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LONG-TERM LAND MANAGEMENT PRACTICES AND THEIR EFFECT ON SOIL HEALTH AND CROP PRODUCTIVITY

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LONG-TERM LAND MANAGEMENT PRACTICES
AND THEIR EFFECT ON SOIL HEALTH AND CROP PRODUCTIVITY

THESIS
A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Agriculture, Food and the Environment at the University of Kentucky

By
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Lexington, Kentucky

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2019

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ABSTRACT OF THESIS

LONG-TERM LAND MANAGEMENT PRACTICES
AND THEIR EFFECT ON SOIL HEALTH AND CROP PRODUCTIVITY

Agricultural intensification reliant on monocrops could change soil health in a way that does not support maximum crop productivity. Twenty-nine-year-old no-till field plots at the University of Kentucky Spindletop research farm showed a significant reduction in corn yields from continuous corn plots compared to those from plots in various types of rotation. The objective of this study was to determine what role soil microbes might play in yield reduction and how management and time effects microbial community structure. Samples were collected from the following treatments: continuous corn (CC), continuous soybean (SS), a 2-year corn/soybean rotation (CCSS), Corn in rotation with soybean with winter wheat cover (C/W/S), and sod controls (SOD). Soil health-related parameters were determined along with microbial community structure using phospholipid fatty acid analysis (PLFA). Results show that there is a strong seasonal dynamic in microbial communities with May, July and September showing the greatest differentiation between treatments. Nonparametric multidimensional analysis (NMDS) shows that microbial communities under SS, CC treatments were significantly different from the CS and CWS treatments across all four years of the study. My findings will prove useful for assessing the contribution of biological indicators to agroecosystem function and will aid in making recommendations of when and how to manage these parameters to improve soil health and maximize yield.

KEYWORDS: PLFA, Microbial Community Structure, Crop Rotation, Corn Yield, Temporal Dynamics

Thomas Joseph Muratore Jr.

4/02/2019
Date
DEDICATION

To my mother and father for their unconditional love and support in all that I do; and
Kate, with love and gratitude.
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The following Thesis, while and individual work, benefitted from the insight and input of several people. First, my Thesis Advisor, David McNear, embodies what it means to be a leader. Thank you for your patience, encouragement, and guidance in all aspects of this project and beyond. Additionally, I would not have been able to complete this project if not for Joe Kupper’s expertise. Next, I wish to thank my Thesis Committee, an outstanding group of mentors that I strive to aspire to one day, for the individual insight that guided and challenged me to grow in ways beyond the scope of this thesis. My many thanks are also extended to Diane Hunter, Frank Sikora, and the many members of U.K. Regulatory Services, Eric Roemmele, and all members of the Rhizolab.
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CHAPTER 1. LONG-TERM LAND MANAGEMENT PRACTICES AND THEIR EFFECT ON SOIL HEALTH AND CROP PRODUCTIVITY

1.1 General Introduction

Soil health is used to describe the “continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans.” (Soils 2018; Lehman et al. 2015) This term was first introduced by (Doran, Sarrantonio, and Liebig 1996) and has been useful in describing soil status. Because living things inherently have health, viewing the soil as a living ecosystem reflects the soil status across the world. Management strategies implemented to maintain or enhance inherent soil ecosystem functions are mediated by the diversity of biological soil communities. Soil biological communities require management and conservation to preserve ecosystem services, which is a defining factor associated with soil health. Yet, there is no common consensus on what an ideal soil microbiome consists of (Anderson 2003).

Intensification of agricultural soil use with time has led to a concern that soil health may be altered for the long-term (Bennett et al. 2012). Numerous factors (e.g. population increase, demand for specialty foods, industrialization (Hobbs 2007; Giller et al. 2015) have caused greater soil use. While productivity increase is associated with soil use intensification, conventional agriculture has raised concerns regarding sustainability, environmental degradation, loss of biodiversity, and ecosystem functioning (Foley et al. 2011). The loss of aboveground biodiversity and associated ecosystem functions are major challenges confronting the world (McDaniel et al. 2014a). Few studies have linked long-term aboveground land management to belowground communities and ecosystem services. Management strategies and their influence on belowground microbial
communities should be investigated to identify temporal dynamics that can better assess soil health from a biological point of view.

Management strategies aim at influencing the three pillars of soil health: chemical, physical, and biological components (NRCS USDA). These properties associated with soil health are dynamic and change at various time scales. The chemical property of soil relates to the mineral composition, the pH of a given soil unit, the cation exchange capacity, and the nutrient status. Soil physical properties include soil texture, structure, aggregate size and distribution, and the density of a soil unit. Soil biological properties are comprised of the organisms found in soil and their activity. The pillars of soil health are not independent of one another. Thus, no single measurement can capture a holistic view of soil health.

1.1.1 Soil Health Measurements

Holistic measurements of soil health are difficult because one must consider the three pillars of soil health and their functioning in the system (Karlen, Ditzler, and Andrews 2003). However, frameworks exist to assess soil health, which rely on measuring the soil chemical, physical, and biological properties and processes that are sensitive to change (Lehman et al. 2015). These assessments measure soil organic matter (SOM), particulate organic matter (POM), microbial biomass carbon (MBC), potentially mineralizable nitrogen (PMN), macro-aggregate stability, electrical conductivity (EC), sodium absorption ratio (SAR), pH, inorganic N, P, potassium (K), calcium (Ca) and magnesium (Mg), available water-holding capacity (AWC), bulk density (BD), topsoil depth, and infiltration rate (Lehman et al. 2015).
Conventional agriculture has attempted to maintain the chemical and physical properties of the soil through adding chemical fertilizers and mechanization with assumptions that by managing the physical and chemical components of the soil, the biological component would be maintained. However, certain land management practices attempt to improve soil biology. Time is a critical in agriculture and determines management decisions including: fertilizer applications (N, P, K), pesticide treatments, and tillage. Yet there are no management practices that establish baseline microbial communities and manage their change throughout seasons or even years.

Soil organic matter (SOM) has been regarded as the single most important indicator for soil health in nearly all soils throughout the world because of the interaction it has with the biological, chemical, and physical environment (Karlen, Ditzler, and Andrews 2003). Commonly, SOM is the most deficient property in degraded soils. Soil organic matter is comprised of a heterogeneous mixture of materials that range from plant material to highly-decomposed material known as humus. Various studies have shown that as the total SOM increases, factors associated with good soil health also increase (e.g. water infiltration porosity, microbial activity, nitrogen turnover, stable aggregate formation, increased water retention, increased cation exchange capacity, and increased internal nutrient cycling) (Schloter, Dilly, and Munch 2003; Karlen, Ditzler, and Andrews 2003; Lal 2016).

The complexity and quality of the carbon structures contributes to the properties and reactivity of organic matter entering the soil environment, which then interact with the microbial biomass. Plant structure (root, stem, leaf) and the species of plant differ in the composition of organic compounds. Organic compounds found in macro and
microorganisms are grouped into broad classes: 1) sugars starches, and simple proteins; 2) crude proteins; 3) hemicellulose; 4) fats and waxes; 5) lignin and phenolic compounds. Carbon compounds can also be grouped based on the decomposition rate in the soil environment. Sugars and starches are easily decomposed. At the other end of the spectrum lignin and phenolic compounds are very slow to decompose because of the highly complex and polymerized structures associated with these compounds. The diversity of C:N ratio is another aspect of carbon that controls how C will interact within the soil system and critical for microbial-plant relationships, like N mineralization. Recent studies linked increases in organic matter diversity to an increase in total microbial biomass by 20%, increase aggregate stability, and increased C respiration by 125% when compared to systems with less C complexity (McDaniel et al. 2014a; Tiemann et al. 2015).

Despite the information known about soil physical and chemical parameter contributions to soil health, there is often a controversy in the discussion and assessment of the biological role in soil health due to the difficulty of a measure that could encompass a biotic-abiotic linkage (Anderson 2003; Lehman et al. 2015; Maul et al. 2014). Studies are needed to establish baseline microbial communities, account for their changes over time, and with various land management practices (Lehman et al. 2015). The lack of understanding of the biological component of soil health has caused concern soil health analysis may be misinterpreted or over represented by greatly accounting for the soil physicochemical properties while soil biological function is suppressed.
1.2 Soil Biology

The extent of soil biodiversity is large. Discoveries of new species and phyla in archaea and bacteria kingdoms has been increasing at a rapid rate. There is a diverse array of bacteria, archaea, fungi, unicellular eukaryotes, and animals (Pace 2009). Soil biology is the total biomass and activity of all organisms interacting with the soil environment. This include macrofauna, mesofauna, and microfauna and an array of trophic levels that create a complex food web. The quantities of organisms in the soils are staggering calculations suggest that the microbial biomass existing underground may approach the sum of all living biomass on the earth surface (Lehman et al. 2015). By viewing the three biological domains, one can begin to account for the biodiversity associated with microbial communities (Figure 1). Prokaryotes comprise bacteria and archaea and the biodiversity found within these domains contributes to extraordinary physiological capabilities, tolerances, and energy sources that are specific to prokaryotes.
Figure 1: Tree of life based on genetic relatedness. Adopted from (Lehman et al. 2015)
Traditional classification of the bacteria and archaea have been based on cultivated organisms on media specific plates. The classifications were typically based on morphology, motility, and biochemical characteristics. However, there has been bias in this approach due to the selectivity of the media, representation of novel groups, and the inability to culture all soil microorganisms. Recent advancements in genotypic and phenotypic molecular and biochemical analyses have led to better taxonomic identification and grouping, but also false discoveries and biases. For examples, there has been an over estimation of microbial diversity and the exclusion of taxa (Delmont et al. 2011).

Within the prokaryotes, the functionality and physiology are staggering. Although the phylogenic diversity is important, understanding of the metabolic capabilities of these organisms is crucial in preserving soil health (Garbeva, van Veen, and van Elsas 2004). Prokaryotes carry out all the metabolism found in plants and animals along with novel energy generating processes that underpin soil function. Without the metabolic capacity of the soil microbial community, soils would become stagnant and not be able to provide many of the ecosystem services that are critical for humanity.

Within the eukaryotes, soil fungi contend with the prokaryotes not in diversity but in biomass and ecological significance. Soil fungi are very competitive in the soil environment due to their vast surface area, ability to produce a wide range of extracellular enzymes, and cause the break down the plethora of organic matter inputs introduced to the soil system. Soil fungi generally represent the largest biomass of the microbial community in the soil ecosystem and convert dead organic matter into biomass, CO₂ and organic acids.
Soil fungi play an integral part in contributing to soil health due to the numerous roles, services, and indicators that soil fungi contribute to the soil environment. There are many different roles of soil fungi based on the species present. There are saprotrophic fungi, mycorrhizal fungi, fungal pathogens, fungal endophytes, and fungal parasites. Fungi are the main decomposers of soil organic matter, critical in nutrient cycling, and aid the formation of soil structure through the production of proteins. There have been very promising results showing that increases in soil fungal biodiversity increase soil health and plant productivity (Frac et al. 2018)

1.3 Land Management

Land use has intensified drastically over the last century and, more recently, the last decade, causing concern over the state of soil health and sustainability. A decrease in the biodiversity of terrestrial ecosystems is accompanied by an increase in arable land. Increases in intensification of soil use has been brought about by numerous factors including increase in population, demand for specialty foods, and the industrialization of countries around the world (Hobbs 2007; Giller et al. 2015). Despite the productivity gains with soil use intensification, conventional agriculture faces challenges including environmental degradation and long-term productivity (Foley et al. 2011). Soil management, an important factor influencing and maintaining soil, aims at changing the intrinsic properties of a soil. Beneficial changes can be difficult to alter significantly on meaningful time scales. However, there are land management practices that aim at preserving or enhancing responsive soil properties including: tillage and crop diversity.
Tillage is a common practice involving the turnover of top soil and mixing of surface soil layers for preparing seedbeds, controlling weeds, increasing soil organic matter decomposition, warming of soil, and nutrient mineralization (Giller et al. 2015). Although tillage is used for preparation of an agricultural field, continuous tillage has been linked to negative effects, including soil compaction, soil erosion, decreased soil organic matter and nutrient contents (Indoria et al. 2017). At the other end of the tillage spectrum, no-till management provides minimal soil disturbances leaving plant residues on soil surfaces. There are well known positive effects between no-till practices and soil organic matter, which lead to increases in structure and infiltration (Raphael et al. 2016). Improvements to herbicides, direct seeding technologies, government incentives, and the improvement of genetically modified crops or enhanced crop breeds have allowed for the efficacious use of herbicides and diminishing the need for tillage. These technological advances have shifted agriculture toward to no-till or minimal till systems (Giller et al. 2015).

1.3.1 Crop Rotation

There is a worldwide trend to grow crops in a short rotation or monocrop (Bennett et al. 2012; Foley et al. 2011; Karlen et al. 2006). Monocropping is the practice of growing the same variety of crop species in the same area over multiple growing seasons. The infrastructure developed and devoted to corn and soybean has increased 500% in harvested area and there has been a 800% increase in soybean production between 1950-2003 (Karlen et al. 2006). The huge increase in production of short rotation crops is primarily due to the mechanization of agriculture, improvements of chemicals, and government incentives.
There is concern regarding the sustainability of monocropping due to the unproportioned depletion of certain soil nutrients, build-up of certain soil pathogens that could be associated with a particular crop type. There are measurable yield penalties seen in crops grown continuously compared to those in rotation. Monocropping has succeeded because of the advances in fertilizers, mechanization, pesticide technologies and these technologies have attempted to replace the benefits of crop rotation and other sustainable agricultural techniques. Monocrop agriculture depends on external inputs to replace the internal nutrient cycling capacities. The widespread adoption of shortened rotations and monocrop may suggest maintenance of productivity. However, evidence indicates that crops grown in short rotation or monocrop experience a yield gap compared to that of crops in rotations and potential yields are not being realized (Seifert, Roberts, and Lobell 2017; Grover, Karsten, and Roth 2009).

1.3.2 Crop Diversity

The widely-adapted practice of growing crops in short rotation or monocrop have contributed to significant aboveground biodiversity losses across the globe leading to questions concerning the consequences on belowground soil properties. It is hypothesized that a shift in above ground plant diversity would affect belowground communities.

Crop rotation is the practice of growing dissimilar crops in the same area over seasons. This practice incorporates ecological concepts to mimic ecosystems creating an agroecosystem that provides increased ecosystem functioning. Crop rotations benefits have been used as early as 6,000BCE without the understanding of biological or chemical concepts. It is recognized that growing crops in rotation helps to manage soil fertility, maintain physical soil properties, and prevent the pathogen buildup in soil. Among other
beneficial services, crops in rotation experience the rotation effect. The rotation effect is often times synonymous with yield increase in crops grown for the first time or in rotation (Bennett et al. 2012; Foley et al. 2011; Hilton et al. 2013). Specific mechanisms of the rotation effect have been hypothesized and include mitigating weeds, insects, and pathogens, increase nutrient cycling, and improved soil physical properties. In many cases, microorganisms are either directly or indirectly the cause. However, an ideal microbiome associated with the rotation effect is not known.

Long-term studies are critical in evaluating the influence of the rotation effect. Understanding the temporal changes in crop yield over many growing seasons permits analysis of yields and yield stability across growing seasons. Without long-term studies, it would be incredibly difficult to identify cropping systems that have low production risks and stable yields. This is vital to the identification of more sustainable cropping systems. Crop rotations that included 3 years of corn and 2 years of a break crop indicated that first and second corn grain yields increased an average of 69 and 68 kg ha\(^{-1}\) yr\(^{-1}\), respectively. In the third year of corn, the benefits of the crop rotation were diminished and yields were only increased 58 kg ha\(^{-1}\) yr\(^{-1}\) (Stanger and Lauer 2008). Another long-term study, investigating year to year variability and average crop yields found that, on average, crop rotations including 2 or more species increased crop yield (Grover, Karsten, and Roth 2009). Furthermore, (Seifert, Roberts, and Lobell 2017)) found that corn yield gap increased each consecutive year corn was planted and plateaued after the third year averaging a 4.3% gap in continuous corn to corn in rotation.

Bennet (2012) noted that the rotation effect is not always seen within similar crops grown in monocrop and rotation, which could be due to soil type and climate
variables. Thus, there are uncertainties on what is the major cause of yield gap and below ground mechanisms remain unclear (McDaniel, Tiemann, and Grandy 2014). To further complicate the role of crop diversity, there is evidence that show crops grown in monocrop and short rotation eventually reach an equilibrium in yield decline and remain consistent but at a lower level than rotation (Stanger and Lauer 2008; Bennett et al. 2012; Seifert, Roberts, and Lobell 2017). Thus, potential yields may not be realized by producers.

Although there is growing evidence that crops grown in short rotation or monocrop experience a yield gap compared to crops in rotation, the relative influence of hypothesized causes are not well understood. There are many proposed causes of yield decline in monocrop systems ranging from biotic to abiotic factors and further data needs to be gathered to determine best management strategies. The biotic factors are hypothesized as being plant pathogens, deleterious rhizosphere microorganisms, mycorrhizas acting as pathogens, and allelopathy or autotoxicity of the crop. Abiotic factors include land management practices and nutrient availability. In many of the cases for potential yield decline, microorganisms are either directly or indirectly involved (Bennett et al. 2012).

1.4 Soil Microbial Communities in the Agroecosystem

Many ecosystem services are influenced by microbial communities (Table 1). Many of ecosystem services mediated by microbial communities also happen to be the same ecosystem functions that are depressed in agricultural soils.
Table 1: Ecosystem Services Provided by Soil Biology. Adapted from (Lehman et al. 2015).

<table>
<thead>
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<th>Ecosystem Services Provided by Soil Biology</th>
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<tr>
<td>Regulation of biogeochemical cycles</td>
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<tr>
<td>Retention and delivery of nutrients to primary producers</td>
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<tr>
<td>Maintenance of soil structure and fertility</td>
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<tr>
<td>Bioremediation of pollutants</td>
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<tr>
<td>Provision of clean drinking water</td>
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<tr>
<td>Mitigation of floods and droughts</td>
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<td>Erosion control</td>
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<tr>
<td>Regulation of atmospheric trace gases</td>
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<tr>
<td>Pest and pathogen control</td>
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<tr>
<td>Regulation of plant production via non-nutrient biochemicals</td>
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There is a need to develop microbial based soil health indicators due to the microbial community interactions with soil function and sustainability. It is well documented that tillage and crop diversity influence biological properties. The affect that these practices have on the microbial community are especially important because of the central role microbial communities play in ecological function and biological stability (Zhang et al. 2014). Microbiomes respond rapidly to environmental change, making them sensitive to management, and therefore could be a useful indicators of soil health. Exactly what the microbiome mean in terms of soil health is challenging due to the variety of climates, soil types, and temporal variability. Thus, information is needed to determine what the microbiome mean in terms of soil health across a variety of climates, soil types, and time scales.

Tillage disturbs the agroecosystem by turning over topsoil, breaking aggregates and compacting soil structure. disrupting the soil environment causes direct effect on microbial community structure. Different tillage regimes (e.g. conventional tillage and minimal tillage) produce different ecological niches that select for different microbial community structures. In conventional tillage, fungi were always decreased. However, bacteria responded to tillage by favoring groups that favored copiotrophic lifestyles potentially caused by their ability to degrade recalcitrant or previously protected compounds. Overall, research indicates the increase in microbial biomass in no-till management in multiple studies (Zhang et al. 2014; Frac et al. 2018; Sun et al. 2016).

Monocrop and crop rotation are management practice that affect microbial community structure, but the effect on members therein and their contribution to crop yields are not fully understood. In one study, microbial biomass measured by
phospholipid fatty acid analysis (PLFA) did not increase between continuous corn and corn/soybean treatment, however, the relative abundance of microbial groups increased (Zhang et al. 2014). A common thread in PLFA studies is the increase in fungal biomass in corn in rotation relative to continuous corn and that crop rotation complexity has less influence on the bacterial communities. (Zhang et al. 2014; Yin et al. 2010; Sun et al. 2016). However, a study showed that the gram positive (G+) biomarker group increased when crop diversity increased (Zhang, Sun, et al. 2019). The occurrence of specific groups and/or the interaction between groups may cause increase nutrient exchange, production of plant growth promoting hormones, and a decrease in pathogens within the soil. Using PLFA, Sun et al. (2016) found there was an increase in the saprophytic fungi, AMF, and general fungi in no-till, maize-soybean rotation in the 0-5 cm depth compared to monocrop corn. Different results between the studies may be attributed to varying climatic conditions, soil types or differing sampling dates.

PLFA has shown that fungal communities tend to be the most sensitive biomarker group to crop rotation. However, when a more detailed analysis of the bacterial community is performed, changes in the relative abundance of taxa within the community change. Recently in a meta-analysis, Venter et al. (2016) showed that the effect of crop rotations on bacterial communities may increase, decrease, or have no significant effect on bacterial communities. Specific studies, like Liu et al. (Liu et al. 2017), using Illumina miseq revealed there was greater bacterial abundance but lower bacterial diversity in the rhizosphere of corn in rotation than the rhizosphere of continuous corn. Twelve phyla/classes were present in the rotational corn versus 17 in the continuous corn. The most important taxa contributing to variations between the two different cropping
systems in bulk and rhizosphere soils of corn the Betaproteobacteria, Acidobacteria, Actinobacteria and Alphaproteobacteria. Furthermore, Lui et al. (2017) also found that there was no significant difference between microbial communities in the bulk soil of rotational treatments and the continuous corn treatment. The meter to micrometer scale could be an important factor influencing microbial community structure but also how these changes occur as the growing season progresses. Additionally, these same taxa were shown to have successional patterns over the growing season and those seasonal patterns may be different depending on cropping sequence (Shi et al. 2015).

As more studies are published, the data suggests that overall microbial biomass may not be a sufficient indicator for soil health and crop yields. Accounting for the overall groups of organisms present and their temporal dynamics may prove more useful in the study of soil health and land management. Sampling microbial communities has often been treated similarly to sampling protocols of physicochemical variables. Common sampling practices for microbial communities tend to be at the beginning or end of the growing season. Yet, the agroecosystem demand is greatest during the middle of the growing season and soil communities likely to experience change with plant growth stage and season. A study of PLFA showed a ~50% increase in the total PLFA from winter to summer in high rotation system, low rotation system and natural environments. (Ferrari et al. 2015). Temporal dynamics of microbial communities across years would be very insightful, yet longer term studies that track changes in microbial communities across different cropping years and climates are lacking.

There has been an identified need to evaluate the health of the agroecosystem from a biological prospective. The objective of this research is to characterize and map
the temporal dynamics of microbial communities’ structure across multiple years and cropping cycles to determine their influence on ecosystem services such as crop yield and well-known soil health measurements.
CHAPTER 2. LONG-TERM LAND MANAGEMENT: LINKS BETWEEN CROP
ROTATION AND SOIL BIOLOGY

2.1 Introduction

There is growing interest in evaluating the contribution of soil biology to the
health and quality of soil resources. Yet there is a fundamental lack of knowledge
pertaining to how agricultural management practices influence soil microbial community
structure and function. Particularly, conversion of complex, native ecosystems to
simplified, single species conventional agroecosystems has raised questions regarding
links between overall biodiversity and ecosystem function (Tilman et al. 2001).
Conventional agricultural practices have resulted in short term productivity and economic
gains, but evidence indicates that these gains many not translate over the long term
(Foley et al. 2011; Bennett et al. 2012; Karlen et al. 2006). There is a clear gap in
knowledge with respect to how management practices alter microbial community
structure and function and how this correlates with yield and the provision of ecosystem
services (Maul et al. 2014; Lehman et al. 2015). Overall, there is still no consensus
around what an *ideal* soil microbiome looks like in an agroecosystem, nor how land
management shapes belowground communities over various time scales.

The term soil health has frequently been used as a term associated with
“sustainable agriculture” and depicts the soil as a vital living system that is dynamic and
capable of functioning within a land use boundary to sustain biological productivity,
maintain soil, air, and water quality, and promote, animal, plant, and human health
(Doran and Zeiss 2000). Challenges arise when trying to balance the need for greater
food, feed, fuel and fiber production with those focused on preserving the environment.
Soil health is comprised of the biological, chemical, and physical soil components, all of which interact at various levels to influence the productivity and function of the system. Conventional agricultural practices have placed considerable emphasis on managing the chemical and physical components of the system, with the expectation that these would maintain the biological component of soil health. However, recent research indicates that this result is not always the case and the impacts on soil biology may be negligible or in many cases detrimental to the agroecosystem (Bennett et al. 2012; Seifert, Roberts, and Lobell 2017; McDaniel and Grandy 2016). The contribution of soil microbiology to soil health and agroecosystem function is still largely unknown primarily due to the lack of studies with adequate temporal resolution and sample site diversity (Anderson 2003; Frac et al. 2018; Lehman et al. 2015).

Aboveground management practices have been shown to influence soil physicochemical properties (Lupwayi, Rice, and Clayton 1998; Peralta et al. 2018; McDaniel et al. 2014a). No-till and minimal till agriculture are accepted agronomic practices yet, there is a contentious nature surrounding tillage’s impact on soil properties. No till monocrop maize treatments increase soil organic C in the 0-5 cm compared to conventional tilled treatments. (Sun et al. 2016; Post et al. 1982). Yet, there is evidence that no-till does not truly sequester stabilized carbon below 30 cm depth while conventional tillage has shown increased soil organic matter accrual below a 30 cm depth (Baker et al. 2007). Decades of research indicate that building up soil organic matter (SOC) is beneficial for the agroecosystem and has feedbacks on many other properties of the soil such as aggregate formation, nutrient cycling, and water retention. Tillage introduces disturbances that disrupts the soil environment and microbial communities can
be directly influenced through habitat modification, loss of connectivity, and increase nutrient loss. No-till fields compared with conventional tilled fields had distinct microbial communities (Smith et al. 2016). Different tillage regimes (conventional versus minimal) created different ecological niches favoring different microbial lifestyles (Degrune et al. 2017).

Edaphic factors, including pH, temperature, and moisture, have a strong influence on the composition and function of soil microbial communities. Plant species and variety are also factors influencing microbial community structure and function (Venter, Jacobs, and Hawkins 2016). The rhizosphere microbiome consists of a collection of microbial species that live in close proximity with plants the composition of which is shaped by and helps shape the plant community. The microbiome is known to assist with nutrient acquisition and host plant protection from pathogen infection and disease. Many of the mechanisms responsible for these interactions are still not understood (Peralta et al. 2018).

While it is generally recognized that an increase in above-ground plant diversity is beneficial for agroecosystem function, the corresponding shift in soil microbial community structure, and the significance of that shift, remains uncertain (Peralta et al. 2018; McDaniel and Grandy 2016; Sun et al. 2016). Whether increasing crop biodiversity has a significant impact on soil microbial communities is still unclear. A meta-analysis by Venter, Jacobs, and Hawkins (2016) showed that increases in plant biodiversity may increase, decrease, or have no significant impact on belowground microbial biodiversity.

Two frequently used methods for identification of microbial communities are phospholipid fatty acid analysis (PLFA) and DNA sequencing of rRNA. Each of these
methods has its own inherent bias. When using PLFA it was shown that crop rotations had little influence on soil bacterial communities but had more of an influence on soil fungal communities, particularly arbuscular mycorrhizal and general fungi communities (Zhang et al. 2014; Zak et al. 2003). In other studies G+ was most significantly influenced by crop rotations (Zhang, Sun, et al. 2019). However, when a 16S DNA sequencing of rRNA was used to identify the bacterial soil communities, higher abundances and diversity were found in crop rotation treatments than monocrop treatments, (Liu et al. 2017). (Ai et al. 2018) found that bacteria abundance increased in N fertilized plots while fungal abundance increased in rotational plots. Contradictory results between methods and sampling could arise from numerous factors including soil type, sample timing, sampling methods, management practices, and seasonal climate.

Time is critical in agroecosystems as it determines management decisions. Management practices reliant on seasonal changes include N, P, and K applications, pesticide treatments, tillage, and harvest. Yet very few studies consider the temporal dynamic of soil biology over the course of the growing season or establish baseline biological measurements. The objective of this research is to characterize and map the temporal dynamics of microbial communities’ structure across multiple years and cropping cycles to determine their influence on ecosystem services such as crop yield and well-known soil health measurements. I sampled from high N input plots to avoid seeing a rotation effect in yield between monocrop and rotational treatments because of an N-limitation. I hypothesize that there will be differences in the microbial community structure and members there in over seasons and cropping treatment.
2.2 Materials and Methods

2.2.1 Field Site

The study was performed using long-term (29 year) no till plots established in 1983 at the University of Kentucky “Spindletop” research farm (38°07’46.4”N 84°29’36.6”W) near Lexington, Kentucky (Figure 2: Plot layout for the long-term no till plots established at the University of Kentucky “Spindletop” research farm (38°07’46.4”N 84°29’36.6”W) near Lexington Kentucky. Red and blue asterisks indicate the high N pots which soil samples were collected.) as part of a no-till crop rotation trial. Annually, the location averages 1147 mm of rain per year and has an average annual air temperature of 13.1°C. In the last five years, the location had an average annual rain fall of 1411 mm and temperature of 14.2°C (Table 2). Soils were primarily the Maury-Bluegrass series developed from phosphatic limestone parent material and classified as a fine, mixed, active, mesic oxyaquic paleudalf. The field trial consists of 4 replicated completely randomized split blocks with two cropping treatments in each split block. The cropping treatments were monocrop corn (CC), monocrop soybean (SS), two years of corn followed by two year of soybean (CS), and corn followed by winter wheat double crop soybean (CWS). A sod (SOD) plot adjacent to the main plots was collected as an uncultivated control. Five random soil samples from each field replicate receiving ~90 kg N ha⁻¹ (depending on rotation) were collected each month in the approximate crop row from May 2015- November 2018 using a 2.5 cm diameter push probe to a depth of ~20 cm. The five random soil samples from each field replication were thoroughly mixed and then portioned into 3 bags depending on the desired analysis as follows: UK regulatory services soil testing bags for soil chemical analysis; sterile Whirlpack™ bags for
biochemical analysis; and 50 ml falcon tubes for microbial community analysis. The latter two samples were immediately placed on dry ice for transport back to the lab and

Table 2: Average annual precipitation and temperature experienced during the experiment.

<table>
<thead>
<tr>
<th>Year</th>
<th>Annual Precipitation (mm)</th>
<th>Mean Annual Temperature (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>1378.46</td>
<td>12.60</td>
</tr>
<tr>
<td>2015</td>
<td>1521.46</td>
<td>13.50</td>
</tr>
<tr>
<td>2016</td>
<td>1127.76</td>
<td>14.60</td>
</tr>
<tr>
<td>2017</td>
<td>1249.93</td>
<td>14.40</td>
</tr>
<tr>
<td>2018</td>
<td>1777.24</td>
<td>14.80</td>
</tr>
</tbody>
</table>
Figure 2: Plot layout for the long-term no till plots established at the University of Kentucky “Spindletop” research farm (38°07'46.4"N 84°29'36.6"W) near Lexington Kentucky. Red and blue asterisks indicate the high N pots which soil samples were collected.
stored at -20°C until analysis. Nested within the rotation treatments were the rotation years. Rotation years are used to indicate the crop that will be going in the ground that growing season. For example, in the CS treatment exists first year corn (CR1), second year corn (CR2), first year soybean (SR1), and second year soybean (SR2). The CWS treatment has the following rotation years nested in it; first year corn alternate (CR1a) and winter wheat-soybean year (W/S). CR1a indicates that it is a first year of corn but is distinctly different from the CR1 rotation year due to the influence of differing previous crops. Knowing the rotation year of the crop is important for evaluating the timing of microbiome transitions when moving from one rotation treatment to the next.

Climate data, including soil temperature (0-5cm and 5-10cm), monthly average air temperature, and monthly precipitation were compiled from the UK Ag weather center (UKAGWC) station located at the Spindletop Research farm.

2.2.2 Soil PhysicoChemical Properties

Soil samples were placed in standard issue soil survey bags distributed by the University of Kentucky’s Regulatory Service Division. Soil survey bags were then placed on drying racks for up to a week to air dry. Dry soil samples were submitted to the Regulatory services lab for routine soil tests which included: Mehlich III extractable nutrient analysis (P, K, Ca, Cu, Mn, Zn), pH, and total C and N. Details on the standard soil test procedures can be found on the University of Kentucky Division of Regulatory Services website (http://soils.rs.uky.edu/tests/methods.php#Detailed).
2.2.3 Phospholipid Fatty Acid Analysis

Total fatty acid methyl esters (FAMES) were extracted from soils using the high-throughput, 96-well plate-based procedure described by (Buyer and Sasser 2012). Briefly, a Bligh-dyer (chloroform/methanol/phosphate buffer) extractant (4.0 ml, 1:2:0.8, v/v/v, 50 mM, pH 7.4) spiked with internal standard (19:0, 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine) was added to 1 g freeze-dried soil sample, sonicated for 10 min followed by rotation on an end-over-end shaker for 2 h. Samples were centrifuged and then the supernatant transferred to 13 x 100 mm glass test tubes with PTFE lined screw caps to which 1 mL of chloroform and water were added. The lower phase containing lipids was collected and then dried using a CentriVap (Labconco). One mL of chloroform was then added to the dry powder and the solution added to a preconditioned 96-well SPE plate (Phenomenex, Torrance, CA, USA) followed by washing with 1 mL of chloroform and 1 mL acetone and then the FAMES were eluted into glass vials using 0.5 mL of methanol:chloroform:H₂O (5:5:1). Finally, 0.2 mL of transesterification reagent was added to the samples and they were incubated at 37 °C for 15 min. After incubation, 0.4 mL of 0.075 M acetic acid and 0.4 mL chloroform were added and the bottom phase removed and dried. The samples were redissolved in 75 μL of hexane, transferred to glass inserts in GC vials, and the FAMES identified using a MIDI system (Microbial Identification System Inc., Newark, DE). The MIDI system consists of an Agilent 7890 gas chromatograph (Agilent Technologies, Wilmington, DE, USA) fitted with a 100 place autosampler, an Agilent 7693 Ultra 2 column, and a flame ionization detector. The carrier gas was ultra-high-purity hydrogen gas with a column split ratio of 30:1 and a flow rate of 1.2 mL min⁻¹. The oven temperature was raised from 190 °C to 285 °C at 10
°C min⁻¹ and then to 310 °C at 60 °C min⁻¹ where 310 °C was held for 2 min. The injector and detector temperatures were 285 °C and 300 °C, respectively.

Total microbial biomass was calculated as the quantity of total extractable FAMEs (nmol g⁻¹ soil). The FAME identities and relative percentages were calculated using MIDI methods (Sherlock Microbial Identification System version 6.2, MIDI Inc., Newark, DE) as described by Buyer and Sasser (2012). The concentration of individual fatty acids were summed into the following biomarker groups: gram-positive bacteria (G+; iso and anteiso branched), gram-negative bacteria (G-; monounsaturated, cyclopropyl 17:0 and 19:0), actinobacteria (10-methyl fatty acids), general fungi (18:2 ω6c), arbuscular mycorrhizae (16:1 ω5c), and eukaryotes (18:3ω6c, 19:3ω3c, 20:5ω3c, 20:2ω6,9c).

2.2.4 Statistical Analysis

For analysis of the PLFA data, concentrations and relative abundance of the microbial biomarker groups (G+, G-, actinobacteria, AM fungi, fungi and eukaryote) were treated as continuous response variables and analyzed using a mixed model (JMP®, Version 14.0 SAS Institute Inc., Cary, NC, 2019). Normal and beta distributions with identity and logit link functions, respectively, were fit to the biomarker group concentration and proportion data, respectively, using the Mixed Model procedure of JMP. Where the influence of cropping treatment, month, year or their interaction were significant, differences between the means were determined using a Student’s t-test at the 95% level of confidence (p < 0.05).
To test how cropping treatment, year, and month influenced microbial community structure, microbial biomarker group concentrations were first relativized using a Hellinger (i.e. square-root) transformation (Ramette 2007) and then a non-metric multidimensional scaling (NMDS) analysis was performed using PC-ORD (version 6.08, MjM Software, Gleneden Beach, OR) in autopilot mode using Sorensen (Bray-Curtis) distances and slow and thorough settings. To determine if microbial community structure differed on the basis of treatment (CC, CS, CWS, SOD), year (2015, 2016, 2017, 2018), or month (Jan-Dec) a multi-response permutation procedure (MRPP) was used with a relative Sorensen distance measure of the PLFA matrix with the hypothesis that these groups would not be different. MRPP is a non-parametric procedure in which the resulting A-value describes how similar samples are within a group (i.e. chance-corrected within group agreement) and the p-value evaluates how likely an observed difference is due to chance. An A-value equals one (1) when samples in a group are identical and zero if their heterogeneity is higher than expected by chance. A low p-value (e.g., \( p < 0.05 \)) and A-statistic > 0.1 indicates that the differences in microbial community structure detected between the predefined grouping variables (e.g. cropping treatment, year, month) are greater than would be expected by chance (McCune and Grace, 2002). The \( p \) values were corrected for multiple comparisons using the (Benjamini and Hochberg 1995) approach with a false discovery rate (FDR) of 0.10.

Soil physicochemical (e.g. pH, total %C) and weather (e.g. precipitation, temperature) parameters were correlated with axis scores in the NMDS ordination to determine relationships between environmental variables and PLFA microbial biomarker groups. Those parameters that had an associated \( r^2 \) value of 0.30 or greater with an axis
were overlaid on the NMDS ordination as a biplot. The direction and length of the biplot vectors indicate the direction (positive or negative) and strength of the correlation while the angle between vectors indicates the correlation between environmental variables (small angles = higher correlation).

A mixed-model was used to evaluate which soil physicochemical and environmental variables were strongly correlated with the response in microbial biomarker group relative abundance and concentration. The mixed model was fit to a sub population predicted from the set of screened physicochemical and environmental variables compiled along with the cropping treatments. Variable screening was done through bivariate correlations between physicochemical and environmental variables. If variables had Pearson’s correlation coefficients greater than |.6| then one of the variables was removed to eliminate multi collinearity. Through this screening process, the following independent variables were chosen: pH, Mehlich III P, K, Cu, Mn, Zn and Ca, total C, average bare soil temperature, and monthly precipitation. An F distribution was assumed when evaluating the lipid concentration and a beta distribution was assumed when evaluating the proportional abundance of PLFAs. In order to account for correlations over time an AR(1) error structure was used. The correlation of biomarker group concentrations with yield were assessed with a LASSO regression using all the biomarker concentrations over the whole year. A generalized additive model was used to account for nonlinear fits. All analyses pertaining to microbial temporal dynamics and figures to describe them were performed/created in SAS 9.4, SAS System for Windows.
2.3  Results

2.3.1  Yield

Twenty-eight years of corn yield data from the plots measured in this study reveal a yield gap between the monocrop corn treatments and the corn yield in rotation (Figure 3a.) Yield data from just the last four years of this study showed the same trend (Figure 3b). Over the 4-year period of this study, yield from the monocrop corn plots were between 5-15% (avg. ~7%) less per year than the corn yields in rotation.
Figure 3: a.) Season average corn yields from the continuous corn (CC), double crop corn-soybean (CS), and corn-wheat-soybean rotations from 1989-2015. b.) Season average corn yields from continuous corn (CC), double crop corn-soybean (CS), and corn-wheat-soybean rotations from 2015-2018.
2.3.2 Soil Physicochemical Properties

All physicochemical parameters assessed in this study showed a strong treatment effect. A seasonal effect was only observed for the total %C, Mehlich K, and Mehlich Mn with no interaction between these main effects (Table 3). The pH was greatest in the SS cropping treatment and significantly different than all the other treatments except CWS (Table 4). The total %C was lowest in the SS cropping treatment and significantly different from the other treatments. Mehlich P in the SS treatment was significantly greater than the CC and CWS treatment but significantly less than the CS or SOD treatments. The Mehlich K in the SS treatment was similar to the CS, CWS and SOD treatments, but significantly less than the CC cropping treatment. Mehlich Ca in the SS treatment was significantly greater than all other treatments. The SS treatment had similar Mehlich Cu and Mehlich Mn concentrations to those in the CWS treatment, both of which were significantly greater than the concentrations in the CC, CS, and SOD treatments. Finally, the SS treatment had significantly greater Mehlich Zn than all other treatments. However, the s.d. of Mehlich Zn in SS was high.

The pH in the CC cropping treatment was significantly less than CS, CWS and SOD but significantly less than SS. The Total %C was the lowest of all the treatments to contain maize and was significantly less than the CS, SS, and SOD treatments but not the CWS. Mehlich P in CC was significantly lower than in the SS, CS, and SOD treatments. Mehlich K measured under CC was greatest across all the treatments and was significantly greater than all the other treatments. Mehlich Ca under CC was significantly greater compared to the SOD treatment and it was significantly less than from the SS, CS, and similar to CWS treatment. Mehlich Cu in the CC treatment similar to CS
Table 3: Mixed model results for the fixed effects of Season, Cropping Treatment, and their interaction on different soil physicochemical properties during the four-year (2015-2018) study. Bold values highlight statistically significant differences (p<0.05)

<table>
<thead>
<tr>
<th>P.C. Variable</th>
<th>Season</th>
<th>Treatment</th>
<th>Season*Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>pH</td>
<td>1.4668</td>
<td>0.2222</td>
<td>109.3211</td>
</tr>
<tr>
<td>Total C %</td>
<td>9.7032</td>
<td>&lt;.0001*</td>
<td>268.292</td>
</tr>
<tr>
<td>Mehlich P</td>
<td>0.2999</td>
<td>0.8255</td>
<td>69.4611</td>
</tr>
<tr>
<td>Mehlich K</td>
<td>7.5222</td>
<td>&lt;.0001*</td>
<td>30.2503</td>
</tr>
<tr>
<td>Mehlich Ca</td>
<td>1.7996</td>
<td>0.1458</td>
<td>23.4921</td>
</tr>
<tr>
<td>Mehlich Cu</td>
<td>0.8676</td>
<td>0.4574</td>
<td>253.0552</td>
</tr>
<tr>
<td>Mehlich Mn</td>
<td>4.6883</td>
<td>0.0030*</td>
<td>83.529</td>
</tr>
<tr>
<td>Mehlich Zn</td>
<td>0.9341</td>
<td>0.4236</td>
<td>8.7072</td>
</tr>
</tbody>
</table>
Table 4: Average (2015-2018) seasonal means(s.d.) for soil physicochemical properties found in continuous soybean (SS), continuous corn (CC), double crop corn-soybean (CS), corn-wheat-soybean (CWS) cropping treatments, and sod control (SOD). Spring = March, April, May; Summer = June, July, August; Fall = September, October, November; Winter = December, January, February. Values in columns within treatment and season not connected by the same letter are significantly different.

<table>
<thead>
<tr>
<th>Physicochemical Parameters</th>
<th>pH</th>
<th>Total %C</th>
<th>MehP (lb/ac)</th>
<th>MehK(lb/ac)</th>
<th>MehCa(lb/ac)</th>
<th>MehCu (lb/ac)</th>
<th>MehMn (lb/ac)</th>
<th>MehZn(lb/ac)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>5.09(0.18)a</td>
<td>1.52(0.18)d</td>
<td>79.7(33.9)b</td>
<td>208.5(37.3)bc</td>
<td>3059.6(568.2)a</td>
<td>4.7(1.1)a</td>
<td>167.2(25.9)a</td>
<td>9.5(2.6)b</td>
</tr>
<tr>
<td>CC</td>
<td>4.99(0.41)b</td>
<td>1.58(0.18)c</td>
<td>68.0(17.8)c</td>
<td>259.4(65.1)a</td>
<td>2735.2(563.8)c</td>
<td>4.1(0.9)c</td>
<td>157(25.4)b</td>
<td>5.6(4.7)c</td>
</tr>
<tr>
<td>CS</td>
<td>4.89(0.22)c</td>
<td>1.63(0.19)b</td>
<td>84.0(25.2)b</td>
<td>219.6(58.6)b</td>
<td>2884(476.4)b</td>
<td>4.1(0.8)c</td>
<td>154.7(27.8)b</td>
<td>5.2(1.6)c</td>
</tr>
<tr>
<td>CWS</td>
<td>4.96(0.40)a</td>
<td>1.59(0.20)b</td>
<td>71.5(24.8)c</td>
<td>198.5(52.6)c</td>
<td>2738.6(488.6)c</td>
<td>4.7(1.3)a</td>
<td>171.5(29.4)a</td>
<td>6.4(3.5)a</td>
</tr>
<tr>
<td>SOD</td>
<td>4.62(0.13)d</td>
<td>2.25(0.41)a</td>
<td>111.2(27.8)a</td>
<td>203.7(63.6)c</td>
<td>2600.3(271.6)d</td>
<td>1.8(0.3)d</td>
<td>119.8(29.6)c</td>
<td>2.2(0.6)d</td>
</tr>
<tr>
<td><strong>Season</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>4.93(0.31)a</td>
<td>1.57(0.28)c</td>
<td>79.3(28.5)a</td>
<td>214.3(47.6)b</td>
<td>2772.7(432.3)a</td>
<td>4.0(1.4)a</td>
<td>159.8(26.6)a</td>
<td>6.4(21.5)a</td>
</tr>
<tr>
<td>Summer</td>
<td>5.02(0.38)a</td>
<td>1.65(0.29)b</td>
<td>78.5(28.3)a</td>
<td>215.6(56.6)b</td>
<td>2919.0(588.4)a</td>
<td>4.0(1.3)a</td>
<td>154(28.9)b</td>
<td>5.6(5.1)a</td>
</tr>
<tr>
<td>Fall</td>
<td>5.00(0.40)a</td>
<td>1.72(0.38)b</td>
<td>78.0(29.4)a</td>
<td>215.1(59.9)b</td>
<td>2903.9(594.1)a</td>
<td>4.1(1.3)a</td>
<td>158.3(32.9)a</td>
<td>5.2(2.3)a</td>
</tr>
<tr>
<td>Winter</td>
<td>4.9(0.31)a</td>
<td>1.66(0.37)b</td>
<td>80.2(30.2)a</td>
<td>233.9(74)a</td>
<td>2749.6(400.3)a</td>
<td>4.1(1.5)a</td>
<td>153.5(40)a</td>
<td>5.5(3.9)a</td>
</tr>
</tbody>
</table>
treatment. CC was significantly greater than the SOD treatment and was significantly lower than the SS and CWS. Mehlich Mn was significantly greater in the CC treatment compared to the SOD and was significantly less than the SS CWS but similar to CS. CC had significantly greater Mehlich Zn and then the SS, CWS, and SOD.

The pH in the CS treatment was the lowest of the cropping treatments and was significantly less than the SS, CC, and CWS but significantly greater than SOD. (Table 4). Total %C under CS was the greatest of all cropping treatments and was significantly greater than the CC and SS treatments but not the CWS. However, total %C between the CC, CS, and CWS ranged only 0.05%. Mehlich P in the CS was significantly greater than the CC and CWS treatments and less than SOD. Mehlich K under CS was significantly less than CC but greater than the CWS. It was not different from the SS. Mehlich Ca in the CS treatment was greater than the SOD and was similar to the CC or CWS treatments. Mehlich Cu and Mehlich Mn in the CS was lowest of the cropping treatments and had a similar level to the CC treatment and but was not lower than the SOD. In both cases, CS was significantly less than SS and CWS and significantly greater than SOD. Mehlich Zn was significantly greater in the CS treatment compared to the SOD however it was significantly less than all the SS and CWS.

Soil pH measured in the CWS was significantly greater than the CC, CS, and SOD treatment but similar to SS. Total %C in CWS was not significantly different than the CC, CS, or SOD treatments and was significantly greater than the SS. Mehlich P was significantly less than the SS, CS and SOD treatments but similar to CC. Mehlich K was
lowest in the CWS treatment and was significantly less than the CC and CS but similar to the SS and SOD. Mehlich Ca under CWS had similar levels measured in CC and was significantly less than the SS and CS treatments. Mehlich Cu in the CWS was the greatest and was equal to the value measured the SS treatment and was significantly different than the CC, CS, and SOD treatments. CWS had the greatest Mehlich Mn and was significantly greater than the SOD, CC, and CS but not the SS. Mehlich Zn in the CWS treatment was significantly greater than the CC, CS, and SOD but lower than the SS.

Physicochemical properties were significantly influenced by land management practice. The SOD control was either the greatest or the least of the treatments and was significantly different from all cropping treatments except for in Mehlich K. SOD had the greatest total %C and the greatest Mehlich P. Overall SOD had the lowest pH, Mehlich Ca, Mehlich Cu (1.8 ± 0.2 lbs. ac⁻¹), Mehlich Mn, and Mehlich Zn (Table 4). In all cases, except for with the Mehlich K, SOD was significantly different than the agroecosystem treatments. Within the agroecosystem treatments, physicochemical variables were influenced as well.
2.3.3 Soil Microbial Communities

2.3.3.1 Overall Monthly Variation in Microbial Community Structure

The sampling month had a significant influence on the microbial community structure under each of the treatments (Table 5; Figure 4 and Figure 5). The NMDS ordination for the monthly samples from the control SOD treatment averaged over 2015-2018 produced a 2D solution with a final stress of 9.72 after 65 iterations (Figure 4). In the orientation shown, axis 1 explains 52.9% and axis 2 explains 41.5% of the variation for a total of 94.4%. Microbial communities for each month separated along axis 1 of the NMDS plot and were no different during the growing season (May - Sept) while communities in the late winter and early spring were significantly different (MRPP A=0.3154, p<0.001). The microbial communities in SOD in the middle of the growing season (June and July) were correlated with greater general fungi (axis 1=0.614, axis 2=0.067), F:B (axis 1=0.712, axis 2 r^2=0.03), protists (axis 1=0.312, axis 2=0.003), G- (axis 1=0.346, axis 2=0.127), G+:G- (axis 1=0.628, axis 2=0.173).

The NMDS ordinations for the cropping treatments showed greater separation in community structure from month to month compared to the SOD treatment. The NMDS ordination for CC treatment (Figure 5a) produced a 2D solution with a final stress of 10.91 after 85 iterations. In the orientation shown, Axis 1 explains 40.5% and axis 2 explains 48.6% of the variation for a total of 89.2%. Microbial communities separated along axis 1 of the NMDS plot and were significantly different (MRPP A=0.3154, p<0.001).
Table 5: Mixed model results testing for the fixed effects of treatment, month, year, and their interaction on the concentration and relative abundance of different soil microbial PLFAs during the four-year (2015-2018) study. Bold values and highlighted main or interactive effects (p<0.05). TMB = total microbial biomass, G+= gram positive, G- = gram negative, AMF = arbuscular mycorrhizal fungi.

<table>
<thead>
<tr>
<th>Parameter (Year)</th>
<th>Protein 0.76 0.685</th>
<th>4.18 0.001</th>
<th>2.77 0.002</th>
<th>2.67 0.002</th>
<th>3.58 0.001</th>
<th>2.69 0.002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>1.8 0.198</td>
<td>1.18 0.198</td>
<td>1.18 0.198</td>
<td>1.18 0.198</td>
<td>1.18 0.198</td>
<td>1.18 0.198</td>
</tr>
<tr>
<td>Relative Abundance</td>
<td>0.00 0.001</td>
<td>0.00 0.001</td>
<td>0.00 0.001</td>
<td>0.00 0.001</td>
<td>0.00 0.001</td>
<td>0.00 0.001</td>
</tr>
<tr>
<td>Treatment (T)</td>
<td>0.18 0.198</td>
<td>1.18 0.198</td>
<td>1.18 0.198</td>
<td>1.18 0.198</td>
<td>1.18 0.198</td>
<td>1.18 0.198</td>
</tr>
<tr>
<td>Month (M)</td>
<td>0.00 0.001</td>
<td>0.00 0.001</td>
<td>0.00 0.001</td>
<td>0.00 0.001</td>
<td>0.00 0.001</td>
<td>0.00 0.001</td>
</tr>
</tbody>
</table>

C = gram negative, AMF = arbuscular mycorrhizal fungi.
Figure 4: NMDS ordination of grouped microbial PLFAs in SOD control. PLFA biomarker group concentrations and soil physicochemical parameters with $r^2 > 0.300$ between the variable and the axis score are displayed as vectors (Scaled to 70%). Vectors indicate strength and direction of the graphed relationships. In each of the figures the numbers 1-12 correspond the months Jan-Dec.
Figure 5: NMDS ordination of grouped microbial PLFAs from a) CC, b) CS, c) CWS, d) SS. PLFA biomarker group concentrations and soil physicochemical parameters with $r^2 > 0.300$ between the variable and the axis score are displayed as vectors (Scaled to 70%). Vectors indicate strength and direction of the graphed relationships. In each of the figures the numbers 1-12 correspond the months Jan-Dec.
Microbial communities in the CC treatment were correlated with fungi (axis 1 $r^2 = 0.02$, axis 2 $r^2 = 0.409$), F:B (axis 1 $r^2 = 0.27$, axis 2 $r^2 = 0.363$), protists (axis 1 $r^2 = 0.00$, axis 2 $r^2 = 0.461$), actinobacteria (axis 1 $r^2 = 0.00$, axis 2 $r^2 = 0.656$), AMF (axis 1 $r^2 = 0.06$, axis 2 $r^2 = 0.706$), G+ (axis 1 $r^2 = 0.002$, axis 2 $r^2 = 0.819$), and G- (axis 1 $r^2 = 0.12$, axis 2 $r^2 = 0.871$).

The NMDS ordination for CS treatment (Figure 5b) produced a 2D solution with a final stress of 6.53 after 61 iterations. In the orientation shown, axis 1 represents 22.7% and axis 2 represented 64.8% of the variability for a final variation of 86.5%. Microbial communities separated primarily along axis 1 of the NMDS plot and were significantly different (MRPP A=0.3353, p<0.001). Microbial communities in the CS treatment were correlated with fungi (axis 1 $r^2 = 0.002$, axis 2 $r^2 = 0.427$), actinobacteria (axis 1 $r^2 = 0.044$, axis 2 $r^2 = 0.612$), AMF (axis 1 $r^2 = 0.005$, axis 2 $r^2 = 0.734$), G+ (axis 1=0.105, axis 2=0.790), and G- (axis 1=0.011, axis 2=.881).

The NMDS ordination for CWS produced a 2D solution with a final stress value of 10.39 after 67 iterations (Figure 5c). In the orientation shown, axis 1 represents 41.3% while axis 2 represents 47.6% of the variability for a total variability of 88.9%. Microbial communities separated primarily along axis 1 of the NMDS plot and were significantly different (MRPP A=0.3411, p<0.001). Microbial communities in the CWS treatment were correlated with fungi (axis 1=0.043, axis 2=0.674), F:B (axis 1=0.042, axis 2 $r^2=0.359$), protists (axis 1=0.071, axis 2=0.361), actinobacteria (axis 1=0.089, axis 2=0.656), and AMF, G+ (axis 1=0.194, axis 2=0.786), and G- (axis 1=0.145, axis 2=0.725).
The NMDS ordination for SS produced a 2D solution with a final stress of 9.63 after 60 iterations (Figure 5d). In the orientation shown, axis 1 represents 43.8% while axis 2 represents 41.0% of the variability for a total variation of 84.9%. Microbial communities separated primarily along axis 1 of the NMDS plot and were significantly different (MRPP A= 0.3233, p<0.001). Microbial communities under the SS treatment were correlated with fungi (axis 1=0.174 axis 2=0.310), F:B (axis 1=0.001, axis 2 $r^2=0.335$), AMF (axis 1=0.003, axis 2=0.766), actinobacteria (axis 1=0.020, axis 2=0.652), G+ (axis 1=0.041, axis 2=0.842), G- (axis 1=0.003, axis 2=0.908) and G+:G- (axis 1=0.020, axis 2=0.339).

Seasonal dynamics were apparent in all treatments (Figure 5). Temporal patterns that were seen in the SOD versus the agroecosystems were different. The microbial communities in the agroecosystem treatments had significantly greater separation between month that continued throughout the late winter, spring and early summer (Figure 4) while the microbial communities in the control SOD treatment only had significant separation during January and February. SOD was had less significant difference between communities throughout the remaining months compared to the agroecosystem treatments. There were no significant differences between months in SOD between 5-12 (Figure 5). Across all treatments, the PLFA biomarker drove differences each month. Biplot indicates that the months that made up the growing season (May-September) were strongly correlated with PLFAs. In each treatment, biplots for each of the biomarkers pointed to growing season due to the overall increase in all PLFA concentration. G+ and G- tended to be the greatest factors across all treatments separating the microbial communities during July.
2.3.3.2 Cropping treatment and seasonal effects on the concentration and relative abundance of PLFA biomarker groups

The concentration of most PLFA biomarkers were significantly influenced by treatment, month, year or their interaction (Table 5). The SS treatment had the lowest TMB of all the treatments which was similar to CC but significantly less than the CS, CWS and nearly half that of the SOD treatment (Table 6). Similarly, the concentration of the G+ and G- biomarker groups were lowest in the SS treatment which were similar to the CC but significantly less than CS, CWS, and SOD treatments. Actinobacteria biomarker concentrations were lowest in the CC treatment and similar to the SS but significantly less than the CS, CWS, and SOD. The general fungal biomarker concentration was 3-4 times greater in the control SOD treatment than in any of the cropping treatments where again the SS treatment was the least. Similarly, the AMF concentration in the SOD control treatment was nearly twice that of the cropping treatments. In this case, the treatment with the lowest AMF concentration was CC which was similar to SS and CS but significantly less than CWS.
Table 6: The effect of treatment, month and year sampled on the mean (s.d.) PLFA biomarker group concentrations (nmol g\(^{-1}\)). Values not connected by the same letter within treatment, month, and year for each biomarker group are significantly different (p < 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(\text{TMB} \pm \text{s.d.})</th>
<th>(\text{G}^+ \pm \text{s.d.})</th>
<th>(\text{G}^- \pm \text{s.d.})</th>
<th>(\text{ActinoB} \pm \text{s.d.})</th>
<th>(\text{Fungi} \pm \text{s.d.})</th>
<th>(\text{AMF} \pm \text{s.d.})</th>
<th>(\text{Protists} \pm \text{s.d.})</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>54.09(9.46)D</td>
<td>17.53(2.54)D</td>
<td>20.18(4.10)C</td>
<td>11.48(1.64)CD</td>
<td>1.72(1.73)B</td>
<td>2.32(0.66)C</td>
<td>0.85(0.47)C</td>
</tr>
<tr>
<td>CC</td>
<td>56.36(14.32)CD</td>
<td>18.02(2.67)CD</td>
<td>21.61(10.76)BC</td>
<td>11.20(1.49)D</td>
<td>2.11(1.9)B</td>
<td>2.25(0.71)C</td>
<td>1.17(0.86)B</td>
</tr>
<tr>
<td>CS</td>
<td>58.22(9.91)C</td>
<td>18.9(2.64)C</td>
<td>22.08(4.79)B</td>
<td>11.95(1.67)BC</td>
<td>1.93(2.33)B</td>
<td>2.36(0.64)C</td>
<td>1(0.82)BC</td>
</tr>
<tr>
<td>CWS</td>
<td>61.62(10.82)B</td>
<td>19.68(2.78)B</td>
<td>23.40(4.89)B</td>
<td>12.30(1.65)B</td>
<td>2.31(2.02)B</td>
<td>2.68(0.69)B</td>
<td>1.24(1.47)B</td>
</tr>
<tr>
<td>SOD</td>
<td>102.37(24.45)A</td>
<td>29.52(5.77)A</td>
<td>41.93(10.89)A</td>
<td>16.68(2.94)A</td>
<td>6.72(4.65)A</td>
<td>5.11(1.53)A</td>
<td>2.42(1.83)A</td>
</tr>
</tbody>
</table>

| Month      | \(\text{PLFAs Concentration (nmol g}^{-1}\) | |
|------------|--------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| January    | 67.46(38.4)A                  | 19.4(7.37)CD   | 27.97(21.26)A  | 11.81(3.87)BC  | 4.09(7.64)A    | 2.86(2.29)A    | 1.33(1.39)     |
| February   | 58.79(22.88)DE                | 18.17(5.04)EF  | 22.92(10.57)BC | 11.59(2.74)C   | 2.6(3.62)B     | 2.41(1.42)AB   | 1.1(0.83)      |
| March      | 56.81(22.11)E                 | 17.6(5.39)F    | 22.11(9.75)C   | 11.39(3.05)C   | 2.21(2.45)B    | 2.55(1.38)AB   | 0.95(0.58)     |
| April      | 63.73(20.43)BCD               | 19.39(5.05)DE  | 24.72(8.84)AB  | 12.35(2.6)AB   | 3.06(3.37)B    | 2.97(1.22)AB   | 1.24(1.3)      |
| May        | 66.02(23.08)ABC               | 20.36(5.72)BC  | 25.64(10.38)AB | 12.72(2.81)A   | 2.77(2.3)B     | 3.15(1.43)AB   | 1.38(2.35)     |
| June       | 65.79(19.34)ABC               | 20.56(4.94)ABC | 25.10(8.41)AB  | 12.77(2.44)A   | 3.07(2.93)B    | 3.08(1.17)BC   | 1.21(0.63)     |
| July       | 64.61(20.2)BC                 | 20.8(5.35)AB   | 24.40(9.11)B   | 12.5(2.59)A    | 2.79(2.05)B    | 2.78(1.22)CD   | 1.35(1.12)     |
| August     | 63.40(17.56)BCD               | 20.74(5.04)AB  | 23.8(7.48)BC   | 12.16(2.49)AB  | 2.79(1.75)B    | 2.68(1.04)CD   | 1.24(0.52)     |
| September  | 66.53(21.32)AB                | 21.19(5.64)A   | 25.41(9.65)AB  | 12.49(2.66)A   | 3.08(2.71)B    | 2.96(1.3)CD    | 1.4(1.23)      |
| October    | 62.78(23.76)CD                | 20.27(6.51)BC  | 23.95(10.36)BC | 12.29(3.34)AB  | 2.37(2.3)B     | 2.62(1.38)D    | 1.28(0.77)     |
| November   | 62.95(20.44)CD                | 19.79(5)CD     | 24.43(10.04)AB | 12.28(2.74)AB  | 2.54(2.45)B    | 2.53(1.27)D    | 1.39(1.88)     |
| December   | 61.71(20.59)CD                | 19.35(4.93)CD  | 23.71(9.03)BC  | 12.15(2.76)AB  | 2.80(3.8)B     | 2.49(1.34)D    | 1.21(0.79)     |

| Year       | \(\text{PLFAs Concentration (nmol g}^{-1}\) | |
|------------|--------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 2015       | 65.02(20.78)A                 | 20.3(5.13)A    | 25.3(9.16)A    | 12.21(2.66)BC  | 2.88(2.42)AB   | 2.75(1.36)     | 1.58(1.77)A    |
| 2016       | 61.59(25.29)B                | 19.42(5.83)A   | 24.17(13.45)AB | 11.68(3)C     | 2.65(3.8)BC    | 2.49(1.47)     | 1.18(0.9)BC    |
| 2017       | 61.49(20.18)B                | 19.67(5.58)A   | 23.36(8.88)B   | 12.3(2.92)AB   | 2.49(2.41)C    | 2.58(1.2)      | 1.1(0.59)C     |
Averaged over all treatments, the greatest concentration of TMB occurred in 2015 and 2018 while the lowest occurred in 2016 and 2017 (Table 6). Averaged over all years and treatments, the greatest concentration of TMB occurred in January, May, June, and September with the lowest in December. 2018 had the greatest G- concentration and was significantly greater than the concentration found in 2017. Similarly, the G- biomarker concentration peaked in January, April, May and September with the least occurring in March. There were no significant differences in G+ concentrations between the 4 years of the study (Table 6). The overall G+ biomarker concentration followed a seasonal pattern peaking in the summer and early fall months of June, July, August and September and reaching a minimum in the winter months (Table 6)

The greatest fungi concentration was measured during the month of January (4.09 ± 7.64) and was significantly greater than all other months. The lowest fungi concentration was measured in March. The year with the greatest fungi was 2018 and the year with the least fungi was 2017.
The AMF biomarker concentration was greatest in May and significantly different than July-December but was not different from the concentrations measured in January-June. 2018 had the greatest AMF concentrations and 2015 had the lowest. AMF concentrations were also affected by the interaction between treatment and month (Table 5 and Figure 6). The AMF biomarker concentration in the SOD control was always greater than the cropping treatments while the CWS was always the greatest of the cropping treatments. There was an interaction between the SS, CC, and CS treatments. The concentration in the CC treatment was only greater than the SS during the 2018 but was always less than the CS treatment.

Protists concentration was greatest in November and May and was the only biomarker to have a peak in the fall and in the spring (Table 7). The lowest protists concentration was measured in March. The year with the greatest protists concentration was 2015 and the lowest concentration was in 2017 which was significantly different from 2018. Protists proportions peaked in the mid-summer and were lowest in the winter. The proportion of protists were greatest in the CWS treatment and lowest in the SS treatment.
Table 7: Mean (s.d.) of Protists concentrations (nmol g⁻¹) measured via PLFA microbial biomarkers concentration (nmol g⁻¹) for each treatment and month sampled. Differences between treatments within the same month were analyzed via ANOVA and a Tukey's post-hoc test. Different letters represent significant differences between the treatments within the same month (p < 0.05). Bold values indicate overall treatments while italicized values represent the rotation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
</tr>
</thead>
</table>

Within the same month (p < 0.05), Bold values indicate overall treatments while italicized values represent the rotation.
Figure 6: Interaction of Treatment*Year for the mean AMF concentration. Treatments include the SS, CC, CS, CWS, and the SOD control.
2.3.3.3 Correlation of Environmental and Physicochemical Variables with Microbial Biomarkers.

Mixed model analysis was used to determine which of the climate and physicochemical variables were best correlated with the response in relative abundance of the individual biomarker groups (Table 8 and Figure 7). Protists were significantly correlated with all physicochemical parameters while the relative abundance of the individual microbial biomarker groups were influenced by a unique set of environmental variables.

The physicochemical variable that had the strongest correlation with the G+ biomarker was the total %C. The interaction between pH and treatment also significantly influenced the proportion of G+. The G+ relative abundance was significantly influenced by the Mehlich K and Mehlich Ca. The G+ relative abundance peaked during the months of the growing season and was lowest during the winter (Figure 7).

The G- proportions were correlated the second least by the physicochemical variables in the study. The G- proportions fluctuated with time due to the increase in the amounts of the other biomarkers, primarily G+ rather than physicochemical parameters. The physicochemical parameter that was most strongly correlated with the relative abundance of G- was total %C. G- proportions were greatest when total %C was lowest in March. Gram negative proportion tended to be lowest in the late summer months and greatest during the winter.
Actinobacteria was correlated the least with the physicochemical variables measured in this study. The only variables that effected actinobacteria were the Mehlich
Table 8: Mixed Model statistical results for the fixed effects of treatment (CC, CS, CWS, and SOD), month, year and their interaction on the relative abundance of the different soil microbial PLFAs during the four years of this study. Bold values highlight significantly significant differences (p<0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Precipitation</th>
<th>Temperature</th>
<th>Total % C</th>
<th>Meth Cm</th>
<th>Meth Cn</th>
<th>Meth Ca</th>
<th>Meth K</th>
<th>Meth P</th>
<th>PH</th>
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<tbody>
<tr>
<td>Control</td>
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<td>37.36 0.0904</td>
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<td>2.07 0.0904</td>
<td>5.95 0.0904</td>
</tr>
<tr>
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<td>37.36 0.0904</td>
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<td>9.32 0.0904</td>
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</tr>
<tr>
<td>CS</td>
<td>2.66 0.0316</td>
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<td>33.08 0.0904</td>
<td>37.36 0.0904</td>
<td>29.87 0.0904</td>
<td>9.32 0.0904</td>
<td>2.07 0.0904</td>
<td>5.95 0.0904</td>
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<td>37.36 0.0904</td>
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<th>% P -</th>
<th>% FUNG</th>
<th>% G</th>
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<tr>
<td>Treatment</td>
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<td>0.29</td>
<td>0.4596</td>
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<td>0.3</td>
<td>0.2</td>
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<td>0.2</td>
<td>0.3</td>
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</tr>
<tr>
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<td>1.00</td>
<td>0.4596</td>
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<td>SOD</td>
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Figure 7: Predicted relative abundance based on physicochemical parameters of a) gram positive, b) gram negative, c) actinobacteria, d) fungi, e) AMF, f) protist PLFA microbial biomarker group proportions in each cropping treatment (SS, CC, CS, and CWS) estimated using physicochemical and climate variables.
P and the total %C. Over the four years of the study, treatment did not significantly influence the actinobacteria proportions.

Mixed Model analysis shows that the yearly changes in the fungi and AMF proportions were predicted from both climate (temperature and precipitation) and environmental variables. The fungi and AMF biomarker were the only two biomarkers to be correlated with both the climatic variables. Bare soil temperature and the monthly precipitation significantly influenced the proportion of the fungi biomarker. Total %C was strongly correlated with the proportion of the fungi biomarker. The interaction between pH and treatment was significant in controlling the fungi biomarker. The proportion of AMF coincided with the growing season.

2.3.4 Changes in Microbial Community Structure over the Growing Season

Months of May, July and September

May, July, and September were selected for NMDS screening of agroecosystem treatments based on observations of data and temporal trends across each of the treatments (Figure 8). NMDS ordinations for each treatment in May produced a 3D solution with a final stress of 6.845 after 76 iterations (Figure 8a). Axis 1 represents 76.2% and axis 2 represents 0.1%, axis 3 represents 9.6% of the variability for a total variation of 85.9%. Microbial communities for the SS, CC, CS, and CWS separated out primarily along axis 1 of the NMDS ordination. The communities under CWS were significantly different (MRPP A=0.413, p<0.001). Rotational communities (CWS and CS) were correlated with AMF (axis 1 $r^2=0.668$, axis 2 $r^2=0.033$), G+(axis 1 $r^2=0.919$, axis 2 $r^2=0.043$), G-(axis 1 $r^2=0.936$, axis 2 $r^2=0.015$), and actinobacteria (axis 1 $r^2=0.789$, axis 2 $r^2=0.024$).
Figure 8: NMDS ordination of grouped microbial PLFAs from soils under the SS, CC, CS, and CWS cropping treatments from a) May, b) July, and c) September (Sept). PLFAs and physicochemical variables with an $r^2 > 0.300$ between the variable and the axis score are shown as vectors (scaled 70%) indicating the strength and direction of the relationship. Within each ordination 1=SS, 2= CC, 3=CS, and 4=CWS.
NMDS ordinations for each treatment in July produced a 3D solution with a final stress of 6.39 after 39 iterations (Figure 8b). Axis 1 represents 51.2% and axis 2 represents 33.5%, axis 3 represents 4.4% of the variability for a total variation of 89.1%. Microbial communities for the SS, CC, separated out along axis 1 and 2 and were significantly different from the communities found under CS and CWS treatments but not each other (MRPP A=0.258, p<0.001). The SS and CC microbial communities were correlated with G+: G- (axis 1 \( r^2 = 0.396 \), axis 2 \( r^2 = 0.093 \)). CS, and CWS separated out primarily along axis 1 of the NMDS ordination. Rotational communities were correlated with fungi (axis 1 \( r^2 = 0.515 \), axis 2 \( r^2 = 0.207 \)), F:B (axis 1 \( r^2 = 0.445 \), axis 2 \( r^2 = 0.179 \)), AMF (axis 1 \( r^2 = 0.483 \), axis 2 \( r^2 = 0.184 \)), G+(axis 1 \( r^2 = 0.623 \), axis 2 \( r^2 = 0.080 \)), G-(axis 1 \( r^2 = 0.711 \), axis 2 \( r^2 = 0.144 \)), and actinobacteria (axis 1 \( r^2 = 0.381 \), axis 2 \( r^2 = 0.097 \)).

NMDS ordinations for each treatment in September produced a 3D solution with a final stress of 6.92 after 91 iterations (Figure 8e). Axis 1 represents 71.8% and axis 2 represents 4.5%, axis 3 represents 6.5% of the variability for a total variation of 82.8%. Microbial communities for the SS, CC, CS, and CWS separated out primarily along axis 1 of the NMDS ordination. The communities in each treatment were significantly different (MRPP A=0.380, p<0.001) CS microbial communities were correlated with AMF (axis 1 \( r^2 = 0.250 \), axis 2 \( r^2 = 0.434 \)), G+(axis 1 \( r^2 = 0.454 \), axis 2 \( r^2 = 0.299 \)), G-(axis 1 \( r^2 = 0.486 \), axis 2 \( r^2 = 0.345 \)), and actinobacteria (axis 1 \( r^2 = 0.290 \), axis 2 \( r^2 = 0.270 \)).

In May, the SS and CC treatment were not significantly different from one another but were different from the rotational treatments (Figure 8a). In July and September all treatments were significantly different from one another (Figure 8b).
During May, the model explained the least amount of variability between the treatments but in July, the model explained more of the variability (Figure 8b). The variability between the treatments decreased in September but was still significant. During July, Fungi, F:B, AMF and G+ were strongly correlated with the rotational treatments while G+:G- was strongly correlated with the monocrop treatments. Fungi biomarkers were strongly correlated with the rotational treatments.

2.3.4.1 Microbial Community Dynamics in Differing Crop Rotation Years in May, July, and September

NMDS ordination for the rotation year in 2015 produced a 3D solution with a final stress of 7.42 after 53 iterations. Axis 1 represents 47.6%, axis 2 represents 36.7%, and axis 3 represents 4.5% of the variability for a total variation of 88.8% (Figure 9a). The microbial communities in each rotation year were significantly different (MRPP A=0.357, p<0.001). Microbial communities separated out primarily along axis 1 of the ordination. SOD was strongly correlated with AMF (axis 1 $r^2=0.581$, axis 2 $r^2=0.377$), G- (axis 1 $r^2=0.525$, axis 2 $r^2=0.430$), G+ (axis 1 $r^2=0.537$, axis 2 $r^2=0.361$), actinobacteria (axis 1 $r^2=0.502$, axis 2 $r^2=0.215$), Fungi (axis 1 $r^2=0.233$, axis 2 $r^2=0.761$), and temperature (axis 1 $r^2=0.021$, axis 2 $r^2=0.309$). Rotational treatment microbial communities, particularly CR1, were strongly correlated with G+:G- (axis 1 $r^2=0.380$, axis 2 $r^2=0.514$) The CC agroecosystem were not strongly correlated with environmental variables (Figure 9a).
Figure 9: NMDS ordination of grouped microbial PLFAs from soils under treatments consisting of CC, CS, and CWS from the years a) 2015, b) 2016, c) 2017, and d) 2018. Within each of the rotational treatments a rotation year is present (CC, CR1, CR2, SR1, SR2, CR1a, and W/S). PLFAs and physicochemical variables with an r^2>0.300 between the variable and the axis score are shown as vectors (scaled 70%) indicating the strength and direction of the relationship. Within each ordination’s legend represents the rotation year followed by the month of the year sampled.
NMDS ordination for the rotation year in 2016 produced a 3D solution with a final stress of 5.55 after 65 iterations. Axis 1 represents 70.4%, axis 2 represents 8.2% and axis 3 represents 1.3% of the variability for a total variation of 79.9% (Figure 9b). The communities in the agroecosystem and SOD separated out along axis 1 and were significantly different (MRPP A=0.620 p<0.001). Microbial communities for the CC were not strongly correlated with environmental variables. SOD separated along axis 1 and 2 and was strongly correlated with AMF (axis 1 r²=0.744, axis 2 r²=0.0.11), G- (axis 1 r²=0.691, axis 2 r²=0.0.016), fungi (axis 1 r²=0.763, axis 2 r²=0.064), protists (axis 1 r²=0.714, axis 2 r²=0.0.006), total %N (axis 1 r²=0.387, axis 2 r²=0.012), total %C (axis 1 r²=0.316, axis 2 r²=0.022), and F:B (axis 1 r²=0.675, axis 2 r²=0.099). The CS treatment (CR2 rotation year) was strongly correlated with G+:G- (axis 1 r²=0.813, axis 2 r²=0.032) and Mehlich Mn (axis 1 r²=0.350, axis 2 r²=0.0.021). (Figure 9b).

NMDS ordination for the rotation year in 2017 produced a 2D solution with a final stress of 11.37 after 41 iterations. Axis 1 represents 65.7% and axis 2 represents 25.1% of the variability for a total variation of 90.8% (Figure 9c). The microbial communities in each rotation year were significantly different (MRPP A=0.502, p<0.001). Microbial communities separated out primarily along axis 1 of the ordination. SOD was strongly correlated with AMF (axis 1 r²=0.627, axis 2 r²=0.287), G- (axis 1 r²=0.558, axis 2 r²=0.296), fungi (axis 1 r²=0.487, axis 2 r²=0.464), G+ (axis 1 r²=0.377, axis 2 r²=0.175), actinobacteria (axis 1 r²=0.355, axis 2 r²=0.153), protists (axis 1 r²=0.501, axis 2 r²=0.317), total %C (axis 1 r²=0.473, axis 2 r²=0.274) F:B (axis 1 r²=0.480, axis 2 r²=0.464), Rotational agroecosystem treatments were strongly correlated with G+:G- (axis 1 r²=0.636, axis 2 r²=0.424), Mehlich Mn (axis 1 r²=0.350, axis 2 r²=0.143), Mehlich Zn
(axis 1 $r^2=0.138$, axis 2 $r^2=0.618$), Mehlich Mg (axis 1 $r^2=0.161$, axis 2 $r^2=0.300$) and pH (axis 1 $r^2=0.084$, axis 2 $r^2=0.359$). The CC agroecosystem were not strongly correlated with environmental variables (Figure 9c).

NMDS ordination for the rotation year in 2018 produced a 3D solution with a final stress of 8.61 after 74 iterations. Axis 1 represents 28.0%, axis 2 represents 44.7% and axis 3 represents 1.9% of the variability for a total variation of 74.5% (Figure 9d). The communities in each rotation year were significantly different (MRPP A=0., p<0.001). Microbial communities for the CC, SR2, and W/S separated out primarily along axis 1 and axis 2 of the NMDS ordination. Sod was strongly correlated with AMF (axis $r^2_1=0.013$, axis 2 $r^2=0.622$), G- (axis 1 $r^2=0.0743$, axis 2 $r^2=0.740$), fungi (axis 1 $r^2=0.090$, axis 2 $r^2=0.686$), G+ (axis 1 $r^2=0.031$, axis 2 $r^2=0.622$), actinobacteria (axis 1 $r^2=0.017$, axis 2 $r^2=0.473$), protists (axis 1 $r^2=0.083$, axis 2 $r^2=0.393$), F:B (axis 1 $r^2=0.036$, axis 2 $r^2=0.462$), and total %C (axis 1 $r^2=0.098$, axis 2 $r^2=0.321$). Rotational communities were strongly correlated with Mehlich Zn (axis 1 $r^2=0.001$, axis 2 $r^2=0.412$), Mehlich Cu (axis 1 $r^2=0.042$, axis 2 $r^2=0.463$), G+:G- (axis 1 $r^2=0.172$, axis 2 $r^2=0.681$) and pH (axis 1 $r^2=0.225$, axis 2 $r^2=0.312$). (Figure 9d).

NMDS analysis of microbial community structure in the rotational treatments (CS and CWS) saw high variability over the course of the growing season (May to September) while the continuous corn treatment shifted significantly from May to July but then remained unchanged at the end of the growing season (Figure 9). The double crop corn-soybean treatment (CS) saw the microbial community structure shift to look similar to the community under CWS and SOD when moved into the first year of
soybean but still significantly different. When the same plots entered their second-year of soybean (SR2) there was not difference between the communities between CWS and SOD. Finally, when the CS treatment entered back into CR1 it returned to a microbial community similar to the CC and became more similar as it entered CR2. The CC treatment remained significantly different and otherwise unchanged throughout all four years of the study while the SOD control had less variability year-to-year and month-to-month (Figure 9). The result provide evidence that microbial communities respond to crop type with time. Corn in rotation had a different community structure in both the CS and CWS compared to CC.

2.3.5 Correlations of Yield and Microbial Biomarker Groups - Yield Regression Analysis

Regression analysis on all PLFA biomarkers revealed that the biomarkers that were strongly correlated with predicting yield were the AMF and fungi biomarkers (Figure 10). AMF had a linear relationship with yield and did not appear to have a lack-of-fit. The model for AMF and Fungi had an $r^2$ of 0.404 and an adjusted $r^2$ of 0.3769. The model for AMF (Figure 10a) was significantly different ($p=0.0289$). The model for fungi (Figure 10b) was significantly different ($p=0.0070$). Fungi had a nonlinear relationship with yield which began to approach a slop of zero after a mean concentration of 2100 pmol g$^{-1}$. Above this threshold, the yield no longer fit a linear relationship.
Figure 10: Predicted corn yield from the CC, CS, and CWS treatments modeled from PLFA biomarkers. Biomarkers that had strong correlations with yield were the a) AMF and b) fungi. AMF was linearly related to yield. Fungi utilized the general additive model and a spline was used to account for the non-linear relationship.
2.4 Discussion

In this study, we set out to answer two primary questions, 1) how does microbial community structure shift over the season and year in different cropping treatments and 2) how does the microbial community, and members there in, respond to different cropping management. My results show that microbial community structure in unmanaged SOD are less variable to change over the course of a year while agroecosystem treatments are more prone to yearly and monthly changes. We showed that the physicochemical parameters were important in distinguishing microbial communities, particularly total %C. Cropping treatment was shown to have an influence on microbial communities as the growing season progressed driving crops of the same type toward a microbial community that was similar. Specifically, the rotation year crop was shown to impact the soil community each month of the growing season, separating rotation years that had differing crops. The PLFAs that were most sensitive to land management and crop rotations were the AMF, fungi, G+, and G- biomarkers. We showed the AMF and fungi biomarkers were correlated to the yield measured in My study. My results provide insight on temporal dynamics of microbial communities, and members within, under various rotations and serve as the baseline structure of microbial communities under similar management practices.

2.4.1 Physicochemical Parameters and the Effect on Soil Microbial Community Biomarkers

Physicochemical parameters in each of the treatments were shown to have a strong influence on microbial community structure (Table 3). The pH and total C% were
shown to be strong predictors of the proportion of microbial PLFAs. Soil pH is a key environmental variable controlling soil microbial community structure (Bartram et al. 2014; Xue et al. 2017; Fierer and Jackson 2006). One study using PLFA found a two-threefold increase in F:B as pH was decreased from 8.5 to 4.5. A recent meta-analysis indicates that previous land management may be a strong driver of soil microbial community composition on a macroecological scale. There were significant differences between pH values in the treatments in My study. Rates of organic matter decomposition and N-fertilization could account for deviations from an optimal pH range. Soil pH, measured in 1:1 soil:water, was greatest in continuous soybean plot and an optimal pH range for soybean is between 6.2-6.8 while corn’s optimal range is less (Extension 2018).

Land management practices (i.e. crop rotation) can significantly alter the total %C, types of C, and quality of C entering the soil ecosystem. Total %C peaked during the Fall, specifically September, and was lowest in the end of winter, March (Table 4). During the four years of this study, the agroecosystems treatments total %C was influenced by treatment and season. CS had greater total %C while the CWS and CC total %C were similar. My results are consistent with the land management influence on soil physicochemical properties. Treatments that were in tillage versus no till saw a significant decrease in total %C in the 0-10 cm depth (Sun et al. 2016), however, when going from a monocrop corn to a corn-soybean rotation in no-till systems there were no significant changes in total %C (West and Post 2002). Soil may have a limited capacity to sequester carbon which may be an important consideration when working in long-term managed agroecosystems that are in no-till (Chung, Grove, and Six 2008). This study took place in long-term no till soil, one might expect the overall %C not to increase, but
the complexity of the carbon entering the system may in fact be different. Total %C may not be an adequate measurement to truly assess the influence of crop rotation on the soil carbon pool. Introducing a crop rotation that contained two or more crops significantly altered the types of carbon by changing the quality of residues entering the soil ecosystem (McDaniel et al. 2014b; McDaniel, Tiemann, and Grandy 2014). Future research would do well by investigating the various fractions of C that contribute to total %C in the agroecosystem especially considering we found that total %C was a strong predictor of PLFA biomarkers.

Total %C was one of the strongest predictors of microbial community’s temporal dynamics (Table 5). The SOC and total C are known to contribute positively to soil health, soil tilth, and fertility and are regarded as one of the most important soil health measurements (Lal 2016; Bauer and Black 1994). Total %C in my study had seasonal dynamics that were always greatest in the Fall (September) and lowest in the spring (March). Concomitantly, analysis of the PLFAs indicate that the greatest concentrations of biomarkers were found in September and tended to be lowest in March. Soil microbial communities in the study correlated to the increase in total %C which likely originated from below ground plant residues such as root exudates, organic acids, and the root biomass itself, as well as leaf litter on the surface. The quality and quantity are two important factors of the C entering the system that influence soil microbial communities. For example, (McDaniel et al. 2014a; McDaniel, Tiemann, and Grandy 2014) showed that diverse C inputs controlled by different crop rotation diversity treatments influenced total microbial biomass. Carbon use efficiency was greater in soils with more diverse higher quality C inputs (Soares and Rousk 2019). When carbon use efficiency increases,
more C that enters the soil agroecosystem can pass into the microbial cell as opposed to being lost as CO₂. Carbon that is incorporated into the cells can then to be incorporated into necromass that can enter the mineral stabilized SOC pool, positively influencing beneficial soil properties and microbial activity (Wieder et al. 2014). My work expands on previous studies by providing evidence that total C% is significant in controlling microbial communities across multiple years and adds evidence that PLFA is a useful measure for capturing microbial communities’ response to soil health measurements such as total %C.

I observed significantly greater total microbial biomass in SOD treatments compared to the agroecosystem treatments, suggesting that SOD is more supportive of microbial biomass, which was likely driven by total %C differences. SOD had significantly greater total %C than any agroecosystem treatment. The observation aligns with previous work comparing long-term unmanaged grassland to managed agroecosystems within the same soil type (Mackelprang et al. 2018). Microbial communities in managed agroecosystems have been shown to have greater richness and diversity when compared to unmanaged SOD and may be the results of agricultural practices creating diverging niches (Barber et al. 2017). Management practices that contribute to niche development include N fertilization, dramatic changes in annual organic carbon inputs, and turnover stemming from greater plant productivity and litter inputs. Notably, my plots were managed based on soil test recommendation and at a N fertilizer rate of 90 kg N acre⁻¹. Analysis of the physicochemical properties of the soil distinguished SOD from the agroecosystem treatments. SOD always had the lowest pH, and Mehlich extractable ions (apart from Mehlich K) (Table 4). The Mehlich extractable
metals tended to be lower in the SOD than the agroecosystem treatments possibly due to a continuous, living cover and the likelihood of SOM binding Mehlich III extractables. SOD is an undisturbed ecosystem, with very little anthropogenic inputs, a highly diverse ecosystem, and maintains a highly productive soil that has a high degree of resistance and resilience (Zak et al. 2003)

2.4.2 Seasonal Changes in Microbial Community Structure and the Influence of Cropping Treatment

PLFAs were extracted from soils collected in long term no-till managed agroecosystems to measure soil microbial community structure to assess how communities in different land management change throughout the season. Microbial community in SOD, which is a low input system with minimal disturbances had less variability over the course of a year (Figure 4) compared to agroecosystem communities’ (Figure 5). A previous study comparing land use type (hay pasture and agricultural soils) showed that shifts in PLFAs in agricultural soils were more pronounced throughout the season and less so in soil in hay pasture (Mackelprang et al. 2018). PLFA biomarkers that were most influenced belonged to the bacteria groupings that aligns with My findings. The G+ and G- had strong seasonal trends (Figure 7) Furthermore, Jangid et al. (2008) showed that undisturbed pasture was also less likely to change due to perturbation from climatic conditions. Managed agroecosystems have greater diversity of bacterial taxa compared to undisturbed pasture by creating unique niches and could therefore be a cause in the different seasonal patterns (Mackelprang et al. 2018). The overall shifting of microbial communities throughout the season could be an indication of the ecosystems resilience to changes and may reflect, to a degree, soil health.
The results indicated that after 29 years of land management microbial communities were selected based on rotational treatment. An examination of the microbial communities during May-September showed a strong treatment effect that was more pronounced as the growing season continued (Figure 8). Microbial communities are hypothesized to contribute to the yield gap in monocrop treatments compared to crops in rotation (Bennett et al. 2012; Hilton et al. 2013; Stanger and Lauer 2008). I showed that microbial communities over the four years of the study were significantly different between monocrops (SS and CC) and crop rotations (CS and CWS). Thus, members within each cropping treatments community may play an important role in the observed yield gap between monocrop and crop rotations. Differences between the communities were greater as the season continued. Differences in microbial communities during the early season could affect seedling establishment and contribute to yield reduction at the end of the season by inhibiting the seeds ability to establish AMF relationships or survive pathogen loads before germination (Bever et al. 2010; Dalling et al. 2011). Common frameworks for understanding plant establishment often neglect microbial-root interactions. For example, AMF colonization in early season agricultural fields is an important mechanism for seedling establishment (Bever et al. 2010), and the ability of seeds to survive pathogens before germination, both of which may affect yields. There were significant differences between all communities during July and this sampling time point coincides with high plant productivity during the growing season and limitations or stresses during the early or middle of the season may cause yield loss (Ashworth et al. 2017; Dixon et al. 1994). These results highlight that microbial communities under
different land management practices (SOD versus agroecosystems) have different temporal dynamics that could be influencing ecosystem services.

2.4.2.1 Rotation Effect on Microbial Community Structure and Yield

The study showed shifting microbial community structures in differing crop rotations and land management through the months of May, July, and September (Figure 9). Agroecosystem treatments had more variability through the growing season than the SOD. During the four-year study, cropping treatment had a significant influence on the microbial community in May, July, and September (Figure 8). This results indicate that different cropping treatments may select for a unique microbiome. I observed diverging microbial communities between the CC and CS treatments. When the CS was in corn (CR1 and CR2), the microbial community resembled the community under CC (Figure 9a,b). However, when the CS treatment entered into soybean years (SR1 and SR2), the microbial community shifted away from CC, becoming significantly different, and was not different than the communities under CWS, or SOD (Figure 9c,d). My results indicate the ability of crop rotations to break microbial community cycles. Planting crops of dissimilar types have been shown to break pathogen cycles in agroecosystem and is a proposed mechanism of the rotation effect (Bennett et al. 2012; Foley et al. 2011; Hilton et al. 2013; Qin et al. 2017).

By increasing the rotational diversity a corresponding increase in the fungal biomass, including AMF (Tiemann et al. 2015; Ai et al. 2018; Sun et al. 2016; Zhang, Sun, et al. 2019; Angus et al. 2015; Lupwayi et al. 2017) and in some cases an increase in fungal diversity occurs (Ai et al. 2018). I also showed that crop rotation increased fungal
and AMF biomass among others (G+ and G-). Agroecosystem that have been planted in soil that was previously in undisturbed SOD experienced greater yields than plots that have been planted in crops continuously (Bennett et al. 2012; Hilton et al. 2013; Mackelprang et al. 2018). Likewise, the increase in yield of a crop following a dissimilar crop is known as the rotation effect. In a study comparing long-term monocrop corn to corn in rotation revealed that the corn yield penalty on average was 4.3% less. The yield penalty increase each year the field was continuously cropped and leveled off after 3 years (Seifert, Roberts, and Lobell 2017).

In my study, long-term no-till continuous corn plots had a 5-15% yield gap when compared to the corn yields from corn that is in rotation. I observed a strong treatment effect on microbial community structure and members within. The biomarkers that were correlated best with yield were the AMF and the fungi. I saw a positive linear relationship between AMF concentration and seasonal yields (Figure 10a). AMF was strongly correlated with rotational treatments and crop yield. My finding is in line with research that shows AMF are important in predicting corn yields (Zhang, Lehmann, et al. 2019; Miller 2000). However, in high input agroecosystems, AMF have been hypothesized as potential reason for yield decline (Johnson et al. 1992). General fungi on the other hand did not fit a linear relationship with yield (Figure 10b). As general fungi concentration increased during the season, yield increased until a threshold was met at ~2,100 pmol g⁻¹. These results indicate that fungi are important at predicting yield until a threshold is met, after which fungi may become negligible or harmful. The plateau seen in respect to fungi and yield may be that PLFA levels of fungi in the soil have reached their potential at predicting yield. AMF biomarker on the other hand, may not have reached a plateau in
yield and may indicate that AMF could be a target for increasing yield or closing yield gaps in long-term managed agroecosystems that are in no-till.

The results don’t draw a causal relationship to microbial communities and yield decline in the CC treatment. I can speculate that the low variability in the continuous corn’s microbial communities over the growing season may indicate the selection of a pathogenic microbial community or lesser microbial functioning compared to the rotational treatments. Pathogen buildup is hypothesized as being a major contribution to the yield decline seen in monocrop corn (Sumner et al. 1990). Research investigating microbial communities in continuous corn and corn-soybean rotation have found greater fungal concentrations and fungal: bacteria in corn soybean rotations. Fungi and AMF were significant in separating the communities. Fungal pathogens that are hypothesized to contribute to yield decline are *Fusarium* spp. and *Pythium* spp. (Mabuza et al. 2018; Marburger et al. 2015). The observed yield gap between the CC and the CS and CWS treatments was greatest in years that had the greatest rainfall, which could be a driver of increased fungal activity in CC treatments. On the other hand, the ecosystem functions provided by the fungal community are numerous. Increase fungal biomass may not always be a sign of pathogen load and fungal biodiversity has been identified as key to soil health by transforming organic matter with enzymes, enhancing soil structure, and biological population regulation. (Frac et al. 2018). My results (Figure 10b) indicate the two-sided influence of fungi in ecosystem services acting as both a pathogen and a beneficial decomposer of organic matter. Future research should investigate the temporal dynamic of specific taxa throughout the growing season in monocrop and crop rotations.
2.5 Conclusion

This study showed that crop treatment influences microbial communities over the long term (years) and within individual growing season in response to crop rotational year. Going from unmanaged SOD to cropping treatments significantly altered overall microbial community structure and individual biomarker groups within. Total %C was a strong predictor of individual biomarkers with the exception of AMF. I also showed that the different crop rotations had significantly different microbial communities compared to monocrop treatments and that these microbial communities responded in different ways throughout the year. The rotational cropping treatments had greater TMB, AMF, fungi, G+ and G- and had microbial communities that diverged from the other treatments throughout the growing season. Corn yield in rotation was greater than monocrop corn. The AMF biomarker showed a strong linear correlation with yield with no evidence of decline even at the greatest AMF concentrations. The general fungi biomarker, however, correlated linearly to a point after which increasing general fungal concentrations had negative impact on yield. SOD acted as an ideal microbiome and any shifts in microbial community similar to SOD was perceived as beneficial to agroecosystem functioning while shifts toward a community like CC resulted in a yield reduction in corn.

Management practices focused on the microbial component of soil health might benefit by shifting toward practices that favor microbial communities that resemble those found under rotation or at best SOD treatments which may be indicative of those with greater productivity. Future work should investigate specific fungal taxa that are present under differing crop rotations in May, July, and September to establish the role of fungal pathogens in the observed yield reduction.
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