial diversity when in rotation (Li et al. 2017; Majchrzak et al. 2010). Brassicas in both cover crop mixtures were seeded at a rate of less than 10% (kg/ha), but their mean biomass substantially increased in proportion to the legumes and grasses in the warmer/drier mid-season cover crops, compared to the warmer/drier early season cover crops. These factors may have driven the observed bacterial community dissimilarity between the early and mid-season cover crop mixtures.

The abundance heatmaps reflect dissimilarities between the mid-season cover crop soils and the early season and fallow soils. The fifty most common sequence variants found in the 2018 samples were better represented in early season and fallow soils than in mid-season soils. However, the mid-season cover crops were characterized by soil bacteria linked to several aspects of soil health. *Sphingomonas* is associated with plant health as an antagonist towards

plant pathogens (Yang et al. 2019). The mid-season cover crop mixture contained more legume species than the early season mixture, potentially increasing support for bacteria associated with nutrient cycling. *Mesorhizobium* fix nitrogen, *Nitrospira* participate in nitrification, and Nitrosomonadaceae and Nocardioidaceae are thought to play a role in carbon and nitrogen cycling (Yang et al. 2019); these were differentially abundant in the mid-season soils compared to the early season and fallow soils.

Effects of Temperature and Moisture Conditions

Environmental conditions strongly affect soil bacterial communities (Maestre 2015; Naylor and Coleman-Derr 2018; Ishaq et al. 2020; Ouyerson et al. 2021). Precipitation in 2019 may have been a stronger influence on bacterial community alpha and beta diversity than the presence and composition of cover crops, termination methods, or simulated climate conditions. The study site received 188mm more rainfall in June 2019 than in June 2018 (Northern Agricultural Research Center 2020); there was 2.5x as much precipitation in the two weeks preceding sample collection than during the same period the year before (Figure S3.5), making it the 20th wettest June for Havre since 1970 (NOAA 2021). It is likely that soils were saturated from cumulative precipitation, as evident by the higher soil moisture readings for 2019 (Figure S3.6). Soils that normally undergo wet-dry cycles host distinct bacterial communities better adapted to these extremes (Fierer et al. 2003). In a study comparing bacterial communities under winter wheat in contrasting cropping systems, community dissimilarity increased toward the end of the growing season when conditions were hotter and

drier (Ishaq et al. 2020). Without the selective pressure of decreased soil moisture, higher precipitation in 2019 may have homogenized soil bacterial communities instead.

In 2018, bacterial richness and diversity were lower in the warmer/drier treatment as hypothesized, though richness was not correlated with soil moisture and soil temperature. The early season cover crops, especially those in the warmer/drier conditions, may have driven the negative correlation between plant biomass and soil moisture and this relationship has been proposed as a mechanism for changes in soil microbial communities (de Vries et al. 2018). Bacterial richness and evenness have been observed to decrease in response to drier conditions and exhibit a lag between drought and recovery (de Vries et al. 2018). Soil bacterial community composition, however, does not have a consistent response to drought. Dominant taxa can either respond poorly to drought or not be affected compared to subordinate taxa (de Vries et al. 2018; Sayer et al. 2017). Also, altered precipitation can lead to shifts in vegetation community composition (Nielsen and Ball 2015), resulting in changes in root structure and depth that in turn affect what root exudates and litter are available throughout the soil profile (van der Putten et al. 2013).

Cover Crop Termination and Soil Bacterial Communities

Based on previous studies (Zhou et al. 2010; Eldridge et al. 2017; Ouerson et al. 2021), cattle grazed cover crop and fallow strip plots were expected to have higher bacterial diversity compared to glyphosate application and haying. However, no differences were found among soil bacterial communities based on cover crop termination method. In previous work, sheep grazing selected for dissimilar soil bacterial communities compared to tillage or chemical

methods for cover crop termination and weed management (Ouverson et al. 2021). Long term exclusive cattle grazing and exclusive sheep grazing have different impacts on soil carbon storage, with no impact from cattle grazing observed in high diversity plant communities (Chang et al. 2018). The cropping system in this study is characterized by brief, targeted grazing, an approach that can negatively impact organic carbon storage without affecting microbial enzymatic activity (Tobin et al. 2020), which may account for the lack of soil bacterial response. Glyphosate application did not reduce bacterial diversity, which is consistent with previous work that found glyphosate application at recommended rates does not significantly change soil bacterial community structure (Rose et al. 2016; Kepler et al. 2020). As observed in other studies (Zhang et al. 2013; Carey et al. 2015), haying also had no effect.

Conclusion

In this study, the presence and composition of cover crops and interactions with soil temperature and moisture conditions had a greater effect on soil bacterial communities than cover crop termination methods. Our results suggest that in warmer and drier conditions, cover crop mixtures with similar composition to the mid-season mixture may encourage more diverse bacterial communities compared to cover crop mixtures composed of species from the early season mix. Future work could assess bacterial response to cover crop phases of single species, testing those used in the early season and mid-season mixtures as well as other species commonly used as cover crops in the region. This would address the question of whether soil bacterial communities are influenced more by species or by the diversity of cover crops

mixtures in a rotation. In either scenario, cover crops can be used to promote diverse soil bacterial communities as an important component of soil health.

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CHAPTER FOUR

SUMMARY OF FINDINGS AND FUTURE RESEARCH

This research examined the impacts of diverse cropping systems on soil bacterial communities in semiarid dryland agriculture under current and predicted climate conditions. The goal was to assess how these communities respond to contrasting cropping systems through temporal and environmental changes, which can further increase understanding of how soil regenerative practices affect biological indicators of soil health. The specific objectives were to evaluate soil bacterial community response to 1) crop phases in contrasting cropping systems over a growing season, and 2) the presence and composition of cover crop mixtures terminated via different methods under current induced warmer and drier conditions simulating regional climate change projections.

The first objective was addressed within a study of three cropping systems characterized by off-farm inputs with no tillage, USDA-certified organic with sheep grazing, or USDA-certified organic with tillage. Samples were taken from each cropping system in the first three phases of a five-year crop rotation at two points over the growing season. We hypothesized that organically managed systems would support higher soil bacterial diversity than chemically managed systems, that the two organic systems would have distinct soil bacterial communities, and that diversity would be the highest in the cover crop phases.

The second objective was addressed with a study comparing winter wheat in rotation with cover crop mixtures of different species richness and phenologies to summer fallow. The

cover crop/fallow phase was terminated via three different methods perpendicular to planting direction. To simulate predicted climate conditions, there were structures increasing soil temperature and decreasing soil moisture compared to ambient soil conditions in each combination. We hypothesized that cover crop mixtures would have more diverse soil bacterial communities than fallow, that ambient soil condition would support higher soil bacterial diversity than warmer/drier conditions, and that there would be a gradient in soil microbial community diversity from chemically terminated warmer/drier fallow soils (lowest) to grazed ambient mid-season soils (highest).

Summary of Findings

With respect to the first and second hypotheses of the first study, the organic grazed system hosted more diverse soil bacterial communities than the chemical no-till system, but there were no differences in alpha diversity between the two organic systems. Bacterial communities became less diverse in all cropping systems throughout the summer. Organic grazed communities were distinct from organic tilled and chemical no-till communities throughout the summer. Within-community dissimilarity of the chemical no-till communities decreased but between-community dissimilarity increased from June to August as they became more disparate from the organic tilled and organic grazed communities. These observations were possibly a response to drier conditions at the end of the growing season. Crop phase alone did not impact bacterial communities as per the third hypothesis but interacted with cropping system on community dissimilarity. This may have been driven by the higher within-

community dissimilarity of organic grazed clover. Overall, this study indicated that system-level differences between cropping systems can have a greater impact on soil bacterial communities than differences within a system such as crop phase. However, changes in the soil environment through the growing season and interactions between cropping systems can influence community dissimilarity.

As per the first and second hypotheses of the second study, bacterial diversity was higher in mid-season cover crops than in early season cover crops and fallow, as well as in ambient conditions compared to warmer/drier conditions. However, bacterial richness responded to an interaction between the presence and composition of cover crops and manipulated environmental conditions, which these hypotheses did not account for. Cover crop termination methods did not impact soil bacterial communities, meaning it was not possible to assess a gradient in diversity for all combinations of treatments for the third hypothesis. The mid-season cover crops may have supported higher bacterial diversity than the early season cover crops because of how species within each mixture responded to warmer and drier conditions. Results of this study indicate that planting date and the composition of cover crop mixtures must be considered especially with respect to available soil moisture. There were some shifts in dominant taxa from 2018 to 2019, but treatments had no discernible impact on bacterial communities in 2019 - potentially due to high levels of precipitation. Cover crop mixtures including species that can tolerate an increase in soil temperature and decrease in soil moisture can foster more diverse soil microbial communities under projected climate change conditions.

Future Directions

Soil bacterial communities are an important component of the soil ecosystem and associated soil health of agricultural systems (Lal 2016). This study contributed to our understanding of how these communities respond to regenerative agricultural practices in the Northern Great Plains. Our results suggest that cropping systems can foster unique soil microbial communities, but these effects may be moderated by environmental and temporal conditions. Environmental conditions can be strong drivers of change in soil bacterial communities, such as a drought at the end of the growing season or heavy rains. Temperature is projected to increase during the growing season in the Northern Great Plains while precipitation is expected to decrease (Weinhold et al. 2018). However, precipitation is predicted to increase before the growing season, though agricultural ecosystems in the region are not likely to benefit from this because of the associated increase in evapotranspiration (Whitlock et al. 2017). An increase in the intensity of wet-dry cycles and other environmental stressors such as heat may put a strain on soil bacterial communities that form an essential component of healthy soils (Fierer et al. 2003; Bérard et al. 2011; Hernandez et al. 2021).

Soil health-building practices need to be tailored for semiarid dryland cropping systems, as they are vulnerable to degradation and climate change (Brewer and Gaudin 2020; Chimwamurombe and Mataranyika 2021). Drylands have the potential to store more soil organic matter (Plaza et al. 2018), but restoring degraded soils is a slow process because low precipitation limits plant biomass production (Thapa et al. 2018). Even so, diversified crop rotations that include cover crops can be implemented, given the use of species that are

appropriate for the region with respect to soil moisture limitations (Smith et al. 2017; Hansen et al. 2016). Though immediate effects on soil bacterial diversity are not always apparent, integrated crop-livestock management can also increase the sustainability of these systems by reducing the use of off-farm inputs and tillage (Miller et al. 2015; LaCanne and Lundgren 2018). Soil bacterial community response to these practices should be viewed as part of a larger framework including other trophic levels such as fungal, weed (Dupre et al. 2021b) and beneficial insect communities (Dupre et al. 2021a) for a more complete understanding of how regenerative practices affect agroecosystems. Future work could assess soil bacterial response to cover crop functional groups compared to the cover crop mixtures used in this study and rotations of single species from those mixtures. The rhizosphere should be sampled rather than bulk soil for this work because the impacts of single species crop phases cannot always be detected in the latter. Overall, we recommend the continued pursuit of including cover crops in crop rotations to strengthen the resiliency of the soil and agricultural ecosystems of the semiarid Northern Great Plains.

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APPENDICES

APPENDIX A

SUPPLEMENTARY INFORMATION FOR CHAPTER TWO

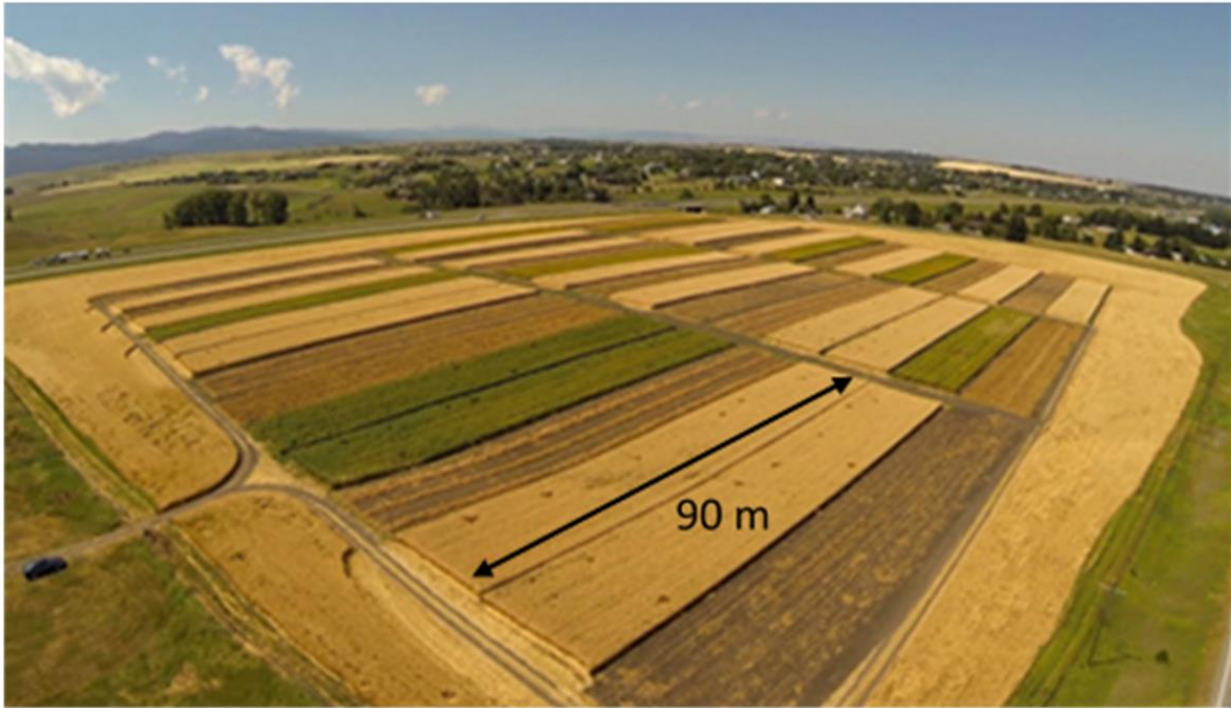


Figure S2.1. Aerial view of the Montana State University Fort Ellis Research and Teaching Center in Bozeman, MT. Each of the three cropping systems has three total field replications, which were randomly arranged with one replication in each of the columns of plots seen here. The columns are arranged north to south, with the north side located on the right side of the photo. Each cropping system plot is subdivided into 5 subplots managed with a 5-year crop rotation.

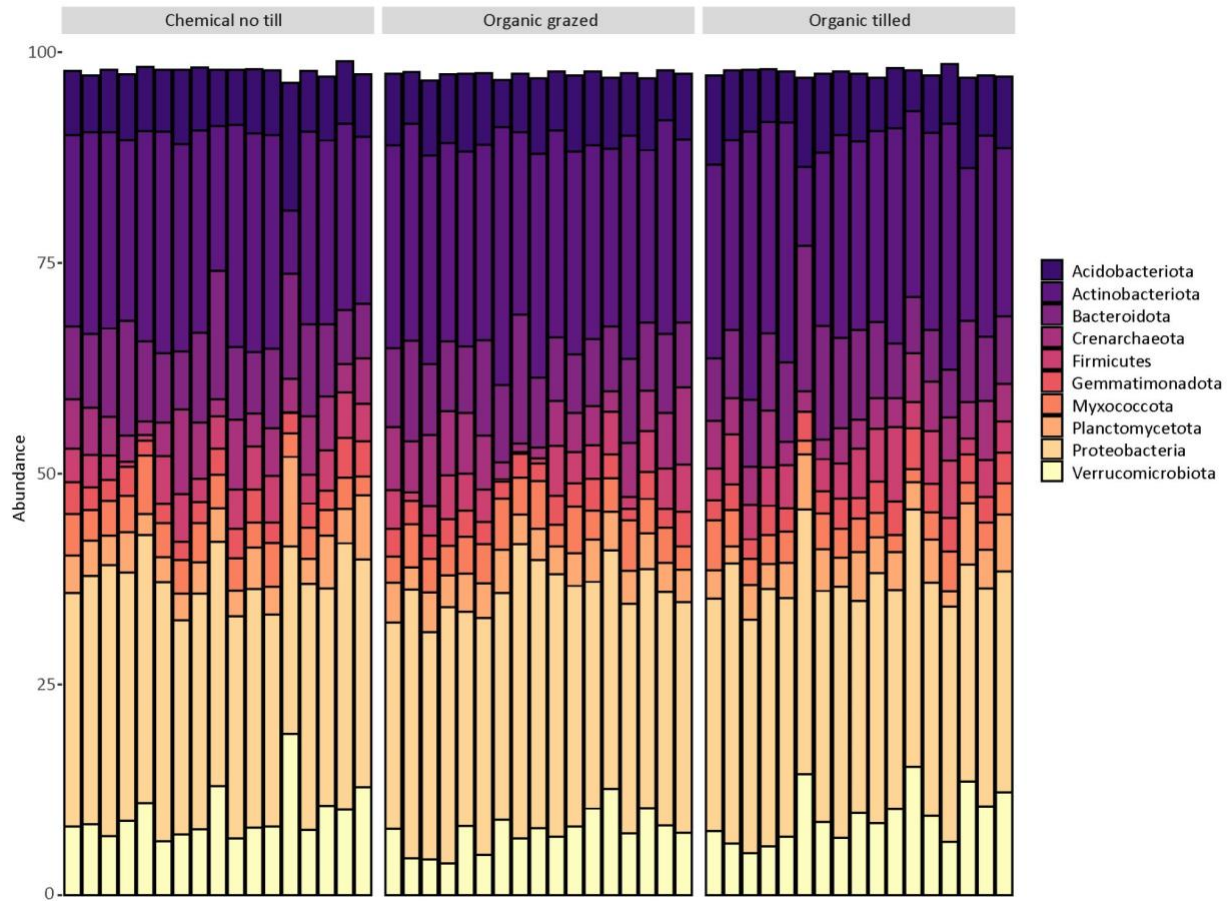


Figure S2.2. Soil bacterial communities at the phylum level of taxonomy in three cropping systems. The ten most abundant phyla are displayed.

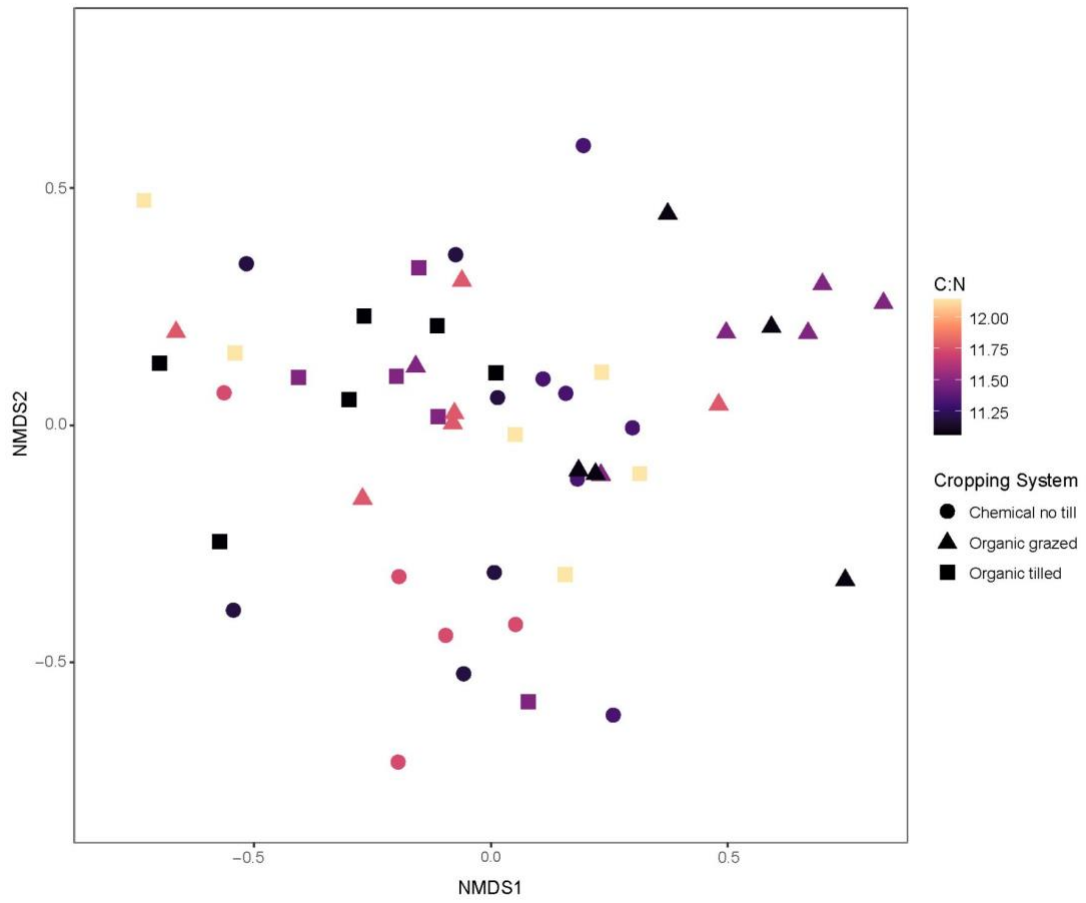


Figure S2.3. Soil bacterial community dissimilarity by cropping system and soil C:N.

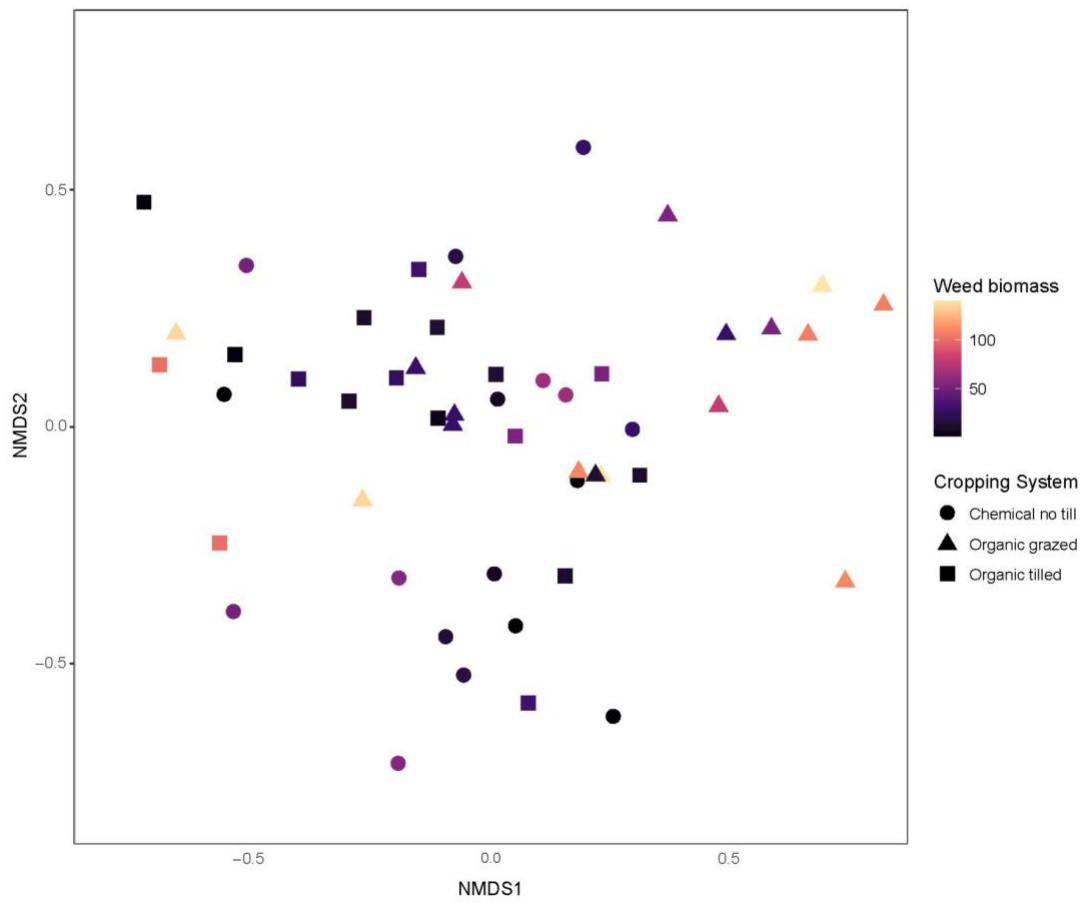


Figure S2.4. Soil bacterial community dissimilarity by cropping system and total weed biomass in grams.

APPENDIX B

SUPPLEMENTARY INFORMATION FOR CHAPTER THREE

Table S3.1. Seeding rates of cover crops for 2018 and 2019

Cover crop species	Seeding rate (Kg/ha)		
	Early	Mid (2018)	Mid (2019)
Oat (<i>Avena sativa</i>)	12.55	14.08	14.08
Turnip (<i>Brassica rapa</i>)	1.72	1.71	1.71
Chickpea (<i>Cicer arietinum</i>)	-	-	56.31
Soybean (<i>Glycine max</i>)	-	8.46	8.46
Lentil (<i>Lens culinaris</i>)	-	4.79	-
Pea (<i>Pisum sativum</i>)	26.59	14.14	-
Radish (<i>Raphanus raphanistrum</i>)	2.77	2.93	2.93
Millet (<i>Setaria italica</i>)	-	-	3.01
Sorghum x sudan grass (<i>Sorghum x drummondii</i>)	-	3.16	-
Hairy Vetch (<i>Vicia villosa</i>)	13.58	-	6.86
Total	57.21	49.27	93.36

Table S3.2. PERMANOVA of the effects of presence and composition of cover crops, termination method, environmental conditions, and the interactions among them on soil bacterial communities.

2018	Df	F Model	R2	Pr(>F)
Cover crop	2	6.102	0.259	0.001
Climate	1	0.678	0.140	0.640
Termination	2	0.917	0.039	0.470
Cover crop:Climate	2	0.572	0.024	0.904
Cover crop:Termination	4	0.778	0.066	0.702
Climate:Termination	2	0.541	0.023	0.926
Cover crop:Climate:Termination	4	0.506	0.043	0.995
Residuals	25		0.531	
Total	42		1.000	
2019				
Cover crop	2	0.878	0.048	0.795
Climate	1	0.968	0.026	0.427
Termination	2	1.020	0.055	0.352
Cover crop:Climate	2	1.064	0.058	0.267
Cover crop:Termination	4	0.840	0.091	0.961
Climate:Termination	2	0.756	0.041	0.994
Cover crop:Climate:Termination	4	0.767	0.083	0.993

	142	
Residuals	22	0.597
Total	39	1.000

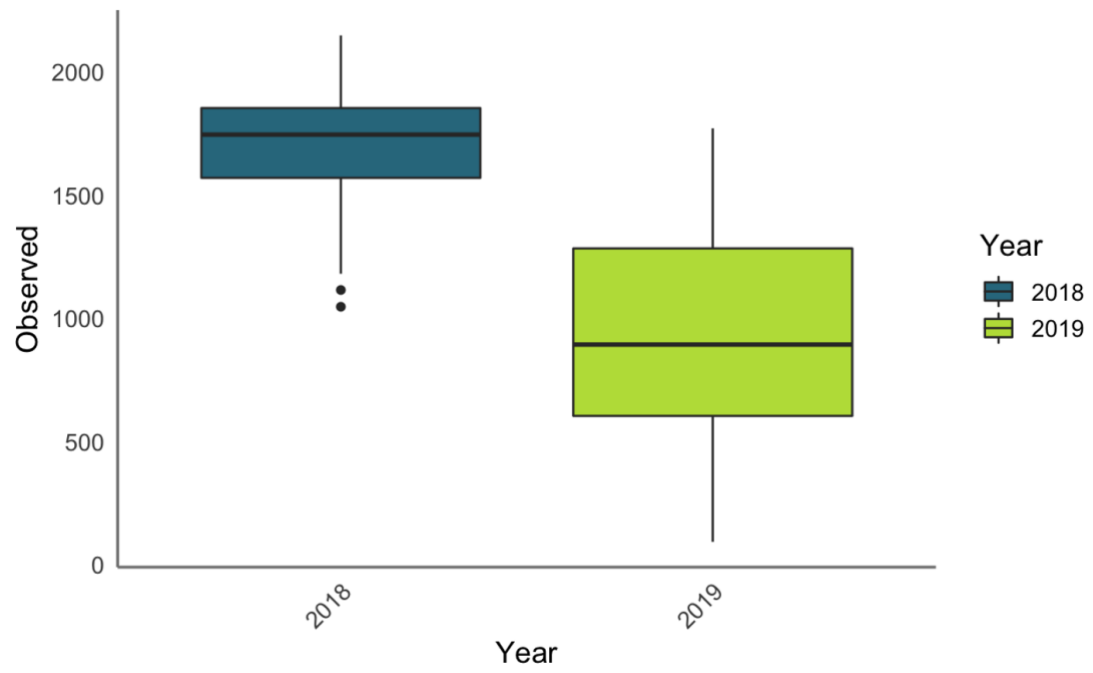


Figure S3.1. Bacterial richness (observed sequence variants) in 2018 and 2019.

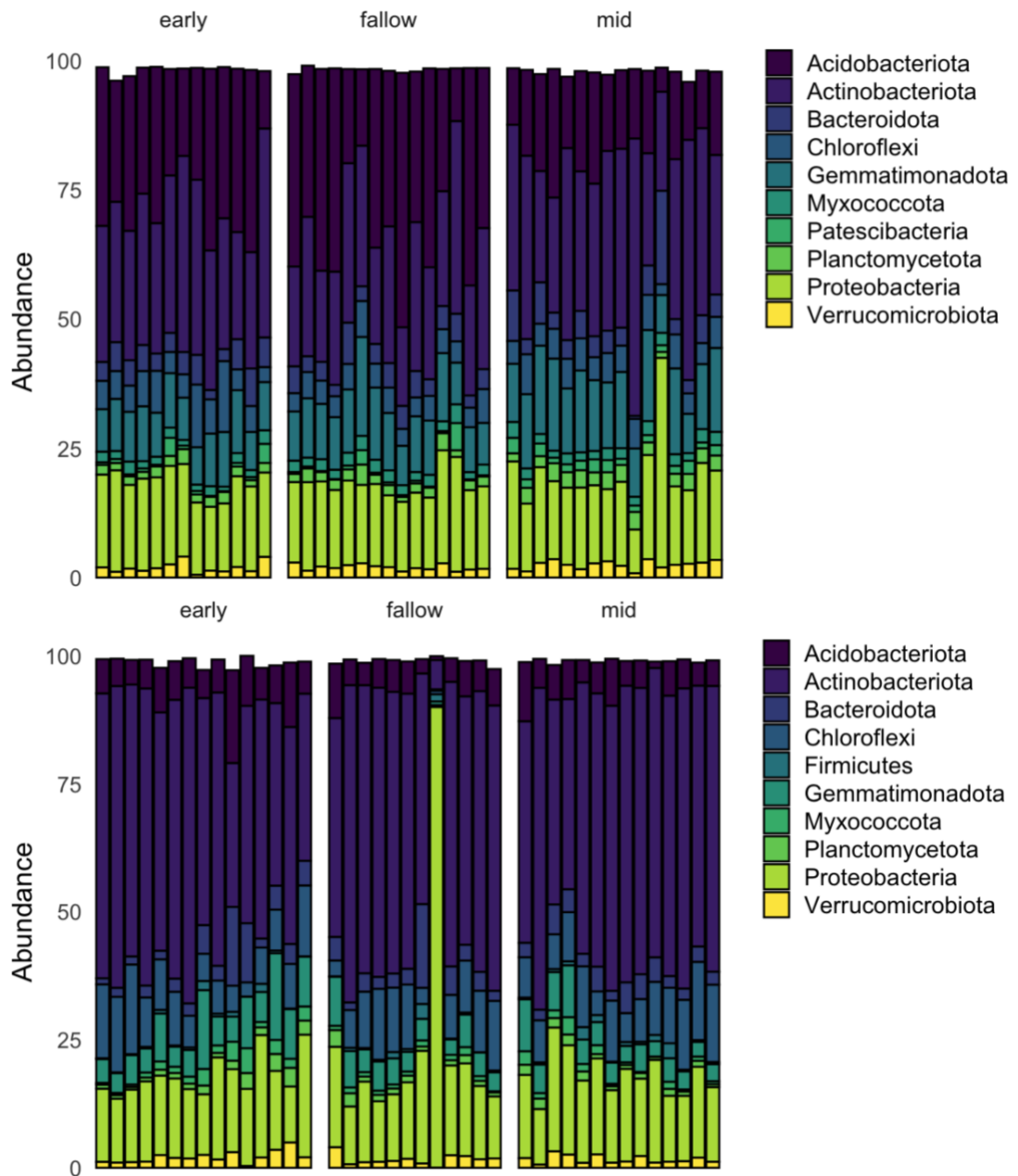


Figure S3.2. Soil bacterial communities at the phylum level of taxonomy in the cover crop/fallow phases for each year, outliers included. The ten most abundant phyla are displayed

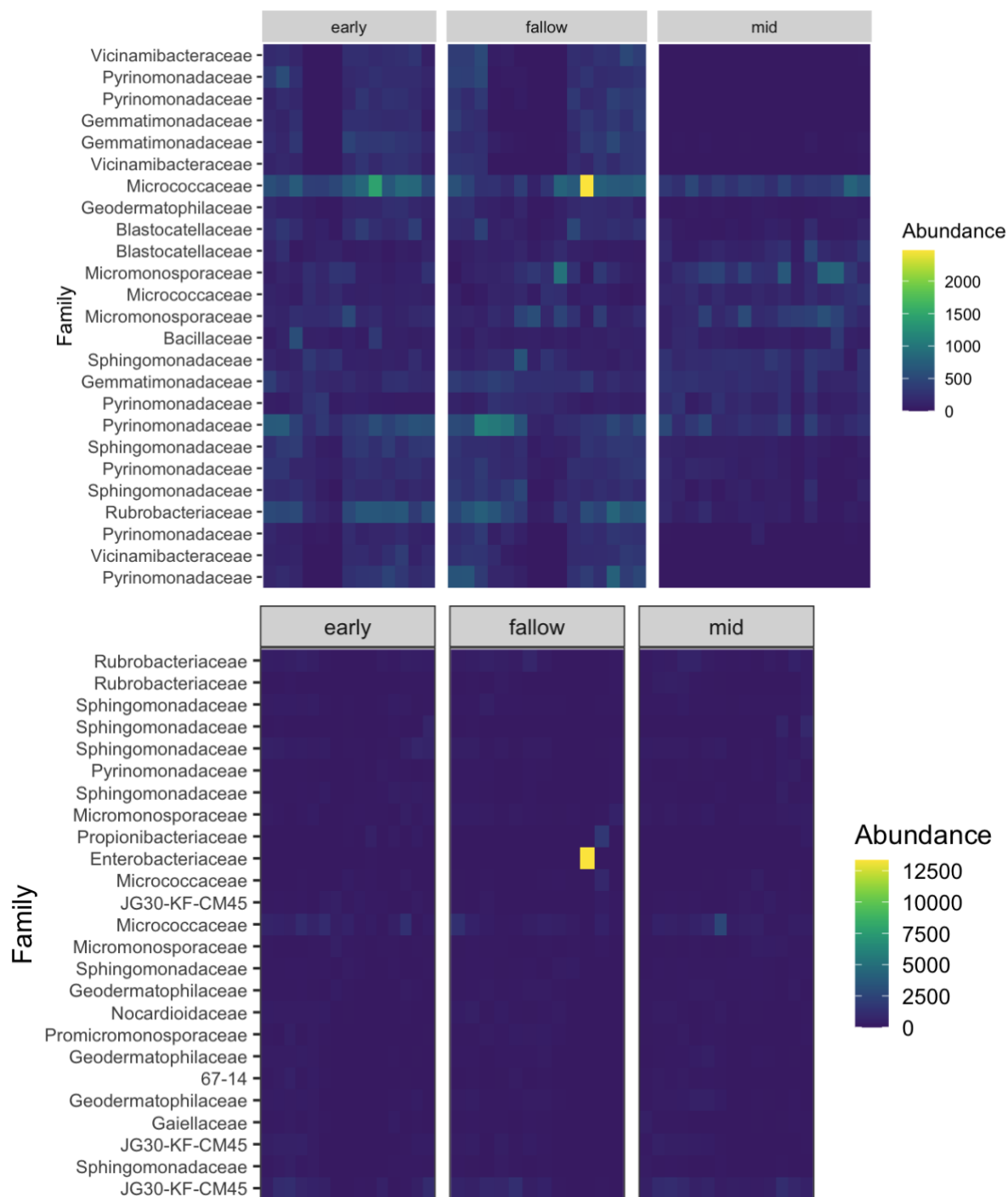


Figure S3.3 Heatmaps of the most abundant sequence variants identified at the family level in the cover crop/fallow phase for each year, outliers included. Taxa not present or at very low abundance in samples were set to display as the closest limit (0) for visual coherence.

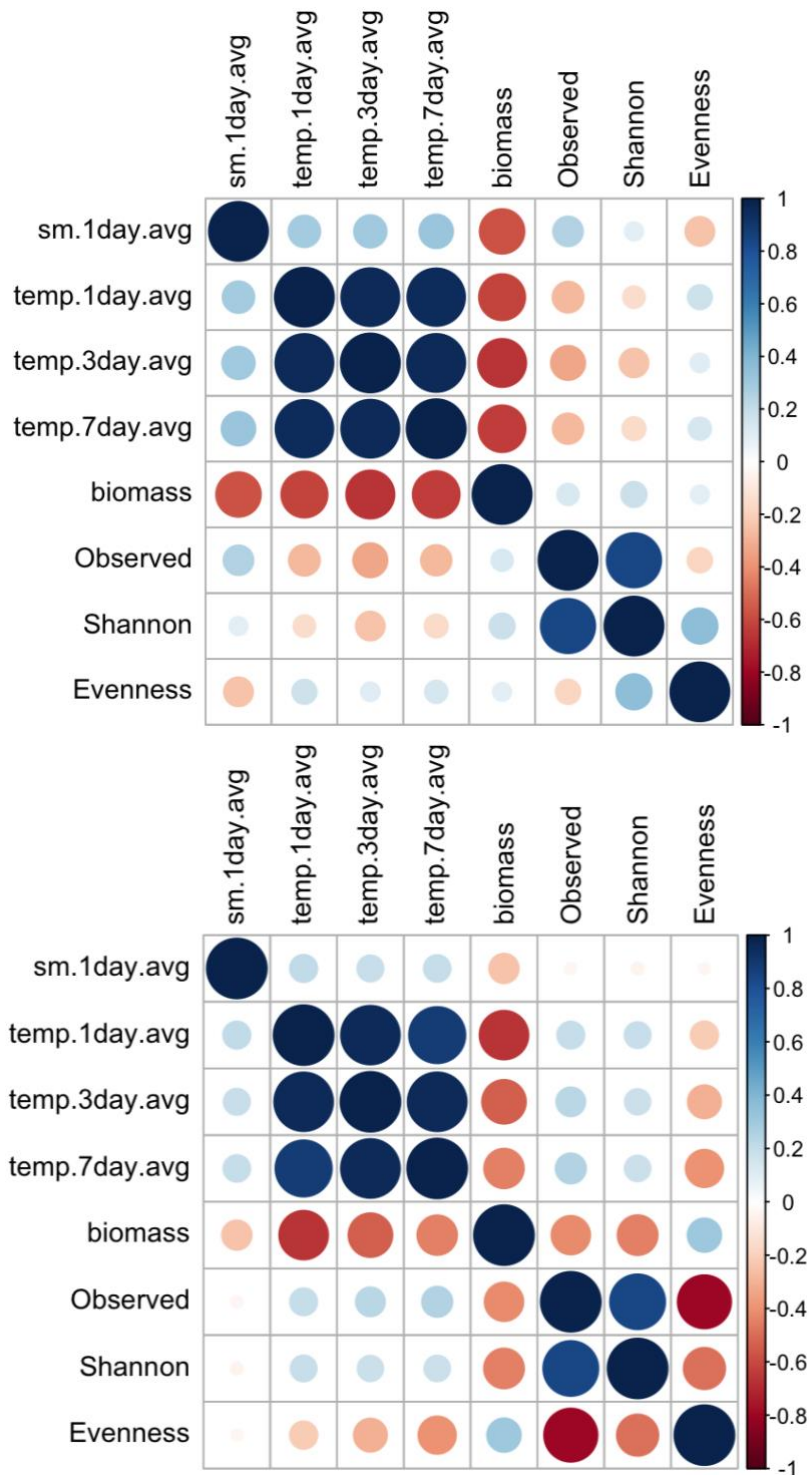


Figure S3.4. Pearson's correlation plots for measures of bacterial alpha diversity against soil moisture, soil temperature averages, and aboveground plant biomass for 2018 (top) and 2019 (bottom).

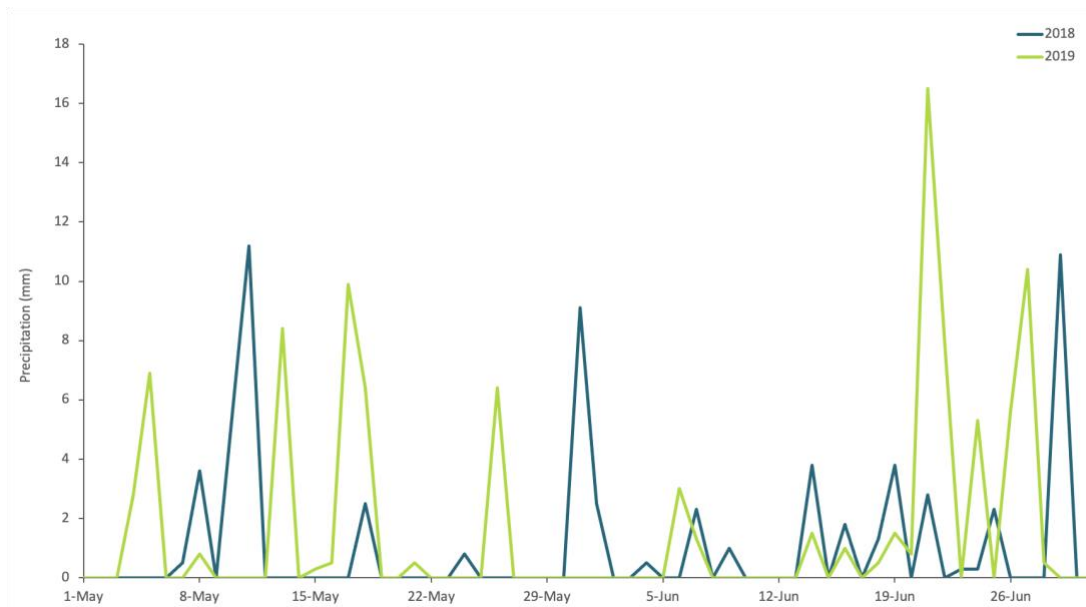


Figure S3.5. Precipitation in millimeters for May 1 to July 1 in 2018 and 2019. Data for the closest weather station to the Northern Agricultural Research Center (Havre City - County Airport, 48.54278°N 109.76333°W) were requested from the National Oceans and Atmosphere Administration.

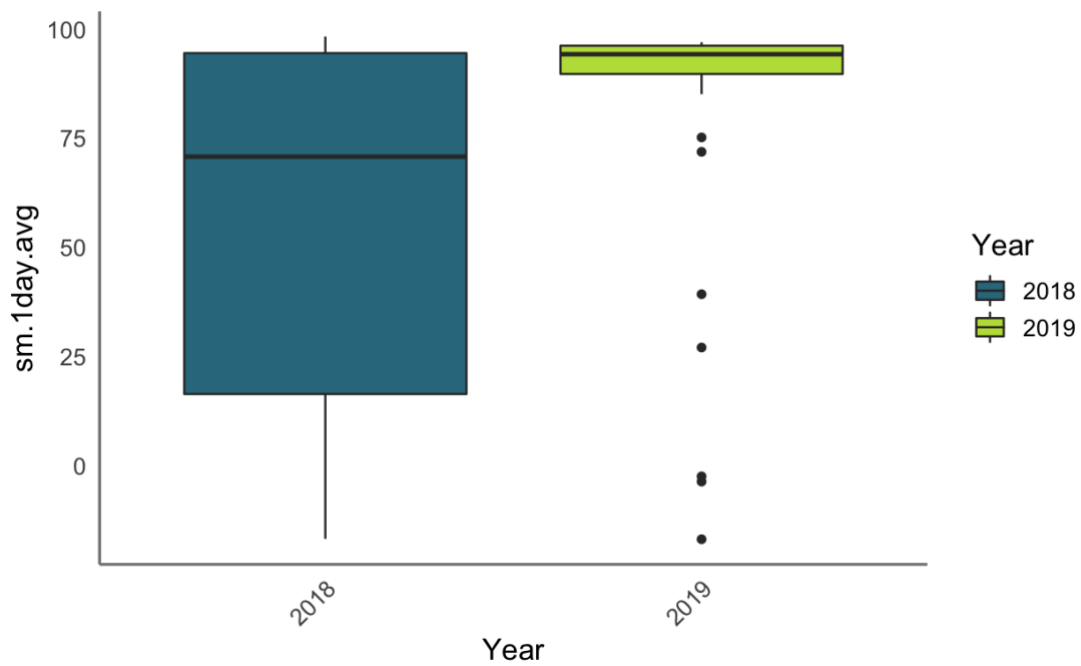


Figure S3.6. Average soil moisture, measured in conductivity, the day prior to sampling the cover crop/fallow phase for 2018 and 2019.