Soil microbial community dynamics in response to cover crop implementation and P fertilizer management

by

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B.S., University of Louisiana at Lafayette, 2011
M.S., University of Nebraska - Lincoln, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Soil microorganisms facilitate nutrient cycling within soils providing a critical component of soil health and serve a key role in maintaining productive agricultural systems. There are many ways to assess soil health and how soil systems respond to agricultural management practices. Some of these methods target either recalcitrant or labile nutrient pools within soils, while others focus on the microorganisms themselves. This study sought to examine a variety of different assays targeting components of soil health and how they were impacted by agricultural management practices. Objectives of this study were to (i) examine carbon (C) and nitrogen (N) soil health metrics; (ii) to explore the microbial community structure using phospholipid fatty acid analysis, and (iii) to identify key microbial functional gene composition, and soil health metrics relate to key soil microbial functional gene composition in the fall 2019 and spring 2020 seasons in response to management practices that include cover crop usage and P fertilizer treatments at an early transition to no-tillage (less than 5 years) site at the Kansas Agricultural Watershed Field Research Facility. Objective one examined soil samples from the spring and fall of 2018 and 2019 at the 0-5 cm soil depth. Objective two examined soil samples from the spring and fall of 2018 and 2019 at the 0-5, 5-10, and 10-15 cm depths. Objective three examined soil samples from fall 2019 and spring 2020 at the 0-5 cm depth. The experiment has a 2 by 3 factorial treatment structure with two levels of cover crop treatments: with cover crops (CC) and without cover crops (NC) and three levels of P fertilizer managements: no P fertilizer (NP), fall broadcast (FB), and spring injected (SI) in a randomized complete block design with three replicates of each treatment combination. When assessing traditional soil health assays, I found assays that targeted soil C nutrient pools were more consistently able to detect differences with the cover crop implementation as compared to those that examined N pools. Assays using

total C, microbial biomass C, active C, dissolved organic C, and enzyme activity were more successful in detecting cover crop implementation as compared to assays that targeted N pools including total N, microbial biomass N, dissolved organic N, and inorganic N. For the second objective I found that PLFA microbial biomass decreases with increasing depth, and that cover crops can significantly increase microbial biomass in several PLFA categories when compared to plots with no cover crop in a no-tillage system with a corn-soybean rotation. The microbial community composition was found to be similar between the CC and NC treatments at the 0-5 cm depth. Bacteria and fungi were not impacted by treatments. The third objective found that genes related to microorganismal nutrient dynamics responded differently based on seasonality with fall samples being more frequently responsive to treatment differences than spring samples. This objective found the greatest gene abundance in the NP*CC treatment in fall within the examined sub-categories of microorganism functional genes.

Soil microbial community dynamics in response to management

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Abstract

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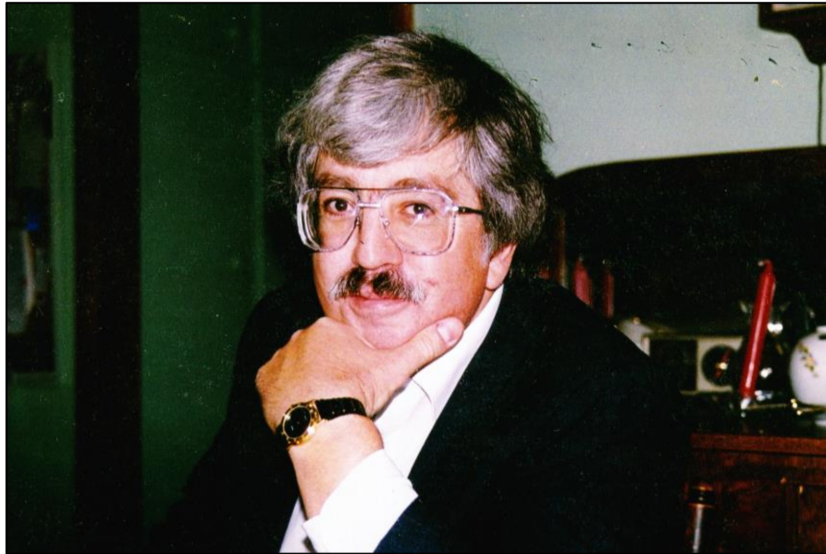
Dedication

This dissertation is dedicated to my parents Johnathan and Pamela Stewart. Thank you for always knowing I could.



Johnathan Stewart

Pamela Stewart

(and Yukon)

Chapter 1 - Literature Review

Introduction

The global population is expected to grow by one third (~2.3 billion people) from 2009 - 2050. This expansion as well as growing environmental concerns must be constructively approached in agriculture. Intensive agriculture has ushered in a vision of the potential for greater global food security (Lehman et al., 2015b); however, it has also contributed to significant environmental damage (EPA, 2005). One aspect of the environmental impact from intensive agriculture is soil degradation. Soil degradation is defined as a change in the soil health status resulting in a diminished capacity of the ecosystem to provide goods and services for its beneficiaries (FAO, 2019). Roughly 25% of soil globally faces degradation (Stavi & Lal, 2015). Soil erosion is a major contributing factor to soil degradation, and tillage contributes to the process of soil erosion. Conservation tillage is any tillage practice that leaves 30% or more of the soil surface covered by crop residues after planting; these practices can reduce the amount of soil erosion due to water and wind. No-till is a type of conservation tillage practice that leaves 50-100% of the soil surface covered by crop residues. No-tillage practices have been found to reduce sediment losses (Chichester & Richardson, 1992; Blevins, Frye, Baldwin, & Robertson, 1990). Soil organic carbon has been found to be increased in conservation tillage as compared to plow tillage (Cambardella, Johnson, & Varvel, 2012; Franzluebbers, 2010). Adoption of conservation tillage practices across the United States is associated with a decline in soil erosion from 3.1 billion tons per year in 1982 to 1.8 billion tons per year in 2001 (NRI, 2003).

Soil biota contribute to soil quality by affecting both physical properties as well as chemical composition. It has been estimated that the amount of microbial biomass underground may be roughly equal to the living biomass found above (Gold, 1992). Higher microbial biomass

has been found with no-till practices as compared to those with conventional tillage (Karlen et al., 1994; Motta, Reevesd, Burmester, & Feng, 2007). The soil biological community is highly diverse with many types of eukaryotes, prokaryotes, and archaea present (Weil & Brady, 2016). It is estimated that soil biodiversity contributes an estimated 1.5 quadrillion U.S. dollars to ecosystem services (Pimentel et al., 1997). It is challenging to prove the presence of a sterile location on the earth exists (Lehman et al., 2015a). Despite the awareness of the importance of soil biota, there remains a great deal to understand their functional contribution to agricultural systems.

A deeper understanding of soil biota may prove useful for mitigating the negative impacts of intensive agriculture. If the soil biota were better understood, they could potentially be managed to provide more optimum agricultural benefits to producers, which in-turn could reduce inputs, and thus lower issues of eutrophication in freshwater systems related to nitrogen (N) and phosphorus (P) losses from fields. It is understood that soil biota provide several important soil functions including nutrient provisioning and cycling, pest and pathogen protection, production of growth factors, enhanced water availability, and the formation of soil aggregates that reduce soil erosion and increase water infiltration (Lehman et al., 2015b). Soil microorganisms modulate the physiochemical character of soil as they play key roles in the biogeochemical cycles including those of carbon, nitrogen, sulfur, phosphorus, and iron. Better understanding the microbial functional capacity of agricultural soils could help inform agricultural management strategies.

Literature Review

The microbiological community can be classified into four major groups: bacteria, archaea, fungi, and protozoa. Some of the roles microorganisms play are more understood than

others, as there are many biota that have not yet been identified due to being difficult or currently impossible to culture (Theis, 2008). Focusing on microorganisms, there are members from Bacteria, Archaea, and Eukarya representing all three branches of the tree of life. A single gram of soil has been estimated to have one billion bacterial cells that can be grouped into one thousand to one million different species (Gans, Wolinsky, & Dunbar, 2005; Schloss & Handelsman, 2006). The role these microorganisms have in agricultural soils is not fully accessed; however, there has been a great deal of work done on this topic, and as research continues to elucidate the activities of microorganisms it becomes possible to understand the impact different agricultural practices impart on soils.

Cover cropping is an agricultural management practice of establishing plants between the cash-crop growing seasons. Cover cropping has been found to have several benefits in terms of soil biological activity, productivity, soil quality, water quality, water use, nutrient management, and pest management. A study by Finney, Buyer, & Kaye (2017) examined eight fall-sown cover crop species and found that cover crop treatments have higher microbial biomass than the no cover crop control (untilled weedy fallow); higher microbial biomass with cover crop use has been found by numerous other studies as well (McDaniel, Tiemann, & Grandy, 2014; Nair & Ngouajio, 2012; Spedding, Hamel, Mehuys, & Madramootoo, 2004). Cover crops have been shown to reduce soil loss by reducing wind and water erosion (Langdale et al, 1991). Cover crops have also been shown to increase the soil organic matter pool, which improves soil structure, increases water infiltration and storage, and prevents surface crusting of the soil (Roberson, Sarig, Shennan, & Firestone, 1995). Some studies have found increased yield after cover crop application; however, cover crops can also impart yield loss. Cover cropping is associated with negatively impacting yield in areas with low precipitation. Cover crops also may

share common pests with the cash crop or increase pest survival. Factors of precipitation and pests should be considered when implementing cover cropping management practices to gain the benefits of this practice. Cover crops have also been found to affect the soil microbial community. Finney et al. (2017) found that different cover crop species enhanced the presence of different microbial functional groups. In their study of 14 cover crop treatments, the authors found that arbuscular mycorrhizal (AM) fungi were present at higher levels in oat and cereal rye cover crop treatments. Non-AM fungi were present at higher levels when hairy vetch was used as a cover crop. As plant material including cover crops deteriorates, it can impact soil microorganisms. Li et al. (2018) found that application of corn straw and wheat straw significantly increased microbial biomass carbon (MBC) and microbial functional diversity in a field study with three different soil types (Ferralic Cambisol, Calcaric Cambisol, and Luvic Phaeozem) as compared to wheat root, corn root, pig manure, and cattle manure. Microorganisms affect soil quality and cover cropping can increase the amount of microorganisms and affect their community structure. Much remains unknown regarding soil microorganisms and how they are impacted by agricultural management practices.

Bacteria and Archaea (also known as Prokaryotes) are known to be diverse and numerous within soils and they contribute to nutrient cycling as well as the physical structure of agricultural soils, as well as all soils (Alexander, 2005). The nutrient transformations performed by Prokaryotes are one of the critical roles they provide to soils. Some organic matter such as sugars, starches, fats, and proteins is broken down more quickly than others (recalcitrant) such as lignins, waxes, and oils (Alexander, 2005). This degradation process of organic matter contributes to the formation of soil organic matter and because of the nutrient, water, and physical benefits is important to agriculture. A fifteen-year field study found that no-tillage when

compared to conventional tillage increased the soil organic matter and microbial biomass within the top 10 cm of the soil (Sapkota, Mazzoncini, Barberi, Antichi, & Silvestri 2012). This is a more general view of the contributions of Prokaryotes; however, this does not address what specific contributions are made by specific Prokaryotes.

In a 2018 study by Delgado-Baquerizo et al. bacteria sampled from varied soils across six continents was found to fall into nine major phylogenetic groups: Proteobacteria, Actinobacteria, Acidobacteria, Planctomycetes, Chloroflexi, Verrucomicrobia, Bacteroidetes, Gemmatimonadetes, and Firmicutes. There is great diversity within these major phylogenetic groups. For instance, within Proteobacteria there is a great deal of diversity in how members attain nutrients, some are saprophytes (energy from dead organisms) while others are symbiotes and they play a role in global carbon, nitrogen, and sulfur cycling (Kersters et al., 2006). Notably there are several genera within this group that are known to be involved in the nitrogen cycle including *Nitrosomonas* and *Nitrobacter*, as well as rhizobia existing inside plant root nodules and fixing nitrogen for the plant host (Kersters et al., 2006). The Firmicutes phylum is known to have many bacteria capable of forming endospores and thus being able to survive for long periods of time within soils (Alexander, 2005). Acidobacteria and Verrucomicrobia are globally distributed having been detected in most DNA soil analysis; however, little is known as to their specific roles especially in an agronomic setting (Giguere et al., 2020). Acidobacteria were first discovered in 1997 by Kuske, Barns, & Busch, from agricultural soil, and since their discovery they have been found to have highly adaptable genetic elements that are thought to contribute to their abilities to utilize many different carbohydrate and nitrogen sources (Eichorst et al., 2018). Archaea are also prokaryotes and are thought to have possibly evolved from Gram-positive bacteria (Gupta, 2000);; however, they are their own distinct branch of life. Within Archaea there

are several phyla thought to be important to soil nutrient cycling including members of Euryarchaeota and Thaumarchaeota. Members of the Euryarchaeota phyla have been found to produce methane under anoxic conditions (methanogens), which could be relevant for agriculture when considering flooding, high compaction, or permafrost conditions. Methanogens have also been found to fix nitrogen in anoxic conditions (Bae, Morrison, Chanton, & OGram, 2018). Other contributors to the N cycle include members of the Thaumarchaeota phyla that are capable of ammonia-oxidation (Leininger et al., 2006; Brochier-Armanet, Boussau, Gribaldo, & Forterre, 2008). Members of Archaea have been found to contribute more to nitrification than bacteria (Leininger et al., 2006). In addition to ammonia-oxidizing archaea (AOA) there are also ammonia-oxidizing bacteria (AOB). This raises the question of whether these organisms exhibit functional redundancy in soils or if they exist in distinct niches. Several studies suggest that the latter is the more common scenario, with AOA preferring acidic soils and low ammonia input and AOB dominating in fertilized soils with a neutral to slightly acid pH (Stempfhuber et al., 2015; Nicol, Leininger, Schleper, & Prosser, 2008). Bacteria in the phylum Actinobacteria are often called 'actinomycetes'; they are highly branched and filamentous in structure, which allows them to be more prolific in no-tillage or undisturbed soils. Some actinomycetes can form symbiotic relationships with plants and are especially skilled at breaking down compounds dominant in later decay stages; compounds such as cellulose, and chitin which are considered more resistant to breakdown processes (Lewin et al., 2016). Some actinomycetes can also perform nitrogen fixation and make nutrients and minerals more plant available (Lewin et al., 2016). They have also been shown to inhibit plant pathogens (Jeffrey et al., 2007; Oskay, Tamer, & Azeri, 2004). A study by Hozzein et al. (2019) inoculated different strains of actinomycetes to soils planted with wheat, barley, oat, maize, and sorghum. When plants had developed mature

seed, the leaves were found to have higher chlorophyll and photosynthetic rates, while the seeds had increased phenolics and sugars. All tested cereals had increased grain yield with barley and maize showing the most significant gains compared to controls (Hozzein et al., 2019). In the pursuit of energy, prokaryotes contribute significantly to nutrient cycling within the soil. There are soil bacteria and Archaea that are known to be involved in all major nutrient cycling processes (carbon, nitrogen, sulfur, phosphorus) within soils, which is a credit to their adaptive metabolism capabilities. Despite the expansive phylogenetic diversity, the vital aspect of the significance of prokaryotes to soils lies in their metabolic diversity. This metabolic diversity allows prokaryotes to intake plant-inaccessible forms of nutrients and turn them into plant-accessible nutrients (Sikorski, 2015).

Fungi are heterotrophs and many are saprophytes, meaning they acquire nutrients from dead organic material (Morton, 2005). Fungi are often especially adept in their ability to degrade even recalcitrant substances such as lignin, celluloses, chitin (Morton, 2005). All of these compounds are found within plant material and celluloses and lignin are the first and second most abundant cell wall material respectively (McNamara, Morgan, & Zimmer, 2015). A study examining carbon transference from wheat straw to soil representative of cultivated soil designed to mimic a no-tillage management found that after five weeks up to five percent of the total soil C originated from the wheat straw (Frey & Elliot, 2003). Frey & Elliot (2003) also found that most of this C was located in macroaggregates where the authors theorized that this C is less susceptible to decay and therefore contributes to a stabilized organic matter pool. Some fungi are not strictly saprophytes, and instead obtain nutritional requirements from living tissues; these fungi can form symbiotic or pathogenic relationships with their living nutrient source.

Mycorrhizal fungi form mutualistic relationships with plants, and some are especially important in agriculture. These fungi provide their plant hosts with inorganic nutrients while plant hosts provide carbohydrates to the mycorrhizae. The two classes of mycorrhizae are ectomycorrhizae, these fungi form a sheath around plant host roots with slight penetration for nutrient exchange that does not extend into the cell walls of the root cortex, and endomycorrhizae which penetrate root cells more extensively (Madigan, Bender, Buckley, & Sattley, 2018). Temperate or semi-arid shrubs and trees (woody plants) can serve as hosts for ectomycorrhizae (Sylvia, 2005; Madigan et al., 2018; Weil & Brady, 2016). An agriculturally significant member of the endomycorrhizae fungi are the arbuscular mycorrhizae (AM) fungi (Weil & Brady, 2016). Most agricultural crops can form associations with AM fungi, including corn, soybeans, rice, wheat, sorghum, and barley. AM fungi are especially beneficial in soils low in phosphorus as well as other nutrients. AM fungi aid plants in nutrient acquisition by being able to extend their growth into spaces that have higher nutrient stores. This is especially relevant to phosphorus plant accumulation, given the low mobility of phosphorus, as well as micronutrients such as copper and zinc (Sylvia, 2005). A meta-analysis of field studies with AM fungi and wheat performed from 1975 to 2013, found that grain yield was positively correlated with AM fungi colonization rate (Pellegrino, Opik, Bonari, & Ercoli, 2015). Pellagrino et al. (2015) also found that field inoculation with AM fungi led to higher aboveground biomass, grain yield, and harvest index. A 2018 study found that an amendment containing half of the typical recommended commercial fertilizer rate supported 60% more AM fungi in sorghum roots while yielding comparable biomass, protein, and mineral content as compared to sorghum grown with the recommended commercial fertilizer (N and P) rate (Cobb, Wilson, & Goad, 2018). These studies highlight how microorganisms like AM fungi can respond to agricultural practices and be

used to potentially lower chemical application while maintaining or enhancing yield. Ultimately the effectiveness of microorganisms to aid agriculture production is contingent upon their interactions with each other.

The term "microbial loop" refers to a framework to discuss microorganism interactions and how they relate to nutrient cycling. The term was first presented by Azam et al. (1983), where it was used to describe the flow of dissolved organic carbon to bacteria and then that carbon being remobilized when protozoa fed upon the bacteria in aquatic systems. This term has been applied to soil systems to describe the process of the release of root exudates that allows bacteria to incorporate mineral N into bacterial biomass, which is then released by protozoan grazers in the form of ammonium, which in turn benefits plant growth leading to increased root exudates (Clarholm, 1985; Bonkowski & Clarholm, 2012). This microbial loop has also been found to be linked to protists and AM fungi. Protists graze on bacteria that have accumulated ammonia from soil organic matter. Then AM fungi intake the ammonia or oxidized forms (nitrite/nitrate) that is then beneficial to their symbiote plants (Koller, Rodriguez, Robin, Scheu, & Bonkowski, 2013; Bukovska et al., 2018). Increasingly it is not the actions of a lone player, but the orchestration of these players interacting together that allows an understanding in how nutrients interact in agricultural soil systems. Two of the most important nutrient cycles in agriculture are the carbon and nitrogen cycles.

The carbon cycle is important to agriculture in that it describes the flow of carbon through the soil, making it accessible for nutrient cycling processes. Agricultural management practices such as no-tillage and cover cropping have been shown to increase soil organic matter (also referred to as soil organic carbon) in the soil. A global meta-analysis examining the interaction between soil organic matter and crop yields of maize and wheat found that on average

a higher soil organic matter was correlated with a higher yield (Oldfield, Bradford, & Wood, 2019). Soil organic matter is broken down by microorganisms in the soil into nutrients that are able to be utilized for plant growth. Examining the functional genes of microorganisms in carbon cycling highlights their involvement in nutrient cycling. In no-tillage systems and especially those that also have cover cropping with 100% residue remaining on soils, there is a great deal of plant material available for breakdown back into the soil. Cellulose, hemicellulose, and lignin (respectively) are the most common structural polysaccharides in plant residues; structural polysaccharides aid plants in cell wall rigidity (Burranov and Mazza, 2008). Starch is also a common polysaccharide found in plant residues that in plants stores energy. Chitin is another type of structural polysaccharide, and it occurs in fungi and arthropods. The breakdown of these structural polysaccharides is dependent upon extracellular enzymes that are excreted from bacteria and fungi, that can eventually break down these complex molecules into monomers, which can be utilized by a larger proportion of microorganisms and plants (Luo, Meng, & Gu, 2017; Utobo & Tewarl, 2014; Eivazi & Tabatabai, 1987). Genes responsible for the production of these enzymes can be identified from microorganisms and thus lead to a functional characterization of the microbial community (Zhou et al., 2015; Xue et al., 2013; Zhang et al., 2013). Some of the functional genes that have been established to be involved in carbon cycling in bacterial, archaeal and fungal systems include: acetylglucosaminidase (nag) (degrades chitin) and α-amylase (amyA) (degrades starch) (Trivedi et al., 2016; Xue et al., 2013).

The nitrogen cycle is especially important in agriculture as plants cannot fix atmospheric N_2 and nitrogen is a limiting nutrient in soils for crop growth and yield. To overcome limited N supply, fertilizers are applied; however, this is costly and can have a negative environmental impact. Nitrification occurs when ammonium (NH_4^+) is transformed into nitrate (NO_3) via

microorganisms. The ammonia monooxygenase gene (*amoA*) has been found in both Archaea and bacteria. This gene oxidizes NH_4^+ to nitrite (NO_2^-), which is the first, rate-limiting step of autotrophic nitrification; it is used as one metric for the functional capacity of nitrification in soils. Currently the distribution of ammonia-oxidizing bacteria and archaea in soils is known to be complex; however, there is an indication of ammonia-oxidizing bacteria responding to N-fertilizer presence (increased numbers) where ammonia-oxidizing archaea do not seem to show a response to N-fertilizer (Zeglin, Taylor, Myrold, & Bottomley, 2011; Taylor, Zeglin, Wanzek, Myrold, & Bottomley, 2012). It is known that the ratio of ammonia-oxidizing bacteria to archaea varies considerably in soils; however, the factors that define these distributions are an area of on-going research (Leininger et al., 2006; Taylor et al., 2012; Zeglin et al., 2011; Habteselassie, Xu, & Norton, 2013). Other microorganism genes that have been studied to investigate N cycling include: *hzo* encodes hydrazine oxidase in anammox, *nirK* and *nirS* encode nitrite reductase, which is a precursor for denitrification, *nifH* which encodes nitrogenase reductase for N fixation (Xue et al., 2013). Understanding nutrient cycling within soils can help characterize what impact microbes are having in the soil and potentially lead to more effective agricultural management recommendations for producers. Habteselassie et al. (2013) found that *amoA* (ammonia monooxygenase, enzyme involved in nitrogen cycling) abundances varied while populations of ammonia oxidizing bacteria and archaea remained stable over a six-year field study examining treatments exposed to seven different nitrogen applications.

Studying microorganisms presents challenges and as such agronomists have relied on analysis of predominately chemical and physical soil properties to gauge the microbial community and its response to agronomic management techniques. As a result of using tests that measure general microbial properties, how microorganisms respond to conservation management

techniques has been broadly generalized and found to have variable responses to these techniques (Bender & van der Heijden, 2015; Xue et al., 2013). This broad generalization of soil microorganisms may contribute to variable results found from implementation of conservation management techniques. As the demands on global food supply continue and sustainability practices becomes increasingly sought after, understanding the roles microorganisms play in nutrient dynamics becomes more necessary.

Previous work examining microbial communities has implemented methods such as polymerase chain reaction (PCR) and phospholipid fatty acid (PLFA) analysis. PCR and PLFA methods have been used to examine microorganismal community composition, abundance, and taxonomic diversity. Microbial communities are impacted by conservation management practices; however, translating this knowledge into meaningful application for producers has proven difficult (Prosser, 2012; Navarro-Noya et al., 2013; Schmidt, Gravuer, Bossange, Mitchell, & Scow, 2018). One significant reason for this is the lack of studies connecting field practices and the functional capabilities of microorganisms at field scale (Lehman et al., 2015b). One of the most critical roles microorganisms play in soil is nutrient cycling, which is especially pertinent for agricultural production. Carbon turnover, soil organic carbon (Six, Frey, Thiet, & Batten, 2006; Nielsen, Ayres, Wall, & Bardgett, 2011; Tardy et al., 2015), and nutrient availability and uptake (Adesemoye & Kloepper, 2009; Bender & van der Heijden, 2015, Alori, Glick, & Babalola, 2017) are shown to be positively related to the soil microbial community; however, this has not been a consistent finding. It is not currently known if the microbial biomass or the microbial community structure is key to accomplish desirable agricultural outcomes; it is also not well established how well species diversity represents actual functional diversity (Lehman et al., 2015a). Some studies examining nutrient cycling point to microbial

community structure as the driving force behind some nutrient cycling processes while other studies indicate that microbial community structure is more significant (Benayas, Newton, Diaz, & Bullock, 2009; Nielsen et al., 2011; Schimel & Schaeffer, 2012; Wagg, Bender, Widmer, & Heijden, 2014). A study by Schmidt et al. (2018) found that long-term use of cover crops resulted in a microbial community with more varied metabolic capabilities and that the forms and amount of organic carbon present increased. Work by Ding, Su, Sun, Wu, & Wei (2018) used a microarray to link an increase in functional gene diversity after adding rice straw and reducing chemical fertilizers to a subsequent increase in rice productivity. Work by Schmidt et al. (2018) and Ding et al. (2018) examined the link between an increase in microbial nutrient cycling services and improved agronomic outcomes.

Given the strategy of no-till and cover crops to broadly change the soil environment to be generally conducive to microbial activity and diversity, a greater understanding of functional rather than taxonomic changes from conservation techniques is important as it could contribute to sustainability. The nature of PCR allows researchers to target one or a few specific sequences, these sequences could be indicative of taxonomic or functional classification, but they are limited by their number of targets, and therefore this has a limited scope in what is able to be examined (Theis, 2008). Given the vast diversity of soil microorganisms techniques that allow greater view of microorganisms can better address the vast nature of their function. PLFA analysis can classify microorganisms present in soil samples into broad community groupings and can at this resolution depict the community structure present. However, PLFA cannot speak to what is functionally occurring in soils. GeoChip is a functional gene microarray that offers a unique opportunity to access microbial functional gene response to the environment, and similarly to PCR methods it targets specific sequences. This analysis has yet to be applied widely to

agricultural management. GeoChip contains over 160,000 distinct probes that target about 1,500 functional gene families. These targeted gene families are involved in many nutrient cycling processes including microbial carbon (degradation, fixation, methane), nitrogen, sulfur, and phosphorus cycling, energy metabolism, metal homeostasis, organic remediation, secondary metabolism, stress responses, and virulence (Glomics, 2014). This type of analysis has not yet been utilized to assess the effects of cover crops or tillage within cropping systems and could serve to better illustrate the role soil microbes play in conservation management practices.

Understanding the functional ability and diversity of the microbiota is critical as it has the power to enhance productivity, provide ecological services and system resiliency, and sustain soil quality (Lehman et al., 2015a; Lehman et al., 2015b; Schmidt et al., 2018). Focusing on functional measurement rather than taxonomic identification will provide the link between conservation practices, optimized nutrient cycling, and ecologically intensified agricultural systems (Bender et al., 2015). Research is needed to further understand how no-till, cover crops, and fertilizer management change microbial function in order to justify and improve implementation of conservation management techniques.

Research Rationale

Better understanding how microbes are influenced by management practices such as no-tillage, cover crop usage, and P fertilizer management will aid in understanding the roles they play in an agricultural system. No-tillage practices have become more common over the past 50 years; however, with any system there are benefits and costs (Huggins & Reganold, 2008). While there has been research looking at the microbial response to no-tillage practices, these studies do not typically examine recent no-tillage sites and how this factor interacts with cover cropping (Karlen et al., 1994; Motta, Reeves, Burmester, & Feng, 2007). This work seeks to

better understand how conservation practices (i.e. cover cropping) at a no-tillage site with the use of different P fertilizer sources and timing impact soil microorganisms. Does cover cropping alter microbial community structure in no-tillage systems? If cover cropping does alter microbial community structure, is this trend also observed in the fall? Do cover crops impact microbial functional gene diversity? Is functional gene redundancy impacted by cover cropping or P fertilizer application? Do differences in microbial community structure or functional gene diversity relate to crop yield in a positive, negative, or neutral way? The relationships between examined treatments, indicators of soil health, and metrics to access the microbial community structure will be analyzed in this study. This study looked to utilize innovative techniques such as the GeoChip to access functional gene presence and abundance. Specific methods employed for this study include microbial biomass C and N, soil respiration, autoclaved citrate extractible protein content, β – glucosaminidase and β – glucosidase enzyme activity, phospholipid fatty acid analysis, and functional gene microarray (GeoChip) analyses.

This research was conducted at the Kansas Agricultural Watershed (KAW) field research facility. This site is a no-tillage site with cover cropping and a corn-soybean rotation. This research strives to analyze the impact of cover crop usage and P fertilizer management on soil microorganisms, with the long-term goal of contributing information on meaningful management practices for Kansas producers.

Hypotheses and Objectives for Research

The hypotheses of this project were: (i) measurements of biological soil properties can detect differences in land management; (ii) the taxonomic microbial community structure will be altered in response to different agricultural management practices; (iii) microbial functional gene composition will be impacted by treatments of cover cropping and P fertilizer. These impacts

will be observable in both a spring and a fall sampling. The objectives of this study were to (i) examine multiple soil health tests as they relate to land management practices examined; (ii) to explore the taxonomic microbial community structure at the KAW research site in response to management practices that include cover crop usage and P fertilizer treatments at an early transition to no-tillage (less than 5 years) no-till field-scale site; (iii) to identify key microbial functional gene composition in the fall 2019 and spring 2020 seasons at a depth of 0-5 cm, to determine how P fertilizer management, cover crop management impact key soil microbial functional gene composition.

References

Adesemoye, A. O., & Kloepper, J. W. (2009, November). Plant-microbes interactions in enhanced fertilizer-use efficiency. *Applied Microbiology and Biotechnology*, Vol. 85, pp. 1–12. https://doi.org/10.1007/s00253-009-2196-0

Alexander, D. B. (2005). Bacteria and archaea. In M. D. Sylvia (Ed.), *Principles and applications of soil microbiology* (2nd ed., pp. 101–139). Upper Saddle River: Pearson.

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017, June 2). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology*, Vol. 8. https://doi.org/10.3389/fmicb.2017.00971

Azam, F., Genchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., & Thingstad, F. (1983). *Marine Ecology-Progress Series The Ecological Role of Water-Column Microbes in the Sea** (Vol. 10).

Bae, H. S., Morrison, E., Chanton, J. P., & OGram, A. (2018). Methanogens are major contributors to nitrogen fixation in soils of the Florida Everglades. *Applied and Environmental Microbiology*, *84*(7). https://doi.org/10.1128/AEM.02222-17

Benayas, J. M. R., Newton, A. C., Diaz, A., & Bullock, J. M. (2009). Enhancement of Biodiversity and Ecosystem Services by Ecological Restoration: A Meta-Analysis. *Science*, *325*(5944), 1121. https://doi.org/10.1126/science.1172460

Bender, S. F., & van der Heijden, M. G. A. (2015). Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. *Journal of Applied Ecology*, *52*(1), 228–239. https://doi.org/10.1111/1365-2664.12351

Blevins, R. L., Frye, W. W., Baldwin, P. L., & Robertson, S. D. (1990). Tillage Effects on Sediment and Soluble Nutrient Losses from a Maury Silt Loam Soil. *J. Environ. Qual.*, *19*, 683–686.

Bonkowski, M., & Clarholm, M. (2012). Stimulation of plant growth through interactions of bacteria and protozoa: Testing the auxiliary microbial loop hypothesis. *Acta Protozoologica*, Vol. 51, pp. 237–247. https://doi.org/10.4467/16890027AP.12.019.0765

Boone, D. R., Liu, Y., Zhao, Z., Balkwill, D. L., Drake, G. R., & Aldrich, H. C. (1995). Bacillus infernus sp. nov., an Fe(II1)-and Mn(1V)-Reducing Anaerobe from the Deep Terrestrial Subsurface. In *INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY*. International Union of Microbiological Societies.

Brochier-Armanet, C., Boussau, B., Gribaldo, S., & Forterre, P. (2008). Mesophilic crenarchaeota: Proposal for a third archaeal phylum, the Thaumarchaeota. *Nature Reviews Microbiology*, *6*(3), 245–252. https://doi.org/10.1038/nrmicro1852

Bukovská, P., Bonkowski, M., Konvalinková, T., Beskid, O., Hujslová, M., Püschel, D., … Jansa, J. (2018). Utilization of organic nitrogen by arbuscular mycorrhizal fungi—is there a specific role for protists and ammonia oxidizers? *Mycorrhiza*, *28*(3), 269–283. https://doi.org/10.1007/s00572-018-0825-0

Cambardella, C. A., Johnson, J. M. F., & Varvel, G. E. (2012). Soil carbon sequestration in central U.S. Agroecosystems. In M. A. Liebig, A. J. Franzleubbers, & R. F. Follet (Eds.), Managing Agricultural Greenhouse Gases: Coordinated Agricultural Research through Gracenet to Address Our Changing Climate (pp. 41–58). San Diego, CA: Academic Press, Elsevier.

Chichester, F. W., & Richardson, C. W. (1992). Sediment and Nutrient Loss from Clay Soils as Affected by Tillage. *Journal of Environmental Quality*, *21*(4), 587–590. https://doi.org/10.2134/jeq1992.00472425002100040010x

Clarholm, M. (1985). Interactions of Bacteria, Protozoa, and Plants Leading to Mineralization of Soil Nitrogen. *Soil Biol. Biochem.*, *17*(2), 181–187.

Cobb, A. B., Wilson, G. W. T., & Goad, C. L. (2018). Linking sorghum nutrition and production with arbuscular mycorrhizal fungi and alternative soil amendments. *Journal of Plant Nutrition and Soil Science*, *181*(2), 211–219. https://doi.org/10.1002/jpln.201700052

Delgado-Baquerizo, M., Oliverio, A. M., Brewer, T. E., Benavent-González, A., Eldridge, D. J., Bardgett, R. D., … Fierer, N. (2018). A global atlas of the dominant bacteria found in soil. *Science Report*, 320–325. Retrieved from http://science.sciencemag.org/

Ding, L. J., Su, J. Q., Sun, G. X., Wu, J. S., & Wei, W. X. (2018). Increased microbial functional diversity under long-term organic and integrated fertilization in a paddy soil. *Applied Microbiology and Biotechnology*, *102*(4), 1969–1982. https://doi.org/10.1007/s00253-017-8704-8

Eichorst, S. A., Trojan, D., Roux, S., Herbold, C., Rattei, T., & Woebken, D. (2018). Genomic insights into the Acidobacteria reveal strategies for their success in terrestrial environments. *Environmental Microbiology*, *20*(3), 1041–1063. https://doi.org/10.1111/1462-2920.14043

Eivazi, F., & Tabatabai, M. A. (1990). Factors affecting glucosidase and galactosidase activities in soils. *Soil Biology and Biochemistry*, *22*(7), 891–897. https://doi.org/10.1016/0038-0717(90)90126-K

EPA. (2005). Nutrient Pollution, The Sources and Solutions: Agriculture. Retrieved December 11, 2020, from https://www.epa.gov/nutrientpollution/sources-and-solutions-agriculture

Ertefai, T. F., Fisher, M. C., Fredricks, H. F., Lipp, J. S., Pearson, A., Birgel, D., … Hinrichs, K. U. (2008). Vertical distribution of microbial lipids and functional genes in chemically distinct layers of a highly polluted meromictic lake. *Organic Geochemistry*, *39*(11), 1572–1588. https://doi.org/10.1016/j.orggeochem.2008.07.009

FAO. (2019). Soil degradation. Retrieved from http://www.fao.org/soils-portal/soil-degradation-restoration/en/

Fichtner, A., von Oheimb, G., Härdtle, W., Wilken, C., & Gutknecht, J. L. M. (2014). Effects of anthropogenic disturbances on soil microbial communities in oak forests persist for more than 100 years. *Soil Biology and Biochemistry*, *70*, 79–87. https://doi.org/10.1016/j.soilbio.2013.12.015

Finney, D. M., Buyer, J. S., & Kaye, J. P. (2017). Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation*, *72*(4), 361–373. https://doi.org/10.2489/jswc.72.4.361

Franzluebbers, A. J. (2010). Achieving Soil Organic Carbon Sequestration with Conservation Agricultural Systems in the Southeastern United States. Soil Science Society of America Journal, 74(2), 347–357. https://doi.org/10.2136/sssaj2009.0079

Frey, S. D., Six, J., & Elliott, E. T. (2003). Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil-litter interface. *Soil Biology and Biochemistry*, *35*(7), 1001–1004. https://doi.org/10.1016/S0038-0717(03)00155-X

Gans, J., Wolinsky, M., & Dunbar, J. (2005). Computational Improvements Reveal Great Bacterial Diversity and High Metal Toxicity in Soil. *Science*, *309*(5739), 1387. https://doi.org/10.1126/science.1112665

Germida, J. J. (2005). Transformation of sulfur. In D. M. Sylvia (Ed.), *Principles and applications of soil microbiology* (pp. 433–462). Upper Saddle River.

Giguere, A. T., Eichorst, S. A., Meier, D. V., Herbold, C. W., Richter, A., Greening, C., & Woebken, D. (2020). Acidobacteria are active and abundant members of diverse atmospheric H2-oxidizing communities detected in temperate soils. ISME Journal. https://doi.org/10.1038/s41396-020-00750-8

Glomics Inc. (2014). GeoChip Technology. Retrieved April 30, 2019, from http://www.glomics.com/gch-tech.html

Gold, T. (1992). *The deep, hot biosphere* (Vol. 89).

Gregorich, E. G., Wen, G., Voroney, R. P., & Kachanoski, R. G. (1990). Calibration of a rapid direct chloroform extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, *22*(7), 1009–1011. https://doi.org/10.1016/0038-0717(90)90148-S

Gupta, R. S. (2000). The natural evolutionary relationships among prokaryotes. *Critical Reviews in Microbiology*, Vol. 26, pp. 111–131. https://doi.org/10.1080/10408410091154219

Habteselassie, M. Y., Xu, L., & Norton, J. M. (2013). Ammonia-oxidizer communities in an agricultural soil treated with contrasting nitrogen sources. *Frontiers in Microbiology*, *4*(NOV). https://doi.org/10.3389/fmicb.2013.00326

Hozzein, W. N., Abuelsoud, W., Wadaan, M. A. M., Shuikan, A. M., Selim, S., Al Jaouni, S., & AbdElgawad, H. (2019). Exploring the potential of actinomycetes in improving soil fertility and grain quality of economically important cereals. *Science of the Total Environment*, *651*, 2787–2798. https://doi.org/10.1016/j.scitotenv.2018.10.048

Huggins, D., & Reganold, J. (2008). No-Till: The Quiet Revolution. *Scientific American*, *299*, 70–77. https://doi.org/10.1038/scientificamerican0708-70

ISO/TS. (2010). Soil quality-determination of soil microbial diversity. Retrieved from https://www.iso.org/standard/45703.html

Jeffrey, L. S. H., Sahilah, A. M., Son, R., Tosiah, S., Lim, J., Heng, S., … Sadi, T. (2007). Isolation and screening of actinomycetes from Malaysian soil for their enzymatic and antimicrobial activities (Pemencilan dan penyaringan aktinomiset dari tanah di Malaysia untuk aktiviti enzim dan anti-mikrob). In *J. Trop. Agric. and Fd. Sc* (Vol. 35).

Jenkinson, D. S., & Powlson, D. S. (1976). The effects of biocidal treatments on metabolism in soil—V: A method for measuring soil biomass. *Soil Biology and Biochemistry*, *8*(3), 209–213. https://doi.org/10.1016/0038-0717(76)90005-5

Karlen, D. L., Wollenhaupt, N. C., Erbach, D. C., Berry, E. C., Swan, J. B., Eash, N. S., & Jordahl, J. L. (1994). Long-term tillage effects on soil quality. *Soil and Tillage Research*, *32*(4), 313–327. https://doi.org/10.1016/0167-1987(94)00427-G

Keen, N. T., & Legrand, M. (1980). Surface glycoproteins : evidence that they may function as the race specific phytoalexin elicitors of Phytophthora megasperma f.sp. glycinea. *Physiological Plant Pathology*, *17*(2), 175–192. https://doi.org/10.1016/0048-4059(80)90050-8

Kesters, K., De Vos, P., Gillis, M., Swings, J., Vandamme, P., & Stackebrandt, E. (2006). Introduction to the Proteobacteria. In M. Dwarkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), The Prokaryotes (3rd ed., Vol. 5, pp. 3–37). New York: Springer.

Klose, S., Bilen, S., Tabatabai, M. A., Dick, W. A., & Dick, R. P. (2011). *Sulfur Cycle Enzymes*. https://doi.org/10.2136/sssabookser9.c7

Koller, R., Rodriguez, A., Robin, C., Scheu, S., & Bonkowski, M. (2013). Protozoa enhance foraging efficiency of arbuscular mycorrhizal fungi for mineral nitrogen from organic matter in soil to the benefit of host plants. *New Phytologist*, *199*(1), 203–211. https://doi.org/10.1111/nph.12249

Kuske, C. R., Barns, S. M., & Busch, J. D. (1997). Diverse Uncultivated Bacterial Groups from Soils of the Arid Southwestern United States That Are Present in Many Geographic Regions. In *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* (Vol. 63). Retrieved from http://www-ls.lanl.gov/lsdiv/LS7/SunsetCrater/SC.html.

Langdale, G. W., Blevins, R. L., Karlen, D. L., McCool, D. K., Nearing, M. A., Skidmore, E. L., … Williams, J. R. (1991). Cover crop effects on soil erosion by wind and water. In W. L. Hargrove (Ed.), *Cover Crops for Clean Water* (pp. 15–22). Retrieved from https://www.researchgate.net/publication/238089979

Lehman, R. M., Acosta-Martinez, V., Buyer, J. S., Cambardella, C. A., Collins, H. P., Ducey, T. F., … Stott, D. E. (2015, January 1). Soil biology for resilient, healthy soil. *Journal of Soil and Water Conservation*, Vol. 70, pp. 12A-18A. https://doi.org/10.2489/jswc.70.1.12A. A

Lehman, R. M., Cambardella, C. A., Stott, D. E., Acosta-Martinez, V., Manter, D. K., Buyer, J. S., … Karlen, D. L. (2015). Understanding and enhancing soil biological health: The solution for reversing soil degradation. *Sustainability (Switzerland)*, *7*(1), 988–1027. https://doi.org/10.3390/su7010988. B

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G. W., … Schleper, C. (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*, *442*(7104), 806–809. https://doi.org/10.1038/nature04983

Li, L., Xu, M., Ali, M. E., Zhang, W., Duan, Y., & Li, D. (2018). Factors affecting soil microbial biomass and functional diversity with the application of organic amendments in three contrasting cropland soils during a field experiment. *PLoS ONE*, *13*(9). https://doi.org/10.1371/journal.pone.0203812

Lin, Q., & Brookes, P. C. (1999). Comparison of substrate induced respiration, selective inhibition and biovolume measurements of microbial biomass and its community structure in unamended, ryegrass-amended, fumigated and pesticide-treated soils. *Soil Biology and Biochemistry*, *31*(14), 1999–2014. https://doi.org/10.1016/S0038-0717(99)00122-4

Madigan MT, Bender KS, Buckley DH, Sattley WM, S. DA. (2018). *Brock biology of microorganisms* (15th ed.). Boston: Pearson.

Mahmoudi, N., Porter, T. M., Zimmerman, A. R., Fulthorpe, R. R., Kasozi, G. N., Silliman, B. R., & Slater, G. F. (2013). Rapid degradation of deepwater horizon spilled oil by indigenous microbial communities in louisiana saltmarsh sediments. *Environmental Science and Technology*, *47*(23), 13303–13312. https://doi.org/10.1021/es4036072

McDaniel, M. D., Tiemann, L. K., & Grandy, A. S. (2014). Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. In *Ecological Applications* (Vol. 24). Retrieved from http://www.metawinsoft.com/

McKinley, V. L., Peacock, A. D., & White, D. C. (2005). Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. *Soil Biology and Biochemistry*, *37*(10), 1946–1958. https://doi.org/10.1016/j.soilbio.2005.02.033

McNamara, J. T., Morgan, J. L. W., & Zimmer, J. (2015, June 2). A molecular description of cellulose biosynthesis. Annual Review of Biochemistry, Vol. 84, pp. 895–921. https://doi.org/10.1146/annurev-biochem-060614-033930

Morton, J. B. (2005). Fungi. In D. M. Sylvia (Ed.), *Principles and applications of soil microbiology* (2nd ed., pp. 141–161). Upper Saddle River: Pearson.

Motta, A. C. V., Reeves, D. W., Burmester, C., & Feng, Y. (2007). Conservation tillage, rotations, and cover crop affecting soil quality in the tennessee valley: Particulate organic matter, organic matter, and microbial biomass. *Communications in Soil Science and Plant Analysis*, *38*(19–20), 2831–2847. https://doi.org/10.1080/00103620701663065

Nair, A., & Ngouajio, M. (2012). Soil microbial biomass, functional microbial diversity, and nematode community structure as affected by cover crops and compost in an organic vegetable production system. *Applied Soil Ecology*, *58*, 45–55. https://doi.org/10.1016/j.apsoil.2012.03.008

Navarro-Noya, Y. E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M. C., Valenzuela-Encinas, C., … Dendooven, L. (2013). Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. *Soil Biology and Biochemistry*, *65*, 86–95. https://doi.org/10.1016/j.soilbio.2013.05.009

Nicol, G. W., Leininger, S., Schleper, C., & Prosser, J. I. (2008). The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environmental Microbiology*, *10*(11), 2966–2978. https://doi.org/10.1111/j.1462-2920.2008.01701.x

Nielsen, U. N., Ayres, E., Wall, D. H., & Bardgett, R. D. (2011, February). Soil biodiversity and carbon cycling: A review and synthesis of studies examining diversity-function relationships. *European Journal of Soil Science*, Vol. 62, pp. 105–116. https://doi.org/10.1111/j.1365-2389.2010.01314.x

NRI (National Resources Institute). (2003). *2003 Annual NRI Soil Erosion*. Washington D.C.

Oldfield, E. E., Bradford, M. A., & Wood, S. A. (2019). Global meta-analysis of the relationship between soil organic matter and crop yields. *SOIL*, *5*(1), 15–32. https://doi.org/10.5194/soil-5-15-2019

Oskay, M., Tamer, A. U., & Azeri, C. (2004). Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. *Applied Microbiology*, *3*(9), 441–446.

Parham, J. A., & Deng, S. P. (2000). Detection, quantification and characterization of β-glucosaminidase activity in soil. *Soil Biology and Biochemistry*, *32*(8–9), 1183–1190. https://doi.org/10.1016/S0038-0717(00)00034-1

Pellegrino, E., Öpik, M., Bonari, E., & Ercoli, L. (2015). Responses of wheat to arbuscular mycorrhizal fungi: A meta-analysis of field studies from 1975 to 2013. *Soil Biology and Biochemistry*, *84*, 210–217. https://doi.org/10.1016/j.soilbio.2015.02.020

Pimentel, D., Wilson, C., Mccullum, C., Huang, R., Dwen, P., Flack, J., … Cliff, B. (1997). Economic and Environmental Benefits of Biodiversity. *BioScience, 47* (11), 747-757, https://doi.org/10.2307/1313097

Prosser, J. I. (2012). Ecosystem processes and interactions in a morass of diversity. *FEMS Microbiology Ecology*, *81*(3), 507–519. https://doi.org/10.1111/j.1574-6941.2012.01435.x

Richmond, P. A. (1991). Occurrence and function of native cellulose. In P. J. Haigler, C. H., Weimer (Ed.), *Biosynthesis and biodegradation of cellulose* (pp. 5–23). New York: Marcel-Dekker.

Roberson, E. B., Sarig, S., Shennan, C., & Firestone, M. K. (1995). Nutritional Management of Microbial Polysaccharide Production and Aggregation in Agricultural Soil. *Soil Sci. Am.*, *59*, 1587–1594.

Romillac, N. (2018). Ammonification. In *Encyclopedia of Ecology* (2nd ed., p. 256). Elsevier.

Sapkota, T. B., Mazzoncini, M., Bàrberi, P., Antichi, D., & Silvestri, N. (2012). Fifteen years of no till increase soil organic matter, microbial biomass and arthropod diversity in cover crop-based arable cropping systems. *Agronomy for Sustainable Development*, *32*(4), 853–863. https://doi.org/10.1007/s13593-011-0079-0

Sikorski, J. (2015). The Prokaryotic Biology of Soil. Soil Organisms, 87, 1–28. Retrieved from www.soil-organisms.de

Schimel, J. P., & Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Frontiers in Microbiology*, Vol. 3. https://doi.org/10.3389/fmicb.2012.00348

Schindelbeck, R.R., Moebius-Clune, B. N., Moebius-Clune, D. J., Kurtz, K. S., & and H.M. van Es. (2016). *Cornell Soil Health Laboratory Comprehensive Assessment of Soil Health Standard Operating Procedures*. Retrieved from http://soilhealth.cals.cornell.edu.

Schloss, P. D., & Handelsman, J. (2006). Toward a census of bacteria in soil. *PLoS Computational Biology*, *2*(7), 0786–0793. https://doi.org/10.1371/journal.pcbi.0020092

Schmid, M. C., Hooper, A. B., Klotz, M. G., Woebken, D., Lam, P., Kuypers, M. M. M., … Jetten, M. S. M. (2008). Environmental detection of octahaem cytochrome c hydroxylamine/hydrazine oxidoreductase genes of aerobic and anaerobic ammonium-

oxidizing bacteria. *Environmental Microbiology*, *10*(11), 3140–3149. https://doi.org/10.1111/j.1462-2920.2008.01732.x

Schmidt, R., Gravuer, K., Bossange, A. V., Mitchell, J., & Scow, K. (2018). Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. *PLoS ONE*, *13*(2). https://doi.org/10.1371/journal.pone.0192953

Six, J., Frey, S. D., Thiet, R. K., & Batten, K. M. (2006, March). Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal*, Vol. 70, pp. 555–569. https://doi.org/10.2136/sssaj2004.0347

Smith, J. L., & Paul, E. A. (1990). The significance of soil microbial biomass estimations. In *Soil biochemistry. Volume 6.* (pp. 357–396). Retrieved from https://www.cabdirect.org/cabdirect/abstract/19911950380

Spedding, T. A., Hamel, C., Mehuys, G. R., & Madramootoo, C. A. (2004). Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. *Soil Biology and Biochemistry*, *36*(3), 499–512. https://doi.org/10.1016/j.soilbio.2003.10.026

Stavi, I., & Lal, R. (2015). Achieving Zero Net Land Degradation: Challenges and opportunities. *Journal of Arid Environments*, *112*(PA), 44–51. https://doi.org/10.1016/j.jaridenv.2014.01.016

Stempfhuber, B., Engel, M., Fischer, D., Neskovic-Prit, G., Wubet, T., Schöning, I., … Schloter, M. (2015). pH as a Driver for Ammonia-Oxidizing Archaea in Forest Soils. *Microbial Ecology*, *69*(4), 879–883. https://doi.org/10.1007/s00248-014-0548-5

Sylvia, D. M. (2005). Mycorrhizal symbioses. In *Principles and applications of soil microbiology* (2nd ed., pp. 263–282). Upper Saddle River: Pearson.

Tardy, V., Spor, A., Mathieu, O., Lévèque, J., Terrat, S., Plassart, P., … Maron, P. A. (2015). Shifts in microbial diversity through land use intensity as drivers of carbon mineralization in soil. *Soil Biology and Biochemistry*, *90*, 204–213. https://doi.org/10.1016/j.soilbio.2015.08.010

Taylor, A. E., Zeglin, L. H., Wanzek, T. A., Myrold, D. D., & Bottomley, P. J. (2012). Dynamics of ammonia-oxidizing archaea and bacteria populations and contributions to soil nitrification potentials. *ISME Journal*, *6*(11), 2024–2032. https://doi.org/10.1038/ismej.2012.51

Theis, J. E. (2008). Molecular methods for studying microbial ecology in the soil and rhizosphere. In *Molecular Mechanisms of Plant and Microbe Coexistence*. Berlin, Germany: Springer-Verlag.

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). Microbial biomass measurements in forest soils: Determination of kC values and tests of hypotheses to explain the failure of the chloroform fumigation-incubation method in acid soils. *Soil Biology and Biochemistry*, *19*(6), 689–696. https://doi.org/10.1016/0038-0717(87)90050-2

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, *19*(6), 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Wagg, C., Bender, S. F., Widmer, F., & Heijden, M. G. A. van der. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, *111*(14), 5266–5270. https://doi.org/10.1073/PNAS.1320054111

Walker, J. M. (1994). The Bicinchoninic Acid (BCA) Assay for Protein Quantitation. In Walker J.M. (eds) *Basic Protein and Peptide Protocols vol 32* (pp. 5-8). Humana Press. https://doi.org/10.1385/0-89603-268-X:5

Wallenstein, M. D., & Burns, R. G. (2011). Ecology of Extracellular Enzyme Activities and Organic Matter Degradation in Soil: A Complex Community-Driven Process. In R. P. Dick (Ed.), *Methods of soil enzymology* (pp. 35–55). https://doi.org/10.2136/sssabookser9.c2

Weil, R. R., & Brady, N. C. (2016). *The nature and properties of soils* (15th ed.). Pearson.

Williams, A., Birkhofer, K., & Hedlund, K. (2014). Above- and below-ground interactions with agricultural management: Effects of soil microbial communities on barley and aphids. *Pedobiologia*, *57*(2), 67–74. https://doi.org/10.1016/j.pedobi.2014.01.004

Wolf, D. C. and Wagner, G. H. (2005). Carbon transformation and soil organic matter formation. In D. M. Sylvia (Ed.), *Principles and applications of soil microbiology* (2nd ed.). Upper Saddle River: Pearson.

Wollum, A. G., & Gomez, J. E. (1970). A conductivity method for measuring microbially evolved carbon dioxide. In *Source: Ecology* (Vol. 51), 155-156.

Wright, S. F. ;, & Upadhyaya, A. (1996). Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science*, *161*(9), 575–586.

Xue, K., Wu, L., Deng, Y., He, Z., Van Nostrand, J., Robertson, P. G., … Zhou, J. (2013). Functional gene differences in soil microbial communities from conventional, low-input, and organic farmlands. *Applied and Environmental Microbiology*, *79*(4), 1284–1292. https://doi.org/10.1128/AEM.03393-12

Zeglin, L. H., Taylor, A. E., Myrold, D. D., & Bottomley, P. J. (2011). Bacterial and archaeal amoA gene distribution covaries with soil nitrification properties across a range of land

uses. *Environmental Microbiology Reports*, *3*(6), 717–726. https://doi.org/10.1111/j.1758-2229.2011.00290.x

Zhang, B., He, H., Ding, X., Zhang, X., Zhang, X., Yang, X., & Filley, T. R. (2012). Soil microbial community dynamics over a maize (Zea mays L.) growing season under conventional- and no-tillage practices in a rainfed agroecosystem. *Soil and Tillage Research*. https://doi.org/10.1016/j.still.2012.05.011

Zhang, H., Ding, W., He, X., Yu, H., Fan, J., & Liu, D. (2014). Influence of 20-year organic and inorganic fertilization on organic carbon accumulation and microbial community structure of aggregates in an intensively cultivated sandy loam soil. *PLoS ONE*, *9*(3). https://doi.org/10.1371/journal.pone.0092733

Zibilske, L. M., Bottomley, P. S., Angle, J. S., & Weaver, R. W. (1994). Carbon Mineralization1. In *SSSA Book Series*. *Methods of Soil Analysis: Part 2—Microbiological and Biochemical Properties* (pp. 835–863). https://doi.org/10.2136/sssabookser5.2.c38

Chapter 2 - Traditional Soil Health Measurements in Response to Cover Crop Implementation and P Fertilizer Management

Abstract

Cover crop usage can result in soil health benefits; however, cover crops can have negative impacts especially in water-limited regions. Soil health can be measured in many ways and this can prove challenging to compare literature of field scale experiments. The objective of this study was to examine an array of soil heath assays including total C, total N, active C, dissolved organic carbon, dissolved organic nitrogen, microbial biomass C and N, soil respiration, the enzyme activities of β-glucosidase and β-glucosaminidase, soil extractable nitrate-N, and soil extractable ammonium-N in response to cover crop usage and P fertilizer management regimes under no-tillage in a field scale study. Soil samples were collected from the Kansas Agricultural Watershed Field Laboratory in spring and fall of 2018 and 2019 at the 0-5 cm soil depth. The experiment has a 2 by 3 factorial treatment structure with two levels of cover crop (with and without) and three levels of P fertilizer management (no P fertilizer, fall broadcast, and spring injected) in a randomized complete block design with three replicates of each treatment combination (total of 18 plots). Total carbon, active C, microbial biomass C, and enzyme activity were the most consistent indices in being able to detect higher C in plots with a cover crop treatment. Measurements examining N pools were, in general, not found to be impacted by cover crop nor P fertilizer treatments. Differences in seasonality were detected in active C and microbial biomass C. Soil extractable ammonium-N was impacted by P fertilizer treatments at all sampling points except fall 2018. The results indicate that cover crop presence continued to have an affect impact on some soil health metrics up to four years after

implementing no-tillage and cover cropping. These results also demonstrate the effect of seasonality on soil carbon nutrient pool dynamics.

Introduction

The global population is expected to grow by one third (~2.3 billion people) from 2009 - 2050. This expansion as well as growing environmental concerns must be constructively approached in agriculture. Intensive agriculture has ushered in a vision of the potential for greater global food security; however, it has also contributed to significant environmental damage. One aspect of the environmental impact from intensive agriculture is soil degradation. Soil degradation is defined as a change in the soil health status resulting in a diminished capacity of the ecosystem to provide goods and services for its beneficiaries (FAO, 2019). Roughly 25% of soil globally faces degradation (Stavi & Lal, 2015). Soil erosion is a major contributing factor to soil degradation, and tillage contributes to the process of soil erosion. The term 'soil health' is often used interchangeably with the term 'soil quality' (Romig, Garyland, Harris, & McSweeny, 1995; Karlen et al., 1997). In this paper the term 'soil health' will be used, because it is similar to the concept of human health (Doran & Parkin 1997; Brennan & Acosta-Martinez, 2017).

Conservation tillage is any tillage practice that leaves 30% or more of the soil surface covered by crop residues after planting; these practices can reduce the amount of soil erosion due to water and wind. No-till is a type of conservation tillage practice that leaves 50-100% of the soil surface covered by crop residues. No-tillage practices have been found to reduce sediment losses (Chichester & Richardson, 1992; Blevins, Frye, Baldwin, & Robertson, 1990). Conventional tillage breaks down the physical structure of soils which can increase soil compaction and thus lower oxygen levels in soils and lower the amount of soil organic matter at the soil surface. Adoption of conservation tillage practices across the United States has been

associated with attributed to a decline in soil erosion from 3.1 billion tons per year in 1982 to 1.8 billion tons per year in 2001 (NRI, 2003). Soil organic matter has also been found to increase with no-tillage practices. In a fifteen-year field study, Sapkota, Mazzoncini, Barberi, Antichi, & Silverstri (2012) found that no-tillage increased the soil organic matter within the top 10 cm of the soil, as well as microbial biomass, when compared to conventional tillage.

Soil erosion was also reduced when cover crops were present; their presence reduces wind and water erosion. Cover crops have been shown to increase the soil organic matter pool, which improves soil structure, increases water infiltration and storage, and prevents surface crusting of the soil (Roberson, Sarig, Shennan, & Firestone 1995). In addition to reducing soil erosion, cover cropping has also been found to have several benefits in terms of soil biological activity, productivity, soil quality, water quality, water use, nutrient management, and pests. Finney, Buyer, & Kaye (2017) examined eight fall-sown cover crop species and found that cover crop treatments had higher microbial biomass than the no cover crop control (untilled weedy fallow). Higher microbial biomass with cover crop use has been found by numerous other studies as well (McDaniel, Tiemann, & Grandy 2014; Nair & Ngouajio, 2012; Spedding, Hamel, Mehuys, & Madramootoo, 2004). Li et al. (2018) found that application of corn stocks and wheat straw significantly increased microbial biomass carbon (MBC) and microbial functional diversity in a field study with three different soil types (Ferralic Cambisol, Calcaric Cambisol, and Luvic Phaeozem collected from the National Field Experimental Station, Henan Academy of Agricultural Sciences and Jilin Academy of Agricultural Sciences respectively in China) as compared to wheat root, corn root, pig manure, and cattle manure. In a study by Brennan & Acosta-Martinez (2017) examining soil health in a vegetable system that studied winter cover crop frequency and cover crop type, found that cover cropping frequency was the main driver of

soil microbial changes over their six-year study. A 2019 study also by Brennan & Acosta-Martinez examining cover cropping and compost influence on microbial biomass and soil enzymes (with the exception of one enzyme tested, aspartase), found that enzyme activity (in β-glucosidase, β-glucosaminidase, alkaline phosphatase, dehydrogenase, and L-asparaginase) and microbial biomass carbon and nitrogen had a positive relationship. They also found that microbial biomass and enzyme activity were mainly positively influenced by the frequency of cover cropping and that the type of cover crop used had relatively little impact.

Examining soil organic matter has been identified as a key component of assessing soil health as it is vital in sustaining the physical, chemical, and biological functions within soils (Dalal, Allen, Chan, & Singh, 2011; Dalal & Chan, 2001; Weil, Islam, Stine, Gruver, & Samson-Liebig, 2003; Gregorich, Carter, Angers, Monreal, & Ellert, 1994; Wander & Drinkwater, 2000). Soil organic matter can exist in different pools that are often characterized by their turnover rate; pools having a fast turnover rate are considered labile and pools that have a slow turnover rate are often termed recalcitrant (Weil et al., 2003; Dalal & Chan, 2001). Total C or total N measurements examine organic matter in soil and examine a total pool that includes both labile and recalcitrant pools (Wright & Bailey, 2011; Awale, Emeson, & Machado, 2017). Labile pools of organic matter primarily originate from the decomposition of plant and faunal biomass, root exudates, and deceased microbial biomass (Bolan et al., 2011; Bongiomo et al., 2019). The labile pool has a great influence on nutrient cycling processes within the soil (Weil et al., 2003). The labile pool is also sensitive to management-induced changes (Bongiomo et al., 2019; Gregorich et al., 1994; Sequeira & Alley, 2011). The significance of soil organic matter in critical processes such as nutrient cycling, cation exchange capacity, and soil structure are well established, and

assays focusing on carbon and nitrogen within these pools are often utilized to assess soil organic matter (Combs & Nathan, 1998; Bolan et al., 2011; Weil et al., 2003).

The labile pool can be measured with several assays that examine compounds that are readily broken down by soil microorganisms and undergo short-term turnover (Haynes, 2005; Zhang et al., 2020). There is no standard method to measure the labile pool, and measurements targeting this pool can be composed of specific assays that target overlapping labile pools. Some of the methods utilized to examine the carbon and / or the nitrogen within labile pools include microbial biomass carbon and nitrogen (MBC and MBN), active C, and dissolved organic carbon and nitrogen (DOC and DON). Microbial biomass C and N assays aim to measure the mass of the living component of soil organic matter, that do not include plant roots or larger organisms. Soil microbial biomass is dynamic in response to abiotic and biotic factors and has been regarded as having greater sensitivity to changes in soil organic matter than total soil organic carbon content (Ferraz de Almeida et al., 2015). Previous work has shown that microbial biomass is higher in treatments with cover crops as opposed to treatments without cover crops (Dabney et al., 2001; Strickland et al., 2020). This effect has been found to be especially pronounced in systems where cover crops are used annually (Brennan and Acosta-Martinez, 2017). The active C pool is also sensitive to management-induced changes (Blair et al., 1997, Blair et al., 2000; Weil et al., 2003). The active C pool has been found to be sensitive to cover crop implementation (Maul, 2007; Culman et al., 2012). DOC and DON have been found to have similar biodegradability (Qualls and Haines, 1992), and DOC has been found at higher levels in studies implementing cover crop usage (Steenwerth and Belina, 2008).

Extracellular enzyme activity and soil respiration have also been found to increase under cover crop implementation (Balota et al., 2014; Surucu et al., 2014; Kim et al., 2020). The

activity of β-glucosidase is measured to examine carbon nutrient cycling, as it acts in the breakdown of cellulose, which is the most abundant polymer on earth (McNarmara, Morgan, & Zimmer, 2015). β-Glucosidase acts in the final step of the breakdown of cellulose to release simple sugars, which are then available as an energy source to soil microorganisms (Ferras de Almdida et al., 2015). β – glucosaminidase is another extracellular enzyme in soils that can degrade chitin, which is found in fungi, insects and arthropod exoskeletons. Chitin and cellulose are similar and only differ by a substitution at C-2 of D-glucose. The enzyme activities of β – glucosidase and β – glucosaminidase have been found to be positively correlated with organic C in soil profiles (Eivazi and Tabatabai, 1990).Although many studies have examined cover cropping and soil health metrics, the benefits to producers of cover crop implementation can prove inconsistent and thus cover crop implementation needs to examined in many different field studies to provide producers with a better articulated map for successful cover crop implementation. Few studies have examined the seasonal impact of annual cover crop adoption across the same field plots under corn and soybean cultivation in the U.S. Midwest with a diverse array of soil health metrics. This study aims to utilize multiple soil health metrics to access the impact of cover crop implementation in no-tillage management under different P fertilizer management treatments with and without cover crops in a corn-soybean rotation in NE Kansas. In this study we hypothesized: (i) that implementation of cover cropping will increase total organic carbon, active C, dissolved organic C, dissolved organic N, and inorganic N; (ii) soil microbial biomass and activity indicators (respiration and enzyme activity assays) are hypothesized to increase within cover crops; (iii) phosphorus fertilizer management strategies will have little direct impact on carbon and nitrogen soil health metrics in the presence or absence of cover crops. The objective of this study was to investigate the impact of cover crop

presence and absence and P fertilizer management strategies using traditional soil health metrics. Soil health metrics employed in this study included: total organic carbon, active C, dissolved organic C and N, and dissolved inorganic N, microbial biomass C and N, soil respiration, enzyme activity of β-glucosidase and β-glucosaminidase.

Materials and Methods

Site Description and Experimental Design

This work was conducted at the Kansas Agricultural Watershed (KAW) Field Laboratory located near Manhattan, KS (39.134, -96.641) from spring of 2018 through fall of 2019. This field laboratory was established in 2014 and designed with the goal of examining how agricultural management practices impact soil health and consequently water, sediment, nutrient, and chemical losses. The KAW is made up of 18 small watersheds that range from 0.49 ha to 0.65 ha in size. Smolan silty clay loam (fine, smectitic, mesic Pachic Argiustoll) is the principal soil type and is the site has an average slope of 6 to 8 %; soil pH at the site ranges from 6-7. The climate for the area is a hot, humid continental climate with a mean annual temperature of 12.7^{o}C and an annual precipitation of 904 mm, with the majority of precipitation occurring in late spring to early fall (Table 2.1).

The treatment design is a 2 by 3 randomized complete block factorial design, with three replicates of each treatment, totaling 18 plots each roughly 0.5 ha. There are two levels of the cover crop treatment: cover crops (CC) and no cover crops (NC). There are three levels of phosphorus fertilizer management: no fertilizer (NP), spring injected (SI), and fall surface broadcast (FB). The KAW has been managed in a continuous no-till, corn-soybean rotation. The last tillage event occurred on November 7th, 2014. All crops grown starting in 2014 have been

under no-till management. Cover crops were first planted at KAW in 2015 and have been planted every year after. Cover crops used have reflected corn-soybean producer cover crop usage in Northwest Kansas. Cover crops have included: winter wheat before soybean in 2016, triticale and rapeseed before corn in 2017, and before soybean in 2018. Every year, the same amount of P fertilizer was applied as either a fall broadcast or spring injected applications. The form of P applied in the fall broadcast treatment was diammonium phosphate (DAP: 18-46-0) at 134.5 kg/ha (27 kgP/ha), and the form of P applied in the spring injected treatment was ammonium polyphosphate (APP: 10-34-0) at 131 L/ha (27 kgP/ha). Nitrogen (N) fertilizer, 28% urea ammonium nitrate, was injected below the surface at a uniform rate of 145.71 kg N/ha for all plots in corn years. In spring and fall of 2018, just before the CC was terminated (spring) and after the cash crop was harvested (fall), soil samples were collected from the 0-5 cm depth.

Soil Sampling and Laboratory Analysis

Soil samples were taken at a depth of 0-5 cm for spring 2018, fall 2018, spring 2019, and fall 2019. These samples were collected by taking 40 cores along a line transect in each plot. Sampling was conducted before the cash crop was planted in the spring and immediately after cash crops were harvested in the fall. Samples for a given plot and depth were sieved using a 2 mm sieve. After sieving, samples were separated into oven-dried, air-dried, freeze-dried, and field-moist divisions based on the methods used for various analysis. Samples were taken in early May of spring 2018, late November in fall 2018, late April in spring 2019, and late September in fall 2019.

Analysis sent to the K-State Soil Testing Laboratory included: soil pH and total C and N. Briefly, soil pH was determined using a 1:1(v:v) soil : water mixture of 10 g of prepared soil with deionized water. Total C and N were analyzed by direct combustion of a 0.35g soil sample

using a C/N analyzer (Model LECO TruSpec, LECO Corporation, St. Joseph, MI) on a weight percent basis.

Microbial Biomass C and N, Dissolved Organic C, Dissolved Organic N, and Inorganic N

Microbial biomass carbon (MB-C) and nitrogen (MB-N) was measured using the chloroform fumigation extraction method, which quantifies an increase in organic C and total dissolved N in a fumigated sample that results from cell lyses during a 24hr chloroform fumigation period compared to a non-fumigated soil sample to quantify the mass of microorganisms within the soil (Brookes et al., 1985; Vance et al. 1987). To prepare the fumigated and unfumigated samples, two 8 g samples of moist soil were weighed out into two 100 mL Erlenmeyer flasks. The fumigated samples were placed in a desiccator that was lined with moist (H_2O) paper towels to prevent soil samples from losing moisture. A beaker containing roughly 30 mL of ethanol-free chloroform and boiling chips was then placed in the center of the desiccator. The desiccator was evacuated for five minutes which started when the chloroform boiled, fumigation with chloroform then continued for 24 hr. After the fumigation period, paper towels and the beaker of chloroform were removed. The desiccator was then evacuated six times, for 3 min followed by allow air to reenter the desiccator and samples to remove residual chloroform. Unfumigated and fumigated samples were extracted by adding 40 mL of 0.5 M K_2SO_4 and the samples were shaken for 30 min. Samples were then filtered through Ahlstrom 74 filter paper (11 cm diameter) into 40 mL borosilicate vials. The filtrate was analyzed for dissolved organic carbon (DOC) (non-purgeable organic carbon) on a Total Organic Carbon (TOC) analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). MB-C was obtained by calculating the difference between the fumigated and unfumigated samples, and negative

controls were used to adjust values for any non-sample carbon introduced into the assay. DOC was determined from the extractant of the unfumigated MBC/MBN samples. DOC was measured through analysis of the non-purgeable organic carbon with a Total Organic Carbon (TOC) analyzer (Shimadzu, Kyoto, Japan) (Jones and Willett, 2006).

Dissolved total N (DTN), was determined by oxidizing an aliquot of the filtrate using the potassium persulfate oxidation method (Cabrera and Beare, 1993). $K_2S_2O_8$ reagent was then added to the samples and then autoclaved for 30 min at 120°C (Cabrera and Beare, 1993). Samples were then allowed to cool to room temperature (22°C), and the digest was then analyzed for nitrogen via colorimetric procedure using the Rapid Flow Analyzer, Model RFA-300 (Alpkem Corporation, Clackamas, OR). MB-N was calculated by finding the difference DTN between the fumigated and the unfumigated samples with negative controls used to account for introduction of non-sample nitrogen into the analysis. The unfumigated samples were also analyzed for inorganic N (NO_3^- and NH_4^+) and DON was determined by calculating the difference between the DTN (data not reported) and inorganic N values.

Active C

Active C was determined using $KMnO_4$ and the method developed by Weil et al. (2003). Samples were air dried and further ground and sieved using a 2mm sieve. Samples (1g) were weighed into polycarbonate centrifuge tubes. Samples were mixed with 20 mL 0.02 M KMnO4, that was adjusted to pH 7.2. Samples were then shaken at 200 rpm for 2 min, followed by centrifugation for 5 min at 3000 rpm. Soil particles were separated from the solution and a 0.20 mL sample of the solution was then diluted with 10.0 mL of DI water in another polycarbonate centrifuge tube. The absorbance of the solution was measured at 550 nm with a Hitachi U-1100 Spectrophotometer. Active C was determined with the following equation:

Equation 2.1

Equation for Active C

Active C (mg/kg) (*or the dependent Y variable*) = [0.02 mol/L – (m * absorbance (x) + b)] * (9000 mg C/mol) * (0.02 L solution/0.0025 kg soil)

Where –

0.02 mol/L =initial concentration of solution

(m * absorbance (x) + b) = the post reaction concentration

9000 mg C (or 0.75 mol) is assumed to be oxidized when 1 mol MnO4 changes from Mn_{7+} Mn_{2+}

0.02 L = volume of reacted $KMnO_4$ solution

0.0025 kg = weight of soil samples used in reaction

Soil Respiration

The method developed by Schindelbeck et al. (2016), was used. In short, 20 g of sieved air-dried soil, was placed in a perforated, aluminum weigh boat, and set on top of two filter papers (VWR 413). An alkali trap containing 9 mL 0.5 M KOH was placed in a glass container that rests on a plastic stand that sits above the soil. The entire set-up from the filter paper to the 9 mL of 0.5 M KOH was inside of a mason jar. Water (7.5 mL) was added to the bottom of the jar, the filter papers allow the water to maintain contact with the perforated bottom of the aluminum weigh boat that contains the soil. Once the water and KOH were placed within each jar, the jar was sealed and incubated at room temperature for four days. During this incubation time, CO_2 respired from the rewetted soil in response to microbial activity within the soil, and the CO_2 was captured in the KOH trap above the soil. A completely saturated KOH solution would occur at 0.25 M K_2CO_3. The electrical conductivity (EC) difference between 0.5 M KOH and 0.25 M

K_2CO_3 is used to determine the amount of CO_2 that was respired and absorbed (Schindelbeck et al., 2016); based on the following equations:

Equation 2.2
Soil respiration calculation

((ECraw – ECsample)/(ECraw – ECsat)) = P

P* (99.025 mg CO2 (trap capacity)) = mg CO2 absorbed by trap

Where ECraw = EC of 0.5 M KOH, ECsample = EC reading of sample, ECsat = EC of 0.25 M K2CO3

P = used proportion of trap

Enzyme Activity

To access the potential activity of β – glucosidase and β D– glucosaminidase enzymes, methods by Eivazi and Tabatabai (1988) and Parham and Deng (2000) were used respectively. This methodology allowed potential enzyme activity to be assessed via colorimetric determination when p-nitrophenol was released after soil was incubated with the respective p¬nitrophenol substrates at a pH found to be optimal for a given enzymatic reaction (Parham and Deng, 2000).

Three 0.5 g subsamples were weighed into 20 mL glass vials labelled A, B, and C. 2 mL of the start buffer was then added to each vial and 0.5 mL of the substrate was added to vials A and B. Vials A, B, and C were then incubated at 37°C for 1 hr. The reaction was terminated by adding 0.5 mL $CaCl_2$ and 2 mL of stop buffer to vials A, B, and C. Substrate (0.5 mL) was then added to vial C. The solution in each vial was then filtered to remove soil particles using 12.5 cm diameter cellulose filter paper within a 2 μm pore size (Ahlstrom 642) for 30 min. Samples were then diluted if necessary, to achieve an absorbance of ≤ 1.3 and analyzed using a spectrophotometer (U-1000, Hitachi High-Tech Corporation, Tokyo, Japan) at 400 nm to

quantify p-nitrophenol (PNP). The enzyme activity was measured as mg PNP per kg soil per hour. In respect to the buffers and substrates used, for the β – glucosaminidase assay the start buffer was 0.1M acetate buffer, and the substrate was 0.01M p-nitrophenyl-N-acetyl-β-D-glucosaminidase, and the reaction was terminated with 0.5M NaOH (stop buffer). For the β – glucosidase assay the start buffer used is the modified universal buffer at pH 6, the substrate used was 0.05M p-Nitrophenyl-β-D-glucopyranoside, and the reaction was terminated with 0.1M THAM (stop buffer).

Statistical Analysis

Statistical analysis was performed using SAS version 9.4 software (Cary, NC, U.S.A.) with a PROC MIXED procedure. The cover crop treatments and the P fertilizer treatments were fixed effects and the blocking factor was random. Analysis of variance (ANOVA) and Tukey-adjusted LSMEANS were used to indicate differences between treatments. The significance threshold used was $p > 0.05$.

Results

Total C and Total N

Fall 2018 total C was affected by a significant interaction between cover crop treatment and fertilizer management (Table 2.2). In this interaction both SI*CC and FB*CC > NP*NC, FB*NC, SI*NC, and NP*CC was statistically equal to all treatment interactions. In spring 2018, fall 2018, and spring 2019 plots with a cover crop treatment were found to be significantly higher in total C than those without the cover crop treatment (Table 2.3). In spring 2019 P fertilizer treatment was found to significantly affect total soil C, with FB = SI > NP. Total N was not found to be significantly different in any treatment interaction nor main effects in any of the tested samples (Table 2.2).

Microbial Biomass C and N, Dissolved Organic C, Dissolved Organic N, and Inorganic N

Microbial biomass carbon was found to be significantly higher in plots with the cover crop treatment as opposed to plots without cover crop treatment in both spring 2018 and spring 2019 (Table 2.3). In fall 2018 and fall 2019, no significance was detected between cover crop treatments (Table 2.3). No significance was detected between P-fertilizer treatments in any season sampled. No significant interaction was detected between cover crop treatments and P-fertilizer treatments in any of the sampled seasons.

Microbial biomass N was not found to be significantly different between cover crop treatments in any sampled seasons. There was also no significance detected in microbial biomass N among P-fertilizer treatments. No significant interaction effect was detected in microbial biomass N between cover crop and P-fertilizer treatments. The ratio of MBC to MBN was also examined, and no significant differences were found.

In spring 2018 the interaction between the fertilizer treatments and cover crop treatments was found to be significant for dissolved organic C (DOC) with: FB*NC > SI*NC > NP*CC, FB*CC, SI*CC, and NP*NC. No other interactions nor main effects were found to be significant for DOC.

In spring 2018 dissolved organic N (DON) was significantly lower in the CC treatment as compared to the NC treatment. No other interactions nor main effects were found to be significant for DON (Table 2.2).

In spring 2018 CC treatment was found to have less soil nitrate (NO_3-N) than plots with NC treatment (Table 2.2). No other treatment interactions nor main effects were found for NO_3-N. Soil ammonium (NH_4-N) was found to be significantly lower in CC treatment as compared to

the NC treatment in spring 2018. In spring 2018 and spring 2019 P fertilizer treatments were found to have a significant impact on NH_4-N with FB and SI > NP. In fall 2019 NH_4-N was found to be significantly impacted by P fertilizer treatments with SI > FB, and NP = SI and NP.

Active C

Active C was found to be significantly higher in the CC treatment as compared to the NC treatment in spring 2018 and spring 2019; no other treatments nor treatment interactions were found to be significant (Table 2.2 and Table 2.3).

Soil Respiration

In spring 2018 the CC treatment had higher soil respiration than the NC treatment. No other interactions nor main effects were found to be significant for soil respiration assays (Table 2.2).

Enzyme Activity

In spring 2019 in β-glucosidase enzyme activity assays interactions between the P fertilizer treatments and the cover crop treatments were found to be significant with FB*CC, NP*CC > SI*CC > FB*NC, NP*NC, and SI*NC = SI*CC, FB*NC, NP*NC. Higher β-glucosidase enzyme activity was found in CC treatment as compared to the NC treatment in spring 2018, fall 2018, and spring 2019 (Fig. 2.1 and Table 2.3)). Higher β- glucosaminidase enzyme activity was found in CC treatment as compared to the NC treatment in spring 2018, fall 2018, spring 2019, and fall 2019 (Fig. 2.2 and Table 2.3). In spring 2018 β-glucosaminidase enzyme activity was significantly impacted by P fertilizer treatments with FB > NP > SI (Table 2.2).

Discussion

Total C and N

Total carbon was found to be significantly higher in the CC treatment as compared to the NC treatment in spring 2018, fall 2018, and spring 2019. Higher total carbon in the CC treatment, regardless of tillage or no-tillage management practices was consistent with several meta-analysis studies (McDaniel et al., 2014; Kim et al., 2020) This result was also consistent with other literature showing that cover crop usage increases soil organic matter (Dabney et al., 2001). Cereal cover crops, which were used in this study have been shown to increase soil organic matter (Snapp et al., 2005; Lehman et al., 2015).

When examining total nitrogen however, there were no significant main effects nor interactions. This contrasted with literature that demonstrates an increase in total N with cover crop implementation (McDaniel et al., 2014; Kim et al., 2020; Snapp et al., 2005). Recalcitrant nutrient pools can take more time to be impacted by management practices (Ghimire et al., 2015; Awale et al., 2017); however, total C did detect differences at some sampling points.

Microbial Biomass C and N, Dissolved Organic C, Dissolved Organic N, and Inorganic N

Microbial biomass C was significantly higher in the CC treatment as compared to the NC treatment in both spring 2018 and spring 2019. This is consistent with literature examining the impact of cover crop usage on MBC (Dabney et al., 2001; Strickland et al., 2019). This impact has been found to be especially pronounced in systems where cover crops are used annually (Brennan and Acosta-Martinez, 2017). The finding of MBC being present at significantly higher levels in the CC treatment is a similar trend to findings with active C. This trend was also observed in spring 2018 in soil respiration.

Temperature coupled with moisture content are the most important environmental factors impacting microbial growth and activity in soils (Paul and Clark, 1996). Temperature is known to impact microbial growth and work by Pietikainen et al. (2005) found that the optimum soil temperature (highest growth rate) for both fungi and bacteria was from 25-30°C when comparing forest and agricultural soils. Labile compounds are cycled more rapidly in microorganisms with increasing temperatures as compared to more recalcitrant ones (Davidson and Janssens, 2006). Microorganisms typically prefer to meet metabolism needs with simpler compounds over those with greater complexity (Bosatta and Agren, 1999; Davidson and Janssens, 2006). Increased temperatures increase substrate cycling and they also increase the microbial need for other resources such as water (ArchMiller and Samuelson, 2016). These factors may serve as an explanation for the difference between spring and fall active C and MBC findings; however, this connection is difficult given there was no difference in soil moisture from soil samples (data not shown) and temperatures at the time of sampling were similar (Table 2.2).

It is also possible there was greater substrate availability at the spring sampling points, when there was living cover crop in the CC treatment, than at the fall sampling points, when there was no living plant material in either the CC nor NC treatments. This greater substrate availability could be related to root exudates that stimulated soil microbial proliferation and activity. Work by Dabney et al. (2001) and Strickland et al. (2019) also finding higher levels of MBC in CC treatments as compared to the NC treatment performed the timing of sampling similar to this study's spring sampling point occurring prior to cover crop termination.

Microbial biomass N as well as the ratio of MBC/N was not significantly impacted by examined treatments. Although literature has found that cover crops increase MBC (Dabney et al., 2001; Strickland et al., 2020), there is other literature that has not observed this and found

that under no-tillage management practices cover crop usage has been found to have a limited impact on soil microbial biomass C and N (Strickland et al., 2019; Liebig et al., 2015; Mbuthia et al., 2015; Acosta-Martinez et al., 2011). Soil microbial biomass has been found to be highly variable in response to environmental factors, management practices, soil and crop types (Carter et al., 1999; Gonzalez-Quinones et al., 2011). Brennan and Acosta-Martinez (2017) found a weaker correlation in changes to agricultural management practices that included cover crop implementation with MBN than MBC.

Treatment effects on DOC and DON were only found to be significant in spring 2018, with DOC being significantly impacted by interaction of treatments and DON being significantly lower in the CC treatment. Observing a singular point of significance for DOC and DON makes it more challenging to understand any trends from these dynamics. Other literature has also found DOC and DON to not detect any difference between CC and NC treatments in no-till on-farm research with soy-corn cropping systems in Virginia (Strickland et al., 2019). The work by Strickland et al. (2019) is unique in that, like this study, it examined the use of cover crops compared to no cover crops with no-till management in corn-soybean systems at field scale.

When examining soil inorganic nitrogen in this study, soil extractable nitrate-N was only found to be significant in spring 2018 and soil extractable ammonium-N was found to be significant in spring 2018, spring 2019, and fall 2019 under P fertilizer treatment. In both spring 2018 and spring 2019, FB and SI treatments were not statistically different and each significantly higher than the NP treatment. Soil extractable ammonium-N was also found to be higher in plots with the cover crop treatment as opposed to those without in spring 2018. Work by Strickland et al. (2019) showed that cover cropping with no-till management in corn-soybean systems can

influence soil N pools. Strickland et al. (2019) observed increased soil extractable ammonium-N and decreased soil extractable nitrate-N in plots with cover crops.

Active C

Labile carbon was higher in plots with a cover crop as compared to plots without a cover crop in both the sampled spring seasons, with no significant difference observed between the sampled fall seasons. This alteration of active C in seasons has been observed by other authors and this difference has been attributed to differences between seasons in temperature, water content, and substrate availability (Schutter and Dick, 2002; Franzluebbers et al., 1995; Sainju et al., 2007).

Soil Respiration

Soil respiration was only found to be significant in spring 2018, with higher respiration found in the CC treatment. A meta-analysis examining the interaction between cover cropping effects on the soil microbiome examined laboratory soil respiration and found that overall cover crops did increase soil respiration rates as well as other indicators of soil microbiome activity (Kim et al., 2020). Kim et al. (2020) also found that the indicators of increased soil microbiome activity observed with cover crop usage were less pronounced under certain conditions including continental climate, chemical cover crop termination, and conservation tillage. This study utilized chemical termination of cover crops (sampling occurred before chemical termination) and in the meta-analysis by Kim et al. (2020) studies that utilized chemical termination of cover crops as opposed to mechanical termination showed a smaller impact with cover crop implementation on soil health parameters. This may at least partially explain why soil respiration was typically not found to be significantly impacted by the cover crop treatment. Some studies have suggested that increased soil respiration with the use of cover crops occurs only when the

cover crops are also increasing the soil organic carbon (Liebig et al., 2010; Kim et al., 2012; Haque et al., 2014); these studies all implemented in situ field chambers. This contrasts with this current study as, the CC treatment was found to have significantly higher total C than plots that received no cover crop treatment. Because this study used laboratory soil respiration it focuses on microbial activity and does not include as other studies (Liebig et al., 2010; Kim et al., 2012; Haque et al., 2014) potential plant root respiration. It may be that in studies using in situ field measurements the microbial respiration is sometime drowned out by the presence of actively growing plants.

Enzyme Activity

β – glucosidase is an extracellular enzyme that degrades cellulose. In soils cellulose can originate from bacteria; however, most cellulose originates from plant material (Richmond, 1991). Plant cellulose typically exists within a matrix of hemicellulose and lignin polymers and contributes to cell wall structure. β – glucosaminidase is one type of glycosidase that can degrade chitin. Chitin is a structural component of fungal cell walls and insect and arthropod exoskeletons, but is not found in plants. Chitin is very similar to cellulose, differing only by a different substitution at C-2 of D-glucose. Both β – glucosaminidase and β – glucosidase have been shown to be positively correlated with organic C in soil profiles (Eivazi and Tabatabai, 1990).

β-glucosidase activity was found to be significantly higher in plots with the cover crop treatment as compared to those without the cover crop treatment for all seasons sampled except fall 2019 and β-glucosaminidase activity was also found to be significantly higher in plots with the cover crop treatment for all seasons sampled. Some studies have found little impact of cover crop implementation on enzyme activity in the short-term (less than 3 yrs) (Rankoth et al., 2019;

Calderon, Nielsen, Acosta-Martinez, Vigil, & Lyon, 2016; Acosta-Martinez et al., 2011). However, Rankoth et al. (2019) suggest that beyond the short-term enzyme activity would increase under no-tillage management with cover crop implementation as compared to no cover crop usage. The Acosta-Martinez et al. (2011) study found an increase in β-glucosidase and β-glucosaminidase activity at three years after cover crop implementation; however, they found at 5 yrs no detectable difference between cover crop and no cover crop usage in no-tillage systems. No-tillage management and cover crops were first implemented in this study in 2015, and although the cover crop treatment resulted in a significantly higher amount of β-glucosidase and β-glucosaminidase activity, this trend may diminish over time.

Conclusions

This study demonstrated that soil health metrics can have varying levels of response to cover crop implementation in a no-till corn-soybean rotation field scale experiment. The first hypothesis that cover crop implementation would increase total organic carbon, active C, DOC, DON, and inorganic N was found in some of these examined nutrient pools but not all. Total organic C was greater in the CC treatment in spring 2018, fall 2018, and spring 2019, but not in fall 2019. Active C was higher in the CC treatment in spring 2018 and spring 2019. Dissolved organic C, DON, and inorganic N were only significantly influenced by the cover crop treatments in spring 2018. The second hypothesis that assays examining microbial biomass and activity indicators (respiration and enzyme activity assays) would increase within cover crops was not entirely in-line with the results of this study. Soil respiration was only greater in the CC treatment in spring 2018. Both β-glucosidase and β-glucosaminidase enzyme activities showed significantly higher levels of activity across seasons sampled, except for fall 2019 which was not significantly different between cover crop treatments for β-glucosidase. This could indicate that

β-glucosaminidase is more reliable in detecting enzyme activity in response to cover crop treatments; however, with only one data point of not observing this trend in β-glucosidase this is not definitive. Microbial biomass C followed a similar trend to active C findings, while MBN was not found to be impacted by any treatments. The third hypothesis that P fertilizer management strategies would have little direct impact on C and N soil health metrics in the presence or absence of cover crops was predominately found with the exceptions of β-glucosaminidase in spring 2018, and NH_4-N in spring 2018, spring 2019, and fall 2019. This study found that assays examining total C, labile C pools, and microbial biomass C and enzyme activity were more successful in detecting cover crop implementation as compared to assays that targeted N pools including total N, labile N, and microbial biomass.

References

Acosta-Martínez, V., Lascano, R., Calderón, F., Booker, J. D., Zobeck, T. M., & Upchurch, D. R. (2011). Dryland cropping systems influence the microbial biomass and enzyme activities in a semiarid sandy soil. *Biology and Fertility of Soils*, *47*(6), 655–667. https://doi.org/10.1007/s00374-011-0565-1

ArchMiller, A. A., & Samuelson, L. J. (2016). Intra-annual variation of soil respiration across four heterogeneous longleaf pine forests in the southeastern United States. *Forest Ecology and Management*, *359*, 370–380. https://doi.org/10.1016/j.foreco.2015.05.016

Awale, R., Emeson, M. A., & Machado, S. (2017). Soil organic carbon pools as early indicators for soil organic matter stock changes under different tillage practices in Inland Pacific Northwest. *Frontiers in Ecology and Evolution*, *5*(AUG). https://doi.org/10.3389/fevo.2017.00096

Balota, E. L., Calegari, A., Nakatani, A. S., & Coyne, M. S. (2014). Benefits of winter cover crops and no-tillage for microbial parameters in a Brazilian Oxisol: A long-term study. *Agriculture, Ecosystems and Environment*, *197*, 31–40. https://doi.org/10.1016/j.agee.2014.07.010

Blair, G. J., Blair, N., Lefroy, R. B. D., Conteh, A., Daniel, H., Lefroy, A., & Conteh. (1997). Relationships between KMNO4 oxidizable c and soil aggregate stability and the derivation of a carbon management index. In J. Drozd, S. S. Gonet, N. Senesi, & J. Weber (Eds.), *The Role of Humic Substances in the Ecosystem and in Environmental Protection. Proceedings 8th Meeting of the International Humic Substances Society Polish Society of Humic Substances* (pp. 227–232). Grunwaldzka, Wroclaw, Poland.

Blair, G., Lefroy, R., Whitbread, A., Blair, N., & Conteh, A. (2000). *The Development of the KMn0 4 Oxidation Technique to Determine Labile Carbon in Soil and Its Use in a Carbon Management Index.*

Blevins, R. L., Frye, W. W., Baldwin, P. L., & Robertson, S. D. (n.d.). *(1990) Tillage Effects on Sediment and Soluble Nutrient Losses from a Maury Silt Loam Soil*.

Bongiorno, G., Bünemann, E. K., Oguejiofor, C. U., Meier, J., Gort, G., Comans, R., … de Goede, R. (2019). Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. *Ecological Indicators*, *99*, 38–50. https://doi.org/10.1016/j.ecolind.2018.12.008

Bosatta, E., & Ågren, G. I. (1999). Soil organic matter quality interpreted thermodynamically. *Soil Biology and Biochemistry*, *31*(13), 1889–1891. https://doi.org/https://doi.org/10.1016/S0038-0717(99)00105-4

Brennan, E. B., & Acosta-Martinez, V. (2017). Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production. *Soil Biology and Biochemistry*, *109*, 188–204. https://doi.org/10.1016/j.soilbio.2017.01.014

Brennan, E. B., & Acosta-Martinez, V. (2019). Cover Crops and Compost Influence Soil Enzymes during Six Years of Tillage-Intensive, Organic Vegetable Production. *Soil Science Society of America Journal*, *83*(3), 624–637. https://doi.org/10.2136/sssaj2017.12.0412

Brookes, P. C., Landman, A., Pruden+, G., & Jenkinson, D. S. (1985). Chloroform Fumigation and the Release of Soil Nitrogen: A Rapid Direct Extraction Method to Measure Microbial Biomass Nitrogen in Soil. In *Journal Issue: 6 Journal Month* (Vol. 17).

Cabrera, M. L., & Beare, M. H. (n.d.). *(1993) Alkaline Persulfate Oxidation for Determining Total Nitrogen in Microbial Biomass Extracts*.

Calderón, F. J., Nielsen, D., Acosta-Martínez, V., Vigil, M. F., & Lyon, D. (2016). Cover Crop and Irrigation Effects on Soil Microbial Communities and Enzymes in Semiarid Agroecosystems of the Central Great Plains of North America. *Pedosphere*, *26*(2), 192–205. https://doi.org/10.1016/S1002-0160(15)60034-0

Carter, M. R., Gregorich, E. G., Angers, D. A., Beare, M. H., Sparling, G. P., Wardle, D. A., … Voroney, R. P. 1999. (1999). Interpretation of microbial biomass measurements for soil quality assessment in humid temperate regions. *Canadian Journal of Soil Science*, *79*(4), 507–520.

Chichester, F. W., & Richardson, C. W. (1992). Sediment and Nutrient Loss from Clay Soils as Affected by Tillage. *Journal of Environmental Quality*, *21*(4), 587–590. https://doi.org/10.2134/jeq1992.00472425002100040010x

Combs, S. M., & Nathan, M. V. (1998). Soil organic matter. *Recommended Chemical Soil Test Procedures for the North Central Region*, (SB 1001), 53–58.

Cookson, W. R., Murphy, D. V., & Roper, M. M. (2008). Characterizing the Relationships Between Soil Organic Matter Components and Microbial Function and Composition Along a Tillage Disturbance Gradient. *Soil Biology and Biochemistry*, *40*(3), 763–777. https://doi.org/10.1016/j.soilbio.2007.10.011

Culman, S. W., Snapp, S. S., Freeman, M. A., Schipanski, M. E., Beniston, J., Lal, R., … Wander, M. M. (2012). Permanganate Oxidizable Carbon Reflects a Processed Soil Fraction that is Sensitive to Management. *Soil Science Society of America Journal*, *76*(2), 494–504. https://doi.org/10.2136/sssaj2011.0286

Dabney, S. M., Delgado, J. A., & Reeves, D. W. (2001). Using Winter Cover Crops to Improve Soil and Water Quality. In *Commun. Soil Sci. Plant Anal* (Vol. 32). Retrieved from www.dekker.com

Dalal, R. C., & Chan, K. Y. (2001). Soil organic matter in rainfed cropping systems of the Australian cereal belt. *Australian Journal of Soil Research*, Vol. 39, pp. 435–464. https://doi.org/10.1071/SR99042

Dalal, R. C., Allen, D. E., Chan, K. Y., & Singh, B. P. (2011). *Soil Organic Matter, Soil Health and Climate Change*. https://doi.org/10.1007/978-3-642-20256-8_5

Davidson, E. A., & Janssens, I. A. (2006, March 9). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, Vol. 440, pp. 165–173. https://doi.org/10.1038/nature04514

Dick, R. P., Breakwell, D. P., & Turco, R. F. (1997). *Soil Enzyme Activities and Biodiversity Measurements as Integrative Microbiological Indicators*. https://doi.org/10.2136/sssaspecpub49.c15

Dilly, O., & Nannipieri, P. (2001). Response of ATP content, respiration rate and enzyme activities in an arable and a forest soil to nutrient additions. *Biology and Fertility of Soils*, *34*(1), 64–72. https://doi.org/10.1007/s003740100375

Doran, J. W., & Parkin, T. B. (1997). Quantitative Indicators of Soil Quality: A Minimum Data Set. In J. W. Doran & A. J. Jones (Eds.), *Methods for Assessing Soil Quality* (pp. 25–37). Madison, WI: Soil Science Society of America.

Eivazi, F., & Tabatabai, M. A. (1988). Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*, *20*(5), 601–606. https://doi.org/10.1016/0038-0717(88)90141-1

FAO. (2019). Soil degradation. Retrieved from http://www.fao.org/soils-portal/soil-degradation-restoration/en/

Ferraz-Almeida, R., Pinheiro Da Mota, R., Ferraz De Almeida, R., & Rezende Naves, E. (2015). *Soil quality: Enzymatic activity of soil β-glucosidase Uso de Nitrogênio na Agricutlura Brasileira View project Global Journal of Agricultural Research and Reviews Soil quality: Enzymatic activity of soil β-glucosidase*. *3*(2), 146–150. Retrieved from http://www.globalscienceresearchjournals.org/

Finney, D. M., Buyer, J. S., & Kaye, J. P. (2017). Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation*, *72*(4), 361–373. https://doi.org/10.2489/jswc.72.4.361

Franzluebbers, A. J., Hons, F. M., & Zuberer, D. A. (1995). Soil Organic Carbon, Microbial Biomass, and Mineralizable Carbon and Nitrogen in Sorghum. *Soil Science Society of America Journal*, *59*(2), 460–466. https://doi.org/10.2136/sssaj1995.03615995005900020027x

Gelderman, R. H., & Beegle, D. (1998). Recommended Chemical Soil Test Procedures for the North Central Region. In *Recommended chemical soil test procedures for the North Central*

region. University of Missouri (pp. 17–20). Columbia, MO: Agricultural Experiment Station.

Ghimire, R., Machado, S., & Rhinhart, K. (2015). Long-term crop residue and nitrogen management effects on soil profile carbon and nitrogen in wheat–fallow systems. *Agronomy Journal*, *107*(6), 2230–2240. https://doi.org/10.2134/agronj14.0601

Gonzalez-Quiñones, V., Stockdale, E. A., Banning, N. C., Hoyle, F. C., Sawada, Y., Wherrett, A. D., … Murphy, D. V. (2011). Soil microbial biomass—Interpretation and consideration for soil monitoring. *Soil Research*, *49*(4), 287–304. Retrieved from https://doi.org/10.1071/SR10203

Gregorich, E. G., Carter, M. R., Angers, D. A., Monreal, C. M., & Ellert, B. H. (1994). Towards a minimum data set to assess soil organic matter quality in agricultural soils. *Canadian Journal of Soil Science*, *74*(4), 367–385. https://doi.org/10.4141/cjss94-051

Haque, M. M., Kim, S. Y., Ali, M. A., & Kim, P. J. (2014). Contribution of greenhouse gas emissions during cropping and fallow seasons on total global warming potential in mono-rice paddy soils. *Plant and Soil*, *387*(1–2), 251–264. https://doi.org/10.1007/s11104-014-2287-2

Haque, M. M., Kim, S. Y., Ali, M. A., & Kim, P. J. (2019). Contribution of greenhouse gas emissions during cropping and fallow seasons on total global warming potential in mono-rice paddy soils. *Plant and Soil*, *387*(1–2), 251–264. https://doi.org/10.1007/s11104-014-2287-2

Haynes, R. (2005). *Labile Organic Matter Fractions as Central Components of the Quality of Agricultural Soils: An Overview*.

Jenkinson, D. S., & Powlson, D. S. (1976). The effects of biocidal treatments on metabolism in soil—V: A method for measuring soil biomass. *Soil Biology and Biochemistry*, *8*(3), 209–213. https://doi.org/10.1016/0038-0717(76)90005-5

Jones, D. L., & Willett, V. B. (2006). Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry*, *38*(5), 991–999. https://doi.org/10.1016/j.soilbio.2005.08.012

Karlen, D. L., Mausbach, M. J., Doran, J. W., Cline, R. G., Harris, R. F., & Schuman, G. E. (1997). Soil Quality: A Concept, Definition, and Framework for Evaluation (A Guest Editorial). *Soil Science Society of America Journal*, *61*(1), 4–10. https://doi.org/10.2136/sssaj1997.03615995006100010001x

Keeney, D. R., & Nelson, D. W. (1982). Nitrogen - Inorganic Forms. In *Methods of Soil Analysis: Part 2* (pp. 643–698). Madison, WI: ASA, SSSA.

Kim, N., Zabaloy, M. C., Guan, K., & Villamil, M. B. (2020). Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biology and Biochemistry*, *142*. https://doi.org/10.1016/j.soilbio.2019.107701

Kim, S. Y., Gutierrez, J., & Kim, P. J. (2012). Considering winter cover crop selection as green manure to control methane emission during rice cultivation in paddy soil. *Agriculture, Ecosystems and Environment*, *161*, 130–136. https://doi.org/10.1016/j.agee.2012.07.026

Koller, R., Rodriguez, A., Robin, C., Scheu, S., & Bonkowski, M. (2013). Protozoa enhance foraging efficiency of arbuscular mycorrhizal fungi for mineral nitrogen from organic matter in soil to the benefit of host plants. *New Phytologist*, *199*(1), 203–211. https://doi.org/10.1111/nph.12249

Lehman, R. M., Acosta-Martinez, V., Buyer, J. S., Cambardella, C. A., Collins, H. P., Ducey, T. F., … Stott, D. E. (2015, January 1). Soil biology for resilient, healthy soil. *Journal of Soil and Water Conservation*, Vol. 70, pp. 12A-18A. https://doi.org/10.2489/jswc.70.1.12A

Li, L., Xu, M., Ali, M. E., Zhang, W., Duan, Y., & Li, D. (2018). Factors affecting soil microbial biomass and functional diversity with the application of organic amendments in three contrasting cropland soils during a field experiment. *PLoS ONE*, *13*(9). https://doi.org/10.1371/journal.pone.0203812

Liebig, M. A., Hendrickson, J. R., Archer, D. W., Schmer, M. A., Nichols, K. A., & Tanaka, D. L. (2015). Short-term soil responses to late-seeded cover crops in a semi-arid environment. *Agronomy Journal*, *107*(6), 2011–2019. https://doi.org/10.2134/agronj15.0146

Liebig, M. A., Tanaka, D. L., & Gross, J. R. (2010). Fallow Effects on Soil Carbon and Greenhouse Gas Flux in Central North Dakota. *Soil Science Society of America Journal*, *74*(2), 358–365. https://doi.org/10.2136/sssaj2008.0368

Maul, J. E. (2007). *The Effects of Plant Traits on Soil Ecology and Biogeochemistry in Agroecosystems* (Ph. D. thesis). Ithica, NY: Cornell Univ.

Mbuthia, L. W., Acosta-Martínez, V., DeBryun, J., Schaeffer, S., Tyler, D., Odoi, E., … Eash, N. (2015). Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. *Soil Biology and Biochemistry*, *89*, 24–34. https://doi.org/10.1016/j.soilbio.2015.06.016

Mcdaniel, M. D., Tiemann, L. K., & Grandy, A. S. (2014). Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. In *Ecological Applications* (Vol. 24). Retrieved from http://www.metawinsoft.com/

McNamara, J. T., Morgan, J. L. W., & Zimmer, J. (2015, June 2). A molecular description of cellulose biosynthesis. *Annual Review of Biochemistry*, Vol. 84, pp. 895–921. https://doi.org/10.1146/annurev-biochem-060614-033930

Nair, A., & Ngouajio, M. (2012). Soil microbial biomass, functional microbial diversity, and nematode community structure as affected by cover crops and compost in an organic vegetable production system. *Applied Soil Ecology*, *58*, 45–55. https://doi.org/10.1016/j.apsoil.2012.03.008

NRI (National Resources Institute). (2003). *2003 Annual NRI Soil Erosion*. Washington D.C.

Parham, J. A., & Deng, S. P. (2000). Detection, quantification and characterization of β-glucosaminidase activity in soil. *Soil Biology and Biochemistry*, *32*(8–9), 1183–1190. https://doi.org/10.1016/S0038-0717(00)00034-1

Paul, E. A., & Clark, F. E. (1996). *Soil Microbiology and Biochemistry* (second ed.). Academic Press, USA.

Pengthamkeerati, P., Motavalli, P. P., & Kremer, R. J. (2011). Soil microbial biomass nitrogen and β-glucosaminidase activity response to surface compaction and poultry-litter application in a claypan soil. *Applied Soil Ecology*, *51*(1), 79–86. https://doi.org/10.1016/j.apsoil.2011.08.013

Pietikäinen, J., Pettersson, M., & Bååth, E. (2005). Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiology Ecology*, *52*(1), 49–58. https://doi.org/10.1016/j.femsec.2004.10.002

Pietikäinen, J., Pettersson, M., & Bååth, E. (2005). Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiology Ecology*, *52*(1), 49–58. https://doi.org/10.1016/j.femsec.2004.10.002

Qualls, R. G., & Haines, B. L. (1992). Biodegradability of Dissolved Organic Matter in Forest Throughfall, Soil Solution, and Stream Water. *Soil Science Society of America Journal*, *56*(2), 578–586. https://doi.org/10.2136/sssaj1992.03615995005600020038x

Rankoth, L. M., Udawatta, R. P., Veum, K. S., Jose, S., & Alagele, S. (2019). Cover crop influence on soil enzymes and selected chemical parameters for a claypan corn-soybean rotation. *Agriculture (Switzerland)*, *9*(6). https://doi.org/10.3390/agriculture9060125

Roberson, E. B., Sarig, S., Shennan, C., & Firestone, M. K. (1995). Nutritional Management of Microbial Polysaccharide Production and Aggregation in Agricultural Soil. *Soil Sci. Am.*, *59*, 1587–1594.

Romig, D. E., Garylynd, M. J., Harris, R. F., & Mcsweeney, K. (n.d.). How farmers assess soil health and quality. In *From: Journal of Soil and Water Conservation* (Vol. 50).

Sainju, U. M., Schomberg, H. H., Singh, B. P., Whitehead, W. F., Tillman, P. G., & Lachnicht-Weyers, S. L. (2007). Cover crop effect on soil carbon fractions under conservation tillage cotton. *Soil and Tillage Research*, *96*(1–2), 205–218. https://doi.org/10.1016/j.still.2007.06.006

Sapkota, T. B., Mazzoncini, M., Bàrberi, P., Antichi, D., & Silvestri, N. (2012). Fifteen years of no till increase soil organic matter, microbial biomass and arthropod diversity in cover crop-based arable cropping systems. *Agronomy for Sustainable Development*, *32*(4), 853–863. https://doi.org/10.1007/s13593-011-0079-0

Schindelbeck, R.R., Moebius-Clune, B. N., Moebius-Clune, D. J., Kurtz, K. S., & and H.M. van Es. (2016). *Cornell Soil Health Laboratory Comprehensive Assessment of Soil Health Standard Operating Procedures*. Retrieved from http://soilhealth.cals.cornell.edu.

Schutter, M. E., & Dick, R. P. (2002). Microbial Community Profiles and Activities among Aggregates of Winter Fallow and Cover-Cropped Soil. *Soil Science Society of America Journal*, *66*(1), 142. https://doi.org/10.2136/sssaj2002.0142

Sequeira, C. H., & Alley, M. M. (2011). Soil Organic Matter Fractions as Indices of Soil Quality Changes. *Soil Science Society of America Journal*, *75*(5), 1766–1773. https://doi.org/10.2136/sssaj2011.0067

Snapp, S. S., Swinton, S. M., Labarta, R., Mutch, D., Black, J. R., Leep, R., … O'Neil, K. (2005). Evaluating Cover Crops for Benefits, Costs and Performance within Cropping System Niches. *Agronomy Journal*, *97*(1), 322–332. https://doi.org/10.2134/agronj2005.0322a

Spedding, T. A., Hamel, C., Mehuys, G. R., & Madramootoo, C. A. (2004). Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. *Soil Biology and Biochemistry*, *36*(3), 499–512. https://doi.org/10.1016/j.soilbio.2003.10.026

Stavi, I., & Lal, R. (2015). Achieving Zero Net Land Degradation: Challenges and opportunities. *Journal of Arid Environments*, *112*(PA), 44–51. https://doi.org/10.1016/j.jaridenv.2014.01.016

Steenwerth, K., & Belina, K. M. (2008). Cover crops enhance soil organic matter, carbon dynamics and microbiological function in a vineyard agroecosystem. *Applied Soil Ecology*, *40*(2), 359–369. https://doi.org/10.1016/j.apsoil.2008.06.006

Strickland, M. S., Thomason, W. E., Avera, B., Franklin, J., Minick, K., Yamada, S., & Badgley, B. D. (2019). Short-Term Effects of Cover Crops on Soil Microbial Characteristics and Biogeochemical Processes across Actively Managed Farms. *Agrosystems, Geosciences & Environment*, *2*(1), 1–9. https://doi.org/10.2134/age2018.12.0064

Surucu, A., Sürücü, A., Arif Özyazici, M., Bayrakli, B., & Kizilkaya, R. (2014). Effects of Green Manuring on Soil Enzyme Activity. *Fresenius Environmental Bulletic*, *23*(9). Retrieved from https://www.researchgate.net/publication/279332437

Tabatabai, M. A. (1994). *Soil Enzymes*. https://doi.org/10.2136/sssabookser5.2.c37

USDA NRCS. (2011). *Carbon to Nitrogen Ratios in Cropping Systems*. Retrieved from http://www.nm.nrcs.usda.gov/Technical/tech-notes/agro/

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, *19*(6), 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Wander, M. M., & Drinkwater, L. E. (2000). Fostering Soil Stewardship through Soil Quality Assessment. *Applied Soil Ecology*, *15*(1), 61–73.

Weil, R. R., Islam, K. R., Stine, M. A., Gruver, J. B., & Samson-Liebig, S. E. (2003). Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. *Article in American Journal of Alternative Agriculture*. https://doi.org/10.1079/AJAA200228

Wright, A. F., & Bailey, J. S. (2001). Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 dry combustion analyzer. *Communications in Soil Science and Plant Analysis*, *32*(19–20), 3243–3258. https://doi.org/10.1081/CSS-120001118

Zhang, L., Chen, X., Xu, Y., Jin, M., Ye, X., Gao, H., … Thompson, M. L. (2020). Soil labile organic carbon fractions and soil enzyme activities after 10 years of continuous fertilization and wheat residue incorporation. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-68163-3

Table 2.1

Monthly temperature averages, maximum, and minimums and precipitation amounts for 2018 and 2019 at the Kansas Agricultural Watershed field site.

Month	**2018**			**2019**		
	High (°C)	Low (°C)	Precipitation (mm)	High (°C)	Low (°C)	Precipitation (mm)
January	5.32	-7.90	10.16	3.58	-7.10	31.24
February	6.84	-7.01	10.16	1.64	-7.48	32.76
March	14.16	0.19	17.52	9.61	-1.48	61.99
April	16.06	1.12	43.43	20.9	6.84	55.88
May	29.38	15.20	83.3	22.16	11.39	307.32
June	32.90	20.1	54.61	29.42	16.63	145.05
July	32.66	19.22	72.63	31.66	19.76	58.42
August	31.02	18.57	168.91	29.25	19.29	218.43
September	26.40	15.35	127.51	29.73	18.38	59.69
October	18.45	5.6	149.35	16.90	3.81	69.34
November	8.83	-3.18	19.05	11.49	-2.04	15.48
December	6.95	-4.61	63	8.44	-3.81	26.92
Total			819.63			1082.52

Table 2.2

ANOVA table showing p-values for spring and fall seasons of 2018 and 2019 for total C, total N, microbial biomass C (MBC), microbial biomass N (MBN), active C, dissolved organic C (DOC), dissolved organic N (DON), β-glucosidase enzyme activity (βG), β-glucosaminidase (βGA), soil respiration (resp), soil extractable nitrate-N (NO_3-N), and soil extractable ammonium-N (NH_4-N). Other table abbreviations include: CC (cover crop) and Fert (fertilizer management practice). Asterix (*) indicates significance ($p<0.05$).

Soil Health Test	Spring 2018			Fall 2018			Spring 2019			Fall 2019		
	Treatment Groups											
	Fert x CC	**Fert**	**CC**	**Fert x CC**	**Fert**	**CC**	**Fert x CC**	**Fert**	**CC**	**Fert x CC**	**Fert**	**CC**
Total C	0.766	0.185	0.008*	0.037*	0.990	<0.0001*	0.19	0.013*	<0.0001*	0.912	0.118	0.424
Total N	0.827	0.827	0.711	0.616	0.264	0.120	0.231	0.311	0.608	0.265	0.235	0.567
MBC	0.741	0.688	0.029*	0.899	0.130	0.712	0.585	0.173	0.029*	0.611	0.295	0.632
MBN	0.111	0.580	0.713	0.938	0.113	0.216	0.427	0.131	0.252	0.817	0.793	0.267
MBC/N	0.269	0.565	0.491	0.977	0.694	0.092	0.550	0.214	0.776	0.580	0.458	0.286
DOC	0.027*	0.352	0.105	0.561	0.594	0.796	0.701	0.062	0.802	0.503	0.961	0.383
DON	0.186	0.178	0.001*	0.251	0.553	0.951	0.658	0.212	0.345	0.714	0.282	0.224
NO_3-N	0.090	0.052	<0.0001*	0.176	0.099	0.770	0.651	0.365	0.444	0.873	0.414	0.207
NH_4-N	0.181	0.0003*	0.036*	0.202	0.579	0.402	0.823	0.011*	0.739	0.278	0.033*	0.947
Active C	0.793	0.085	0.001*	0.787	0.831	0.176	0.805	0.129	0.033*	0.76	0.333	0.175
Resp	0.752	0.377	0.024*	0.186	0.903	0.810	0.752	0.396	0.234	0.642	0.722	0.925
βG	0.143	0.198	<0.0001*	0.057	0.632	0.0002*	0.035*	0.783	<0.0001*	0.325	0.537	0.087
βGA	0.445	0.022*	<0.0001*	0.429	0.595	0.0008*	0.815	0.248	0.005*	0.837	0.419	0.038*

Table 2.3

Total C, microbial biomass C, active C, β-glucosidase (βG), β-glucosaminidase (βGA) treatment (trt) means, standard error (SE) and p-values in cover crop treatments in spring 2018, fall 2018, spring 2019, and fall 2019. An asterisk (*) is used to indicate significance of p < 0.05.

Crop growing prior to sampling	**Spring 2018** *Triticale & Rapeseed*		**Fall 2018** *Soybean*		**Spring 2019** *Winter Wheat & Rapeseed*		**Fall 2019** *Corn*	
	CC	**NC**	**CC**	**NC**	**CC**	**NC**	**CC**	**NC**
Total C								
Trt Means	1.45	1.29	1.72	1.45	1.60	1.38	1.57	1.52
SE	0.04		0.04		0.07		0.06	
p-value	*0.008**		*<0.0001**		*<0.0001**		*0.424*	
MBC								
Trt Means	339.13	314.49	281.09	273.87	96.72	81.47	363.32	370.68
SE	55.27		72.11		12.0		14.11	
p-value	*0.029**		*0.712*		*0.030**		*0.629*	
Active C								
Trt Means	328.34	268.88	326.86	277.77	321.30	275.70	421.69	386.93
SE	19.10		48.13		13.05		16.83	
p-value	*0.001**		*0.755*		*0.033**		*0.175*	
βG								
Trt Means	36.87	24.88	29.03	22.18	21.63	15.77	57.52	49.89
SE	3.00		0.93		3.36		3.38	
p-value	*<0.0001**		*0.0002**		*<0.0001**		*0.087*	
βGA								
Trt Means	11.59	7.84	11.09	8.07	18.07	13.41	21.52	18.09
SE	0.73		0.79		3.01		1.39	
p-value	*<0.0001**		*0.0008**		*0.005**		*0.038**	

Figure 2.1

Enzyme activity of β-Glucosidase. Vertical bars represent standard error. Abbreviations are cover crop treatment (CC) and no cover crop treatment (NC). Letters represent difference between treatments at $p<0.05$.

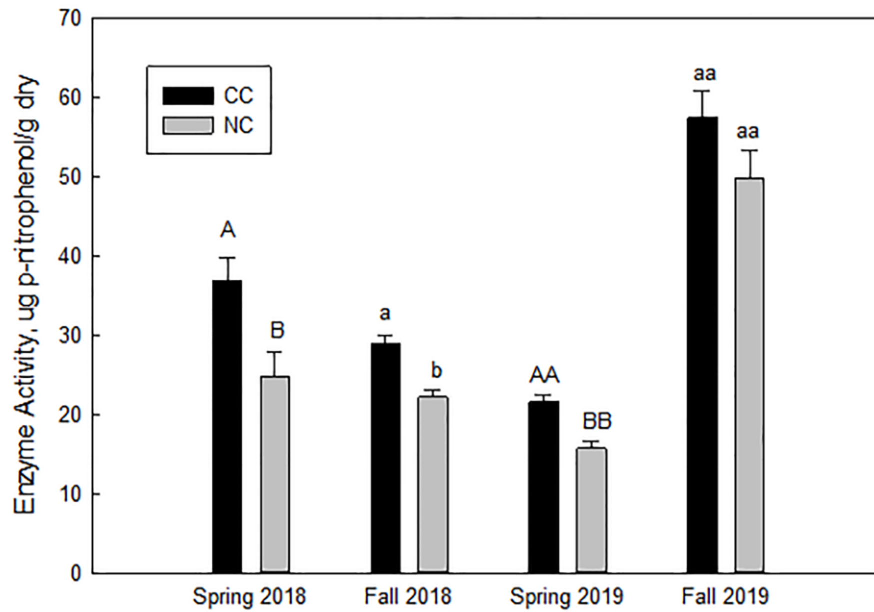
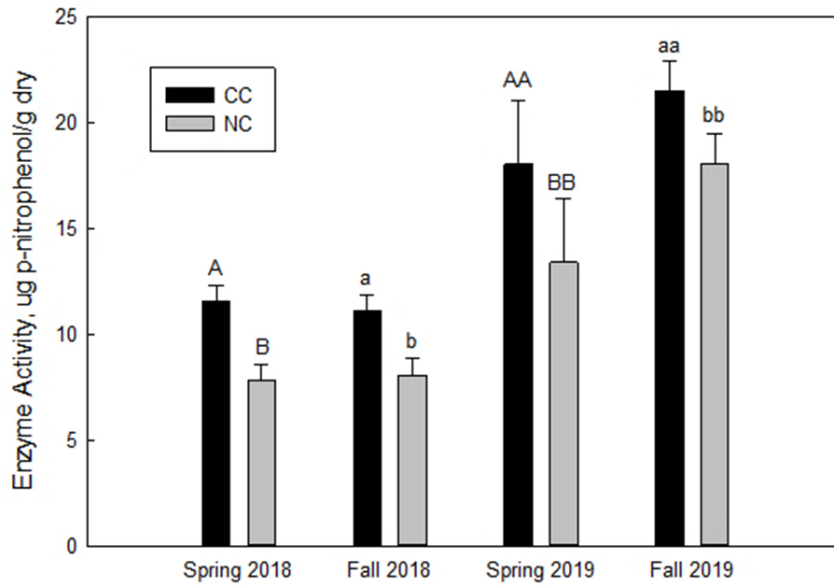


Figure 2.2

Enzyme activity of β-Glucosaminidase. Vertical bars represent standard error. Abbreviations are cover crop treatment (CC) and no cover crop treatment (NC). Letters represent difference between treatments at p<0.05.



Chapter 3 - Soil Microbial Phospholipid Fatty Acid Profiling Response to Cover Crop and Phosphorus Fertilizer Usage in a No-Till Corn-Soybean System

Abstract

Currently there remains much to understand regarding how soil microorganisms respond to agricultural management practices, and studies that have examined this rarely do so at field scale. Understanding how sustainable agricultural management practices like cover crop implementation in a no-tillage corn-soybean system impact soil microorganisms can help to expand current understanding in soil microorganisms. The objective of this study was to examine how soil microorganisms respond to cover crop usage and P fertilizer management regimes under no-tillage in a field scale study using phospholipid fatty acid (PLFA) profiles. Soil samples were collected from the Kansas Agricultural Watershed Field Laboratory in spring 2018 at the 0-5 cm depth and fall 2018, spring 2019, and fall 2019 at the 0-5, 5-10, and 10-15 cm soil depths. The experiment had a 2 by 3 factorial treatment structure with two levels of cover crop including with cover crop (CC) and without cover crop (NC) and three levels of P fertilizer management including no P fertilizer (NP), fall broadcast (FB), and spring injected (SI) in a randomized complete block design with three replicates of each treatment combination. All PLFA categories (Gram-negative bacteria, Gram-positive bacteria, actinomycetes, AM fungi, fungi, and eukaryotes) decreased in biomass with increasing depth. Spring and fall 2018 had a higher biomass in the CC treatment as compared to the NC treatment across all PLFA categories. This was also observed in spring 2019 for Gram-positive bacteria, actinomycetes, and AM fungi. Mean percent community composition was found to be consistent at the 0-5 cm depth across

PLFA categories and between the CC and NC treatments, except in actinomycetes in spring 2019. Fungi were significantly impacted by P fertilizer treatments in fall 2019. This study highlights that soil microorganisms can vary in their response to agricultural management practices by organismal group, depth, and season.

Introduction

Soil biota provide several important soil functions including nutrient provision and cycling, pest and pathogen protection, production of growth factors, water availability, and the formation of soil aggregates that lower soil erosion and increase water infiltration (Lehman et al., 2015b). Soil microorganisms modulate the physiochemical character of soil as they play key roles in the biogeochemical cycles including those of carbon, nitrogen, sulfur, phosphorus, and iron. Better understanding the microbial functional capacity of agricultural soils could help inform agricultural management strategies. By gaining greater insight into the impact of agricultural management practices on the microbial community, more informed management practices could be adopted that enhance soil health.

In addressing questions related to characterization of the soil microbial community, there are a variety of culture-dependent and culture-independent methods. Culture-dependent methods allow soil microorganisms to be isolated and characterization of physical attributes; however, these methods have been estimated to capture less than 0.1% of soil microorganisms, as they are not all able to be cultured or proper methodology to culture them has yet to be determined (Torsvik, Goksoyr, & Daae. 1990). Several culture-independent methods utilized in soil health metrics are polymerase chain reaction (PCR) / DNA based methods and phospholipid fatty acid (PLFA) analysis. The methods of PCR (through 16S analysis) and PLFA have been used to examine microbial community composition, abundance, and taxonomic diversity. Nucleic acids

utilized in PCR methods do not degrade as rapidly after cell death as PLFA biomarkers (Carini et al., 2016; Pinkart, Ringellber, Piceno, Macnaughton, & White, 2002; ISO/TS, 2010). Because PLFA biomarkers degrade rapidly upon cell death they provide a snapshot of the living microbial biomass and community structure (Pinkart et al., 2002).

Phospholipid fatty acid profiles have been linked to soil quality (Bossio et al., 1998). Phospholipid fatty acid profiles have also been found to reveal differences in soil microbial communities in response to a variety of agricultural management practices including crop rotations, cover crops, soil depths, and tillage management (Mbuthia et al., 2015; Nievell et al., 2016; Fierer et al., 2007). Some studies have found a higher microbial biomass with cover crop use (McDaniel, Tieman, & Grandy, 2014; Nair & Ngouajio, 2012; Spedding, Hamel, Mehuys, & Madramootoo, 2004). Bossio & Scow (1998) examined how the soil microbial community was affected in organic and conventional management systems at different sampling periods (April and July). Bossio & Scow (1998) found that PLFA profiles significantly differed between these time points, indicating the need to take sampling time into consideration in agricultural systems. Finney et al. (2017) found that different cover crop species enhanced the presence of different microbial functional groups. In their study of 14 cover crop treatments, the authors found that arbuscular mycorrhizal (AM) fungi were present at higher levels in oat and cereal rye cover crop treatments. Other studies have also found that cover crop implementation can impact soil microbial community make-up (Lehman et al., 2012; Kabir and Koide 2002; White and Weil 2010). Engelhardt et al. (2018) looked at how precipitation and soil depth affected microbial activity in a plant-soil system and found that depth had a more significant effect on microbial activity than precipitation, with greater microbial activity at the 0-5 cm depth than the other depths examined (10-15 and 30-35 cm).

Microbial communities are impacted by conservation management practices; however, translating this knowledge into meaningful application for producers has proven difficult (Prosser et al., 2012; Navarro-Noya et al., 2013; Schmidt, Gravuer, Bossange, Mitchell, & Scow, 2018). One significant reason has been the lack of studies connecting field practices and the functional capabilities of microorganisms at field scale (Lehman et al., 2015b). One of the most critical roles microorganisms play in soils is nutrient cycling, which is especially pertinent for agricultural production. Carbon turnover, soil organic carbon (Six, Frey, Thiet, & Batten, 2006; Nielsen, Ayres, Wall, & Bardgett, 2011; Tardy et al., 2015), and nutrient availability and uptake (Adesemoye & Kloepper, 2009; Bender & van der Heijden, 2015, Alori, Glick, & Babaloa, 2017) are shown to be positively related to the soil microbial community; however, this is not a consistent finding.

Understanding how soil microbial communities are influenced by management practices such as no-tillage, cover crop adoption, and P fertilizer management will aid in understanding the roles microorganisms play in an agricultural system. No-tillage practices have become more common over the past 50 years; however, with any system there are benefits and costs (Huggins & Reganold, 2008). While there has been research examining the microbial response to no-tillage practices, these studies do not typically examine recent no-tillage sites and how this factor interacts with cover cropping (Karlen, et al., 1994; Motta, Reeves, Burmester, & Feng, 2007). Other studies examining long-term cover crop implementation have not examined multiple soil depths (Mbuthia et al., 2015; Rankoth et al., 2019; Arcand, Helgason, & Lemke, 2016). This work seeks to better understand how utilizing cover cropping at a no-tillage site with the use of P fertilizer impact soil microorganisms across spring and fall seasons and at multiple soil depths (0-5, 5-10, and 10-15 cm). The hypotheses of this study were that microbial biomass would

decrease with increasing depth, that addition of cover crops would result in an increase in microbial biomass across all PLFA categories, and that the community structure would be dominated by fungi in the presence of cover crops, and that P fertilizer treatments would not significantly impact PLFA profiles.

Materials and Methods

Site Description

This work was conducted at the Kansas Agricultural Watershed (KAW) Field Laboratory located near Manhattan, KS (39.134, -96.641) from spring of 2018 through fall of 2019. This field laboratory was established in 2014 and designed with the goal of examining how agricultural management practices impact soil health and consequently water, sediment, nutrient, and chemical losses. The KAW is made up of 18 small watersheds that range from 0.49 ha to 0.65 ha in size. Smolan silty clay loam (fine, smectitic, mesic Pachic Argiustoll) is the principal soil type and the site has an average slope of 6 to 8 %. The climate for the area is a hot, humid continental climate with a mean annual temperature of 12.7^{o}C and an annual precipitation of 904 mm, with the majority of precipitation occurring in late spring to early fall (Table 3.1).

The treatment design was a 2 by 3 randomized complete block factorial design, with three replicates of each treatment (18 plots). There were two levels of cover crop treatments: cover crops (CC) and no cover crops (NC). There were three levels of phosphorus fertilizer management: no fertilizer (NF), spring injected (SI), and fall surface broadcast (FB). The KAW has been managed in a continuous no-till, corn-soybean rotation. The last tillage event occurred on November 7th, 2014. All crops grown starting within CC planting in 2014 have been under no-till management. Cover crops were first planted at KAW in 2015 and have been planted every year after. Cover crops used have reflected corn-soybean producer cover crop usage in

Northwest Kansas. Cover crops have included: winter wheat before soybean in 2016, triticale and rapeseed before corn in 2017, and before soybean in 2018. Every year, the same amount of P fertilizer was applied as either a fall broadcast or spring injected applications. The form of P applied in the fall broadcast treatment was diammonium phosphate (DAP) at 134.5 kg/ha (27 kg P/ha), and the form of P applied in the spring injected treatment was ammonium polyphosphate at 131 L/ha (27 kg P/ha). Nitrogen (N) fertilizer, 28% urea ammonium nitrate, was injected below the surface at a uniform rate of 145.71 kg N/ha for all plots in corn years.

Soil Sample Collection

Soil samples were collected at a depth of 0-5 cm for Spring 2018 and at depths of 0-5, 5-10, and 10-15 cm for Fall 2018, Spring 2019, and Fall 2019. These samples were collected by taking 40 cores measuring 18 mm in diameter along a line transect in each plot. Sampling was conducted before the cash crop was planted in the spring and immediately after cash crops were harvested in the fall. Samples for a given plot and depth were sieved using a 2 mm sieve. After sieving, samples were separated into oven dried, air dried, freeze dried, and field moist divisions based on the methods used for various analysis.

PLFA

After soil was passed through a 2 mm mesh sieve, sub-samples were frozen, and freeze dried prior to being sent to the Soil Health Assessment Center at the University of Missouri for phospholipid fatty acid (PLFA) analysis. Soil Health Assessment Center at the University of Missouri extracted 1-2 g of the samples with Bligh-Dyer extractant and then used gas chromatography to analyze (Buyer & Sasser, 2012).

Statistical Analysis

Statistical analysis was performed using SAS version 9.4 software (Cary, NC, U.S.A.) with a PROC MIXED procedure for spring 2018, which only had the 0-5 cm depth. The cover crop treatments and the P fertilizer treatments were fixed effects and the blocking factor was random. Analysis of variance (ANOVA) and Tukey-adjusted LSMEANS were used to indicate differences between treatments. The significance threshold used was $p > 0.05$.

A statistical model that took into account depth was used for fall 2018, spring 2019, and fall 2019 seasons, which was also performed using SAS version 9.4 software (Cary, NC, U.S.A.) with a PROC GLIMMIX procedure, repeated measures of variance procedure, the cover crop treatments and the P fertilizer treatments were fixed effects and the blocking factor was random. Analysis of variance (ANOVA) was used to indicate differences between treatments. The significance threshold used was $p > 0.05$.

Results

Total Biomass

In spring 2018 total biomass in the CC treatment had significantly higher microbial biomass than the NC treatment, with the CC treatment being 26% greater than the NC treatment (Table 3.2). In spring 2018, no other treatment effects were found (Table 3.2). In fall 2018 an interaction between the cover crop treatment and depth was found, with the CC treatment having a greater biomass than the NC treatment at the 0-5 cm depth, and there was no difference statistically detected between the CC and NC treatments at the 5-10 and 10-15 cm depths; however, biomass was found to decrease with increasing depth (Table 3.3 and Fig. 3.1). In fall 2018 at the 0-5 cm depth total biomass in the CC treatment was 28% greater than total biomass in the NC treatment. No other effects from treatments or treatment interactions were detected for

fall 2018 in total biomass. In spring 2019, depth was found to significantly impact total biomass with biomass decreasing with increasing depth (Table 3.2 and Fig. 3.1). In spring 2019 neither the P fertilizer or cover crop treatments nor interactions between treatments were found to significantly impact total biomass (Table 3.2). In fall 2019 depth was found to significantly impact total biomass with biomass decreasing with increasing depth (Table 3.2). In fall 2019 neither the P fertilizer or cover crop treatments nor interactions between treatments were found to significantly affect total biomass (Table 3.2).

Bacterial Categories

There were multiple PLFA categories targeting bacteria including: Gram-negative bacteria, Gram-positive bacteria, and actinomycetes. In spring 2018 the biomass of Gram-negative bacteria was 30% significantly greater in the CC treatment as compared to the NC treatment. In spring 2018 no other treatment effects affected Gram-negative bacteria biomass (Table 3.2). In fall 2018 gram-negative bacteria had a significantly greater biomass in the CC treatment as compared to the NC treatment at the 0-5 cm depth (Table 3.2). In fall 2018 Gram-negative bacteria had 26% significantly greater biomass in the CC treatment as compared to the NC treatment at the 0-5 cm depth (Table 3.4). The biomass of Gram-negative bacteria decreased with increasing depth with no difference between CC and NC treatments at the 5-10 and 10-15 cm depths (Table 3.4). In spring 2019 and fall 2019 the biomass of Gram-negative bacteria decreased with increasing depth, and no significant difference was found due to treatments or treatment interactions (Table 3.2).

In spring 2018 the biomass of Gram-positive bacteria was significantly greater, by 26%, in the CC treatment as compared to the NC treatment. No other significant impact was found from treatments or treatment interactions in Gram-positive bacteria in spring 2018 (Table 3.2 and

Table 3.4). In fall 2018 gram-positive bacteria had a significantly greater biomass in the CC treatment as compared to the NC treatment across depths (Table 3.2). In fall 2018 the Gram-positive bacteria had a significantly greater biomass in the CC treatment as compared to the NC treatment at the 0-5 cm depth, with 15% greater biomass in the CC treatment as compared to the NC treatment. Gram-positive bacteria biomass decreased with increasing depth with no difference between CC and NC treatments at the 5-10 and 10-15 cm depths (Table 3.4). In spring 2019 and fall 2019 the biomass of gram-positive bacteria decreased with increasing depth, and no significant difference was found due to treatments or treatment interactions (Table 3.2).

In spring 2018 the biomass of actinomycetes was significantly greater in the CC treatment as compared to the NC treatment, with a 24% greater biomass in the CC treatment as compared to the NC treatment. No other significant impact was found from treatments or treatment interactions in actinomycetes in spring 2018 (Table 3.2 and Table 3.4). In fall 2018 and spring 2019 actinomycetes had a significantly greater biomass in the CC treatment as compared to the NC treatment across depths (Table 3.2). In both fall 2018 and spring 2019, actinomycete biomass decreased with increasing depth with no difference between CC and NC treatments at individual depths, and no other difference between treatments or treatment interactions was found (Table 3.2). In fall 2019 actinomycete biomass decreased with increasing depth, and no significant difference was found due to treatments or treatment interactions (Table 3.2).

Eukaryotic Categories

There were multiple PLFA categories targeting eukaryotic organisms including: AM fungi, fungi, and other eukaryotes. The fungi category does not include the AM fungi. The other eukaryotes category does not include AM fungi or fungi. In spring 2018 the biomass of AM fungi was significantly greater in the CC treatment as compared to the NC treatment, with a 33%

greater biomass in the CC treatment as compared to the NC treatment. In fall 2018 the cover crop treatment significantly increased biomass of AM fungi at 0-5. 5-10, and 10-15 cm depth. No other significant effect was found from treatments or treatment interactions in AM fungi in spring 2018 (Table 3.2 and Table 3.5). In fall 2018 the CC treatment significantly increased biomass of AM fungi at 0-5, 5-10, and 10-15 cm depths relative to the NC treatment (Table 3.2 and Table 3.5). At the 0-5 cm depth in fall 2018 AM fungi biomass was 14% greater in the CC treatment as compared to the NC treatment. No other treatment effects or treatment interactions were found in fall 2018. In spring 2019 the biomass of AM fungi was significantly effected by the cover crop treatments (Table 3.2). Biomass of AM fungi in spring 2019 and fall 2019 was found to decrease with increasing depth (Table 3.2). No other treatment effects or treatment interactions were found in spring 2019 or fall 2019.

In spring 2018 fungi biomass was significantly effected by the cover crop treatments with a greater biomass in the CC treatment as compared to the NC treatment (Table 3.2 and Table 3.5). The fungi biomass was 39% greater in the CC treatment in spring 2018. In fall 2018, spring 2019, and fall 2019 fungi biomass was significantly affected by depth with a decrease in biomass found with increasing depth, and there were no treatment effects or treatment interactions detected for fall 2018 and spring 2019 (Table 3.2). In fall 2019 fungi biomass was significantly affected by the P fertilizer treatments at depth with the greatest biomass in the SI treatment at 0-5 cm, followed by NP and FB at the 0-5 cm depth, and all other P fertilizer treatments at the 5-10 cm and 10-15 cm depths having the least fungi biomass and being statistically equal to each other (Table 3.2 and Fig. 3.2).

In spring 2018 the biomass of the other eukaryotes was significantly affected by the cover crop treatments with the greatest biomass found in the CC treatment as compared to the NC

treatment (Table 3.2 and Table 3.5). In spring 2018 the CC treatment had 42% greater other eukaryote biomass than the NC treatment. In fall 2018 the other eukaryote biomass was significantly affected by the cover crop treatments with a greater biomass in the CC treatment as compared to the NC treatment at the 0-5 cm depth, with the CC treatment having 14% greater biomass than the NC treatment. Other eukaryotic biomass decreased with increasing depth in fall 2018 (Table 3.2 and Table 3.5). No other treatment effects or treatment interactions were found to impact other eukaryote biomass in fall 2018 (Table 3.2). In spring 2019 and fall 2019 other eukaryote biomass was found to decrease with increasing depth, and no treatment effects or treatment interactions were found (Table 3.2).

Community Composition

The soil microbial community composition of spring 2018, fall 2018, spring 2019, and fall 2019 at the 0-5 cm depth was fairly similar between the CC and NC treatment within a given season, with all but two categories within a season differing by a single percent or less between the CC and NC treatments (Table 3.6). The two PLFA categories found to differ greater than a percent between CC and NC treatments were the gram-positive bacteria (2.5% difference) and the actinomycetes (1.1% difference) in spring 2019 (Table 3.6).

Spring 2018 had the lowest percent composition of Gram-negative bacteria based on biomass with 31.4% in the CC treatment and 30.6% in the NC treatment (Table 3.6). Fall 2018, spring 2019, and fall 2019 had very similar gram-negative bacteria percent compositions in both the CC and NC treatments ranging from 32% - 33.4% (Table 3.6). The percent composition of the biomass of gram-positive bacteria out of the total biomass in spring 2018, fall 2018, and fall 2019 ranged from 24.2% - 25.6% in the CC and NC treatments (Table 3.6). Spring 2019 had the lowest percent composition of the biomass of gram-positive bacteria in both the CC treatment at

21.8% and in the NC treatment at 19.3%. The percent composition of biomass made up of actinomycetes in spring 2018, fall 2018, and fall 2019 ranged from 13.6% - 12.4% in the CC and NC treatments (Table 3.6). Spring 2019 had the lowest percent composition of the total biomass of actinomycetes in both the CC treatment at 11.4% and in the NC treatment at 10.3%. The percent composition of AM fungi was the lowest in spring 2018 with 3.6% in the CC treatment and 3.4% in the NC treatment, and fall 2018, spring 2019, and fall 2019 were all at 4.2% in the CC treatment and ranged from 3.7 – 4.4% in the NC treatment (Table 3.6). Fungi percent composition in spring 2018, fall 2018, and fall 2019 ranged from 2.0% - 3.0% in the CC treatment and from 2.8% - 2.2% in the NC treatment (Table 3.6). In spring 2019 the fungi percent composition was 4.3% in the CC treatment and 4.6% in the NC treatment (Table 3.6). The percent composition of other eukaryotes ranged from 1.5% - 1.9% in the CC treatment and 1.3% - 1.7% in the NC treatment across spring 2018, fall 2018, spring 2019, and fall 2019.

In all seasons sampled, microbial community make-up from greatest percent composition went as followed: gram-negative bacteria > gram-positive bacteria > actinomycetes > AM fungi > fungi > other eukaryotes (Table 3.6). The fungi to bacteria ratio was examined at the 0-5 cm depth; however, no treatment main effects or treatment interaction effects were found in any of the examined PLFA categories. The community composition did not total to 100%, and this is due to uncharacterized PLFAs that were a part of the total biomass but could not be assigned to a specific PLFA category.

Discussion

Total Biomass

Total microbial biomass at the 0-5 cm depth was higher in CC treatment compared to the NC treatment in both the spring and fall of 2018; however, there was no difference between the

CC and NC treatments in the spring and fall of 2019. Other studies have found that cover crop implementation can increase microbial biomass. Finney, Buyer, & Kaye (2017) examined eight fall-sown cover crop species and found that cover crop treatments had higher microbial biomass than the no cover crop controls (1 yr untilled weedy fallow). Higher microbial biomass with cover crop use has been reported in numerous other studies as well (McDaniel, Tieman, & Grandy, 2014; Nair & Ngouajio, 2012; Spedding, Hamel, Mehuys, & Madramootoo, 2004). A study by Rankoth et al. (2019) examining the impact of cover crop usage in a corn – soybean rotation found higher microbial biomass in two of three years studied, and attributed the year where cover crop treatment was equal to the no cover crop treatment to be due to a dense weed ground cover present in the no cover treatment plots.

A meta-analysis by Kim, Zabaloy, Guan, & Villamil (2020) examining the relationship between cover cropping and the soil microbiome found that the affects of cover cropping were not as significant under certain conditions including continental climate (climate characteristic of central North America), chemical cover crop termination, and conservation tillage across 60 studies. All these factors would be relevant for this study, potentially minimizing the impact of cover crop adoption over time; however, given data presented here examines sampling points from 2018 and 2019 it is not possible to draw this as a definitive conclusion. A study by Mbuthia et al. (2015) characterizing the impact of long-term tillage (31 yrs), no-tillage, and cover crop adoption of hairy vetch and winter wheat on microbial biomass (measured by FAME) and other factors under continuous cotton production in West Tennessee found no significant difference between winter wheat and no cover treatments.

Another factor to consider is the annual variability in temperature and precipitation; 2018 experienced less precipitation (Table 3.1) and produced a lower crop yield than 2019.

Temperature coupled with soil moisture content are the most important environmental factors impacting microbial growth and activity in soils (Paul and Clark, 1996). Differences in environmental conditions between years and seasons could impact the microbial response to treatments. However, when looking at total biomass across all sampling events, the CC and NC treatments in fall 2019 had the lowest biomass and 2019 had more precipitation than 2018. Engelhardt et al. (2018) looked at the impact of precipitation and soil depth on microbial activity in a plant-soil system and found that depth played a more significant role on microbial activity than precipitation with greater microbial activity at the 0-5 cm depth than the other depths examined (10-15 and 30-35 cm). The results from Engelhardt et al. (2018) are in-line with findings presented here that depth had a greater impact on microbial activity than precipitation. The findings in Engelhardt et al. (2018) are also consistent with this study's findings of higher microbial biomass across all PLFA categories to decrease with increasing depth.

Bacterial Categories

This study found some differences between examined bacterial categories to treatments. Gram-positive bacteria and actinomycete categories had a greater biomass in CC treatments as compared to the NC treatment in spring 2018, fall 2018, and spring 2019. Gram-negative bacteria had this same trend in spring 2018 and fall 2018. A study by Zhang, Sun, Wang, Li, & Qu (2019) examined the impact of corn-soybean rotation on the soil microbiome using PLFA profiles, and found that Gram-positive bacteria and actinomycetes were more greatly impacted by soil aggregation and organic matter than Gram-negative bacteria and fungi. While the study by Zhang et al. (2019) did not look at cover cropping, cover crop implementation has been found to increase soil organic matter (Dell, Salon, Franks, Benham, & Plowden, 2008; Sullivan, 2004), which may contribute to greater biomass of Gram-positive bacteria and in the CC treatment as

compared to the NC treatment at the 0-5 cm depth. This is consistent with this study where total C was found to be significantly greater in the CC treatment as compared to the NC treatment in spring 2018, fall 2018, and spring 2019, but this difference was not detected in fall 2019 (chapter 2). Other studies have shown that cover crops including oat, radish, and vetch could increase bacterial PLFA categories especially Gram-positive bacteria (Chavarria et al., 2016). This study found increased biomass of Gram-positive bacteria under conservation agricultural practices, other studies have also found this(Bossio et al., 2005; Vargas Gil et al., 2011).

Eukaryotic Categories

All eukaryotic PLFA categories (AM fungi, fungi, and eukaryotes) had a higher biomass in the spring 2018 CC treatment as compared to the spring 2018 NC treatment (Table 3.1). In fall 2018 at the 0-5 cm depth all eukaryotic categories had greater biomass in CC treatment as compared to the NC treatment (Table 3.2). This is consistent with other findings of total microbial biomass being greater in the presence of cover crops (McDaniel et al., 2014; Nair & Ngouajio, 2012; Spedding et al., 2004). In fall 2019 the AM fungi biomass was significantly influenced by the P fertilizer treatments across depth (Fig. 3.2). This finding is challenging to compare to other literature, as often literature targeting fungi focuses on AM fungi; however, in this study the fungi category excludes AM fungi. Also, this was observed at a single sampling point. Ultimately this highlights the opportunities for greater understanding through research into the response of microorganisms to agricultural management practices. In addition to the spring 2018 and fall 2018 0-5 cm findings, the AM fungi biomass was higher in the CC treatment as compared to the NC treatment at the 0-5 cm depth in spring 2019, the 5-10 cm depth in fall 2018 and spring 2019, and at the 10-15 cm depth in spring 2019. The AM fungal biomass was greater in the CC treatment as compared to the NC treatment at the 5-10 cm treatment in fall 2019. AM

fungi has been shown to increase under adoption of no-tillage and cover crops (Rankoth et al., 2019; Chavarria et al., 2018; Martínez-García, Korthlands, Brussard, Jorgensem, & De Deyn, 2018). Somewhat similarly to AM fungi, the biomass of the eukaryote category was found to be higher in the CC treatment as compared to the NC treatment at the 5-10 depth in both fall 2018 and spring 2019 and in the 10-15 cm depth in spring 2019. These results are in contrast to work by Rankoth et al. (2019) that did not find a consistent trend of cover crop treatment increasing eukaryotic biomass. However, work by Rankoth et al. (2019) specifically examined protozoa, and the eukaryote category in this paper includes generic eukaryotic markers that could include algae, nematodes, earthworms, arthropods, and protozoa which may contribute to different findings.

Community Composition

All sampling points in this study found greater percent community composition of Gram-negative bacteria than Gram-positive bacteria but no cover crop treatment difference. This find is consistent with Rankoth et al. (2019) which found no difference in percent community composition between CC and NC treatments for Gram-positive and Gram-negative bacteria as detected by PLFA between the first year of sampling (2016) and the last year of sampling (2018). Gram-positive bacteria have been found to be more successful than Gram-negative bacteria in stressful environmental conditions such as low oxygen or lack of nutrients (Guckert et al., 1986; Pennanen et al., 1996; Kaur et al., 2005). This study found the largest percent composition difference between CC and NC treatments in spring 2019 in Gram-positive bacteria. With one data point demonstrating this it is hard to draw conclusions and highlights the need for more examination of how microbial communities are impacted by agricultural management practices. In general the bacterial category findings in this study are also consistent with work by

Chamerlain et al. (2020) that examined bacterial community composition in response to crop rotation and cover crop implementation (1 yr establishment) in a corn-soybean system in Wisconsin, and found that crop rotation impacted the bacterial community make-up however cover crops did not.

Actinomycetes were also found to have a consistent percent composition across both the CC and NC treatments for a given season. When looking across seasons, spring 2019 stood out with a percent community composition of 11.4% (CC treatment) and 10.3% (NC treatment) while in other seasons and CC and NC treatments the percent composition was above 12%. It is also in spring 2019 that fungi have a higher percent composition at over 4% in both CC and NC treatments, while other seasons are not above 3% in CC and NC treatments. It may be that fungi were able to dominate more of shared niches with actinomycetes at the 0-5 cm depth in spring 2019. Actinomycetes are able to form mycelium similarly to many fungi, and it was not until the 1950's they were recognized as bacteria and not fungi (Williams, 1990).

Community composition between the CC and NC treatments within eukaryotic categories within a given season at the 0-5 cm depth were consistent, varying by a percent or less in all instances. This finding is consistent with Rankoth et al. (2019), that found no difference in percent community composition between CC and NC treatments for fungi and protozoa biomass as detected by PLFA between the first year of sampling (2016) and the last year of sampling (2018). Although the category used in Rankoth et al. (2019) is specific to protozoa, the other eukaryotic category used in this study would include protozoa. Chavarria et al. (2018) examined microbial community response to agroecological and conventional systems of agriculture with CC and NC treatments used in a soybean system in Argentina. Chavarraia et al. (2018) found less percent fungi in the NC treatment as compared to the CC. Systems with greater organic

matter have been found to have greater fungi than bacteria due to fungi's increased C assimilation abilities (Baily, Smith, & Bolton, 2002). In this study greater total C was found in CC treatment as compared to the NC treatment in all seasons except fall 2019 (chap 2). While fungi and bacteria biomasses were found to increase in the CC treatment as compared to the NC treatment in all seasons except fall 2019, the percent difference in the community make-up of those groups within a given sampling point between CC and NC treatments was very similar.

In all seasons sampled, percent community composition went from the greatest to least as: Gram-negative bacteria > Gram-positive bacteria > actinomycetes > AM fungi > fungi > eukaryotes. This finding was consistent with work by Rankoth et al. (2019) showing PLFA biomass from greatest to least as: Gram-positive bacteria > Gram-negative bacteria > actinomycetes > fungi > protozoa, and this was found across all three years sampled (2016, 2017, and 2018). Wang, Han, & Zhang (2020) also found a similar trend with bacteria > fungi > eukaryotes in a study examining the impact of winter cover crops on soil microorganisms in norther China. Finding no difference between CC and NC treatments in the fungi to bacteria ratio was consistent with Rankoth et al. (2019) that found no difference between CC and NC treatments in the fungi to bacteria ratio between the first (2016) and last (2018) years of their study examining PLFA profiles in a corn-soybean system in Missouri. However, Chavarria et al. (2018) found a higher fungi to bacteria ratio in the CC treatment as compared to the NC treatment. More long-term studies are needed to better establish clear relationships between fungi to bacteria ratios and CC implementation.

Conclusions

This study shows that cover crops can significantly increase microbial biomass compared to plots with no cover crop in a no-tillage system in a corn-soybean rotation. However, this study

also shows that this is not a consistent result. This study supported the hypothesis that decreasing microbial biomass occurs with increasing depth. The hypothesis that the CC treatment would have significantly greater microbial biomass than the NC treatment across PLFA categories was somewhat supported in this study, particularly at the 0-5 cm depth;; however, not all PLFA categories were different between CC and NC treatments at the 0-5 cm depth. Only AM fungi having a significantly greater biomass in the CC treatment at the 5-10 and 10-15 cm depths. The percent community structure was not found to vary more than 1% between the CC and NC treatments at the 0-5 cm depth except in spring 2019 in the Gram-positive bacteria and actinomycetes. Fungi were impacted by P fertilizer treatments in fall 2019; however, no other PLFA categories or sampling points were significantly impacted by P fertilizer treatments. This study highlights the need for more work to better understand microbial biomass responses to cover crop adoption over long time periods, especially long-term studies in corn – soybean rotations in the Midwest.

References

Adesemoye, A. O., & Kloepper, J. W. (2009, November). Plant-microbes interactions in enhanced fertilizer-use efficiency. *Applied Microbiology and Biotechnology*, Vol. 85, pp. 1–12. https://doi.org/10.1007/s00253-009-2196-0

Ali, W., Nadeem, M., Ashiq, W., Zaeem, M., Gilani, S. S. M., Rajabi-Khamseh, S., … Cheema, M. (2019). The effects of organic and inorganic phosphorus amendments on the biochemical attributes and active microbial population of agriculture podzols following silage corn cultivation in boreal climate. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-53906-8

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017, June 2). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology*, Vol. 8. https://doi.org/10.3389/fmicb.2017.00971

Arcand, M. M., Helgason, B. L., & Lemke, R. L. (2016). Microbial crop residue decomposition dynamics in organic and conventionally managed soils. *Applied Soil Ecology*, *107*, 347–359. https://doi.org/10.1016/j.apsoil.2016.07.001

Benayas, J. M. R., Newton, A. C., Diaz, A., & Bullock, J. M. (2009). Enhancement of Biodiversity and Ecosystem Services by Ecological Restoration: A Meta-Analysis. *Science*, *325*(5944), 1121. https://doi.org/10.1126/science.1172460

Bender, S. F., & van der Heijden, M. G. A. (2015). Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. *Journal of Applied Ecology*, *52*(1), 228–239. https://doi.org/10.1111/1365-2664.12351

Bossio, D. A., Girvan, M. S., Verchot, L., Bullimore, J., Borelli, T., Albrecht, A., … Osborn, A. M. (2005). Soil microbial community response to land use change in an agricultural landscape of western Kenya. *Microbial Ecology*, *49*(1), 50–62. https://doi.org/10.1007/s00248-003-0209-6

Buyer, J. S., & Sasser, M. (2012). High throughput phospholipid fatty acid analysis of soils. *Applied Soil Ecology*. https://doi.org/10.1016/j.apsoil.2012.06.005

Buyer, J. S., Teasdale, J. R., Roberts, D. P., Zasada, I. A., & Maul, J. E. (2010). Factors affecting soil microbial community structure in tomato cropping systems. *Soil Biology and Biochemistry*, *42*(5), 831–841. https://doi.org/10.1016/j.soilbio.2010.01.020

Carini, P., Marsden, P. J., Leff, J. W., Morgan, E. E., Strickland, M. S., & Fierer, N. (2016). Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nature Microbiology*, *2*. https://doi.org/10.1038/nmicrobiol.2016.242

Chavarria, D. N., Pérez-Brandan, C., Serri, D. L., Meriles, J. M., Restovich, S. B., Andriulo, A. E., … Vargas-Gil, S. (2018). Response of soil microbial communities to agroecological versus conventional systems of extensive agriculture. *Agriculture, Ecosystems and Environment*, *264*, 1–8. https://doi.org/10.1016/j.agee.2018.05.008

Chavarría, D. N., Verdenelli, R. A., Serri, D. L., Restovich, S. B., Andriulo, A. E., Meriles, J. M., & Vargas-Gil, S. (2016). Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability. *European Journal of Soil Biology*, *76*, 74–82. https://doi.org/10.1016/j.ejsobi.2016.07.002

Dell, C. J., Salon, P. R., Franks, C. D., Benham, E. C., & Plowden, Y. (2008). No-till and Cover Crop Impacts on soil Carbon and Associated Properties on Pennsylvania Dairy Farms. *Journal of Soil and Water Conservation*, *63*(3), 136–142.

Ding, L. J., Su, J. Q., Sun, G. X., Wu, J. S., & Wei, W. X. (2018). Increased microbial functional diversity under long-term organic and integrated fertilization in a paddy soil. *Applied Microbiology and Biotechnology*, *102*(4), 1969–1982. https://doi.org/10.1007/s00253-017-8704-8

Finney, D. M., Buyer, J. S., & Kaye, J. P. (2017). Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation*, *72*(4), 361–373. https://doi.org/10.2489/jswc.72.4.361

Huggins, D., & Reganold, J. (2008). No-Till: The Quiet Revolution. *Scientific American*, *299*, 70–77. https://doi.org/10.1038/scientificamerican0708-70

ISO/TS. (2010). Soil quality-determination of soil microbial diversity. Retrieved from https://www.iso.org/standard/45703.html

Karlen, D. L., Wollenhaupt, N. C., Erbach, D. C., Berry, E. C., Swan, J. B., Eash, N. S., & Jordahl, J. L. (1994). Long-term tillage effects on soil quality. *Soil and Tillage Research*, *32*(4), 313–327. https://doi.org/10.1016/0167-1987(94)00427-G

Kim, N., Zabaloy, M. C., Guan, K., & Villamil, M. B. (2020). Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biology and Biochemistry*, *142*. https://doi.org

Lehman, R. M., Acosta-Martinez, V., Buyer, J. S., Cambardella, C. A., Collins, H. P., Ducey, T. F., … Stott, D. E. (2015, January 1). Soil biology for resilient, healthy soil. *Journal of Soil and Water Conservation*, Vol. 70, pp. 12A-18A. https://doi.org/10.2489/jswc.70.1.12A. A

Lehman, R. M., Cambardella, C. A., Stott, D. E., Acosta-Martinez, V., Manter, D. K., Buyer, J. S., … Karlen, D. L. (2015). Understanding and enhancing soil biological health: The solution for reversing soil degradation. *Sustainability (Switzerland)*, *7*(1), 988–1027. https://doi.org/10.3390/su7010988. B

Martínez-García, L. B., Korthals, G., Brussaard, L., Jørgensen, H. B., & De Deyn, G. B. (2018). Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties. *Agriculture, Ecosystems and Environment*, *263*, 7–17. https://doi.org/10.1016/j.agee.2018.04.018

Mbuthia, L. W., Acosta-Martínez, V., DeBryun, J., Schaeffer, S., Tyler, D., Odoi, E., … Eash, N. (2015). Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. *Soil Biology and Biochemistry*, *89*, 24–34. https://doi.org/10.1016/j.soilbio.2015.06.016

McDaniel, M. D., Tiemann, L. K., & Grandy, A. S. (2014). Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. In *Ecological Applications* (Vol. 24). Retrieved from http://www.metawinsoft.com/

Motta, A. C. V, Reeves, D. W., Burmester, C., & Feng, Y. (2007). Conservation Tillage, Rotations, and Cover Crop Affecting Soil Quality in the Tennessee Valley: Particulate Organic Matter, Organic Matter, and Microbial Biomass. *Communications in Soil Science and Plant Analysis*, *38*, 2831–2847. https://doi.org/10.1080/00103620701663065

Nair, A., & Ngouajio, M. (2012). Soil microbial biomass, functional microbial diversity, and nematode community structure as affected by cover crops and compost in an organic vegetable production system. *Applied Soil Ecology*, *58*, 45–55. https://doi.org/10.1016/j.apsoil.2012.03.008

Navarro-Noya, Y. E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M. C., Valenzuela-Encinas, C., … Dendooven, L. (2013). Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. *Soil Biology and Biochemistry*, *65*, 86–95. https://doi.org/10.1016/j.soilbio.2013.05.009

Nielsen, U. N., Ayres, E., Wall, D. H., & Bardgett, R. D. (2011, February). Soil biodiversity and carbon cycling: A review and synthesis of studies examining diversity-function relationships. *European Journal of Soil Science*, Vol. 62, pp. 105–116. https://doi.org/10.1111/j.1365-2389.2010.01314.x

Paul, E. A., & Clark, F. E. (1996). *Soil Microbiology and Biochemistry* (second ed.). Academic Press, USA.

Pereira, N. C. M., Galindo, F. S., Gazola, R. P. D., Dupas, E., Rosa, P. A. L., Mortinho, E. S., & Filho, M. C. M. T. (2020). Corn Yield and Phosphorus Use Efficiency Response to Phosphorus Rates Associated With Plant Growth Promoting Bacteria. *Frontiers in Environmental Science*, *8*. https://doi.org/10.3389/fenvs.2020.00040

Pinkart, H. C., Ringelberg, D. B., Piceno, S. J., Macnaughton, S. J., & White, D. C. (2002). Biochemical Approaches to Biomass Measurements and Community Structure Analysis. In

C. J. Hurst, R. L. Crawford, G. R. Knudsen, M. J. McInerney, & L. D. Steetzenbach (Eds.), *Manual of Environmental Microbiology* (2nd ed., pp. 101–113). Washington D.C.

Prosser, J. I. (2012). Ecosystem processes and interactions in a morass of diversity. *FEMS Microbiology Ecology*, *81*(3), 507–519. https://doi.org/10.1111/j.1574-6941.2012.01435.x

Rankoth, L. M., Udawatta, R. P., Gantzer, C. J., Jose, S., Veum, K., & Dewanto, H. A. (2019). Cover crops on temporal and spatial variations in soil microbial communities by phospholipid fatty acid profiling. *Agronomy Journal*, *111*(4), 1693–1703. https://doi.org/10.2134/agronj2018.12.0789

SAS Institute. (2013). *SAS user's guide*. Cary, NC: SAS Inst.

Schimel, J. P., & Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Frontiers in Microbiology*, Vol. 3. https://doi.org/10.3389/fmicb.2012.00348

Schmidt, R., Gravuer, K., Bossange, A. V., Mitchell, J., & Scow, K. (2018). Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. *PLoS ONE*, *13*(2). https://doi.org/10.1371/journal.pone.0192953

Six, J., Frey, S. D., Thiet, R. K., & Batten, K. M. (2006, March). Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal*, Vol. 70, pp. 555–569. https://doi.org/10.2136/sssaj2004.0347

Spedding, T. A., Hamel, C., Mehuys, G. R., & Madramootoo, C. A. (2004). Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. *Soil Biology and Biochemistry*, *36*(3), 499–512. https://doi.org/10.1016/j.soilbio.2003.10.026

Sullivan, T. J., Moore, J. A., Thomas, D. R., Mallery, E., Snyder, K. U., Wustenberg, M., … Moore, D. L. (2007). Efficacy of vegetated buffers in preventing transport of fecal coliform bacteria from pasturelands. *Environmental Management*, *40*(6), 958–965. https://doi.org/10.1007/s00267-007-9012-3

Tardy, V., Spor, A., Mathieu, O., Lévèque, J., Terrat, S., Plassart, P., … Maron, P. A. (2015). Shifts in microbial diversity through land use intensity as drivers of carbon mineralization in soil. *Soil Biology and Biochemistry*, *90*, 204–213. https://doi.org/10.1016/j.soilbio.2015.08.010

Torsvik, V., Goksoyr, J., & Daae, F. L. (1990). High Diversity in DNA of Soil Bacteria. In *Applied and Environmental Microbiology* (Vol. 56).

Vargas Gil, S., Meriles, J., Conforto, C., Basanta, M., Radl, V., Hagn, A., … March, G. J. (2011). Response of soil microbial communities to different management practices in surface soils of a soybean agroecosystem in Argentina. *European Journal of Soil Biology*, *47*(1), 55–60. https://doi.org/10.1016/j.ejsobi.2010.11.006

Wagg, C., Bender, S. F., Widmer, F., & Heijden, M. G. A. van der. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, *111*(14), 5266–5270. https://doi.org/10.1073/PNAS.1320054111

Zaeem, M., Nadeem, M., Pham, T. H., Ashiq, W., Ali, W., Gilani, S. S. M., … Thomas, R. (2019). The potential of corn-soybean intercropping to improve the soil health status and biomass production in cool climate boreal ecosystems. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-49558-3

Zhang, B., Li, Y., Ren, T., Tian, Z., Wang, G., He, X., & Tian, C. (2014). Short-term effect of tillage and crop rotation on microbial community structure and enzyme activities of a clay loam soil. *Biology and Fertility of Soils*, *50*(7). https://doi.org/10.1007/s00374-014-0929-4

Zhang, P., Sun, J., Li, L., Wang, X., Li, X., & Qu, J. (2019). Effect of soybean and maize rotation on soil microbial community structure. *Agronomy*, *9*(2). https://doi.org/10.3390/agronomy9020042

Table 3.1

Spring 2018, fall 2018, spring 2019, and fall 2019 p-values of the PLFA categories. Treatments include no P fertilizer treatments (Fert) and cover crop treatments (Cover). An asterisk (*) indicates significance of $p < 0.05$.

Effect	PLFA Categories						
	Total	Gram Neg	Gram Pos	Actinomycetes	AM Fungi	Fungi	Other Eukaryotes
Spring 2018[†]							
Fert	0.4294	0.4033	0.3573	0.6942	0.5192	0.4556	0.1298
Cover	0.0001*	<.0001*	0.0001*	0.0002*	0.0005*	0.0077*	<.0001*
Fert*Cover	0.4193	0.475	0.4396	0.3748	0.7483	0.6919	0.0693
Fall 2018							
Fert	0.5475	0.5754	0.7734	0.8693	0.4945	0.9295	0.1957
Cover	0.0226*	0.0671	0.0095*	0.0499*	0.002*	0.0507	0.0786
Fert*Cover	0.304	0.358	0.4607	0.3492	0.1798	0.1871	0.1119
Depth	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Depth*Fert	0.68	0.8995	0.5917	0.6968	0.6405	0.807	0.3432
Depth*Cover	0.0078*	0.0451*	0.0031*	0.0629	0.0207*	0.0132*	0.0447*
Depth*Fert*Cover	0.3879	0.5036	0.4479	0.2105	0.7852	0.3049	0.2527
Spring 2019							
Fert	0.4283	0.5171	0.4748	0.5116	0.8416	0.1957	0.2788
Cover	0.1664	0.1291	<.0001*	<.0001*	0.0002*	0.9669	0.0541
Fert*Cover	0.4075	0.6594	0.1005	0.0326	0.5464	0.8232	0.9354
Depth	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Depth*Fert	0.9815	0.8435	0.9015	0.9557	0.868	0.2411	0.3488
Depth*Cover	0.8416	0.475	0.1174	0.177	0.1638	0.986	0.8582
Depth*Fert*Cover	0.7918	0.8774	0.6922	0.3542	0.7516	0.9087	0.9856
Fall 2019							
Fert	0.1564	0.1553	0.1261	0.4011	0.0573	0.0295*	0.6825
Cover	0.0847	0.0946	0.0372	0.0864	0.0458	0.7177	0.2599
Fert*Cover	0.7523	0.6394	0.6870	0.6764	0.8817	0.3346	0.2291
Depth	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Depth*Fert	0.2615	0.5860	0.1662	0.6985	0.1787	0.0471*	0.8508
Depth*Cover	0.4274	0.4200	0.6035	0.5159	0.1840	0.3458	0.4336
Depth*Fert*Cover	0.6970	0.5736	0.9092	0.8966	0.8747	0.1067	0.3520

[†]Spring 2018 only had samples taken at the 0-5 cm depth

Table 3.2

Cover crop treatment by depth across seasons and PLFA categories. An asterisk (*) and letters are used to indicate significance of p < 0.05. All values are in nmol/g soil x 10^-3.

Crop growing prior to sampling	**Spring 2018**† *Triticale & Rapeseed*		**Fall 2018** *Soybean*		**Spring 2019** *Winter Wheat & Rapeseed*		**Fall 2019** *Corn*	
	CC	**NC**	**CC**	**NC**	**CC**	**NC**	**CC**	**NC**
Total Biomass								
Depth 0-5	109.11 A	78.33 B	88.48 A	77.50 B	84.58	81.14	65.19	62.92
Depth 5-10	N/A	N/A	58.78 C	55.81 C	55.96	48.42	40.73	35.25
Depth 10-15	N/A	N/A	48.60 D	45.73 D	42.20	38.94	35.34	34.81
SE	5.25		3.16		4.75		26.43	
p-value	*0.0001**		*0.0078**		*0.8416*		*0.4274*	

†Spring 2018 only had samples taken at the 0-5 cm depth

Table 3.3

Cover crop treatment by depth across seasons and bacterial PLFA categories. An asterisk (*) and letters are used to indicate significance of $p < 0.05$. All values are in nmol/g soil x 10^-3.

Crop growing prior to sampling	**Spring 2018†** *Triticale & Rapeseed*		**Fall 2018** *Soybean*		**Spring 2019** *Winter Wheat & Rapeseed*		**Fall 2019** *Corn*	
	CC	**NC**	**CC**	**NC**	**CC**	**NC**	**CC**	**NC**
Gram Neg								
Depth 0-5	34.22 A	23.96 B	28.30 A	25.38 B	27.84	27.08	20.84	20.78
Depth 5-10	N/A	N/A	17.65 C	16.72 C	17.03	13.32	12.05	10.17
Depth 10-15	N/A	N/A	13.23 D	12.46 D	11.19	10.17	10.11	9.18
SE	1.81		1.13		1.44		0.88	
p-value	*<0.0001**		*0.0451**		*0.4750*		*0.4200*	
Gram Pos								
Depth 0-5	27.19 A	20.02 B	22.31 A	18.97 B	18.45	15.65	16.28	15.24
Depth 5-10	N/A	N/A	16.07 C	14.90 CD	14.54	12.33	10.90	9.59
Depth 10-15	N/A	N/A	13.89 DE	13.06 E	11.46	10.69	9.75	9.26
SE	1.17		0.85		0.66		0.59	
p-value	*0.0001**		*0.0031**		*0.1174*		*0.6035*	
Actinomycetes								
Depth 0-5	14.04 A	10.66 B	11.16	9.81	9.70	8.36	8.33	7.78
Depth 5-10	N/A	N/A	9.55	8.89	8.36	7.53	6.53	5.82
Depth 10-15	N/A	N/A	8.06	7.54	6.97	6.56	5.84	5.66
SE	0.56		0.55		0.33		0.41	
p-value	*0.0002**		*0.0629*		*0.1770*		*0.516*	

†Spring 2018 only had samples taken at the 0-5 cm depth

Table 3.4

Cover crop treatment by depth across seasons and eukaryotic organism PLFA categories. An asterisk (*) and letters are used to indicate significance of $p < 0.05$. All values are in nmol/g soil x 10^-3.

	Spring 2018†		**Fall 2018**		**Spring 2019**		**Fall 2019**	
Crop growing prior to sampling	*Triticale & Rapeseed*		*Soybean*		*Winter Wheat & Rapeseed*		*Corn*	
	CC	**NC**	**CC**	**NC**	**CC**	**NC**	**CC**	**NC**
AM Fungi								
Depth 0-5	3.96 A	2.67 B	3.71 A	3.19 B	3.59	3.01	2.75	2.75
Depth 5-10	N/A	N/A	2.07 C	1.86 D	1.75	1.34	1.38	1.08
Depth 10-15	N/A	N/A	1.54 E	1.31 F	1.20	1.05	1.14	1.03
SE	0.22		0.12		0.12		0.10	
p-value	*0.0005**		*0.0207**		*0.1638*		*0.1840*	
Fungi								
Depth 0-5	3.28 A	1.99 B	2.64 A	2.18 B	3.67	3.73	1.29	1.39
Depth 5-10	N/A	N/A	0.79 C	0.83 C	0.66	0.59	0.54	0.46
Depth 10-15	N/A	N/A	0.49 D	0.48 D	0.40	0.36	0.34	0.38
SE	0.40		0.09		0.52		0.09	
p-value	*0.0077**		*0.0132**		*0.986*		*0.3458*	
Other Eukaryotes								
Depth 0-5	1.69 A	0.98 B	1.66 A	1.42 B	1.63	1.41	1.05	1.00
Depth 5-10	N/A	N/A	1.05 C	0.93 CD	0.87	0.66	0.63	0.50
Depth 10-15	N/A	N/A	0.80 D	0.84 D	0.69	0.56	0.46	0.47
SE	0.10		0.058		0.100		0.078	
p-value	*<0.0001**		*0.0447**		*0.8582*		*0.4336*	

†Spring 2018 only had samples taken at the 0-5 cm depth

Table 3.5

Mean percent community composition out of total biomass within CC and NC treatments in fall and spring of 2018 and 2019 at the 0-5 cm depth.

	2018				**2019**			
	Spring		**Fall**		**Spring**		**Fall**	
Crop growing prior to sampling	*Triticale & Rapeseed*		*Soybean*		*Winter Wheat & Rapeseed*		*Corn*	
	CC	**NC**	**CC**	**NC**	**CC**	**NC**	**CC**	**NC**
	(% of total biomass)							
Gram-Neg	31.4	30.6	32.0	32.8	32.9	33.4	32.0	33.0
Gram-Pos	24.9	25.6	25.2	24.5	21.8	19.3	25.0	24.2
Actinomycetes	12.9	13.6	12.6	12.7	11.4	10.3	12.8	12.4
AM Fungi	3.6	3.4	4.2	4.1	4.2	3.7	4.2	4.4
Fungi	3.0	2.6	3.0	2.8	4.3	4.6	2.0	2.2
Other Eukaryotes	1.5	1.3	1.9	1.8	1.9	1.7	1.6	1.6

Table 3.6

Monthly temperature average highs and lows and precipitation for 2018 and 2019 at the Kansas Agricultural Watershed field site.

Month	2018			2019		
	High (°C)	Low (°C)	Precipitation (mm)	High (°C)	Low (°C)	Precipitation (mm)
January	5.32	-7.90	10.16	3.58	-7.10	31.24
February	6.84	-7.01	10.16	1.64	-7.48	32.76
March	14.16	0.19	17.52	9.61	-1.48	61.99
April	16.06	1.12	43.43	20.9	6.84	55.88
May	29.38	15.20	83.3	22.16	11.39	307.32
June	32.90	20.1	54.61	29.42	16.63	145.05
July	32.66	19.22	72.63	31.66	19.76	58.42
August	31.02	18.57	168.91	29.25	19.29	218.43
September	26.40	15.35	127.51	29.73	18.38	59.69
October	18.45	5.6	149.35	16.90	3.81	69.34
November	8.83	-3.18	19.05	11.49	-2.04	15.48
December	6.95	-4.61	63	8.44	-3.81	26.92
Total			819.63			1082.52

Figure 3.1

Total microbial biomass in CC and NC treatments across depth in fall 2018 (A), spring 2019 (B), and fall 2019 (C). Different letters within a graph indicate significance of p<0.05.

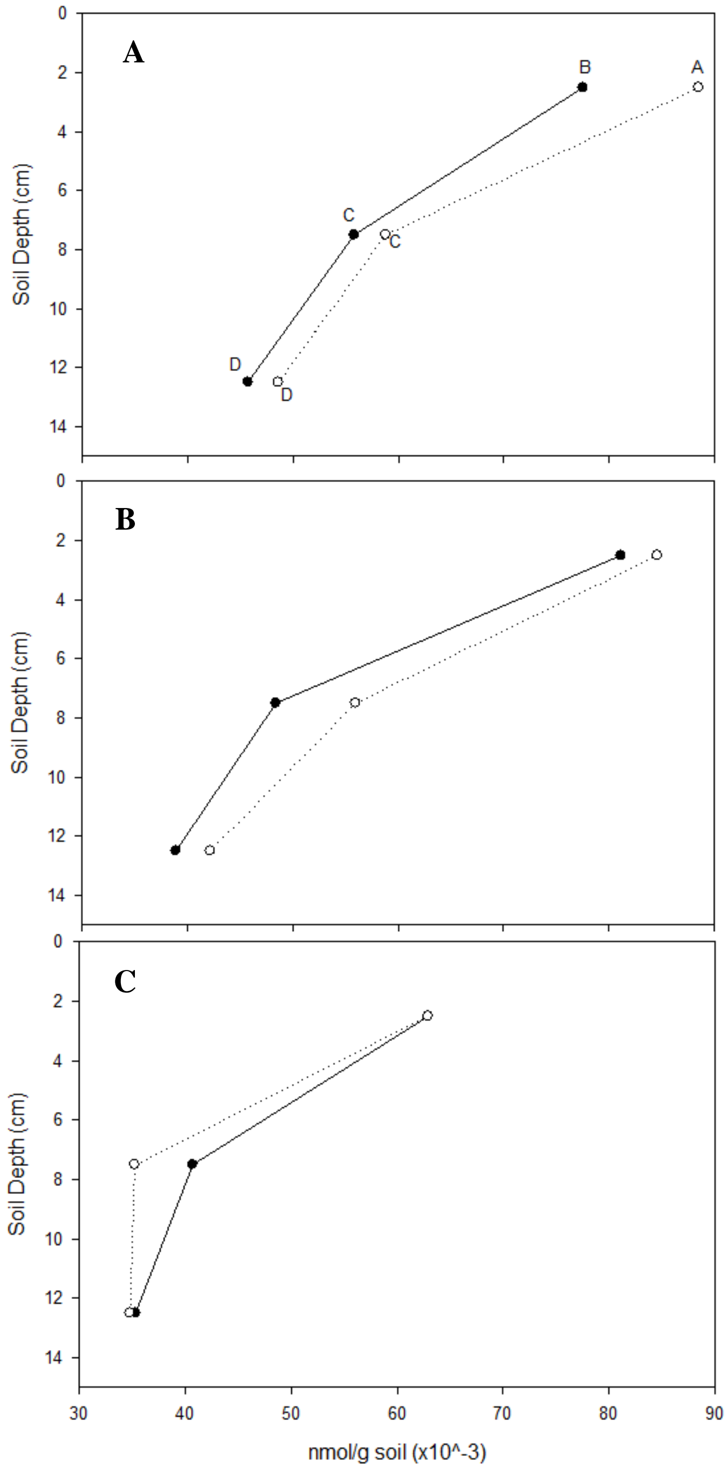
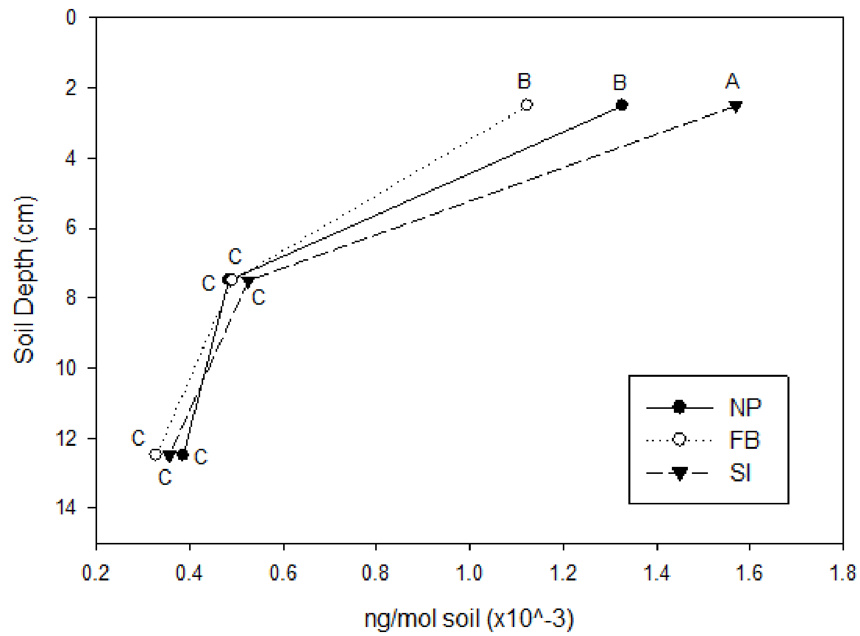


Figure 3.2

Fungi biomass in NP, FB, and SI treatments across depth in fall 2019. Different letters within a graph indicate significance of p<0.05.



Chapter 4 - Microbial Functional Gene Response to Cover Crop and Phosphorus Fertilizer Usage in a No-Till Corn-Soybean System

Abstract

Currently there remains much to understand regarding how soil microorganisms respond to agricultural management practices. Understanding how sustainable agricultural management practices like cover crop implementation in a no-tillage corn-soybean system impact soil microorganisms can help to expand current understanding of soil microbial diversity and function. The objective of this study was to examine the functional genes associated with carbon (C), nitrogen (N), and phosphorus (P) nutrient cycling responses to cover crop usage and P fertilizer management regimes under no-tillage in a field scale study. Soil samples were collected from the Kansas Agricultural Watershed Field Laboratory in the fall of 2019 and spring of 2020 at the 0-5 cm soil depth. Samples were examined using the GeoChip-based functional gene array that targets microbial genes involved in nutrient cycling processes. The experiment has a 2 by 3 factorial treatment structure with two levels of cover crop, with cover crops (CC) and without cover crops (NC) and three levels of P fertilizer management: no P fertilizer (NP), fall broadcast (FB), and spring injected (SI) in a randomized complete block design with three replicates of each treatment combination (total of 18 plots). Within C targets, *amyA* was the most consistently responsive to treatments with greater abundance in the CC treatment as compared to the NC treatment. Diverse treatment effects were found in N cycling genes, with the NP*CC treatment typically having the greatest abundance of N cycling genes. P cycling nutrient cycling processes were found to be fairly consistent across examined subcategories of P cycling processes, *ppK* being responsive to treatments in both fall and spring samples. In P cycling, the NP*CC treatment was found to have the greatest abundance when a treatment effect was detected.

Ultimately this study sheds light on the functional gene potential of the soil microbial community and demonstrates diverse microorganismal responses to the examined agricultural management practices.

Introduction

Soil biota contribute to soil quality in both physical properties as well as chemical composition. It has been estimated that the amount of microbial biomass underground may be roughly equal to the living biomass found above (Gold, 1992). Higher microbial biomass has been found in treatments with no-till practices as compared to those with conventional tillage (Karlen et al., 1994; Motta, Reevesd, Burmester, & Feng, 2007). The soil environment is highly diverse with many types of eukaryotes, prokaryotes, and archaea present. Organisms have been found in diverse and extreme environments; bacteria have been isolated from two miles below the earth's surface (Boone et al., 1995). It is estimated that soil biodiversity contributes an estimated 1.5 quadrillion U.S. dollars to ecosystem services (Pimente et al., 1997). A single gram of soil is estimated to have one billion bacterial cells that can be grouped into one thousand to one million different species (Gans, Wolinsky, & Dunbar, 2005; Schloss & Handelsman, 2006). Yet,the role of microorganisms in agricultural soils is not fully understood.

Studying microorganisms presents challenges and as such agronomists have relied on analysis of predominately chemical and physical soil properties to gauge the microbial community and its response to agronomic management techniques. As a result of using tests that measure general microbial properties, microbial responses to conservation management techniques has been broadly generalized and found to be variable (Bender & van der Heijden, 2015; Xue et al., 2013). This broad generalization of soil microorganisms may contribute to variable results found from implementation of conservation management techniques. Previous

work examining microbial communities has also implemented methods examining nucleic acids using polymerase chain reaction (PCR) and phospholipid fatty acid (PLFA) analysis. Nucleic acid and PLFA methods have been used to examine microorganismal community composition, abundance, and taxonomic diversity. The nature of PCR allows researchers to target one or a few specific gene sequences that could be indicative of taxonomic or functional classification, but this technique is limited by the number of available targets. Therefore, PCR-based techniques are applied to specific questions addressed by examining typically a handful of sequences. While other techniques, such as PFLA, use less specific criteria to broadly access microbial communities. Phospholipid fatty acid analysis can classify microorganisms present in soil samples into broad community groupings and can, at this resolution, depict community structure. However, PLFA cannot speak to what is functionally occurring in soils (Theis, 2008). In contrast, GeoChip is a functional gene microarray that offers a unique opportunity to access microbial functional gene responses to the environment. This analysis has yet to be applied widely to agricultural management. GeoChip contains over 160,000 distinct probes that target about 1,500 functional gene families. These targeted gene families are involved in many nutrient cycling processes including microbial carbon (C), nitrogen (N), and phosphorus (P) cycling (Glomics, 2014). This type of analysis has not yet been utilized to assess the effects of cover crops in a no -tillage management system and could serve to better illustrate the role soil microorganisms play in conservation management practices.

Examining the functional genes of microorganisms in carbon cycling highlights their involvement in nutrient cycling. In no-tillage systems and especially those that also have cover crops with 100% of the residue remaining on the soil surface, there is a great deal of plant material available for breakdown. Cellulose, hemicellulose, and lignin (respectively) are the most

common structural polysaccharides in plant residues; structural polysaccharides aid plants in cell wall rigidity (Burranov & Mazza, 2008). The breakdown of the structural polysaccharides is dependent upon extracellular enzymes that are excreted from bacteria and fungi, that can eventually break down these complex molecules into monomers, which can be utilized by a larger proportion of microorganisms and plants (Luo, Meng, & Gu, 2017; Utobo & Tewarl, 2014; Eivazi & Tabatabai, 1987). Genes responsible for the production of these enzymes can be identified from microorganisms and thus lead to a functional characterization of the microbial community (Zhou et al., 2015; Xue et al., 2013; Zhang et al., 2013). Some of the functional genes that have been established to be involved in carbon cycling in bacterial, archaeal and fungal systems include: acetylglucosaminidase (nag) (degrades chitin) and α-amylase (*amyA*) (degrades starch) (Trivedi et al., 2016; Xue et al., 2013). Within the GeoChip 5.0 microarray targeting C cycling include broad categories such as carbon and chitin degradation, and more specific ones targeting enzymes including acetylglucosaminidase and *amyA*.

The nitrogen cycle is especially important in agriculture as plants cannot fix atmospheric N_2 and nitrogen is a limiting nutrient in soils for crop growth and yield. To overcome limited N supply, fertilizers are applied; however, this is costly and can have a negative environmental impact. Forms of nitrogen that are plant available are inorganic and include nitrate (NO_3) and ammonium (NH_4^+). Within the process of nitrification, ammonia monooxygenase gene (*amoA*) oxidizes NH_4^+ to nitrite (NO_2^-) and has been found in both archaea and bacteria. This gene catalyzes the first, rate-limiting step of autotrophic nitrification. It is used as one metric for the functional capacity of nitrification in soils and is included in the GeoChip 5.0 microarray. Habteselassie et al. (2013) found that *amoA* abundances varied while populations of ammonia oxidizing bacteria and archaea remained stable over a six-year field study of plots exposed to

seven different nitrogen applications that differed in amounts and sources of N. Currently the distribution of ammonia-oxidizing bacteria and archaea in soils is known to be complex. However, there is an indication of ammonia-oxidizing bacteria responding to N-fertilizer presence (increased numbers), while ammonia-oxidizing archaea do not seem to show a response to N-fertilizer (Zeglin, Taylor, Myrold, & Bottomley, 2011; Taylor, Zeglin, Wanzek, Myrold, & Bottomley, 2012; Zhang et al., 2013). The ratio of ammonia-oxidizing bacteria to archaea varies considerably in soils; however, the factors that define these distributions are an area of on-going research (Leininger et al., 2006; Taylor et al., 2012; Zeglin et al., 2011; Habteselassie, Xu, & Norton, 2013).

Nitrite reductase (*nir*) is the primary enzyme of denitrifiers because it catalyzes the transformation of an ion (NO_2^-) to a gas (NO). There are two known forms of genes that code for nitrite reductase: *nirK* and *nirS* (Xue et al., 2013; Zhang et al., 2013). Work by Xue et al. (2013) found that *nirK* and *nirS* were positively correlated with N_2O flux. Both genes have been widely studied; however, current data is conflicting as to which environmental factors impact changes in *nirK* and *nirS* gene abundances (Hallin et al. 2009; Enwall et al. 2010; Clark et al. 2012). Both *nirK* and *nirS* are included in the GeoChip 5.0 microarray.

Phosphorus cycling processes are also important in agriculture, and soil microbes contribute to providing plant available phosphorus. To address limited P supply, fertilizers are applied however this is costly and can have a negative environmental impact. Availability of P in soils is facilitated through the mineralization and immobilization from organic fractions, and the sorption/desorption and precipitation/solubilization processes are mediated from the inorganic fractions (Frossard, Condron, Oberson, Sinaj, & Fardeau, 2000). Microorganisms can mineralize organic compounds in pursuit of C and in doing so release P associated with C, and

microorganisms can also release phosphatase enzymes that specifically target P (McGill & Cole, 1981). A meta-analysis of published field studies that focused on the interactions between cover crops and P cycling examining the impact of cover crops and plant-microbe interactions (Hallama, Pekrun, Lambers, & Kandeler, 2018). The meta-analysis by Hallama et al. (2018) found that in general cover crops were most effective at increasing P availability in systems with low available P and that they may increase access to 'unavailable' pools. While numerous studies have highlighted the potential significance of the soil microbial P contribution to providing plant-available P (Macklon et al., 1997; He, Wu, O'Donnel, & Syers, 1997; Oehl et al., 2001; Oberson, 2001; Richardson, 2003; Gerke, 2015), the actual contribution of microorganisms to providing plant-available P through P-turnover remains to be determined (Richardson, 2003; Gerke, 2015). Phytate is a significant form of organic P in soils, and phytases catalyze P release from phytate (Sparvoli & Cominelli, 2015; Rodriguez, Fraga, Gonzalez, & Bashan, 2006). Phytases are produced by many soil bacteria and fungi and examining phytase potential within soils may help to indicate a soils potential to utilize organic P pools within agricultural systems (Balaban et al., 2017, Bolan, 1991, Yao, Feng, & Christie, 2001); phytase is included on the GeoChip 5.0 analysis. An enzyme important in soil microbial cycling of P that is present in the GeoChip 5.0 analysis is acid phosphatase, which catalyzes the hydrolysis of organic phosphorus to inorganic phosphorus (Ho, 1979; Privat de Garilhe, 1967; Acosta-Martinez, Tabatabai, & Dick 2011). Two other genes involved in soil microbial P cycling included in the GeoChip 5.0 analysis are ppk (polyphosphate kinase) and ppx (exopolyphosphatase). Both ppk and ppx are genes in phosphate solubilizing enzymes (Liu et al., 2018; Van Dien, Keyhani, Yang, & Keasling, 1997; Tzeng & Kornberg, 2000).

Understanding the functional ability and diversity of the microbiota is critical as it has the power to enhance productivity, provide ecological services and system resiliency, and sustain soil quality (Lehman et al., 2015a; Lehman et al., 2015b; Schmidt, Gravuer, Bossange, Mitchell & Scow, 2018). Focusing on functional measurement rather than taxonomic identification will provide the link between conservation practices, optimized nutrient cycling, and ecologically intensified agricultural systems (Bender & van der Heijden, 2015). Research is needed to further understand how no-till, cover crops, and fertilizer management change microbial function in order to improve implementation of conservation management techniques. The objective of this study was to examine the functional genomics associated with C, N, and P nutrient cycling response to cover crop usage and P fertilizer management regimes under no-tillage in a field scale study. We hypothesized that the functional gene abundances in nutrient cycling processes of soil microbial communities would be significantly affected by agricultural management practices with C, N, and P gene abundances being elevated in the CC treatment compared to the NC treatment. We hypothesized C and N gene abundances would not be affected significantly by P fertilizer treatments in either the fall or spring sampling points. We also hypothesized the P specific gene abundances would be significantly altered by the P fertilizer treatments with the greatest abundances in the NP treatments and the lowest abundance in the SI and FB treatments in both fall and spring sampling points.

Materials and Methods

Site Description and Experimental Design

This work was conducted at the Kansas Agricultural Watershed (KAW) Field Laboratory located near Manhattan, KS (39.134, -96.641) from fall of 2019 to spring of 2020. This field laboratory was established in 2014 and designed with the goal of examining how agricultural

management practices impact soil health and consequently water, sediment, nutrient, and chemical losses. The KAW has 18 small watersheds that range from 0.49 ha to 0.65 ha in size. Smolan silty clay loam (fine, smectitic, mesic Pachic Argiustoll) is the principal soil type and the site has an average slope of 6 to 8 %. The climate for the area is a hot, humid continental climate with a mean annual temperature of 12.7^{o}C and an annual precipitation of 904 mm, with the majority of precipitation occurring in late spring to early fall. Samples were collected on November 11th, 2019 (fall 2019 samples) and May 1st, 2020 (spring 2020). The environmental data (Table 4.1) was obtained from Kansas State University's Kansas Mesonet located at Ashland Bottoms (39.126, -96.677).

The treatment design is a 2 by 3 randomized complete block factorial design, with three replicates of each treatment (18 plots). There are two levels of the cover crop treatment: cover crops (CC) and no cover crops (NC). There are three levels of phosphorus fertilizer management: no fertilizer (NP), spring injected (SI), and fall surface broadcast (FB). The KAW has been managed in a continuous no-till, corn-soybean rotation. The last tillage event occurred on November 7th, 2014. All crops grown starting in 2015 have been under no-till management. Cover crops were first planted at KAW in fall 2014 and have been planted every year after. Cover crops used have reflected corn-soybean producer cover crop usage in Northwest Kansas. Cover crops have included: winter wheat before soybean in 2016, triticale and rapeseed before corn in 2017, and before soybean in 2018, winter wheat and rapeseed before corn in 2019, and cereal rye before soybean in 2020. Every year the same amount of P fertilizer was applied as either a fall broadcast or spring injected applications. The form of P applied in the fall broadcast treatment was diammonium phosphate (DAP) ($(NH_4)_2HPO_4$) at 134.5 kg/ha (61.65 kg P/ha), and the form of P applied in the spring injected treatment was ammonium polyphosphate

($[NH_4PO_3]n(OH)_2$) at 131 L/ha (61.65 kg P/ha). Nitrogen fertilizer, 28% urea ammonium nitrate (NH_4NO_3), was injected below the surface at a uniform rate of 145.71 kg N/ha for all plots in corn years. The fall broadcast treatment was discontinued after the 2019 cash crop then the treatment received no P prior to the spring 2020 soil sampling. Soil pH at the site ranges from 6-7.

Soil Sampling

Soil samples were taken at a depth of 0-5 cm in fall 2019 and spring 2020. These samples were collected by taking 40 cores along a line transect in each plot. Sampling was conducted before the cash crop was planted and before the cover crop was terminated in the spring and immediately after cash crops were harvested and before cover crops were planted in the fall. Samples for a given plot were sieved using a 2 mm sieve, and after sieving, samples were placed at -80^oC.

GeoChip Hybridization and Data Processing

Soil DNA extraction followed the manufacturer's instructions using a DNeasy PowerSoil Kit (Qiagen, Germantown, MD, USA), and 150 to 250 mg of soil were used per DNA extraction. Three separate DNA extractions were performed on each sample. The three DNA extracts were then combined to form one composite DNA sample for each sample and were kept at -80^oC until they were sent frozen for GeoChip 5.0s analysis at Glomics (Norman, OK). GeoChip 5.0s was used to analyze DNA samples as described previously (Wang et al., 2014). In short, florescent dye (Cy-3) was used to label DNA (1µg) using a random priming method. Labeled DNA was then purified with the QIA quick purification kit (Qiagen, Germantown, MD, USA) as indicated by the manufacturer's instructions. Labeled DNA was then hybridized with the Agilent platform based GeoChip 5.0s arrays at 67^OC plus 10% formamide for 24 h. GeoChip microarrays were

then scanned with a 100% laser power and 100% photomultiplier tube with a NimbleGen MS 200 Microarray Scanner (Roche, Basel, Switzerland). Spots with a signal-to-noise ratio <2.0, signals <150, or <1.3 times the background were discarded prior to statistical analyses. Raw data was then quantified and processed using the analysis pipeline as previously described (He et al., 2010; Tu et al., 2014). Then processed GeoChip data was examined for targeted genes of interest, for C (Table 4.2), N (Table 4.3), and P (Table 4.4). Examined categories are found in Table 4.2 (C), Table 4.3 (N), and Table 4.4 (P). Probes specific to given nutrient cycling categories as well as specific genes were defined in the output from the GeoChip analysis. Signal intensity values were summed for each functional group and then statistical analysis performed. The Eukaryota category was examined without inclusion of any fungi, as fungi was analyzed as a separate category.

Statistical Analysis

Statistical analysis was performed using SAS version 9.4 software (Cary, NC, U.S.A.) with a PROC MIXED repeated measures of variance procedure. Analysis of variance (ANOVA) and Tukey-adjusted LSMEANS were used to indicate differences between treatments.

Results

Abundance of Functional Genes: Carbon

GeoChip results are presented in terms of signal intensity and this is representative of abundance, as a greater signal intensity is synonymous with a greater abundance of a given transcript. When examining all probes targeting carbon cycling there was no significance found among treatments in fall nor spring samples. When examining these probes at the Archaea, Bacteria, Eukaryota, and fungi levels, the only significant effect found was in fall sampling within the fungi category where a significant interaction between fertilizer and cover crop

treatments was detected (Table 4.2); the NP*CC treatment combination had significantly higher abundance of C cycling genes than all other treatment combinations (Fig. 4.1A).

No significant differences among treatment combinations were found in either fall or spring for probes specific to carbon degradation. When examining Archaea, Bacteria, Eukaryota, and fungi categories within probes targeting carbon degradation, significant treatment effects were found. Archaea and fungi were found to have a significant interaction between the P fertilizer and cover crop treatments in the fall (Table 4.2). In the Archaea category, the NP*CC, FB*CC, and FB*NC categories were statistically similar with the highest abundance. The FB*NC treatment was also not statistically different from the NP*NC and SI*CC treatments which had the lowest abundances. The FB*NC treatment was not statistically different than all treatment combinations (Fig. 4.1B). No significant differences among treatment combinations were found in spring samples. Within the fungi category the NP*CC treatment was found to have significantly higher abundance than all other treatment combinations in fall and No significant differences among treatment combinations were found in spring samples (Fig. 4.1C). No significant differences among treatment combinations were found in the chitin degradation category (Table 4.2).

There was a significant interaction of fertilizer and cover crop treatments affecting all probes targeting acetylglucosaminidase as well as bacteria-specific probes in the fall season. It should be noted that in this instance out of the 373 total probes, only two target archaea and one target is uncategorized, meaning that this significant effect was directly related to the bacteria probes. Probes targeting acetylglucosaminidase were found to have a significantly higher signal intensity in the NP*CC treatment as compared to the FB*NC and SI*CC treatments with the

FB*CC, SI*NC, and NP*NC treatments being equal to all other treatments in fall and no significance was found in spring (Fig. 4.2A and Table 4.2).

There was a significant interaction of fertilizer and cover crop treatments affecting all probes targeting *amyA* as well as bacteria and Archaea-specific probes in the fall season. When examining all the targets for *amyA* NP*CC was found to have a significantly higher signal intensity than all other treatments (Table 4.2). In the *amyA* categories of both Archaea and Bacteria the treatment NP*CC was significantly higher than the NP*NC, SI*CC, SI*NC, and FB*CC treatments. The FB*NC treatment was equal to all other treatments (Table 4.2, Fig. 4.2B, and Fig. 4.2C).

Abundances of Functional Genes: Nitrogen

There was a significant interaction of fertilizer and cover crop treatments affecting all probes targeting nitrogen cycling, as well as within the Archaea and Eukaryota groupings in the fall season. In the Archaea grouping within the overall N cycling group, the NP*CC treatment had the greatest abundance of N cycling genes and all other treatments were lower and statistically equivalent to each other (Table 4.3 and Fig. 4.3A). In the Eukaryota grouping the treatments were as follows: NP*CC > SI*NC > NP*NC, and FB*CC, FB*NC, SI*CC = SI*NC and NP*NC (Fig 4.3B). No significant differences were found in spring samples (Table 4.3).

When grouped together probes targeting ammonification were not significantly different among treatments in either fall or spring. No significant treatment effect was found in probes targeting Eukaryota within ammonification in fall samples. Probes specific to Eukaryota within ammonification had a significantly higher abundance in the NC treatment as compared to the CC treatment in spring samples. When examining the other groups of Archaea, Bacteria, and fungi

within ammonification, no significant difference between treatments were detected in either of the sampled seasons (Table 4.3).

Probes targeting the process of anammox (Bacteria only) from spring samples were found to have a higher signal intensity in plots with CC as compared to those with NC. No significant differences between treatments were detected in the fall (Table 4.3).

In all probes targeting denitrification no significant difference between treatments was found. In the Archaea subgroup abundance in the fall was found to have a significant interaction between the P fertilizer and cover crop treatments, with NP*CC > FB*CC and SI*CC, and NP*NC, FB*NC, and SI*NC were equal to all treatments (Fig. 4.4A); no significance was found in spring samples. Within the targets for denitrification, *nirK* and *nirS* were also examined (Table 4.3). When examining all the probes targeting *nirK*, the interaction between the P fertilizer and cover crop treatments was found to be significant within fall samples, with NP*CC and FB*CC > NP*NC and SI*CC, with FB*CC, FB*NC, and SI*NC all equal to each other (Table 4.3 and Fig. 4.4B), no other significant treatment effects were detected in *nirK* categories for fall and spring samples. No significance was detected in response to treatments within the *nirS* probes in neither the fall nor spring samples (Table 4.3)

All probes as well as those specific to Bacteria targeting nitrogen fixation (*nifH*) were significantly impacted in response to P fertilizer treatments in fall samples. The FB and NP treatments were statistically equivalent while the SI treatment was significantly lower, and no significant difference between treatments was found in spring. The other grouping examined (Archaea) with probes targeting N fixation was not found to be significantly impacted by any treatments in neither the fall nor the spring samples (Table 4.3).

All probes targeting the process of N assimilation were examined and found to be significantly impacted by the interaction between the P fertilizer and the cover crop treatments in the fall samples. The NP*CC treatment significantly higher than the other treatments which were all be not statistically different from each other. The Eukaryota grouping within probes targeting N assimilation in fall samples was significantly impacted by the cover crop treatment and found to have higher signal intensity when cover crops were present as opposed to when they were not implemented. The grouping of fungi within N assimilation was not found to be significantly impacted by any treatments in neither the fall nor spring samples. There was no significance found in any spring samples from probes targeting N assimilation (Table 4.3).

The Bacteria group within the *amoA* probes was also significantly impacted by the cover crop treatment with a greater abundance found in the CC treatment in fall. (Table 4.3). In the spring samples a significant interaction between the P fertilizer and cover crop treatments was found when considering all *amoA* targets, with the FB*CC treatment having the greatest abundance as compared to the other treatments and FB*NC and SI*NC having the lowest (Fig. 4.5). The Archaea group within *amoA* probes was also found to have the same trend in spring samples as the total *amoA* probe readings from spring samples (Table 4.3). No other significant interactions were observed within the *amoA* probes (Table 4.3).

Abundance of Functional Genes: Phosphorus

When looking at all of the probes targeting P cycling from fall samples, both the total and the Bacteria groupings were significantly impacted by the interaction between P fertilizer and cover crop treatments (Table 4.4). Both categories had the same trend in how treatments compared to each other; the NP*CC treatment had a significantly greater abundance than all other treatments which were all statistically equal (Bacteria Fig. 4.6A). Within the probes

targeting P cycling, there were no significant effects of the treatments in the spring (Table 4.4). Probes targeting phytase genes within those specific to fungi had a greater abundance in the NP and FB treatments than the SI treatment in the fall season; no other significance was detected (Table 4.4). All probes targeting *ppK* as well as those within the Bacteria sub-group were significantly impacted by the interaction of the P fertilizer and the cover crop treatments in fall samples with the NP*CC treatment being significantly higher than all other treatments which were not significantly different than one another (Bacteria Fig. 4.6B, Table 4.4). Examining all probes targeting *ppk* as well as those specific to Bacteria from spring samples found greater abundance in the CC treatment as compared to the NC treatment (Table 4.4). All probes targeting *ppX* as well as those within the Bacteria grouping from fall samples were found to be significantly impacted by the interaction between the P fertilizer and cover crop treatments with the NP*CC treatment having the greatest abundance and all other treatments being significantly less and statistically equal to each other (Bacteria Fig. 4.6C); no significance was found in the spring samples of *ppX* nor the Bacteria group within *ppX* (Table 4.4). No significance was detected in fall samples within *ppX* specific to fungi (Table 4.4). However, in spring samples *ppX* within the fungi category were found significantly impacted by the cover crop treatment with a greater abundance in the NC treatment (Table 4.4). The Archaea group within probes targeting *ppX* was also examined and no significant impact from treatments were detected in neither fall nor spring samples (Table 4.4).

Discussion

Abundance of Functional Genes: Carbon

With respect to C cycling in soils, I found that fungi from fall samples were significantly affected by the interaction of the P fertilizer and cover crop treatments. This was also observed in

probes specific to C degradation from fall samples, with the highest signal intensity in the NP*CC treatment. Within the C degradation category, Archaea were also significantly affected by the P fertilizer and cover crop treatment interaction; however, the NP*CC, FB*CC, and FB*NC treatments were statistically equivalent to one another (Fig. 4.1ABC).

Work by Xue et al. (2013) examined functional gene differences between conventional management, low-input management (received one-third of synthetic N fertilizer as compared to the conventional management and a red clover cover crop), and organic management (no synthetic N fertilizer and no compost or manure) in a corn-soy-wheat rotation in Michigan, and performed soil sampling in fall when corn was harvested. Xue et al. (2013) found that microbial genes involved in nutrient cycling (C/N/P) were consistently higher in the low-input and organic systems as compared to the conventionally managed system. Xue et al. (2013) attributed this to low-input systems potentially enhancing soil microbial nutrient cycling. Gene upregulation in response to nutrient acquisition is common in fungi (Korripally et al., 2015; Coman, Mot, Gal, Parvu, & Silaghi-Dumitrescu, 2013, Suzuki et al., 2012).

A study by Schmidt et al. (2018) examining soil microbial functional diversity in response to tillage and no-till management as well as to cover crop implementation, found that no-till shifted the microbial community towards stress tolerators and cover crop implementation shifted the microbial community to ruderals. Stress tolerators were defined as organisms that are able to persist under unfavorable resource-limiting conditions (Schmidt et al., 2018; Krause et al., 2014; Ho et al., 2013). This study found greater gene abundance in the NP*CC treatment in some targeted categories in the fall season. The NP treatment may encourage enzyme production in soil microorganisms to compensate for the lack of P fertilizer. This result would also be in line with findings discussed above by Xue et al. (2013), finding low-input systems enhancing

soil microbial nutrient cycling. When examining the abiotic factor of soil moisture there was no significant difference between soil moisture from fall 2019 and soil moisture from spring 2020 (data not shown). Air temperature between fall and spring samples did vary somewhat; however, when examining the soil temperature at the 5 cm depth this variation was very small at the time of sampling (Table 4.1). Xue et al. (2013) attributed finding enhanced microbial nutrient cycling in low-input systems to potentially the N fertilizer having a suppressing effect on the soil microbial community. Yang, Yao, Hu, and Qi (2000) used random amplified polymorphic DNA analysis and found that N fertilizer lowered soil microbial diversity at the genotype level. N fertilizer application has also been found to diminish soil microbial metabolism; however, this impact was not observed in the examined P fertilizer (Sarathchandra, Ghani, Yeates, Burch, and Cox, 2001).

When examining the sub-division of C cycling targeting acetylglucosaminidase, in respect to all probes as well as those specific to Bacteria within this category for fall samples, the interaction between the P fertilizer and cover crop treatments was again found to be significant. In both categories the NP*CC, FB*CC, and SI*NC treatments had the statistically highest signal intensities; however, the FB*CC and SI*NC treatments were statistically equal to all the treatments (Fig. 4.1). Acetylglucosaminidase is involved in chitin breakdown, and because chitin is only found in arthropods and fungi this suggests the breakdown of fungi within the aforementioned treatments. Greater fungi presence has been found with cover crop implementation (Muhammad et al., 2020; Finney et al. 2017). The finding that the NP*CC treatment was higher than the SI*CC treatment may be due to the nutrient inputs to the SI treatment resulting in less of a stress response.

Starch is a more labile form of carbon than lignins and celluloses and one enzyme that degrades starch is α-amylase (*amyA*) (Yu, Luo, Xu, Gou, & Wang, 2020). This study examined *amyA* and found when examining all probes targeting this gene as well as in the Archaea and Bacteria groups in fall that there was a significant interaction between the P fertilizer and cover crop treatments (Fig. 4.1) and in spring samples all examined categories (all, Archaea, Bacteria and fungi) were significantly impacted by the cover crop treatment. When looking at all probes targeting *amyA* the NP*CC treatment had higher signal intensity than any of the other treatments in the fall, a trend also observed in the fungi category of C degradation and when examining all probes targeting C nutrient cycling. In the study by Xue et al. (2013) *amyA* was present at a greater abundance in the organically managed (no synthetic N fertilizer and no compost or manure) plots as compared to conventionally managed (standard chemical inputs) system, suggesting a consistent finding in this study in that the NP*CC treatment was a reduced input treatment (in respect to P fertilizer and N assorted with the forms of P fertilizer). The contrast found between the fall and spring samples could be attributed to the impact of having a living plant in the soil in the cover crop plots, allowing for greater microbial activity overall (Finney et al. 2017; Muhammad et al., 2020). Some studies have found little impact of cover crop implementation on enzyme activity in the short-term (less than 3 yrs) (Rankoth. Udawatta, Veum, Jose, & Alagele, 2019; Calderon et al., 2016; Acosta-Martinez et al., 2011). However, Rankoth et al. (2019) suggest that beyond the short-term enzyme activity would increase under no-tillage management with cover crop implementation as compared to no cover crop usage. Acosta-Martinez et al. (2011) found an increase in β-glucosidase and β-glucosaminidase activity at three years after cover crop implementation; however, they found at 5 yrs no detectable difference between cover crop and no cover crop usage in no-tillage systems. No-tillage

management and cover crops were first implemented in this study in 2015, and although the cover crop treatment resulted in significantly higher β-glucosidase and β-glucosaminidase activity (chapter 2), this trend may diminish over time.

Abundance of Functional Genes: Nitrogen

Zhang et al. (2013) examined the response of soil microbial N-cycling genes to environmental changes in a Mongolian steppe ecosystem and found overall that the abundance of different microbial genes involved in N nutrient cycling responded differently to various environmental changes. Zhang et al. (2013) concluded mechanisms controlling these varied responses were likely due to complex nutrient feedbacks within soils. Looking at the N nutrient cycling probes examined in this study, a similar finding emerges, that N nutrient cycling genes and microorganism groups have different responses to different treatments; however, this study did find the P fertilizer interaction with the cover crop to be the most frequent treatment effect observed with similar trends as observed in C cycling.

When examining the genetic potential of N nutrient cycling and looking at all probes targeting N cycling the Archaea and Eukaryota had a significant interaction between the P fertilizer and cover crop treatments and had a significantly greater abundance in the NP*CC treatment as compared to other treatments in the fall samples (Table 4.2 and Fig. 4.3). Other categories examined within N nutrient cycling that also were found to have significant treatment interaction between the P fertilizer and cover crop treatments in the fall samples include: all probes targeting assimilation and Archaea within denitrification. In both of these categories the NP*CC treatment is either the treatment group with the highest abundance or one of the highest abundances. In the functional gene differences examining conventional, low-input, and organic farmlands study by Xue et al. (2013), conventionally managed plots were found to have a lower

abundances of genes involved in N cycling processes as compared to both the low-input and organically managed plots. Xue et al. (2013) found no significant difference between the low-input and the organically managed plots in the genes involved in N cycling. This study found a similar trend of higher abundance in the NP*CC treatment than in other treatments in several of the C cycling areas discussed previously. Although the study by Xue et al. (2013) is different, it did find higher abundance in the plots managed with reduced inputs (organic and low-input) which has similarity to the NP*CC treatment in this study would have reduced P and associated N inputs. The other significant interaction found in the probes specific to N assimilation was within the Eukaryota grouping that had significantly higher abundance in plots with the CC treatment in fall samples.

When examining ammonification as a subcategory of N nutrient cycling, this study found that the only significant interaction occurred in the spring samples in the Eukaryota grouping where significantly higher abundances were found in the NC treatment. This trend was not observed in other N cycling probes examined and is difficult to draw conclusions with this finding not being found in other ammonification categories. Ammonification has been found to be correlated with archaeal and bacterial *amoA* gene presence in different land uses (Zeglin et al., 2011). In this study, probes specific to *amoA* and ammonification had different points of significance across sub-categories and seasons.

When further examining denitrification results, as mentioned above, the Archaea grouping within probes specific to denitrification found the NP*CC treatment to have the highest abundance in fall samples. A similar trend was also observed in probes specific to the gene *nirK* (denitrification) in fall samples. The FB*CC treatment was also statistically equal to the NP*CC treatment for abundance specific to all probes targeting *nirK*. Work by Xue et al. (2013) found

that *nirK* (similar to all genes involved in N cycling processes) had the lowest abundance in the conventionally manage plots as compared to the low-input and organically managed plots which were not significantly different in respect to N cycling gene abundances (including *nirK*). In this study the denitrification gene *nirS* was not found to be significantly impacted by any treatment. Work by Zhang et al. (2013) found *nirS* to have a greater sensitivity than *nirK* to examined environmental changes. Work by Zhang et al. (2013) was a very different experiment than what has been presented for this study; however, the finding in this study of *nirK* having a greater sensitivity to field treatments than *nirS* highlights the need for further research to better understand how soil microbial genes involved in nutrient cycling respond to various environmental and agricultural conditions.

The P fertilizer management treatments impacted the N cycling categories in the fall: all probes specific to N fixation (*nifH*), the Bacteria category within *nifH*, and the Bacteria grouping within probes targeting *amoA*. What is interesting about this finding is the consistency that both of these groups are bacteria; however, their responses are somewhat inverted to each other, which is not all together surprising given they target different components of N cycling. The Bacteria grouping of N fixation has the highest target abundance in plots with the FB and NP treatments and the lowest in plots with the SI treatment, while the Bacteria grouping within probes specific to *amoA*, had the highest signal intensity in plots with either the NP or the SI treatments and the lowest in plots with the FB treatment. In both of these instances the NP treatment is one of the treatments with the highest abundance. Work by Zhang et al. (2013) (detailed above) found that *amoA* from bacteria responded to P in the presence of N (fertilizers used in this study had N applied with P fertilizer treatments, N was also applied at time of corn planting) shifted the bacteria to being P limited from being N limited, and this response in

ammonia-oxidizing bacteria has been observed in other studies (Zeglin, et al., 2011; Taylor et al., 2012; Zhang et al., 2013). Although the amount of N added in the FB and SI treatments is the same, the timing of the P fertilizer treatments would mean the SI treatment would have been applied more recently and thus less depleted than the FB treatment. However, it does not help articulate why the NP and SI treatments are statistically equal in abundance within the *amoA* Bacteria group, nor why this trend was not reversed in spring samples. When looking at the results found in targets for *amoA* specific to Archaea, the interaction between the P fertilizer and cover crop treatments was found to have a significant impact on these abundances. In this interaction, the FB*CC treatment was found to have the highest abundance with the FB*NC and SI*NC treatments having the lowest abundance (same trend as observed for all *amoA* specific probes in spring samples, Fig. 4.3). Work by Mao, Yannarell, & Mackie (2011) that studied bioenergy feedstock crops on N cycling soil microorganisms, found a significant correlation between the nitrification rate and the quantity of ammonia-oxidizing archaea and did not find this correlation in ammonia-oxidizing bacteria, indicating that archaea were the major ammonia oxidizers. Also, ammonia-oxidizing archaea have been found to have a higher genetic / metabolic diversity than other N-cycling microbial groups and therefore may be more resistant to environmental stressors than ammonia-oxidizing bacteria (Zhang et al., 2013; Pester, Schleper, & Wagner 2011). While the ammonia-oxidizing archaea may have had higher abundance in respect to nitrification when conditions were not under as much stress i.e. spring, more in-line with findings by Mao et al. (2011) as well as others (Zhang et al. 2010; Offre, Prosser, & Nicol, 2009; Tourna, Freitag, Nicol, & Prosser, 2008), that found archaeal ammonia-oxidizers were more correlated to nitrification than bacterial ammonia-oxidizers. However other work has shown that ammonia-oxidizing archaea are more stable and less responsive to environmental

differences than ammonia-oxidizing bacteria (Jia & Conrad, 2009; Di et al., 2009; Zeglin et al., 2011).

The Bacteria group within probes specific to N fixation was found to have significantly greater abundance in plots with either the FB or the NP treatments as compared to plots with the SI treatment. All N fixation probes targeted the *nifH* gene. Work by Pereg, Morugan-Coronado, McMillan, & Garcia-Orenes (2018) examined the response of N cycling soil microbial genes and their response to organic fertilization which included additions of grape prunings, combined with sheep manure or leguminous cover to conventional inorganic N fertilizers in a grapevine system in the Mediterranean over ten years. Pereg et al. (2018) found that ammonia and nitrate fertilizers may decrease *nifH* – carrying microorganisms. This could potentially support findings from this study in that fall samples received the most recent P fertilizer treatment SI (ammonium polyphosphate) and had the lowest abundance of *nifH*. Work by Zhang et al. (2013) found that *nifH* abundance decreased with N addition. And the study by Xue et al. (2013) found that in-line with all N cycling genes examined, *nifH* had the lowest abundance in conventionally managed plots as compared to low-input and organically managed plots. These results highlight the impact of N application on *nifH* abundance.

In the spring probes specific to anammox (only had members of the Bacteria kingdom) were significantly more abundant in plots with cover crop treatment. Anammox probes were specific to either *hzo* or *hzsa* genes. A rye winter cover crop has been found to reduce evaporation from the soil surface in a corn-soybean rotation in Minnesota (Baker, Ochsner, & Griffis, 2007). Cover crops have been found to allow more rainfall infiltration into soil and reduce the volume of runoff (Dabney, 1998; Kasper & Singer, 2008), reduced runoff has been observed at the KAW. The process of anammox is anaerobic ammonium oxidation and because

of this it is possible that plots with the cover crop treatment see higher abundance of these genes, given plots with a cover crop treatment can retain greater moisture providing a greater likelihood of anoxic conditions especially in the spring when precipitation would be greater than the fall; however, as mentioned above in this study there was no difference in soil moisture between fall and spring at the time of sampling.

Abundance of Functional Genes: Phosphorus

When examining all the P nutrient cycling probes, the fall samples of all the probes as well as all the P probes specific to bacteria were significantly impacted by the P fertilizer and cover crop treatments, in both cases with the NP*CC treatment having significantly greater abundance than all other treatments and all other treatments being equal to each other (Fig. 4.4). Subdivisions of P cycling genes examined included phytase, *ppK*, and *ppX*. Phytase was significant in the fungi category in the fall in response to P fertilizer treatment, with the NP and FB treatments having significantly higher abundance than the SI treatment. Sometimes it can be difficult to know whether or not phytase activity in soils is attributed to plants or microorganisms as both can produce forms of phytase (Nannipieri, Giagnoni, Landi, & Renella, 2011). However, this study found probes specific to fungi phytases to be responsive to P fertilizer treatments, suggesting they do play a role in a no-tillage managed corn-soybean cropping system. A meta-analysis examining over 600 published field studies on cover crops and P cycling that focused on plant-microbe interactions by Hallama et al. (2019) found that members of the Poaceae family were especially good at increasing mycorrhizal abundance and microbial P as well as increasing phosphatase activity. However, Hallama et al. (2019) also found that over long-term studies there was no significant increase in fungi in plots in no-till management with cover crop implementation. Which is more in-line with the finding of genes specific for phytase from fungi

being significantly impacted by P fertilizer treatments. All probes targeting the genes *ppK* and *ppX* in the fall and those specific to bacteria in *ppK* were found to have significantly higher abundance in the NP*CC treatments with all other treatments being equal and statistically less than the NP*CC treatment. Work by Xue et al. (2013), found lower abundances of genes specific to P cycling in the conventionally managed plots as compared to the low-input and organically managed plots. In spring samples the abundance of bacteria specific probes targeting *ppK* were significantly higher in the NC treatment as compared to the CC treatment, and this trend was also observed in the fungi group of probes specific to *ppX*, suggesting a potential stress response, as microorganisms able to better scavenge nutrients would have an advantage and potentially proliferate. A study by Soltangheisi, Teles, Sartor, & Pavinato (2020) examining P dynamics under long-term fertilizer addition found when inorganic P fertilizers were applied that the amount of labile P pools under fallow conditions were higher than under cover crops at the 5-10 cm depth. In this study the CC treatment may be depleting P nutrients increasing nutrient stress; however, work by Starr (2020) examining these same plots found greater labile P present at the 0-5 cm depth in the CC treatment in the spring and fall of 2018 and 2019.

There are limitations of GeoChip that must be considered when interpreting results. One of these is that it can be a challenge to obtain a true representative sample, which is a struggle in any type of soil examination given the intrinsic spatial and temporal heterogeneity of soils. In DNA extraction this struggle is especially present given the small amount of soil used for these methods, typically for metagenomic sequencing (including GeoChip) a few hundred nanoGrams of DNA (Myrold, Zeglin, & Jansson, 2013). DNA extraction also contributed to large variation in metagenomic studies (Delmont et al., 2012; Delmot et al., 2011; Myrold et al., 2013).

Conclusions

This study examined functional microbial genes associated with C, N, and P nutrient cycling in response to cover crop usage and P fertilizer management regimes under no-tillage in a field scale study. This study found the NP*CC treatment in fall to be the most consistent treatment to affect functional gene abundance, suggesting that reduced P fertilizer resulted in a stress response from some microorganisms. This finding did not support the hypothesis that P fertilizer treatments would have no effect on C and N functional genes; however, it did support the hypothesis that P functional genes would have the greatest abundance in the NP treatment. Sub-categories within C, N, and P functional genes were found to have diverse responses to treatments and I found different responses to treatments at different sampling points (fall and spring). This study highlights the impacts of cropping systems and management practices on the microbial community. Ultimately this study suggests the diverse microorganismal responses to the examined agricultural management practices. This study identifies soil microorganism nutrient cycling processes that are active after a corn harvest and then right before cover crop termination, demonstrating temporal dynamics in soil nutrient cycling processes.

References

Acosta-Martínez, V., Tabatabai, M. A., & Dick, R. P. (2011). *Phosphorus Cycle Enzymes*. https://doi.org/10.2136/sssabookser9.c8

Adesemoye, A. O., & Kloepper, J. W. (2009, November). Plant-microbes interactions in enhanced fertilizer-use efficiency. *Applied Microbiology and Biotechnology*, Vol. 85, pp. 1–12. https://doi.org/10.1007/s00253-009-2196-0

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017, June 2). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology*, Vol. 8. https://doi.org/10.3389/fmicb.2017.00971

Baker, J. M., Ochsner, T., & Griffis, T. J. (2007). Impact of a cover crop on carbon and water balance of corn/soybean systems. *In The Fourth USDA Greenhouse Gas Conference*. Baltimore, MD.

Balaban, N. P., Suleimanova, A. D., Valeeva, L. R., Chastukhina, I. B., Rudakova, N. L., Sharipova, M. R., & V. Shakirov, E. (2017). Microbial Phytases and Phytate: Exploring Opportunities for Sustainable Phosphorus Management in Agriculture. *American Journal of Molecular Biology*, *07*(01), 11–29. https://doi.org/10.4236/ajmb.2017.71002

Benayas, J. M. R., Newton, A. C., Diaz, A., & Bullock, J. M. (2009). Enhancement of Biodiversity and Ecosystem Services by Ecological Restoration: A Meta-Analysis. *Science*, *325*(5944), 1121. https://doi.org/10.1126/science.1172460

Bender, S. F., & van der Heijden, M. G. A. (2015). Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. *Journal of Applied Ecology*, *52*(1), 228–239. https://doi.org/10.1111/1365-2664.12351

Bolan, N. S. (1991). A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil*, *134*, 189–207.

Boone, D. R., Liu, Y., Zhao, Z., Balkwill, D. L., Drake, G. R., & Aldrich, H. C. (1995). Bacillus infernus sp. nov., an Fe(II1)-and Mn(1V)-Reducing Anaerobe from the Deep Terrestrial Subsurface. In *INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY*. International Union of Microbiological Societies.

Bugg, R. L., & Waddington, C. (1994). Using cover crops to manage arthropod pests of orchards: A review. *Agriculture, Ecosystems & Environment*, *50*(1), 11–28. https://doi.org/https://doi.org/10.1016/0167-8809(94)90121-X

Buranov, A. U., & Mazza, G. (2008, November). Lignin in straw of herbaceous crops. *Industrial Crops and Products*, Vol. 28, pp. 237–259. https://doi.org/10.1016/j.indcrop.2008.03.008

Calderón, F. J., Nielsen, D., Acosta-Martínez, V., Vigil, M. F., & Lyon, D. (2016). Cover Crop and Irrigation Effects on Soil Microbial Communities and Enzymes in Semiarid Agroecosystems of the Central Great Plains of North America. *Pedosphere*, *26*(2), 192–205. https://doi.org/10.1016/S1002-0160(15)60034-0

Coman, C., Moţ, A. C., Gal, E., Pârvu, M., & Silaghi-Dumitrescu, R. (2013). Laccase is upregulated via stress pathways in the phytopathogenic fungus Sclerotinia sclerotiorum. *Fungal Biology*, *117*(7–8), 528–539. https://doi.org/10.1016/j.funbio.2013.05.005

Connell, J. ., & Vossen, P. . (2007). Organic Olive Orchard Nutrition. In P. M. Vossen (Ed.), *Organic Olive Production Manual* (Vol. 3503, pp. 37–43). Oakland, CA.

Dabney, S. M. (1998). Cover crop impacts on watershed hydrology. *From: Journal of Soil and Water Conservation*, *53*(3), 207–213.

Delmont, T. O., Prestat, E., Keegan, K. P., Faubladier, M., Robe, P., Clark, I. M., … Vogel, T. M. (2012). Structure, fluctuation and magnitude of a natural grassland soil metagenome. *ISME Journal*, *6*(9), 1677–1687. https://doi.org/10.1038/ismej.2011.197

Delmont, T. O., Robe, P., Cecillon, S., Clark, I. M., Constancias, F., Simonet, P., … Vogel, T. M. (2011). Accessing the Soil Metagenome for Studies of Microbial Diversity †. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*, *77*(4), 1315–1324. https://doi.org/10.1128/AEM.01526-10

Di, H. J., Cameron, K. C., Shen, J. P., Winefield, C. S., Ocallaghan, M., Bowatte, S., & He, J. Z. (2009). Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geoscience*, *2*(9), 621–624. https://doi.org/10.1038/ngeo613

Ding, L. J., Su, J. Q., Sun, G. X., Wu, J. S., & Wei, W. X. (2018). Increased microbial functional diversity under long-term organic and integrated fertilization in a paddy soil. *Applied Microbiology and Biotechnology*, *102*(4), 1969–1982. https://doi.org/10.1007/s00253-017-8704-8

Eivazi, F., & Tabatabai, M. A. (1988). Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*, *20*(5), 601–606. https://doi.org/10.1016/0038-0717(88)90141-1

Finney, D. M., Buyer, J. S., & Kaye, J. P. (2017). Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation*, *72*(4), 361–373. https://doi.org/10.2489/jswc.72.4.361

Frossard, E., Condron, L. M., Oberson, A., Sinaj, S., & Fardeau, J. C. (2000). Processes governing phosphorus availability in temperate soils. *Journal of Environmental Quality*, *29*(I), 15-un

Gans, J., Wolinsky, M., & Dunbar, J. (2005). Computational Improvements Reveal Great Bacterial Diversity and High Metal Toxicity in Soil. *Science*, *309*(5739), 1387. https://doi.org/10.1126/science.1112665

Gerke, J. (2015, June 1). Phytate (Inositol hexakisphosphate) in soil and phosphate acquisition from inositol phosphates by higher plants. A review. *Plants*, Vol. 4, pp. 253–266. https://doi.org/10.3390/plants4020253

Glomics Inc. (2014). GeoChip Technology. Retrieved April 30, 2019, from http://www.glomics.com/gch-tech.html

Gold, T. (1992). *The deep, hot biosphere* (Vol. 89).

Grime, J. P. (1977). Evidence for the Existence of Three Primary Strategies in Plants and its Relevance to Ecological and Evolutionary Theory. In *Amer. Natur* (Vol. 111).

Habteselassie, M. Y., Xu, L., & Norton, J. M. (2013). Ammonia-oxidizer communities in an agricultural soil treated with contrasting nitrogen sources. *Frontiers in Microbiology*, *4*(NOV). https://doi.org/10.3389/fmicb.2013.00326

Hallama, M., Pekrun, C., Lambers, H., & Kandeler, E. (2019, January 15). Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant and Soil*, Vol. 434, pp. 7–45. https://doi.org/10.1007/s11104-018-3810-7

He, Z. L., Wu, J., O'Donnel, A. G., & Syers, J. K. (1997). Seasonal responses in microbial biomass carbon, phosphorus and sulphur in soils under pasture. *Biol. Fert. Soils*, *24*, 421–428.

He, Z., Deng, Y., Van Nostrand, J. D., Tu, Q., Xu, M., Hemme, C. L., … Zhou, J. (2010). GeoChip 3.0 as a high-throughput tool for analyzing microbial community composition, structure and functional activity. *ISME Journal*, *4*(9), 1167–1179. https://doi.org/10.1038/ismej.2010.46

Ho, A., Di Lonardo, D. P., & Bodelier, P. L. E. (2017, March 1). Revisiting life strategy concepts in environmental microbial ecology. *FEMS Microbiology Ecology*, Vol. 93. https://doi.org/10.1093/femsec/fix006

Ho, I. (1979). Acid Phosphatase Activity in Forest Soil. *Forest Sci.*, *25*(4), 567–568.

Jia, Z., & Conrad, R. (2009). Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. *Environmental Microbiology*, *11*(7), 1658–1671. https://doi.org/10.1111/j.1462-2920.2009.01891.x

Karlen, D. L., Wollenhaupt, N. C., Erbach, D. C., Berry, E. C., Swan, J. B., Eash, N. S., & Jordahl, J. L. (1994). Long-term tillage effects on soil quality. *Soil and Tillage Research*, *32*(4), 313–327. https://doi.org/10.1016/0167-1987(94)00427-G

Kaspar, T. C., & Singer, J. W. (2008). *in UMRSHNC (Upper Mississippi River Sub-basin Hypoxia Nutrient Committee).*

Korripally, P., Hunt, C. G., Houtman, C. J., Jones, D. C., Kitin, P. J., Cullen, D., & Hammel, K. E. (2015). Regulation of gene expression during the onset of ligninolytic oxidation by Phanerochaete chrysosporium on spruce wood. *Applied and Environmental Microbiology*, *81*(22), 7802–7812. https://doi.org/10.1128/AEM.02064-15

Krause, S., Le Roux, X., Niklaus, P. A., van Bodegom, P. M., Lennon T., J. T., Bertilsson, S., … Bodelier, P. L. E. (2014). Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. *Frontiers in Microbiology*, Vol. 5. https://doi.org/10.3389/fmicb.2014.00251

Lehman, R. M., Acosta-Martinez, V., Buyer, J. S., Cambardella, C. A., Collins, H. P., Ducey, T. F., … Stott, D. E. (2015, January 1). Soil biology for resilient, healthy soil. *Journal of Soil and Water Conservation*, Vol. 70, pp. 12A-18A. https://doi.org/10.2489/jswc.70.1.12A. A

Lehman, R. M., Cambardella, C. A., Stott, D. E., Acosta-Martinez, V., Manter, D. K., Buyer, J. S., … Karlen, D. L. (2015). Understanding and enhancing soil biological health: The solution for reversing soil degradation. *Sustainability (Switzerland)*, *7*(1), 988–1027. https://doi.org/10.3390/su7010988. B

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G. W., … Schleper, C. (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*, *442*(7104), 806–809. https://doi.org/10.1038/nature04983

Li, L., Xu, M., Ali, M. E., Zhang, W., Duan, Y., & Li, D. (2018). Factors affecting soil microbial biomass and functional diversity with the application of organic amendments in three contrasting cropland soils during a field experiment. *PLoS ONE*, *13*(9). https://doi.org/10.1371/journal.pone.0203812

Liu, J., Cade-Menun, B. J., Yang, J., Hu, Y., Liu, C. W., Tremblay, J., … Bainard, L. D. (2018). Long-term land use affects phosphorus speciation and the composition of phosphorus cycling genes in agricultural soils. *Frontiers in Microbiology*, *9*(JUL). https://doi.org/10.3389/fmicb.2018.01643

Lu, Y.-C., Watkins, K. B., Teasdale, J. R., & Abdul-Baki, A. A. (2000). Cover Crops in Sustainable Food Production. In *Food Rev. Int* (Vol. 16). Retrieved from www.dekker.com

Luo, L., Meng, H., & Gu, J. D. (2017, July 15). Microbial extracellular enzymes in biogeochemical cycling of ecosystems. *Journal of Environmental Management*, Vol. 197, pp. 539–549. https://doi.org/10.1016/j.jenvman.2017.04.023

Macklon, A. E. S., Grayston, S. J., Shand, C. A., Sim, A., Sellars, S., & Ord, B. G. (1997). Uptake and transport of phosphorus by Agrostis capillaris seedlings from rapidly hydrolysed organic sources extracted from 32P-labelled bacterial cultures. *Plant Soil*, *190*, 163–167.

Mao, Y., Yannarell, A. C., & Mackie, R. I. (2011). Changes in N-Transforming Archaea and Bacteria in Soil during the Establishment of Bioenergy Crops. *PLoS ONE*, *6*(9), 24750. https://doi.org/10.1371/journal.pone.0024750

McDaniel, M. D., Tiemann, L. K., & Grandy, A. S. (2014). Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. In *Ecological Applications* (Vol. 24). Retrieved from http://www.metawinsoft.com/

McGill, W. B., & Cole, C. V. (1981). Comparative aspects of cycling of organic C, N, S and P through soil organic matter. *Geoderma*, *26*(4), 267–286.

Motta, A. C. V, Reeves, D. W., Burmester, C., & Feng, Y. (2007). Conservation Tillage, Rotations, and Cover Crop Affecting Soil Quality in the Tennessee Valley: Particulate Organic Matter, Organic Matter, and Microbial Biomass. *Communications in Soil Science and Plant Analysis*, *38*, 2831–2847. https://doi.org/10.1080/00103620701663065

Muhammad, I., Wang, J., Sainju, U. M., Zhang, S., Zhao, F., & Khan, A. (2021). Cover cropping enhances soil microbial biomass and affects microbial community structure: A meta-analysis. *Geoderma*, *381*. https://doi.org/10.1016/j.geoderma.2020.114696

Myrold, D. D., Zeglin, L. H., & Jansson, J. K. (2014). The Potential of Metagenomic Approaches for Understanding Soil Microbial Processes. *Soil Science Society of America Journal*, *78*(1), 3–10. https://doi.org/10.2136/sssaj2013.07.0287dgs

Nair, A., & Ngouajio, M. (2012). Soil microbial biomass, functional microbial diversity, and nematode community structure as affected by cover crops and compost in an organic vegetable production system. *Applied Soil Ecology*, *58*, 45–55. https://doi.org/10.1016/j.apsoil.2012.03.008

Nannipieri, P., Giagnoni, L., Landi, L., & Renella, G. (2011). *Role of Phosphatase Enzymes in Soil*. https://doi.org/10.1007/978-3-642-15271-9_9

Navarro-Noya, Y. E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M. C., Valenzuela-Encinas, C., … Dendooven, L. (2013). Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. *Soil Biology and Biochemistry*, *65*, 86–95. https://doi.org/10.1016/j.soilbio.2013.05.009

Nielsen, U. N., Ayres, E., Wall, D. H., & Bardgett, R. D. (2011, February). Soil biodiversity and carbon cycling: A review and synthesis of studies examining diversity-function

relationships. *European Journal of Soil Science*, Vol. 62, pp. 105–116. https://doi.org/10.1111/j.1365-2389.2010.01314.x

Oberson, A. (2001). Phosphorus transformations in an oxisol under contrasting land-use systems: the role of the microbial biomass. *Plant Soil*, *237*, 197–210.

Oehl, G., Oberson, A., Probst, M., Fliessbach, A., Roth, H. R., & Frossard, E. (2001). Kinetics of microbial phosphorus uptake in cultivated soils. *Biol. Fert. Soil*, *34*, 32–41.

Offre, P., Prosser, J. I., & Nicol, G. W. (2009). Growth of ammonia-oxidizing archaea in soil microcosms is inhibited by acetylene. *FEMS Microbiology Ecology*, *70*(1), 99–108. https://doi.org/10.1111/j.1574-6941.2009.00725.x

Oldfield, E. E., Bradford, M. A., & Wood, S. A. (2019). Global meta-analysis of the relationship between soil organic matter and crop yields. *SOIL*, *5*(1), 15–32. https://doi.org/10.5194/soil-5-15-2019

Paul, E. A., & Clark, F. E. (1996). *Soil Microbiology and Biochemistry* (second ed.). Academic Press, USA.

Pereg, L., Morugán-Coronado, A., McMillan, M., & García-Orenes, F. (2018). Restoration of nitrogen cycling community in grapevine soil by a decade of organic fertilization. *Soil and Tillage Research*, *179*, 11–19. https://doi.org/10.1016/j.still.2018.01.007

Pester, M., Schleper, C., & Wagner, M. (2011). The Thaumarchaeota: An emerging view of their phylogeny and ecophysiology. *Current Opinion in Microbiology*, *14*(3), 300–306. https://doi.org/10.1016/j.mib.2011.04.007

Pimente, D., Wilson, C., Mccullum, C., Huang, R., Dwen, P., Flack, J., … Cliff, B. (1997). *Economic and Environmental Benefits of Biodiversity The annual economic and environmental benefits of biodiversity in the United States total approximately $300 billion*. Retrieved from https://academic.oup.com/bioscience/article-abstract/47/11/747/229880

Privat de Garilhe, M. (1967). *Enzymes in nucleic acid research*. San Francisco.

Prosser, J. I. (2012). Ecosystem processes and interactions in a morass of diversity. *FEMS Microbiology Ecology*, *81*(3), 507–519. https://doi.org/10.1111/j.1574-6941.2012.01435.x

Rankoth, L. M., Udawatta, R. P., Veum, K. S., Jose, S., & Alagele, S. (2019). Cover crop influence on soil enzymes and selected chemical parameters for a claypan corn-soybean rotation. *Agriculture (Switzerland)*, *9*(6). https://doi.org/10.3390/agriculture9060125

Richardson, A. E. (2003). Making microorganisms mobilize soil phosphorus. In *First International Meeting on Microbial Phosphate Solubilization* (pp. 85–90).

Roberson, E. B., Sarig, S., Shennan, C., & Firestone, M. K. (1995). Nutritional Management of Microbial Polysaccharide Production and Aggregation in Agricultural Soil. *Soil Sci. Am.*, *59*, 1587–1594.

Rodríguez, H., Fraga, R., Gonzalez, T., & Bashan, Y. (2006, September). Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil*, Vol. 287, pp. 15–21. https://doi.org/10.1007/s11104-006-9056-9

Sarathchandra SU, Ghani A, Yeates GW, Burch G, Cox NR. 2001. Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils. *Soil Biol. Biochem.* (33). 953–964.SAS Institute. (2013). *SAS user's guide*. Cary, NC: SAS Inst.

Schimel, J. P., & Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Frontiers in Microbiology*, Vol. 3. https://doi.org/10.3389/fmicb.2012.00348

Schloss, P. D., & Handelsman, J. (2006). Toward a census of bacteria in soil. *PLoS Computational Biology*, *2*(7), 0786–0793. https://doi.org/10.1371/journal.pcbi.0020092

Schmidt, R., Gravuer, K., Bossange, A. V., Mitchell, J., & Scow, K. (2018). Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. *PLoS ONE*, *13*(2). https://doi.org/10.1371/journal.pone.0192953

Shi, Z., Yin, H., Van Nostrand, J. D., Voordeckers, J. W., Tu, Q., Deng, Y., … Zhou, J. (2019). Functional Gene Array-Based Ultrasensitive and Quantitative Detection of Microbial Populations in Complex Communities. *MSystems*, *4*(4). https://doi.org/10.1128/msystems.00296-19

Six, J., Frey, S. D., Thiet, R. K., & Batten, K. M. (2006, March). Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal*, Vol. 70, pp. 555–569. https://doi.org/10.2136/sssaj2004.0347

Soltangheisi, A., Teles, A. P. B., Sartor, L. R., & Pavinato, P. S. (2020). Cover Cropping May Alter Legacy Phosphorus Dynamics Under Long-Term Fertilizer Addition. *Frontiers in Environmental Science*, *8*. https://doi.org/10.3389/fenvs.2020.00013

Sparvoli, F., & Cominelli, E. (2015, December 1). Seed biofortification and phytic acid reduction: A conflict of interest for the plant? *Plants*, Vol. 4, pp. 728–755. https://doi.org/10.3390/plants4040728

Spedding, T. A., Hamel, C., Mehuys, G. R., & Madramootoo, C. A. (2004). Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. *Soil Biology and Biochemistry*, *36*(3), 499–512. https://doi.org/10.1016/j.soilbio.2003.10.026

Starr, L. M.(2020). *Linkages between cover crops, phosphorus fertilizer management, soil health, and phosphorus bioavailability in replicated research watersheds*. Retrieved from K-State Research Exchange.

Suzuki, H., MacDonald, J., Syed, K., Salamov, A., Hori, C., Aerts, A., … Master, E. R. (2012). Comparative genomics of the white-rot fungi, Phanerochaete carnosa and P. chrysosporium, to elucidate the genetic basis of the distinct wood types they colonize. *BMC Genomics*, *13*(1). https://doi.org/10.1186/1471-2164-13-444

Tardy, V., Spor, A., Mathieu, O., Lévèque, J., Terrat, S., Plassart, P., … Maron, P. A. (2015). Shifts in microbial diversity through land use intensity as drivers of carbon mineralization in soil. *Soil Biology and Biochemistry*, *90*, 204–213. https://doi.org/10.1016/j.soilbio.2015.08.010

Taylor, A. E., Zeglin, L. H., Wanzek, T. A., Myrold, D. D., & Bottomley, P. J. (2012). Dynamics of ammonia-oxidizing archaea and bacteria populations and contributions to soil nitrification potentials. *ISME Journal*, *6*(11), 2024–2032. https://doi.org/10.1038/ismej.2012.51

Theis, J. E. (2008). Molecular methods for studying microbial ecology in the soil and rhizosphere. In *Molecular Mechanisms of Plant and Microbe Coexistence*. Berlin, Germany: Springe-Verlag.

Tourna, M., Freitag, T. E., Nicol, G. W., & Prosser, J. I. (2008). Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environmental Microbiology*, *10*(5), 1357–1364. https://doi.org/10.1111/j.1462-2920.2007.01563.x

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I. C., Jeffries, T. C., … Singh, B. K. (2016). Microbial regulation of the soil carbon cycle: Evidence from gene-enzyme relationships. *ISME Journal*, *10*(11), 2593–2604. https://doi.org/10.1038/ismej.2016.65

Tzeng, C.-M., & Kornberg, A. (2000). *The Multiple Activities of Polyphosphate Kinase of Escherichia coli and Their Subunit Structure Determined by Radiation Target Analysis**. Retrieved from http://www.jbc.org/

Utobo, E. B., & Tewari, L. (2015). Soil enzymes as bioindicators of soil ecosystem status. *Applied Ecology and Environmental Research*, *13*(1), 147–169. https://doi.org/10.15666/aeer/1301_147169

Van Dien, S. J., Keyhani, S., Yang, C., & Keasling, J. D. (1997). Manipulation of Independent Synthesis and Degradation of Polyphosphate in Escherichia coli for Investigation of Phosphate Secretion from the Cell. In *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* (Vol. 63).

Wagg, C., Bender, S. F., Widmer, F., & Heijden, M. G. A. van der. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, *111*(14), 5266–5270. https://doi.org/10.1073/PNAS.1320054111

Wang, C., Wang, X., Liu, D., Wu, H., Lü, X., Fang, Y., … Bai, E. (2014). Aridity threshold in controlling ecosystem nitrogen cycling in arid and semi-Arid grasslands. *Nature Communications*, *5*. https://doi.org/10.1038/ncomms5799

Xue, K., Wu, L., Deng, Y., He, Z., Van Nostrand, J., Robertson, P. G., … Zhou, J. (2013). Functional gene differences in soil microbial communities from conventional, low-input, and organic farmlands. *Applied and Environmental Microbiology*, *79*(4), 1284–1292. https://doi.org/10.1128/AEM.03393-12

Yang, W., Feng, G., Read, J. J., Ouyang, Y., Han, J., & Li, P. (2020). Impact of cover crop on corn–soybean productivity and soil water dynamics under different seasonal rainfall patterns. *Agronomy Journal*, *112*(2), 1201–1215. https://doi.org/10.1002/agj2.20110

Yang, Y.-H., Yao, J., Hu, S., Qi, Y. (2000). Effects of Agricultural Chemicals on DNA Sequence Diversity of Soil Microbial Community: A Study with RAPD Marker. *Microb Ecol* (39), 72-79.

Yao, Q., Li, X., Feng, G., & Christie, P. (2001). Mobilization of sparingly soluble inorganic phosphates by the external mycelium of an abuscular mycorrhizal fungus. In *Plant and Soil* (Vol. 230).

Yu, L., Luo, S., Xu, X., Gou, Y., & Wang, J. (2020). The soil carbon cycle determined by GeoChip 5.0 in sugarcane and soybean intercropping systems with reduced nitrogen input in South China. *Applied Soil Ecology*, *155*. https://doi.org/10.1016/j.apsoil.2020.103653

Zeglin, L. H., Taylor, A. E., Myrold, D. D., & Bottomley, P. J. (2011). Bacterial and archaeal amoA gene distribution covaries with soil nitrification properties across a range of land uses. *Environmental Microbiology Reports*, *3*(6), 717–726. https://doi.org/10.1111/j.1758-2229.2011.00290.x

Zhang, L. M., Offre, P. R., He, J. Z., Verhamme, D. T., Nicol, G. W., & Prosser, J. I. (2010). Autotrophic ammonia oxidation by soil thaumarchaea. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(40), 17240–17245. https://doi.org/10.1073/pnas.1004947107

Zhang, X., Liu, W., Schloter, M., Zhang, G., Chen, Q., Huang, J., … Han, X. (2013). Response of the Abundance of Key Soil Microbial Nitrogen-Cycling Genes to Multi-Factorial Global Changes. *PLoS ONE*, *8*(10). https://doi.org/10.1371/journal.pone.0076500

Zhou, J., He, Z., Yang, Y., Deng, Y., Tringe, S. G., & Alvarez-Cohen, L. (2015). High-throughput metagenomic technologies for complex microbial community analysis: Open and closed formats. *MBio*, *6*(1). https://doi.org/10.1128/mBio.02288-14

Table 4.1

Monthly temperature average highs and lows and precipitation for 2019 and 2020 at the Kansas Agricultural Watershed from the Kansas Mesonet, (N/A) was indicated for weather points that occurred after soil sampling in spring 2020.

Month	2019					2020				
	High (°C)		Low (°C)		Precipitation (mm)	High (°C)		Low (°C)		Precipitation (mm)
	Air	Soil (5 cm)	Air	Soil (5 cm)		Air	Soil (5 cm)	Air	Soil (5 cm)	
January	3.58	0.79	-7.10	-0.45	31.24	5.07	1.88	-5.46	0.55	46.21
February	1.64	0.24	-7.48	-1.23	32.76	7.60	3.00	-4.94	0.90	19.81
March	9.61	7.16	-1.48	2.63	61.99	14.42	10.27	2.77	6.53	58.92
April	20.9	18.06	6.84	10.56	55.88	18.77	15.07	4.47	9.72	60.96
May	22.16	21.55	11.39	15.31	307.32	21.80	20.30	10.77	15.60	189.23
June	29.42	28.07	16.63	21.14	145.05	N/A	N/A	N/A	N/A	N/A
July	31.66	31.40	19.76	24.82	58.42	N/A	N/A	N/A	N/A	N/A
August	29.25	28.79	19.29	24.25	218.43	N/A	N/A	N/A	N/A	N/A
September	29.73	26.21	18.38	22.54	59.69	N/A	N/A	N/A	N/A	N/A
October	16.90	15.1	3.81	11.87	69.34	N/A	N/A	N/A	N/A	N/A
November	11.49	7.64	-2.04	4.32	15.48	N/A	N/A	N/A	N/A	N/A
December	8.44	4.64	-3.81	2.19	26.92	N/A	N/A	N/A	N/A	N/A
Total					1082.52					375.13

Table 4.2

Carbon Nutrient Cycling Categories. Treatment groups include P fertilizer and cover crop treatment interactions (Fert x CC), P fertilizer treatments (Fert), and cover crop treatments (CC). An asterisk (*) indicates significant differences at $p < 0.05$.

Carbon Categories	Fall 2019			Spring 2020		
	Treatment Groups					
	Fert x CC	**Fert**	**CC**	**Fert x CC**	**Fert**	**CC**
C cycling						
All	0.095	0.158	0.115	0.617	0.529	0.183
Archaea	0.107	0.157	0.533	0.530	0.819	0.899
Bacteria	0.124	0.194	0.130	0.620	0.556	0.131
Fungi	0.049*	0.080	0.076	0.708	0.398	0.984
Eukaryota	0.311	0.397	0.234	0.401	0.212	0.405
C Degradation						
All	0.063	0.202	0.137	0.702	0.507	0.158
Archaea	0.032*	0.133	0.078	0.110	0.933	0.162
Bacteria	0.078	0.274	0.177	0.704	0.561	0.096
Fungi	0.049*	0.0780	0.076	0.708	0.398	0.984
Eukaryota	0.117	0.577	0.087	0.362	0.213	0.688
Chitin Degradation						
All	0.689	0.102	0.549	0.529	0.512	0.499
Archaea	0.372	0.760	0.783	0.400	0.528	0.145
Bacteria	0.741	0.108	0.576	0.574	0.507	0.501
Fungi	0.174	0.193	0.257	0.282	0.524	0.694
Acetyl						
All	0.031*	0.654	0.066	0.691	0.473	0.091
Archaea	0.968	0.648	0.658	0.7674	0.162	0.929
Bacteria	0.030*	0.651	0.066	0.688	0.474	0.089
amyA						
All	0.015*	0.551	0.106	0.677	0.680	0.041*
Archaea	0.027*	0.059	0.049*	0.067	0.201	0.035*
Bacteria	0.013*	0.732	0.110	0.670	0.6582	0.041*
Fungi	0.320	0.425	0.396	0.502	0.797	0.011*

Table 4.3

Nitrogen Nutrient Cycling Categories. Treatment groups include P fertilizer and cover crop treatment interactions (Fert x CC), P fertilizer treatments (Fert), and cover crop treatments (CC). An asterisk (*) indicates significant differences at p < 0.05.

Nitrogen Categories	Fall 2019			Spring 2020		
	Treatment Groups					
	Fert x CC	Fert	CC	Fert x CC	Fert	CC
N Cycling						
All	0.053	0.085	0.293	0.667	0.490	0.353
Archaea	0.005*	0.025*	0.296	0.708	0.379	0.724
Bacteria	0.072	0.093	0.313	0.671	0.493	0.318
Fungi	0.235	0.092	0.978	0.415	0.423	0.320
Eukaryota	0.004*	0.069	0.062	0.252	0.878	0.136
Ammonification						
All	0.114	0.090	0.441	0.570	0.286	0.090
Archaea	0.087	0.194	0.263	0.744	0.327	0.911
Bacteria	0.144	0.093	0.438	0.559	0.249	0.102
Eukaryota	0.065	0.091	0.876	0.343	0.668	0.011*
Anamox						
Bacteria (All)	0.973	0.449	0.844	0.134	0.279	0.030*
Denitrification						
All	0.174	0.112	0.324	0.717	0.480	0.512
Archaea	0.040*	0.147	0.983	0.448	0.222	0.280
Bacteria	0.197	0.117	0.312	0.695	0.468	0.533
Fungi	0.925	0.209	0.467	0.543	0.298	0.497
nirK						
All	0.037*	0.074	0.035*	0.765	0.443	0.730
Archaea	0.157	0.450	0.999	0.845	0.678	0.707
Bacteria	0.116	0.762	0.060	0.252	0.752	0.270
nirS						
All	0.149	0.072	0.146	0.480	0.865	0.537
Bacteria	0.160	0.066	0.115	0.467	0.884	0.478
Fixation (*nifH*)						
All	0.155	0.041*	0.108	0.595	0.398	0.497
Archaea	0.231	0.417	0.807	0.904	0.510	0.354
Bacteria	0.205	0.033*	0.087	0.670	0.299	0.579
Assimilation						
All	0.027*	0.080	0.029*	0.571	0.876	0.636
Fungi	0.773	0.221	0.240	0.753	0.485	0.727
Eukaryota	0.090	0.202	0.036*	0.698	0.990	0.552
amoA						
All	0.278	0.147	0.706	0.026*	0.034*	0.0001*
Archaea	0.359	0.161	0.893	0.017*	0.055	<.0001*
Bacteria	0.069	0.049*	0.022*	0.107	0.170	0.078

Table 4.4

Phosphorus Nutrient Cycling Categories. Treatment groups include P fertilizer and cover crop treatment interactions (Fert x CC), P fertilizer treatments (Fert), and cover crop treatments (CC). An asterisk (*) indicates significant differences at $p < 0.05$.

Phosphorus Categories	Fall 2019			Spring 2020		
	Treatment Groups					
	Fert x CC	**Fert**	**CC**	**Fert x CC**	**Fert**	**CC**
P Cycling						
All	0.026*	0.510	0.032*	0.459	0.537	0.052
Archaea	0.620	0.973	0.688	0.890	0.312	0.148
Bacteria	0.028*	0.505	0.033*	0.466	0.551	0.053
Fungi	0.151	0.059	0.885	0.814	0.168	0.628
Phytase						
All	0.177	0.322	0.370	0.481	0.752	0.193
Bacteria	0.210	0.325	0.399	0.4918	0.8055	0.176
Fungi	0.164	0.031*	0.513	0.677	0.184	0.288
ppK						
All	0.016*	0.347	0.046*	0.363	0.613	0.032*
Bacteria	0.015*	0.301	0.042*	0.387	0.613	0.036*
ppX						
All	0.028*	0.685	0.022*	0.560	0.482	0.053
Archaea	0.335	0.946	0.288	0.849	0.440	0.131
Bacteria	0.028*	0.690	0.021*	0.556	0.4823	0.054
Fungi	0.250	0.193	0.523	0.456	0.699	0.009*

Figure 4.1

Abundance of Functional Genes: C, specific to fungi from fall samples within all carbon cycling (A), Archaea from fall samples within C degradation (B), fungi from fall samples within C degradation (C). Treatments include no P fertilizer (NP), fall broadcast (FB), spring injected (SI), cover crop implemented (CC) and no cover crop implemented (NC). Lower case letters indicate significant differences at $p < 0.05$.

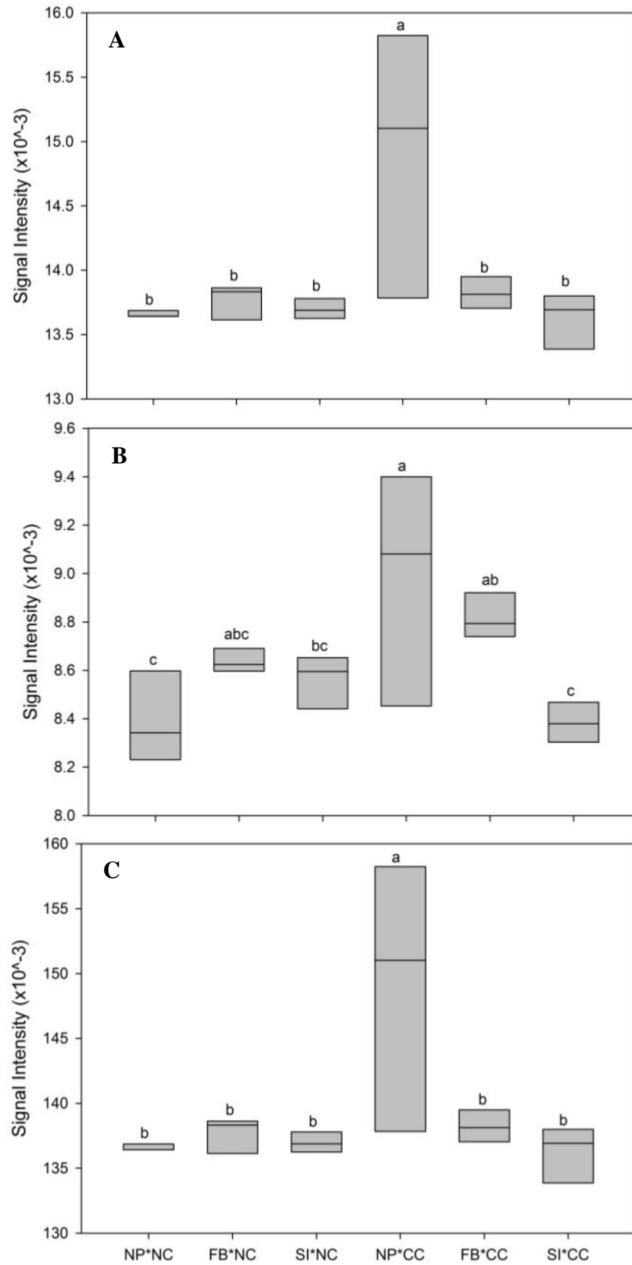


Figure 4.2

Abundance of Functional Genes: C, specific to bacteria from fall samples within acetylglucosidase (A), Bacteria from fall within amyA (B), Archaea from fall within amyA (C). Treatments include no P fertilizer (NP), fall broadcast (FB), spring injected (SI), cover crop implemented (CC) and no cover crop implemented (NC). Lower case letters indicate significanct differences at $p < 0.05$.

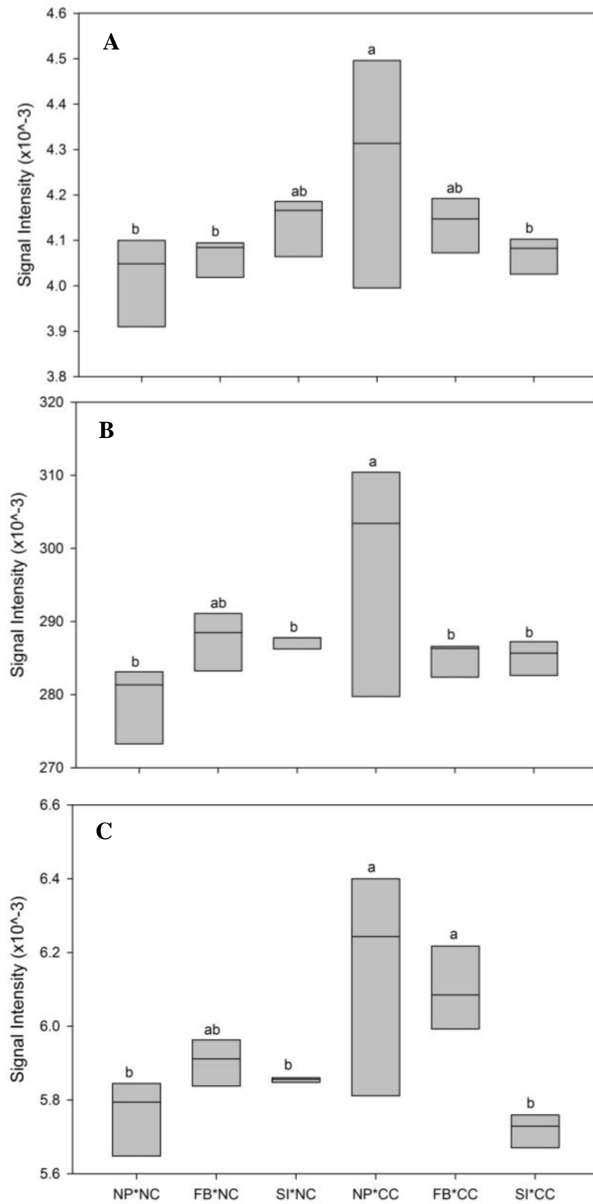


Figure 4.3

Abundance of Functional Genes: N, specific to Archaea (A) and Eukaryota (B) from fall taken from N cycling. Treatments include no P fertilizer (NP), fall broadcast (FB), spring injected (SI), cover crop implemented (CC) and no cover crop implemented (NC). Lower case letters indicate significant differences at $p < 0.05$.

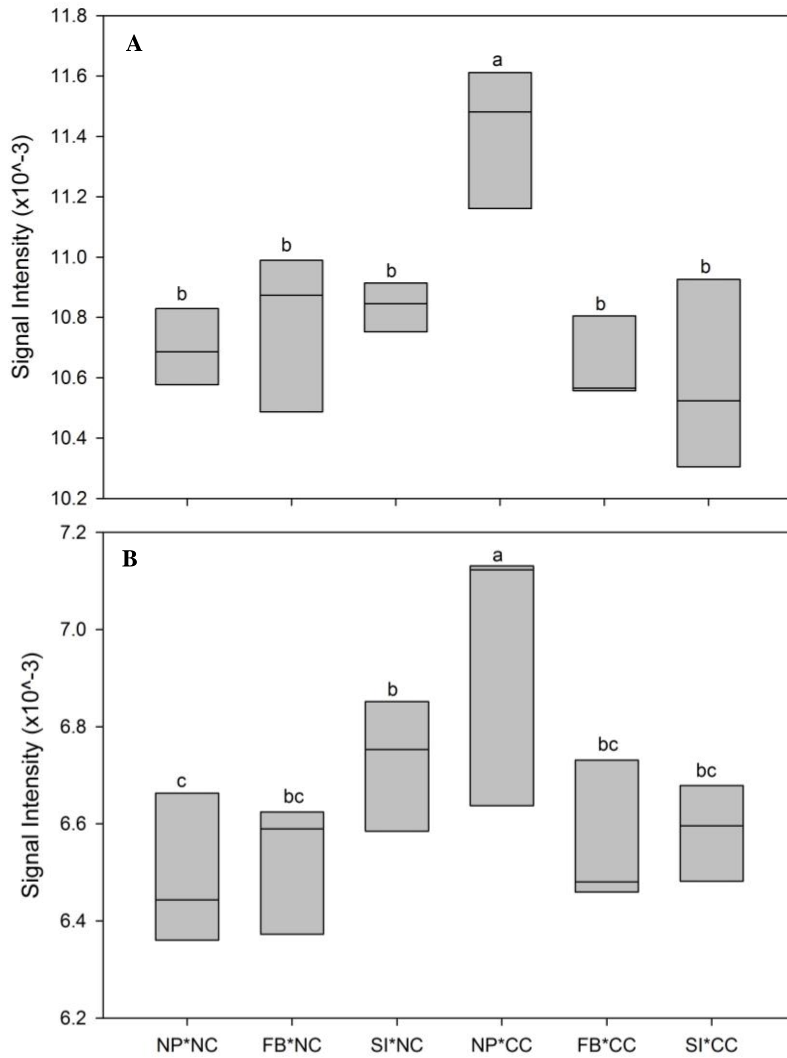


Figure 4.4

Abundance of Functional Genes: N, specific to Archaea from fall and within denitrification (A), all nirK probes from fall samples (B). Treatments include no P fertilizer (NP), fall broadcast (FB), spring injected (SI), cover crop implemented (CC) and no cover crop implemented (NC). Lower case letters indicate significant differences at $p < 0.05$.

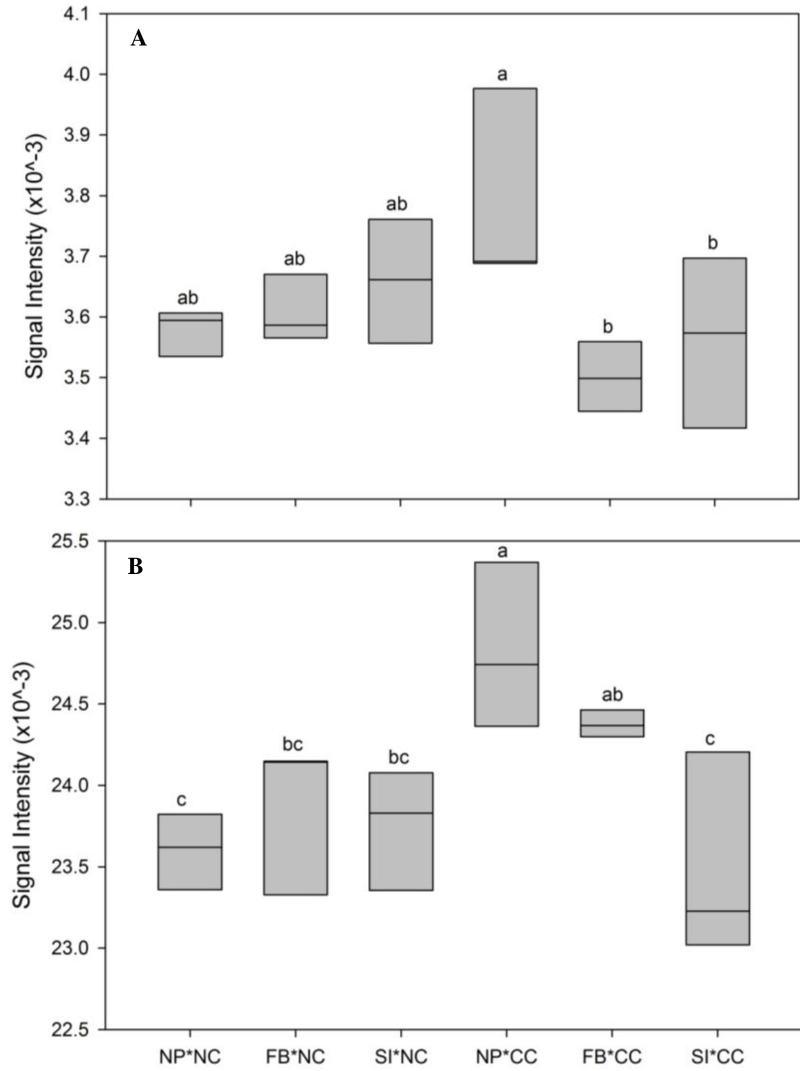


Figure 4.5

Abundance of Functional Genes: N, all amoA probes from spring. Treatments include no P fertilizer (NP), fall broadcast (FB), spring injected (SI), cover crop implemented (CC) and no cover crop implemented (NC). Lower case letters indicate significant differences at $p < 0.05$.

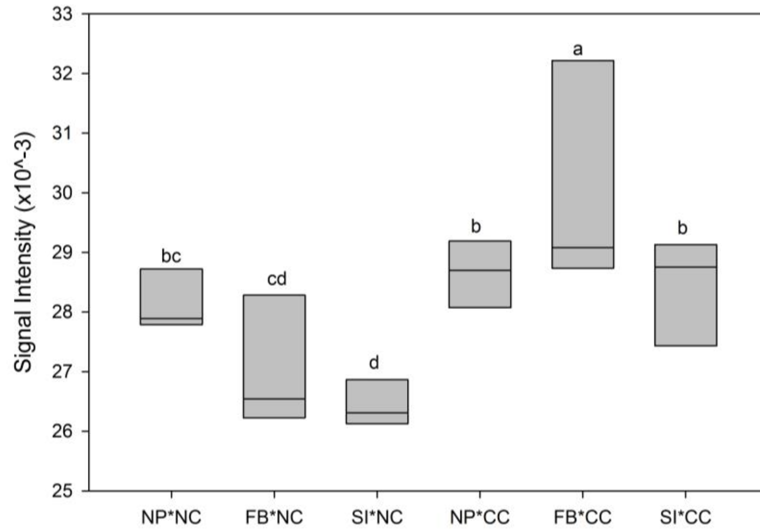
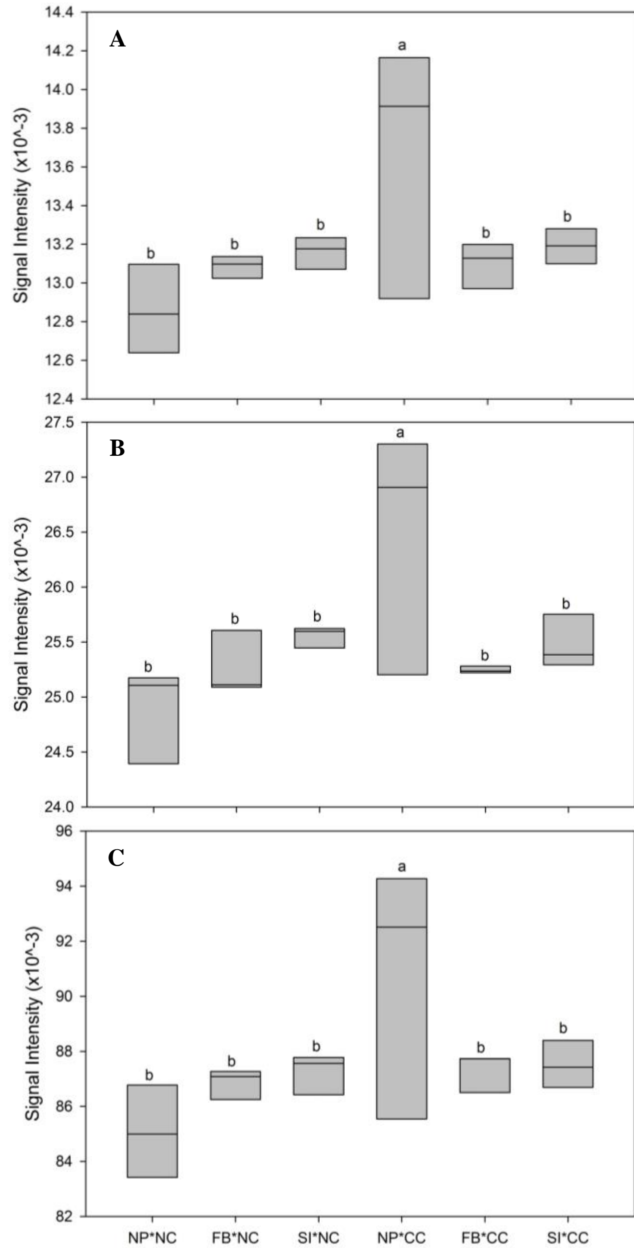


Figure 4.6

Abundance of Functional Genes: P, specific to fall. Bacteria from fall taken from P cycling (A), Bacteria within ppK from fall (B), Bacteria within ppX from fall samples (C). Treatments include no P fertilizer (NP), fall broadcast (FB), spring injected (SI), cover crop implemented (CC) and no cover crop implemented (NC). Lower case letters indicate significant differences at $p < 0.05$.



Chapter 5 - Conclusion

The primary goal of this dissertation research was to examine how aspects of soil health were impacted by cover crop implementation in a no-till corn-soybean rotation system at field scale. The research objectives of this dissertation were (i) to examine carbon (C) and nitrogen (N) soil health metrics; (ii) to explore the taxonomic microbial community structure using phospholipid fatty acid (PLFA) analysis, and (iii) to identify key microbial functional gene composition, and how soil health metrics relate to key soil microbial functional gene composition in response to contrasting management practices. To do this, I sampled soils in the fall 2019 and spring 2020 seasons in management practices that included cover crop usage and P fertilizer treatments at an early transition to no-tillage (less than 5 years) no-till field-scale site at the Kansas Agricultural Watershed Field Research Facility. These research objectives facilitated examination of how various soil health measurements including traditional measurements, broad taxonomic microbial groupings, and microbial genetic functional capacity respond in a field-scale experiment that examines cover crop use and different P fertilizer management practices in a corn-soybean rotation system. The first objective was accomplished through examining soil samples taken at the 0-5 cm depth in the spring and fall of 2018 and 2019, and the second objective examined soil taken at the 0-5, 5-10, and 10-15 cm depths also taken in the spring and fall of 2018 and 2019. The last objective utilized samples taken from fall 2019 and spring 2020 at the 0-5 cm depth.

Examining traditional soil health metrics addressed questions related to traditional measurements of C and N pools targeting soil health and their response to cover crop implementation and P fertilizer management. The objective of this study was to investigate the impact of cover crop presence and absence and P fertilizer management strategies using

traditional soil health metrics. Soil health metrics employed in this study included: total organic carbon, active C, dissolved organic C and N, and dissolved inorganic N, microbial biomass C and N, soil respiration, enzyme activity of β-glucosidase and β-glucosaminidase. Implementation of cover cropping was hypothesized to increase total organic carbon, active C, dissolved organic C, dissolved organic N, and inorganic N. Assays examining soil microbial biomass and activity indicators (respiration and enzyme activity assays) were hypothesized to increase within cover crops. Phosphorus fertilizer management strategies were hypothesized to have little direct impact on carbon and nitrogen the presence or absence of cover crops are expected to influence soil health metrics. The first hypothesis that cover crop implementation would increase total organic carbon, active C, dissolved organic C (DOC), dissolved organic N (DON), and inorganic N was partially supported, with the expected responses in some of these examined nutrient pools but not all. Total organic C was found to be greater in the CC (with cover crops) treatment in spring 2018, fall 2018, and spring 2019, but this was not observed in fall 2019. Active C was higher in CC treatment in spring 2018 and spring 2019. Dissolved organic C, DON, and inorganic N were only significantly impacted by the cover crop treatments in spring 2018. Microbial biomass C aligned with the second hypothesis in both spring samples but not in fall samples. Microbial biomass C followed a similar trend to active C findings with greater biomass found in CC treatment, with greater biomass in the CC treatment in spring 2018 and spring 2019. Microbial biomass N did not agree with the second hypothesis and was not impacted by any treatments. Soil respiration results did not support the second hypothesis and was only found at greater amounts in the CC treatment in spring 2018. Enzyme activity assays aligned with the second hypothesis, with both β-glucosidase and β-glucosaminidase enzyme activities showed significantly higher levels of activity across seasons sampled, except for fall 2019 which was not

found to be significantly different between cover crop treatments for β-glucosidase. This may indicate that β-glucosaminidase is more reliable in detecting enzyme activity in response to cover crop treatments; however, with only one data point of not observing this trend in β-glucosidase this remains unclear. The third hypothesis that P fertilizer management strategies would have little direct impact on C and N soil health metrics in the presence or absence of cover crops was predominately found with the exceptions of β-glucosaminidase in spring 2018, and NH4-N in spring 2018, spring 2019, and fall 2019. This study found that assays examining total C, labile C pools, and microbial biomass C and enzyme activity were more successful in detecting cover crop implementation as compared to assays that targeted N pools including total N, labile N, and microbial biomass.

The second objective utilized PLFA to examine multiple seasons as well as multiple depths at the research site for broad taxonomic microorganism classification. Where the first objective served to provide traditional soil health metrics on this study and found the labile C nutrient pools as being especially responsive to management techniques, the second objective sought to examine the broad taxonomic identification of soil microorganisms, which contribute to soil labile nutrients. The hypotheses of this study were that microbial biomass would decrease with increasing depth, addition of cover crops would result in an increase in microbial biomass across all PLFA categories, that community structure would be dominated by fungi in the presence of cover crops, and P fertilizer treatments would not significantly impact PLFA profiles. Work specific to this objective found that cover crops can increase the total microbial biomass as indicated by PLFA in a no-tillage corn-soybean cropping system. This study also demonstrated that the impact of cover crops may decrease over time, especially at the 0-5 cm depth. There was also a decrease in biomass with increasing depth across examined PLFA

categories and sampling points. This study covered a field scale site that was managed under no-tillage starting in spring of 2015 and samples taken from spring and fall of 2018 and 2019, and thus highlights the potential impact of cropping and timing in respect to cover crops and observed soil microorganism impacts. The percent community make-up of PLFA profiles at a given sampling point at the 0-5 cm depth was relatively consistent across microbial sampling points regardless of CC or NC (without cover crops) treatment.

The third objective implemented microarray technologies to address questions related to the genetic functional capacity of the soil microbial communities to carry out nutrient cycling. This study examined fall 2019 and spring 2020 soil samples taken at the 0-5 cm depth, which allowed examination after a corn crop (fall 2019) and at the end of a living cover crop (spring 2020). Genes related to nutrient dynamics were more frequently responsive to treatment differences in fall samples than in spring samples. In fall, the NP*CC treatment most frequently elicited greater gene abundance within GeoChip sub-categories than other treatments. Spring samples were more often impacted by the CC and NC treatments, with some nutrient cycling categories demonstrating a higher abundance in plots with the NC treatment and others demonstrating a higher abundance in plots with the CC treatment. When examining C nutrient cycling, probes specific to *amyA* and the organismal groupings within *amyA* were the most consistently responsive to treatment effects, with greater abundance in the CC treatment in the spring and greater abundance in the NP*CC treatment. Probes targeting N nutrient cycling processes had diverse treatment effects when broken down into functional and organismal groups. P cycling processes were found to be fairly consistent across examined subcategories of P cycling processes, and probes specific to *ppK* were found to be responsive to treatments in both fall and spring samples. Ultimately this study revealed diverse microorganismal responses

to the examined agricultural management practices and temporal differences in soil nutrient cycling processes between post corn harvest and before termination of a cover crop.

The main objectives of this dissertation were to (i) examine C and N soil health metrics; (ii) to explore the taxonomic microbial community structure using PLFA, and (iii) to identify key microbial functional gene composition, and soil health metrics relate to key soil microbial functional gene composition in the fall 2019 and spring 2020 seasons in response to management practices that include cover crop usage and P fertilizer treatments at an early transition to no-tillage (less than 5 years) no-till field-scale site at the Kansas Agricultural Watershed Field Research Facility. In summary these results indicate that C soil health metrics that target labile pools, enzyme activity assays, and PLFA analysis across organismal categories were especially sensitive to CC implementation. PLFA analysis showed that broad organismal community composition remained relatively unchanged between CC and NC treatments. GeoChip analysis allowed a greater resolution of the microbial community that possible with PLFA and found that microbial genes involved in functional nutrient cycling have diverse responses, but trends did emerge with the NP*CC treatment in fall as the most frequently observed treatment effect attributed to increased gene abundance in C, N, and P nutrient cycling.

Appendix A - SAS Codes

Single Depth SAS Code

```
TITLE "SingleDepth";
DATA SingleDepth;
INPUT SAMP $ BLOC $ FERT $ COVER $ Response;
DATALINES;

;
PROC Print DATA= SingleDepth;
Run;

PROC mixed Data= SingleDepth;
Class BLOC FERT COVER;
Model Response = FERT|COVER;
Random BLOC;
LSMEANS FERT|COVER / PDIFF adjust=tukey;
ods output diffs=diffs;
run;

data calc_lsd;
set diffs;
lsd=stderr*tinv(1-.05/2,df);
run;

proc print data=calc_lsd;
var effect lsd estimate stderr df;
run;
```

Multi-Depth SAS Code

```
TITLE "MultiDepth";

data MultiDepth;
input depth rep fert $ cover $ response;
datalines;

PROC Print DATA= MultiDepth;
;
proc glimmix data = MultiDepth;
class depth rep fert cover;
model response = fert|cover|depth/ddfm = satterth;
random rep rep*cover*fert;
lsmeans fert|cover|depth/lines cl;

Run;
```

Appendix B - Extra Soil Data

Arylsulfatase 0-5 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	8.16	5.49	17.91	N/A
	FB	No	4.37	2.27	13.12	N/A
	NP	Yes	6.26	4.25	14.24	N/A
	NP	No	3.69	2.10	10.70	N/A
	SI	Yes	6.31	4.16	15.09	N/A
	SI	No	5.13	2.10	15.27	N/A
Main Effect		Yes	6.91	4.64	15.74	N/A
		No	4.40	2.16	13.03	N/A
	FB		6.27	3.88	15.51	N/A
	SI		5.72	3.13	15.8	N/A
	NP		4.98	3.18	12.47	N/A
		P – Value $_{Int}$	0.0442	0.7702	0.251	N/A
		Std Error $_{Int}$	0.5126	0.90	1.90	N/A
		P – Value $_{CC}$	<0.001	0.0064	0.0446	N/A
		Std Error $_{CC}$	0.361	0.55	1.49	N/A
		P – Value $_{Fert}$	0.047	0.6468	0.1191	N/A
		Std Error $_{Fert}$	0.4043	0.65	1.60	N/A

β-Glucosaminidase 5-10 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	7.42	13.03	12.57
	FB	No	N/A	7.87	9.50	11.60
	NP	Yes	N/A	9.52	14.89	13.88
	NP	No	N/A	9.69	10.51	13.69
	SI	Yes	N/A	8.20	13.19	13.75
	SI	No	N/A	7.56	9.72	10.23
Main Effect		Yes	N/A	8.38	13.70	13.40
		No	N/A	8.37	9.91	11.84
	FB		N/A	7.64	11.26	12.08
	SI		N/A	7.88	11.45	11.99
	NP		N/A	9.61	12.70	13.79
		P – Value $_{Int}$	N/A	0.7867	0.9204	0.185
		Std Error $_{Int}$	N/A	0.92	1.25	0.879
		P – Value $_{CC}$	N/A	0.9921	0.004	0.053
		Std Error $_{CC}$	N/A	0.64	0.72	0.520
		P – Value $_{Fert}$	N/A	0.0688	0.4863	0.114
		Std Error $_{Fert}$	N/A	0.72	0.88	0.629

β-Glucosidase 5-10 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	13.39	5.18	19.61
	FB	No	N/A	13.82	4.16	17.22
	NP	Yes	N/A	13.98	5.68	20.88
	NP	No	N/A	12.34	3.95	17.88
	SI	Yes	N/A	13.47	4.10	20.81
	SI	No	N/A	13.51	4.06	20.08
Main Effect		Yes	N/A	13.61	4.98	20.43
		No	N/A	13.22	4.06	18.39
	FB		N/A	13.60	4.67	18.42
	SI		N/A	13.49	4.08	20.45
	NP		N/A	13.16	4.81	19.38
		P – Value $_{Int}$	N/A	0.0583	0.2278	0.676
		Std Error $_{Int}$	N/A	0.73	0.66	1.380
		P – Value $_{CC}$	N/A	0.2535	0.0324	0.085
		Std Error $_{CC}$	N/A	0.65	0.54	0.879
		P – Value $_{Fert}$	N/A	0.5309	0.2825	0.339
		Std Error $_{Fert}$	N/A	0.67	0.57	1.030

Arylsulfatase 5-10 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	5.04	11.68	N/A
	FB	No	N/A	4.60	8.97	N/A
	NP	Yes	N/A	4.70	10.67	N/A
	NP	No	N/A	4.22	8.22	N/A
	SI	Yes	N/A	4.57	10.42	N/A
	SI	No	N/A	5.21	8.95	N/A
Main Effect		Yes	N/A	4.77	10.92	N/A
		No	N/A	4.67	8.71	N/A
	FB		N/A	4.82	10.32	N/A
	SI		N/A	4.89	9.69	N/A
	NP		N/A	4.46	9.44	N/A
		P – Value $_{Int}$	N/A	0.2815	0.7525	N/A
		Std Error $_{Int}$	N/A	0.45	0.94	N/A
		P – Value $_{CC}$	N/A	0.7638	0.0099	N/A
		Std Error $_{CC}$	N/A	0.33	0.64	N/A
		P – Value $_{Fert}$	N/A	0.4891	0.5827	N/A
		Std Error $_{Fert}$	N/A	0.37	0.73	N/A

Acid Phosphatase 5-10 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	18.13	141.81	150.43
	FB	No	N/A	19.74	109.86	145.30
	NP	Yes	N/A	21.66	143.30	131.70
	NP	No	N/A	21.49	122.52	138.71
	SI	Yes	N/A	20.38	138.97	160.83
	SI	No	N/A	19.30	123.84	143.34
Main Effect		Yes	N/A	20.05	141.36	147.66
		No	N/A	20.17	118.74	142.45
	FB		N/A	18.93	125.84	147.87
	SI		N/A	19.84	131.41	152.08
	NP		N/A	21.58	132.91	135.21
		P – Value $_{Int}$	N/A	0.3248	0.710	0.422
		Std Error $_{Int}$	N/A	1.18	10.06	12.68
		P – Value $_{CC}$	N/A	0.868	0.020	0.491
		Std Error $_{CC}$	N/A	0.95	5.81	10.38
		P – Value $_{Fert}$	N/A	0.0335	0.770	0.195
		Std Error $_{Fert}$	N/A	1.01	7.12	11.00

Alkaline Phosphatase 5-10 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	13.83	24.13	37.70
	FB	No	N/A	13.90	22.45	33.07
	NP	Yes	N/A	21.60	32.58	55.03
	NP	No	N/A	14.01	24.42	36.54
	SI	Yes	N/A	14.77	23.83	39.08
	SI	No	N/A	14.32	23.12	37.23
Main Effect		Yes	N/A	16.73	26.85	43.94
		No	N/A	14.08	23.33	35.61
	FB		N/A	13.87	23.29	35.38
	SI		N/A	14.55	23.48	38.16
	NP		N/A	17.80	28.50	45.78
		P – Value $_{Int}$	N/A	0.049	0.6054	0.375
		Std Error $_{Int}$	N/A	2.34	4.39	6.82
		P – Value $_{CC}$	N/A	0.0539	0.3005	0.123
		Std Error $_{CC}$	N/A	2.00	2.98	4.70
		P – Value $_{Fert}$	N/A	0.0529	0.3632	0.253
		Std Error $_{Fert}$	N/A	2.09	3.38	5.31

Phosphodiesterase 5-10 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	16.55	26.75	28.20
	FB	No	N/A	15.57	18.40	20.26
	NP	Yes	N/A	22.00	28.65	42.56
	NP	No	N/A	15.74	19.64	25.01
	SI	Yes	N/A	16.15	22.85	30.50
	SI	No	N/A	18.85	18.30	20.37
Main Effect		Yes	N/A	18.23	26.08	33.75
		No	N/A	16.72	18.78	21.88
	FB		N/A	16.06	22.57	24.23
	SI		N/A	17.50	20.58	25.44
	NP		N/A	18.87	24.15	33.78
		P – Value $_{Int}$	N/A	0.0878	0.6611	0.769
		Std Error $_{Int}$	N/A	2.72	2.60	6.86
		P – Value $_{CC}$	N/A	0.3273	0.0063	0.060
		Std Error $_{CC}$	N/A	2.29	1.50	3.96
		P – Value $_{Fert}$	N/A	0.3349	0.4186	0.355
		Std Error $_{Fert}$	N/A	2.40	1.84	4.85

MBC 5-10 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	100.65	152.38	318.21
	FB	No	N/A	98.51	181.58	322.28
	NP	Yes	N/A	110.47	186.95	401.25
	NP	No	N/A	107.53	133.19	351.45
	SI	Yes	N/A	109.63	137.1	391.67
	SI	No	N/A	113.32	139.5	362.96
Main Effect		Yes	N/A	106.92	158.81	370.38
		No	N/A	106.45	151.42	345.56
	FB		N/A	99.58	166.98	320.25
	SI		N/A	111.47	138.3	377.31
	NP		N/A	109	160.07	376.35
		P – Value $_{Int}$	N/A	0.6749	0.2617	0.744
		Std Error $_{Int}$	N/A	9.18	25.93	37.43
		P – Value $_{CC}$	N/A	0.89	0.7157	0.402
		Std Error $_{CC}$	N/A	8.58	16.85	24.42
		P – Value $_{Fert}$	N/A	0.0328	0.4892	0.220
		Std Error $_{Fert}$	N/A	8.73	19.52	28.24

MBN 5-10 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	35.64	45.49	N/A
	FB	No	N/A	35.56	49.05	N/A
	NP	Yes	N/A	35.59	50.00	N/A
	NP	No	N/A	35.50	51.77	N/A
	SI	Yes	N/A	35.56	56.00	N/A
	SI	No	N/A	35.58	45.92	N/A
Main Effect		Yes	N/A	35.60	50.50	N/A
		No	N/A	35.55	48.91	N/A
	FB		N/A	35.60	47.27	N/A
	SI		N/A	35.57	50.96	N/A
	NP		N/A	35.55	50.88	N/A
		P – Value $_{Int}$	N/A	0.5813	0.4091	N/A
		Std Error $_{Int}$	N/A	0.06	5.73	N/A
		P – Value $_{CC}$	N/A	0.2926	0.7218	N/A
		Std Error $_{CC}$	N/A	0.03	3.76	N/A
		P – Value $_{Fert}$	N/A	0.6654	0.7356	N/A
		Std Error $_{Fert}$	N/A	0.04	4.34	N/A

Active Carbon 5-10 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	355.01	287.08	289.56
	FB	No	N/A	282.98	258.38	273.61
	NP	Yes	N/A	305.75	281.03	266.49
	NP	No	N/A	252.56	249.85	258.06
	SI	Yes	N/A	324.28	287.83	299.58
	SI	No	N/A	271.12	270.29	287.17
Main Effect		Yes	N/A	328.34	285.32	285.21
		No	N/A	268.88	259.51	272.94
	FB		N/A	318.99	272.73	281.59
	SI		N/A	297.7	279.06	293.37
	NP		N/A	279.15	265.44	262.27
		P – Value $_{Int}$	N/A	0.7931	0.8531	0.982
		Std Error $_{Int}$	N/A	23.05	13.48	20.14
		P – Value $_{CC}$	N/A	0.001	0.033	0.461
		Std Error $_{CC}$	N/A	13.10	8.53	12.24
		P – Value $_{Fert}$	N/A	0.0852	0.5837	0.319
		Std Error $_{Fert}$	N/A	20.16	10.00	14.63

Soil Respiration 5-10 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	6.04	27.75	22.48
	FB	No	N/A	3.90	26.65	21.16
	NP	Yes	N/A	5.15	27.24	22.50
	NP	No	N/A	3.87	26.66	21.12
	SI	Yes	N/A	6.12	27.98	22.36
	SI	No	N/A	4.97	26.27	22.20
Main Effect		Yes	N/A	5.77	27.66	22.45
		No	N/A	4.25	26.53	21.50
	FB		N/A	4.97	27.20	21.82
	SI		N/A	5.55	27.13	22.28
	NP		N/A	4.51	26.95	21.81
		P – Value $_{Int}$	N/A	0.7518	0.517	0.853
		Std Error $_{Int}$	N/A	0.70	0.57	1.358
		P – Value $_{CC}$	N/A	0.0243	0.0157	0.358
		Std Error $_{CC}$	N/A	0.41	0.42	0.935
		P – Value $_{Fert}$	N/A	0.3764	0.8658	0.909
		Std Error $_{Fert}$	N/A	0.50	0.47	1.06

β-Glucosaminidase 10-15 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	6.887	13.72	13.077
	FB	No	N/A	10.003	11.08	13.333
	NP	Yes	N/A	10.637	16.59	15.238
	NP	No	N/A	10.317	11.83	15.986
	SI	Yes	N/A	9.76	17.14	15.465
	SI	No	N/A	7.383	9.30	9.662
Main Effect		Yes	N/A	9.094	15.82	14.593
		No	N/A	9.234	10.74	12.994
	FB		N/A	8.445	12.40	13.205
	SI		N/A	8.572	13.22	12.564
	NP		N/A	10.477	14.21	15.612
		P – Value $_{Int}$	N/A	0.106	0.3901	0.195
		Std Error $_{Int}$	N/A	1.1220	2.20	1.856
		P – Value $_{CC}$	N/A	0.886	0.0065	0.316
		Std Error $_{CC}$	N/A	0.763	1.63	1.071
		P – Value $_{Fert}$	N/A	0.199	0.6218	0.269
		Std Error $_{Fert}$	N/A	0.90	1.79	1.312

β-Glucosidase 10-15 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	11.413	1.52	15.712
	FB	No	N/A	10.95	2.01	15.804
	NP	Yes	N/A	12.97	3.18	17.312
	NP	No	N/A	10.477	2.29	16.385
	SI	Yes	N/A	10.953	1.98	16.919
	SI	No	N/A	11.80	2.60	16.316
Main Effect		Yes	N/A	11.779	2.23	16.648
		No	N/A	11.076	2.30	16.168
	FB		N/A	11.182	1.76	15.758
	SI		N/A	11.377	2.29	16.617
	NP		N/A	11.723	2.74	16.849
		P – Value $_{Int}$	N/A	0.15	0.3519	0.919
		Std Error $_{Int}$	N/A	1.078	0.55	1.263
		P – Value $_{CC}$	N/A	0.297	0.8711	0.652
		Std Error $_{CC}$	N/A	0.867	0.32	0.729
		P – Value $_{Fert}$	N/A	0.787	0.2518	0.672
		Std Error $_{Fert}$	N/A	0.924	0.39	0.893

Arylsulfatase 10-15 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	3.258	9.32	N/A
	FB	No	N/A	3.476	8.13	N/A
	NP	Yes	N/A	3.277	9.45	N/A
	NP	No	N/A	2.563	7.52	N/A
	SI	Yes	N/A	2.193	9.22	N/A
	SI	No	N/A	3.003	7.55	N/A
Main Effect		Yes	N/A	2.909	9.33	N/A
		No	N/A	3.014	7.74	N/A
	FB		N/A	3.367	8.73	N/A
	SI		N/A	2.598	8.39	N/A
	NP		N/A	2.919	8.49	N/A
		P – Value $_{Int}$	N/A	0.125	0.8703	N/A
		Std Error $_{Int}$	N/A	0.364	0.74	N/A
		P – Value $_{CC}$	N/A	0.713	0.0193	N/A
		Std Error $_{CC}$	N/A	0.238	0.46	N/A
		P – Value $_{Fert}$	N/A	0.123	0.8703	N/A
		Std Error $_{Fert}$	N/A	0.275	0.54	N/A

Acid Phosphatase 10-15 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	13.0433	105.92	113.47
	FB	No	N/A	12.5867	113.55	103.35
	NP	Yes	N/A	17.6233	117.98	105.22
	NP	No	N/A	13.1067	114.12	100.40
	SI	Yes	N/A	13	115.98	120.98
	SI	No	N/A	15.18	104.65	96.20
Main Effect		Yes	N/A	14.56	113.29	113.23
		No	N/A	13.62	110.77	99.98
	FB		N/A	12.82	109.73	108.41
	SI		N/A	14.09	110.32	108.59
	NP		N/A	15.37	116.05	102.81
		P – Value $_{Int}$	N/A	0.3213	0.327	0.791
		Std Error $_{Int}$	N/A	2.69	9.25	15.50
		P – Value $_{CC}$	N/A	0.6012	0.620	0.303
		Std Error $_{CC}$	N/A	2.06	7.83	9.56
		P – Value $_{Fert}$	N/A	0.5067	0.534	0.909
		Std Error $_{Fert}$	N/A	2.24	8.208	11.34

Alkaline Phosphatase 10-15 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	N/A	14.79	25.76
	FB	No	N/A	N/A	22.93	32.71
	NP	Yes	N/A	N/A	25.99	47.46
	NP	No	N/A	N/A	18.93	37.80
	SI	Yes	N/A	N/A	14.79	39.99
	SI	No	N/A	N/A	26.33	34.95
Main Effect		Yes	N/A	N/A	18.52	37.74
		No	N/A	N/A	22.73	35.15
	FB		N/A	N/A	18.86	29.23
	SI		N/A	N/A	20.56	37.47
	NP		N/A	N/A	22.46	42.63
		P – Value $_{Int}$	N/A	N/A	0.0065	0.366
		Std Error $_{Int}$	N/A	N/A	3.68	5.87
		P – Value $_{CC}$	N/A	N/A	0.0554	0.594
		Std Error $_{CC}$	N/A	N/A	3.12	3.54
		P – Value $_{Fert}$	N/A	N/A	0.356	0.110
		Std Error $_{Fert}$	N/A	N/A	3.27	4.24

Phosphodiesterase 10-15 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	11.603	14.04	16.50
	FB	No	N/A	11.04	12.72	20.76
	NP	Yes	N/A	13.663	20.05	35.89
	NP	No	N/A	11.597	13.12	24.05
	SI	Yes	N/A	7.590	14.85	29.34
	SI	No	N/A	7.037	10.98	17.75
Main Effect		Yes	N/A	10.952	16.31	27.24
		No	N/A	9.891	12.27	20.85
	FB		N/A	11.322	13.38	18.63
	SI		N/A	7.313	12.92	23.55
	NP		N/A	12.63	16.58	29.97
		P – Value $_{Int}$	N/A	0.982	0.308	0.416
		Std Error $_{Int}$	N/A	4.588	1.72	6.66
		P – Value $_{CC}$	N/A	0.782	0.0166	0.267
		Std Error $_{CC}$	N/A	2.649	0.99	3.85
		P – Value $_{Fert}$	N/A	0.503	0.1161	0.278
		Std Error $_{Fert}$	N/A	3.244	1.22	4.71

MBC 10-15 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	38.014	59.27	272.95
	FB	No	N/A	20.117	54.01	294.59
	NP	Yes	N/A	30.495	59.82	304
	NP	No	N/A	50.794	65.48	296.42
	SI	Yes	N/A	46.852	60.66	224.58
	SI	No	N/A	39.218	58.31	283.34
Main Effect		Yes	N/A	38.454	59.92	267.18
		No	N/A	36.71	59.26	291.45
	FB		N/A	29.065	56.64	283.77
	SI		N/A	43.035	59.48	253.96
	NP		N/A	40.644	62.65	300.21
		P – Value $_{Int}$	N/A	0.014	0.5586	0.436
		Std Error $_{Int}$	N/A	5.393	5.09	24.737
		P – Value $_{CC}$	N/A	0.70	0.8782	0.257
		Std Error $_{CC}$	N/A	3.3114	2.94	14.282
		P – Value $_{Fert}$	N/A	0.058	0.52	0.216
		Std Error $_{Fert}$	N/A	3.814	3.60	17.492

MBN 10-15 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	4.48	26.52	25.10
	FB	No	N/A	0.213	38.64	22.66
	NP	Yes	N/A	0.542	46.12	18.15
	NP	No	N/A	9.106	52.46	22.84
	SI	Yes	N/A	9.056	45.03	24.86
	SI	No	N/A	7.58	36.18	23.30
Main Effect		Yes	N/A	4.693	39.22	22.71
		No	N/A	5.633	42.43	22.93
	FB		N/A	2.347	32.58	23.88
	SI		N/A	8.318	40.61	24.08
	NP		N/A	4.824	49.29	20.50
		P – Value $_{Int}$	N/A	0.142	0.3019	0.881
		Std Error $_{Int}$	N/A	3.626	9.46	7.69
		P – Value $_{CC}$	N/A	0.717	0.5645	0.972
		Std Error $_{CC}$	N/A	2.604	7.78	4.44
		P – Value $_{Fert}$	N/A	0.202	0.0832	0.873
		Std Error $_{Fert}$	N/A	2.894	8.24	5.44

Active Carbon 10-15 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	242.87	355.01	162.1
	FB	No	N/A	220.01	282.98	156.77
	NP	Yes	N/A	240.41	305.75	159.38
	NP	No	N/A	221.12	252.56	146.4
	SI	Yes	N/A	191.68	324.28	182.53
	SI	No	N/A	227.42	271.12	176.77
Main Effect		Yes	N/A	224.99	328.34	168.00
		No	N/A	222.85	268.88	159.98
	FB		N/A	231.44	318.99	159.43
	SI		N/A	209.55	297.70	179.65
	NP		N/A	230.77	279.15	152.89
		P – Value $_{Int}$	N/A	0.428	0.7931	0.972
		Std Error $_{Int}$	N/A	26.28	23.05	18.14
		P – Value $_{CC}$	N/A	0.916	0.001	0.60
		Std Error $_{CC}$	N/A	17.36	19.10	10.47
		P – Value $_{Fert}$	N/A	0.604	0.0852	0.346
		Std Error $_{Fert}$	N/A	19.97	20.16	12.83

Soil Respiration 10-15 cm

	Treatment		Soybean		Corn	
	P Treatment	Cover Crop	SP18	FL18	SP19	FL19
Treatment Means	FB	Yes	N/A	30.82	6.04	22.58
	FB	No	N/A	31.17	3.90	17.85
	NP	Yes	N/A	31.17	5.15	19.54
	NP	No	N/A	30.65	3.87	19.24
	SI	Yes	N/A	30.70	6.12	19.58
	SI	No	N/A	30.72	4.97	17.92
Main Effect		Yes	N/A	30.89	5.77	20.56
		No	N/A	30.84	4.25	18.33
	FB		N/A	30.99	4.97	20.22
	SI		N/A	30.71	5.55	18.75
	NP		N/A	30.91	4.51	19.39
		P – Value $_{Int}$	N/A	0.064	0.7518	0.04
		Std Error $_{Int}$	N/A	0.173	0.70	0.75
		P – Value $_{CC}$	N/A	0.713	0.0243	0.005
		Std Error $_{CC}$	N/A	0.112	0.41	0.43
		P – Value $_{Fert}$	N/A	0.246	0.3764	0.196
		Std Error $_{Fert}$	N/A	0.130	0.50	0.53