

Microbial properties of soils: Effects of Management and pedogenesis

by

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B.S., National Taiwan University, 2006

M.S., National Taiwan University, 2008

AN ABSTRACT OF A DISSERTATION

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Department of Agronomy
College of Agriculture

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Abstract

Soil microorganisms are a critical component of ecosystem services provided by soil. Soil management drives soil physical, chemical, and biological properties. Pedogenesis and management interact to change microbial structure and function in the soil profile. Soil microbial properties may vary temporally with crop development and crop species. The objective of this study was to explore the pedogenetic and anthropogenic controls on key soil microbial properties by (i) assessing the profile of a claypan soil under conventional tillage (CT), no-till (NT), and hay meadow (HM); (ii) assessing seasonal changes of soil microbial properties in a corn/winter wheat/soybean rotation under CT and NT; and (iii) assessing vertical changes of soil microbial properties in response to long-term (28 yrs) tillage and mineral and organic fertilization. Selected microbial properties included extracellular enzyme activity, microbial structure as measured by phospholipid fatty acid (PLFA), as well as soil chemical properties. Soil C, enzyme activities, and microbial biomass were greatest in HM soils, followed by NT and then CT in the claypan soil. Wheat in the rotation increased hydrolase activity and bacterial biomass more than corn, while microbial activities were stable during soybean growth. Increased enzyme activities in the claypan layer resulted from the combination of clay-enzyme interaction and impacts from management practices. In a Mollisol soil, an increase in C-acquiring enzyme activity and microbial PLFAs in a buried A horizon was a result of root growth under no-till practice and mineral fertilization. Surprisingly, long-term mineral fertilizer applications had little effect on enzyme activities and microbial biomass. Long-term organic fertilization increased soil C, enzyme activities, and PLFAs but decreased arbuscular mycorrhizal fungi (AMF) throughout the soil profile to a depth of 90 cm. Microbial properties are controlled by crop and soil management at the soil surface and by the interaction of management and pedogenetic properties deeper in the

soil profile. Incorporating grasses in the crop rotation may allow nutrients to be extracted from deeper within the soil profile, enhancing the utilization of the entire soil profile and providing additional nutrient resources to cash crops. Incorporating wheat in the crop rotation supports greater microbial activity and biomass after corn harvest, especially in no-till management.

Additional research is required to delineate further causative factors impacting enzyme activity in the claypan layer, a finer resolution in soil microbial community at the species level to explore the linkage between ecological function and microbiome structure, and a network analysis for the soil-plant-microbe interactions.

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Additional research is required to delineate further causative factors impacting enzyme activity in the claypan layer, a finer resolution in soil microbial community at the species level to explore the linkage between ecological function and microbiome structure, and a network analysis for the soil-plant-microbe interactions.

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Dedication

I would like to dedicate this dissertation to my parents, my mother, LiLan Lin and TieFu Hsiao. They are strong, intelligent, and generous. They taught me the value in education and inspiring my interest in learning. Their love and support are the greatest gift in my life. Thank you for everything. All my success is impossible without you!

Chapter 1 - Introduction

Soil is the foundation on which our world depends, one of the major components in ecology, and the link between air and water. Soils are rich ecosystems. In agroecosystems, soils work in concert with climate, management practices, and inherent characteristics such as soil texture and parent material to determine the overall yield of a crop. Good soil functionality also improves the resilience of the agronomic production system. People have come to understand that soil is both biotic and abiotic. The term “soil health”, sometimes referred to as soil quality, is defined as “the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health” (Soil Science Terms committee, 2008). From an environmental perspective, soil health is “the capacity of the soil to promote the growth of plants, protect watersheds by regulating the infiltration and partitioning of precipitation, and prevent water and air pollution by buffering potential pollutants such as agricultural chemicals, organic wastes, and industrial chemicals” (Bünemann et al., 2018). Healthy soil creates a better habitat for plants and soil microorganisms, consequently providing higher productivity. Healthy soil also results in greater environmental functionality and biodiversity.

Although the concept of soil health is more focused on the dynamic soil properties that can be strongly affected by human management in the surface horizon, the inherent soil properties, soil-plant interactions, and other climatic factors also influence the soil health. Multiple soil parameters have been selected for soil health assessment based on expert judgment, multivariate analysis, and multiple correlation and regression (Bünemann et al., 2018). Soil organic matter and pH are the most frequently proposed soil health indicators, followed by available phosphorous (P), water storage capacity, bulk density, and soil texture (Bünemann et

al., 2018). Due to the large size and heterogeneity of the soil organic matter pool, the fraction of organic matter including permanganate-oxidizable C (active C; Weil et al., 2003), particulate organic matter (Cambardella and Elliott, 1992), and dissolved organic C (Filep et al., 2015) are also suggested to be indicators of soil health. However, it is still unclear which components of the organic matter are characterized by these C fractions, and the correlation with other soil functions is limited (Bünemann et al., 2018). Moreover, microbial properties are still underrepresented in this soil health assessment. However, microorganisms are critical to the overall function of the soil, and are important to connect the changes in management practices to soil biogeochemical features and even crop health (Lagomarsino et al., 2009; Adhikari and Hartemink, 2016; Bünemann et al., 2018). Understanding the linkage between ecological function and microbiome structure is still a significant challenge. Recent developments in microbiology and statistics, such as multi-omics, network analysis, structural equation modeling, and machine learning, provide a good opportunity to connect soil functions to soil health indicators, further changing the future soil health assessment (Bünemann et al., 2018).

In this chapter, the fundamental changes in soil microbial properties in conjunction with soil physical and chemical properties in agroecosystems are reviewed, starting with the importance of microbes on soil functions and the approaches to quantify microbial community structure and activity. The interactions between crops, soils, and microbes are discussed in the next section as crop physiology and crop rotation strongly influence the soil microbial properties. Agricultural practices, especially tillage and fertilization, impact soil health directly and indirectly through changes in soil structure and nutrient redistribution. Finally, the pedogenesis of claypan and post-settlement alluvium soils are discussed since the inherent soil properties also affect soil physical, chemical, and biological properties.

Soil microbial properties

Soil microbes decompose organic matter, allowing growth of plants and more microbes. Microbial communities can respond to environmental changes more rapidly than plant communities (Cong et al., 2015). Therefore, soil microbial properties can potentially indicate how well the soil microenvironment supports root growth and hence the productive capacity of the soil (Sinsabaugh and Shah, 2011). Soil biological functions are studied at multiple levels either focusing on broad physiological properties including soil respiration or mineralization, or on specific biochemical reactions including enzyme production or kinetics (Saiya-Cork et al., 2002). About 100 soil enzymes have been identified (Burns et al., 2013), but the major challenge is to determine their actual activities in the natural environment and how to relate the activities to ecological functions.

The increases in soil microbial activity and diversity are expected to have beneficial effects on agroecosystem sustainability, resulting from higher functional redundancy, functional diversity, and niche differentiation (Venter et al., 2016). Although conservation management practices may enhance the soil microbial diversity, the assumption that they are beneficial for agroecosystems needs further investigation. The relationships between above and belowground diversity, and between biodiversity and ecosystem function are complicated. Enhanced knowledge of soil microbial properties will help delineate the factors that control community structure, and manipulate or monitor the soil for biocontrol, nutrient cycling, and C sequestration. Currently, three of the main research topics in soil microbiology are 1) which microorganisms are present, 2) the functions of microorganisms in the field, and 3) the connection between community structure, particular genes, and ecological functions (Fuhrman, 2009; Burns et al., 2013).

Microbial activity

Enzyme activities are essential for organic matter decomposition and nutrient cycling. Microorganisms produce enzymes to assimilate available nutrients immediately (intracellular enzymes) and secrete enzymes into the environment for future nutrient decomposition (extracellular enzymes). Most extracellular enzymes are glycosylated and structurally modified from their intracellular counterparts (Burns et al., 2013). Different microorganisms in the same ecological niches can express similar extracellular enzymes (Fakruddin and Mannan, 2013). Expression of extracellular enzymes is regulated by environmental signals, but once they are released from the cell, enzyme activity is determined by environmental interactions (Sinsabaugh and Shah, 2011). In general, the activity of most hydrolases increases with greater soil organic matter content but decreases with depth in the soil profile (Burns et al., 2013; Stone et al., 2014; Fierer, 2017). However, interactions with clay and soil organo-minerals change enzyme activities (Hsiao et al., 2018). It is noted that although specific enzymes cannot indicate the nutrient dynamics in soils since they over-simplify the many interactions between enzymes, microorganisms, and other abiotic or biotic factors, the extracellular enzyme activity is useful in comparing the biogeochemical properties with different treatments (Fansler et al., 2005; Nannipieri et al., 2012).

Some common soil extracellular enzymes included cellulose (EC 3.2.1.4), xylanase (EC 3.2.1.8), β -glucosidase (EC 3.2.1.21), chitinase (N-acetyl- β -D-glucosaminidase, EC 3.2.1.30), aminopeptidases (e.g. leucine-aminopeptidase, EC 3.4.11.1), urease (EC 3.5.1.5), phosphatases (e.g. acid phosphatase, EC 3.1.3.2; alkaline phosphatase, EC 3.1.3.1), and oxidases (e.g. phenol oxidase, EC 1.10.3.2; peroxidase, EC 1.11.1.7). Cellulose hydrolyzes the 1,4- β -glucosidic bond in cellulose and lichenin, xylanase hydrolyzes the same bond from hemicellulose, and β -

glucosidase hydrolyzes the terminal glucosidic bond with the release of β -D-glucose. Chitinase, also known as N-acetyl- β -D-glucosaminidase, cleaves the amino sugar N-acetyl- β -D-galactosamine from chitin. Aminopeptidase cleaves N-terminal residues of peptides and proteins. Leucine-aminopeptidase is the most widely measured aminopeptidase because it hydrolyzes the most abundant substrate (Sinsabaugh and Follstad Shah, 2012). Urease hydrolyzes urea to CO_2 and NH_4^+ . Phosphatases release inorganic P from phosphosaccharides and phospholipids. Depending on soil pH, the predominant phosphatase could either be acid phosphatase or alkaline phosphatase. Phenol oxidase and peroxidase use oxygen and peroxide, respectively, as an electron acceptor. Oxidases are expressed for various purposes including detoxification, oxidative stress, and acquisition of C from lignin. Dehydrogenases are an intracellular enzyme group also commonly measured because they are found only in living organisms and are formed under optimal conditions (Sinsabaugh and Follstad Shah, 2012).

The extracellular enzyme activity assay measures the potential activity, which is the capability of hydrolase to decompose complex organic molecules or the capability of oxidases to destabilize phenolic compounds from natural organic matter in soil. The resulting activity data are correlated with microbial activity and substrate decomposition rates, and can be used as indicators of the environmental nutrient limitation status for microbes (Zeglin et al., 2007; Sinsabaugh et al., 2009; German et al., 2011; Burns et al., 2013). However, the currently employed enzyme activity assay is not measuring the actual activity in the soil. By adding sufficient artificial substrates either linked to a fluorescent molecule (fluorophore) or substrates that form a colored compound (chromophore) into a dilute homogenized soil slurry, researchers can examine enzyme activities by measuring the increase in fluorescence or absorbance of visual light over a specific incubation time. Although there are some limitations in the method,

including only a limited number of commercially available fluorescently label substrates and the lack of information about enzyme distribution (German et al., 2011; Wallenstein and Weintraub, 2008), the high sensitivity of the fluorometric enzyme activity assay provide an opportunity to detect microbial activity in small samples and subsoils. The fluorescent substrate can also be combined with a zymography technique as an *in-situ* method for two-dimensional enzyme distribution, allowing identification of hotspots of enzyme activity and revealing their spatial and temporal dynamics in soil (Spohn and Kuzyakov, 2014; Razavi et al., 2016; York et al., 2016).

Microbial diversity

Most of the biodiversity in the agroecosystems occurs in the soil. Soil microbial diversity is critical to fulfilling soil ecosystem services (Roger-Estrade et al., 2010). The microbial diversity consists of two components, richness and evenness, within the genetic, species, and community diversity levels (Fakruddin and Mannan, 2013). Microbial diversity typically relates to the degree of stability of the community and therefore can be used to monitor the effect of a perturbation (Fakruddin and Mannan, 2013). The number of soil microorganisms, with up to 10^9 bacteria, 10^5 fungi, and 10^4 algae within one teaspoon of topsoil, makes microbial diversity a central topic in soil ecology (Cong et al., 2015). Any niche within an ecosystem, such as soil particle size, pH, salinity, substrate availability, water availability, aeration, physical disturbance, and biological factors including phages, plasmids, and protozoans, may all change the microbial diversity at different levels. On the continental scale, soil microbial diversity and richness could be largely explained by soil pH (Fierer and Jackson, 2006) and salinity (Rath and Rousk, 2015), in contrast to site temperature, latitude, and other variables that predict plant and animal community composition.

Most soil microbial community structure studies are now using culture-independent approaches. Phospholipid fatty acid (PLFA) assay and molecular-based techniques, especially the Next Generation Sequencing, are the two major categories. Phospholipid fatty acids compose the cell membranes of all organisms. Fatty acids include fully saturated and unsaturated C chain with one or two double bonds; different species prefer using different types of fatty acids. The taxonomic specificity of some PLFA biomarkers are sometimes controversial, but some biomarkers are widely accepted: 1) branched-chain fatty acids (iso, anteiso) as indicators for Gram-positive bacteria; 2) cyclopropyl fatty acids for Gram-negative bacteria; 3) 18:2 ω 6,9 for fungi since 18:2 ω 6,9 is well correlated to ergosterol, a sterol found in fungal cell membranes; and 4) saturated fatty acids which are common in most samples (Frostegård et al., 2011). Some PLFA biomarkers overlap within some groups, meaning they can only be used under certain conditions. Biomarker 16:1 ω 5 co-occurs in both arbuscular mycorrhizal fungi and bacteria, making it only useful for determining the presence of arbuscular mycorrhizal fungi in systems with low bacterial abundance (Aldrich-wolfe et al., 2000; Fakruddin and Mannan, 2013; Frostegård et al., 2011). PLFA can also be used as indicators of microbial biomass (Frostegård et al., 2011). Compared to molecular-based methods, PLFA composition is stable and independent of plasmids or mutation. Additionally, PLFA is a good indicator of active microbial biomass because cell membranes are degraded rapidly upon cell death. Studies indicate that PLFA analysis might be more sensitive to shifts in microbial community composition than molecular-based methods, which usually include both active and dormant microbial communities in the soil (Duncan et al., 2016, Frostegård et al., 2011). However, PLFA cannot identify microorganisms at the species level. Archaea cannot be identified as well since they do not use PLFA as cell

membrane components. Although PLFA is a culture-independent analysis, the PLFA biomarkers are interpreted according to microorganisms from pure cultures (Kaur et al., 2005).

Analysis of nucleic acids provides valuable information about changes in overall microbial community composition at the species level. The minor organisms can also be detected using molecular techniques. Compared with the Sanger sequencing method, the Next Generation Sequencing is much faster and cheaper, with smaller sample sizes, yet provides more reliable results (Mardis, 2008). Nucleic acids may be analyzed directly (shotgun metagenomic sequencing) or after polymerase chain reaction (PCR) amplification (amplicon sequencing). In PCR-based approaches, 16S ribosomal RNA genes (rDNA) in prokaryotes, 18S rRNA genes in eukaryotes, and the internal transcribed spacer (ITS) DNA region in fungi are the common targets (Hill et al., 2000). These small subunit rDNA are found to be universal, highly conserved, but vary greatly between species (Aldrich-wolfe et al., 2000; Fakruddin and Mannan, 2013; Shade, 2017). The PCR biases have been widely identified in molecular-based methods, including polymerase error, lower amplification efficiency due to poorly designed primers, and PCR duplicates due to primer amplification (Hill et al., 2000). Sequence defects are also amplified by PCR. These limitations could be reduced by using well-known primers, lower annealing temperatures, shorter extension time, and processing the smallest possible PCR cycles or other modified PCR protocols (Kurata et al., 2004; Acinas et al., 2005; Aird et al., 2011). An advance in PCR that allows gene quantification is quantitative PCR (qPCR, also known as real-time PCR). A fluorogenic probe is added to the primers, which are used to count the copy numbers of target genes. The qPCR techniques have widely quantified specific functional genes including denitrification related *narG*, *napA*, *nirK*, *nirS* and *nosZ* genes (Philippot et al., 2009),

ammonia oxidation (Bier et al., 2015), as well as 16S rRNA and ITS genes in soil (Acinas et al., 2005; Wallenstein et al., 2014; Castaño et al., 2017; Carson and Zeglin, 2018).

The combined application of stable isotope labeled substrate with PLFA and nucleic acids help link soil processes to specific components of the microbial community (Wang et al., 2015; Watzinger, 2015; Jansson and Hofmockel, 2018). Stable Isotope Probing (SIP) is used to follow the flow of soil nutrients into specific groups of the microbial community. For example, application of ^{14}C plant labeling in conjunction with the ^{13}C natural abundance track the C route from photosynthetic fixation in plants to the C dynamics in soil microorganisms. PLFA-SIP has been used to identify the bacteria involved in sulfate reduction and acetate oxidation in sediments (Boschker et al., 1998), and to identify *Planctomyces* as the predominant decomposer of exopolysaccharide in soil (Wang et al., 2015).

Multi-omics

Multi-omics is an analysis approach aimed at all the genome, transcriptome, proteome, and microbiome datasets from soil microbial communities in a natural environment (Myrold et al., 2014; Jansson and Hofmockel, 2018). The use of PLFA is regarded as one metabolomics strategy (Watzinger, 2015). Besides metabolomics targeting metabolites, multi-omics approaches also study microbial communities by using metagenomics targeting DNA, metatranscriptomics targeting RNA, and metaproteomics targeting the proteins (Wallenstein and Weintraub, 2008). Progress in metagenomics has provided a comprehensive view of the genetic diversity, species composition, phylogenetics, and interactions with environmental conditions (Fakruddin and Mannan, 2013). Connections between metagenomics and metaproteomics further decode the soil microbial community functions (Myrold et al., 2014; Jansson and Hofmockel, 2018). However, there remain significant challenges including the difficulty of extracting metabolites and proteins

from the soil, annotation of functional genes, and the spatial and temporal variability in soils and its influence on microbial communities (Jansson and Hofmockel, 2018).

Plant, soil, and microbe interactions

Crop development

Plants have the capacity to shape the soil microorganisms in the rhizosphere and thereby optimize growth conditions for themselves (Cavaglieri et al., 2009; Li et al., 2014a; McDaniel and Grandy, 2016). Symbiotic relationships between soil microbes and plants enhance plant growth and productivity. Conversely, antagonistic relationships between the soil microbes and plants limit plant productivity. For example, nematodes, bacteria, or fungi, such as *Macrophomina phaseolina*, a fungus responsible for charcoal rot, can be harmful to crop growth and yield. Mutualistic rhizosphere microbes provide plants with nutrients, phytohormones, and protection against pathogens (Li et al., 2014a). Rhizosphere communities are more similar in microbial activity and nutrient use efficiency between different plant species than at different developmental stages (Schmalenberger and Tebbe, 2002; Houlden et al., 2008). Plant growth influences root physiology, changes the quality and quantity of root exudates, and impacts the microbial community (Houlden et al., 2008; Cavaglieri et al., 2009; Li et al., 2014; McDaniel and Grandy, 2016; Degruene et al., 2017). Root exudates are considered one of the major drivers of microbial dynamics (Bardgett and Van DerPutten, 2014). Overall, plants secrete up to 20% of fixed C and 15% of N into the rhizosphere (Sasse et al., 2018). Wheat and corn root exudates include substantial amounts of benzoxazinoids, an indole-3-glycerol-phosphate derived secondary metabolites, which can trigger colonization by the plant-growth-promoting rhizobacterium *Pseudomonas putida* and inhibit host recognition of the pathogenic

Agrobacterium tumefaciens (Hu et al., 2018). Defense-related proteins such as chitinases show enhanced secretion by roots during the flowering period (De-la-Peña et al., 2010). Arabidopsis exudes less sugar but more amino acid and phenolic compounds with increasing age (Sasse et al., 2018). Studies on rhizosphere microbiology reveal that bacteria on young roots prefer to use simple amino acids, whereas bacteria on mature roots utilize more complicated polysaccharides, suggesting a temporal change in the composition of root exudates (Houlden et al., 2008; Sasse et al., 2018).

Even within a given plant, root tip, mature root, and lateral root zones produce distinct border cells and mucilage, resulting in distinct microbial communities (Sasse et al., 2018). Root tips are crucial for plant-soil feedback and are typically associated with the highest numbers of active microbes compared to other root tissues. *Bacillus subtilis*, one of the model organisms used in biotechnology research with an excellent capacity for secreted enzyme production, colonize primarily in the root elongation zone (Sasse et al., 2018). Negative plant-microbe interactions result in plant defense, including higher root border cell productions and higher mucilage production released into the rhizosphere by border cells. The mucilage contains a complex of extracellular DNA and antimicrobial proteins that can neutralize threats by trapping pathogens, thereby changing the behavior of soil microflora (Hawes et al., 2016). Mucilage is also produced under nonpathogenic conditions, serving as a lubricant as the root tips penetrate through the soil (Traoré et al., 2000; Sasse et al., 2018). Additionally, mucilage also serves as a C source for microbes (Hawes et al., 2016). Border cells may release signaling molecules to trigger symbiosis with beneficial microorganisms, including the release of flavonoids that attract rhizobia and induce colonization by mycorrhizal fungi (Sasse et al., 2018).

Changes in the rhizosphere community associated with crop growth stage are significant at lower taxonomic levels (family, genus, and operational taxonomic unit (OTU); Houlden et al., 2008). The copiotrophic or r-selected bacterial populations, including members of phyla Proteobacteria and Bacteroidetes, are predominant in the rhizosphere during the seedling and vegetative growth stages in wheat and corn, though oligotrophic or k-selected populations predominate after flowering (Cavaglieri et al., 2009; Li et al., 2014a). Some consistent trends have been observed across various plant systems. For example, an increase in the number of *Alphaproteobacteria subphylum* and a decrease of the abundance of Actinobacteria in the rhizosphere are reported during plant development (Zhalnina et al., 2018). Interestingly, a study in wild oat (*Avena barbata*) report consistent succession patterns in the chemical composition of root exudates. These plant exudation traits, combined with microbial substrate preference traits, provide an ideal mechanism to manipulate the rhizosphere microbiome for crop benefits (Zhalnina et al., 2018).

In addition to plants, soil type and other environmental variables may also influence the microbial community structure (Li et al., 2014; McDaniel and Grandy, 2016; Ashworth et al., 2017). The short-term effects of root detritus decomposition and soil water distribution are reported to be larger than the effect of living roots on microbial activity (Spohn and Kuzyakov, 2014). The sudden increases in soil water availability following a rainfall event after a prolonged drought cause a sequential revival of microorganisms over timescales ranging from minutes to hours or days (Bardgett and Van DerPutten, 2014). Constant drying-rewetting and freezing-thawing events create stresses and change the activity of the soil microbial community (Evans and Wallenstein, 2012). The question of whether plant development versus environmental

diversity has a stronger influence on soil microbiology remains a critical question in terrestrial ecology (McDaniel and Grandy, 2016).

Crop rotation

In agriculture, plant-soil interactions are manipulated through crop rotation, where the sequence of crops is adjusted to provide optimal soil conditions for crop yield and environmental sustainability (Hu et al., 2018). Soils under a higher diversity of crops generally have distinct microbial properties compared to soils that have been grown in a monoculture (Lauber et al., 2013; Ashworth et al., 2017). Increasing the diversity of residue inputs into soil organic matter over time produce more habitable resource niches (McDaniel et al., 2014), thereby enhancing microbial diversity (Eisenhauer et al., 2010; Venter et al., 2016), microbial C use efficiency (McDaniel and Grandy, 2016), and total biomass (McDaniel et al., 2014). Increased ground cover enhances the soil physical properties including higher water-use efficiency and lower temperature fluctuations (Venter et al., 2016). Studies also report crop rotations together with reduced tillage has positive effects on C sequestration (Ashworth et al., 2014) and plant pathogen inhibition (Krupinsky et al., 2002). Soil from cropping systems with a more diverse rotation have higher soil microbial biomass N and N-acetyl-glucosaminidase activity, indicating soil microbes can better support crops with a higher N mineralization rate (McDaniel and Grandy, 2016).

Detailed mechanisms of the benefits of crop rotations on soil health are still unknown.

Despite the evidence of rotational crop benefits, there are no distinguishable crop combinations or cultivars that are more beneficial than others (Venter et al., 2016). The addition of legumes to a crop rotation has no consistent effects on microbial properties. The host-specific symbiotic rhizobia are low in diversity, and the pH increase associated with leguminous rotations may affect microbial diversity (Eisenhauer et al., 2010; Yin et al., 2010; Venter et al., 2016).

Benefits to microbial properties with different crop rotation sequences are also inconsistent between locations and duration of rotation practice. Microbial community composition for soils in a corn-soybean rotation is intermediate between soils in continuous corn and continuous soybean cropping systems, but this is only observed after four yrs of the corn/soybean rotation (Balota et al., 2004). Effects of the quality and quantity of crop residue on soil microbial properties take time to accrue, while microbial growth and nutrient use efficiency may acclimate to new conditions (Slaughter et al., 2015). Some microbes can recover quickly after disturbance (Shade et al., 2013), and the prevalence of dormancy in a microbial community also reduces the degree of changes in soil microbial properties after the disturbance (Duncan et al., 2016). Additionally, soil spatial variability, soil type, and the complicated climate-plant-microbe interactions may overshadow the direct effects of various crop residues on soil microbial properties in the first few years after implementing a rotation (Balota et al., 2004; Ashworth et al., 2017).

Effects of tillage and fertilization on soil microbial properties

Farmers can use tillage and fertilization to manage soil health. Tillage buries the crop residue, warms the seedbed in the spring, temporarily reduces surface compaction, and may temporarily reduce weeds and diseases (Barber, 1971; Fernández et al., 2015). However, the tillage operation leaves low amounts of residue on the soil surface, resulting in soil erosion, and loss of soil organic matter and soil water (Krupinsky et al., 2002). Conservation tillage, in which farmers minimize the frequency and intensity of tillage operations on the field while leaving the previous crop residue on the soil surface instead of mixing into the soil, is a well-known strategy to promote environmental benefits. Conservation tillage practices range from zero-tillage (no-

till), reduced tillage, mulch tillage, ridge tillage, to contour tillage (Busari et al., 2015). Multiple studies indicate that no-till practices provide some benefits including soil and water conservation, reduced erosion, improved aggregate stability, increased fertility, and greater microbial activity compared to conventional tillage practice (Alvarez and Steinbach, 2009; Franzluebbers, 2005; Mbutia et al., 2015; Roger-Estrade et al., 2010; Smith et al., 2016).

Tillage can change soil water availability and aggregate stability directly. The surface residue reflects solar radiation and conserves soil water (Johnson and Lowery, 1985). Therefore, soils under no-till management have lower temperatures and higher moisture contents during most of the growing season, with the greatest differences occurring in the surface soil (Drury et al., 1999; Alvarez and Steinbach, 2009). Mikha and Rice (2004) reported that soils under long-term no-till developed more macroaggregates ($>2000 \mu\text{m}$) in the top 5 cm layer than in conventional tillage. Accumulation of labile C and associated macroaggregates may be one of the important mechanisms by which no-till practices enhance soil health (Mikha and Rice, 2004; Aziz et al., 2013). No-till can significantly increase soil aggregation and aggregate-protected C and N, while no-till and organic fertilization together further accelerate this pathway, forcing the newly-added C to be translocated downward to the C-depleted subsoil layers (Nicoloso et al. 2018).

Tillage also has impacts on root distribution. Soils under no-till have greater water storage capacity, higher bulk density and penetration resistance, and a lower proportion of small aggregates than conventional tillage (Osunbitan et al., 2005; Soane et al., 2012; Guan et al., 2015). All features encouraged a higher root length density in the top 10 cm of the soil but a lower density in deeper layers (Chassot et al., 2001; Baker et al., 2007). In contrast, intense tillage generally increased root depth and decreased water content in surface soils (Dwyer et al.,

1996; Baker et al., 2007). However, different soil type and the spatial variation in soil water distribution, temperature, and mechanical resistance explained the inconsistent net effect of tillage on root depth and development with sites or with time (Chassot et al., 2001; Guan et al., 2015).

Tillage injures soil microorganism or exposes them to the risk of heat, drought, and predation. Although tillage breaks soil aggregation and releases C and N from protected organic matter that can be used as nutrient resources over a short period, the quantity of soil organic matter decreases after long-term tillage, leading to lower microbial biomass (Roger-Estrade et al., 2010). A meta-analysis of 62 studies, including 139 observations from around the world, points out that overall, microbial biomass and most extracellular enzyme activities were higher under no-till compared to conventional tillage (Zuber and Villamil, 2016). Tillage decreases soil strength, accompanied by decreased macropore continuity and biochannels (Dwyer et al., 1996). White and Rice (2009) found longer retention of plant residue in macroaggregates, higher biomass of Gram-positive and Gram-negative bacteria, fungal phospholipid fatty acids, and higher biological activity under no-till than conventional tillage in the Central Great Plains area. Tillage leads to lower mycorrhizal fungi (Roger-Estrade et al., 2010). Mbutia et al. (2015) also demonstrated that Gram-positive bacteria, arbuscular mycorrhizal fungi, and activities of C-, N-, and P-acquiring enzymes were associated with changes in soil moisture, C, N, and soil pH in no-till systems. Macro-aggregates protected by fungal hyphae typically produce key extracellular enzymes (Cenini et al., 2016; Strickland and Rousk, 2010). However, consistent evidence delineating the impacts of tillage on fungal to bacterial ratios have not been established (Strickland and Rousk, 2010). Potential reasons include an overall increase in both bacterial and fungal biomass when tillage practices are converted from conventional tillage to no-till. Other

soil factors such as pH, moisture, and texture may also change the abundance of fungi and bacteria (Rousk et al., 2010).

There are potential disadvantages of no-till systems including excess water, lower soil temperature, and increased weeds especially on fine-textured soils (Drury et al., 1999; Soane et al., 2012). Although with controversy, delayed seedling emergence is reported in corn and soybean under no-till production, presumably because of the lower soil temperature (Chassot et al., 2001). No-till increases the risk of foliar disease because the higher soil water levels and many pathogens survive longer in crop residue on the soil surface than when they are buried (Krupinsky et al., 2002). Reduced tillage, incorporating a deeply rooted crop in the rotation, or the application of fertilizer and herbicide are alternatives or add-ons to no-till practices in these soil systems (Fernández et al., 2015). Corn emergence and productivity are increased after red clover was used as a cover crop with no-till in clayey soil (Drury et al., 1999). A potential explanation is that the red clover residue had a lower C:N ratio and accelerated the wheat straw decomposition by microbes, resulting in less surface residue. Notably, soil organic C and total N are higher in no-till with a significant reduction in corn yield variability in clayey soil (Yost et al., 2016).

Fertilization is used to increase nutrient availability to crops, and can change the soil physicochemical and microbial properties significantly (Mandal et al., 2007; Li et al., 2014b; Gu et al., 2017). Fertilization adds N to soils, which stimulates the nitrification and denitrification pathways by soil microbes. These pathways change the community dominance in favor of aerobes, facultative anaerobes, and denitrifiers (Mackelprang et al., 2018). The type and composition of fertilizer lead to changes in soil properties. Although information is still conflicting, most studies demonstrated that the long-term application of organic fertilizer leads to

an increase in microbial biomass, potential enzyme activities, and microbial diversity compared to mineral fertilizers or unfertilized soils (Marschner et al., 2003; Zhong et al., 2010; Francioli et al., 2016). The increases in microbial biomass and enzyme activities, especially in β -glucosidase and N-acetyl-glucosaminidase, are mainly due to an increase in soil organic C from fertilizer input (Francioli et al., 2016; Lupwayi et al., 2018). Conversely, application of mineral fertilizer may stimulate activities of nitrification, C- and P-acquiring related hydrolases, and reduce the activities of oxidase and N-acquiring hydrolases including N-acetyl-glucosaminidase and chitinase (Baldrian, 2014; Jian et al., 2016). Both organic and mineral fertilizers shift soil microbial community composition, in which organic fertilization stimulated copiotrophic organisms adapted to nutrient-rich environments but mineral fertilizer stimulated more oligotrophic organisms (Francioli et al., 2016). Application of scarce N and P to soils provide more microbial niches, driving a fertilizer-associated increase in diversity in each site (alpha diversity), and creating cultivation-specific microbiomes compared to native landscapes (Mackelprang et al., 2018). Abundances of Nitrospiraceae and Nitrosomonadaceae families, microbes involved in ammonia oxidation to nitrite and nitrite oxidation to nitrate, are increased after long-term application of ammonia-based fertilizer, while the abundance of N fixation Rhizobiales associate microbes are decreased (Mackelprang et al., 2018). Application of mineral N fertilizer may also affect microbial properties indirectly by changing soil pH and increasing crop yields, thus promoting an active root system and exudates (Zhong et al., 2010). A mixture of organic and mineral fertilizers increase crop yield and microbial biomass and diversity even more than just applying organic fertilizer (Ding et al., 2016). When a plant is rapidly taking up N, organic fertilizer may not be mineralized fast enough to meet plant needs. Consequently, crops may be under slight N stress during periods of high uptake which could limit yield.

Addition of small amounts of N fertilizer to cover the periods of high N uptake and avoid N stress would be beneficial to crop growth, and also potentially increase the microbial community diversity and biomass (Ding et al., 2016; 2017).

Soil profiles

Inherent soil properties, such as clay, silt or sand composition and parent material, impact microbial properties directly or indirectly through nutrient distribution and stabilization (Solly et al., 2015). The dynamics of carbon in topsoil has been relatively well described, but half of the soil C is located in soil layers below 30 cm and has received less research attention (Balesdent et al., 2018). Two common soil types, claypan soils and post-settlement alluvium, were chosen to analyze further how management practices work together with pedogenetic processes on soil microbial properties in this study. Claypan soils have a claypan layer located in the subsurface soil, while post-settlement alluvium is often hidden in the landscape after vegetation establishment. The soil microbial properties and biogeochemistry throughout the soil profiles of these two soil types are still poorly characterized.

Claypan soil and claypan genesis

Claypan soils are characterized by a dense, compact, and slowly permeable subsurface layer with higher clay content than adjacent horizons, from which it is separated by a sharp boundary (Soil Science Terms committee, 2008). The claypan layer is usually hard when dry and sticky when wet. An eluvial (E) horizon above the claypan layer is typical but not necessary. Although “claypan” is not a soil taxonomic term, the prefix “albic”, which means an abrupt change in soil texture, is one necessary condition for identification of a claypan soil in the soil taxonomic system. Claypan soil is recognized as a clay-enriched illuvial B horizon in the

Canadian classification system, and as Planosols in the World Reference Base (WRB) soil classification system (Soil Science Terms committee, 2008). Although without a specific diagnostic subsurface horizon, most claypan soils fall into the groups of Albaqualfs, Albaqualts, and Argialbolls in Soil Taxonomy (Schaetzl and Thompson, 2015). In the United States, claypan soils are abundant in Major Land Resource Area 113A, Central Claypan Area, in southern Illinois and west-northern Missouri; 112, Cherokee Prairies in southwestern Missouri and southeastern Kansas; and 87AB, Texas Claypan Area in northwestern Texas.

Claypan soils may form in different ways. A change in parent material stratigraphy is the major mechanism in claypan soil formation. For example, the development of a claypan soil in Oklahoma is recognized chiefly from inherent fine clays in the upper layer. The Bt and C horizons are formed in similar parent materials, but the A horizon may have been associated with deposition in a different time period (Culver and Gray, 1968a, 1968b). Ferrollysis and associated clay translocation also help claypan soil genesis. Ferrollysis is a two-stage destructive process in clay involving soil solution and interaction of redoximorphic metal (Fe). In the anaerobic stage, Fe^{3+} is reduced to Fe^{2+} and dissolves into the soil solution, and then Fe^{2+} displaces exchangeable cations on the clay surface. In the aerobic stage, Fe^{2+} is oxidized to Fe^{3+} , precipitates as goethite while creating H^+ , and the increased pH leads to the hydrolysis of clay (Schaetzl and Thompson, 2015). On flat landscapes, flow with downward movement facilitates clay translocation. Once the Bt layer passes its intrinsic threshold, it begins to accumulate more clay by sieving. As time goes by, the A and E horizon exhibit leaching of their base cations and clay, creating an acidic and coarser E horizon. At the same time, the Bt horizon becomes clayey and less permeable, creating a perched water table periodically. This promotes more intensive ferrollysis and enhances the formation of the claypan layer (Schaetzl and Thompson, 2015).

In southeastern Kansas, the claypan soils are formed by clay translocation and loess deposition on top of clayey alluvium or residuum weathered mainly from Permian and Pennsylvanian sandstone, shale, and limestone (Hartley et al., 2014). However, claypan soils can be formed from various parent materials such as alluvium, calcareous loess, glacial drift, and residuum under precipitation ranging from 380 to 1100 mm (Nikiforoff and Drosdoff, 1943a). Such a range of parent materials and climates suggests that the local conditions include an old, flat landscape where periodically saturated conditions contribute to the development of the claypan soil.

The genesis of claypan soil directly influences its physical, chemical, and biological properties. In claypan soil with a typical A_E_Bt soil profile, the organic matter, CEC, and soil pH decline from the A to E horizon, increasing at the upper layer of the Bt horizon then declining with depth (Fanning and Gray, 1959; Schaetzl and Thompson, 2015). A study of soybean root systems in claypan soils also found the root length density is reduced above the claypan layer and increased to a secondary maximum within the argillic horizon (Myers et al., 2007). Declining root length density in the E horizon is due to nutrient deficiency and acidic conditions. In contrast, increasing root length below the clay-maximum layer correlated with less aluminum toxicity, and increased nutrient availability and pH.

Post-settlement alluvium

Post-settlement alluvium (PSA) is a layer of alluvium found on many valley floodplain surfaces. During the process of erosion, soil travels downstream and buries fields, filling wetlands and river ecosystems. Combined with soil erosion, PSA deposition is a major environmental concern worldwide as it has been associated with upstream erosion resulting from

land-use changes such as vegetation clearance, agriculture, mining, grazing, or urbanization (Portenga et al., 2016b; a).

Post-settlement alluvium is typically described as a lighter colored layer above a darker, organic pre-settlement soil horizon with a distinct boundary. Because the accumulation rate of the original sediment is generally an order of magnitude lower than the PSA, substantial portions of the PSA are anthropogenic in origin. Post-settlement alluvium is a category of legacy sediment, referring to anthropogenically eroded soil deposits (James, 2013). After land-use pressures decrease, either due to soil conservation practices or dam construction upstream, sediment deliveries are reduced, and river channels are incised or widened over time. This cycle may continue to deliver PSA to lower positions in basins for centuries (James, 2013). Though PSA alters the original landscape by reducing the original relief, PSA is not a well-researched topic. This is likely because a PSA is hidden in the landscape after vegetation establishment (Portenga et al., 2016a). As a result, the soil microbial properties and biogeochemistry in the pre-settlement soil horizon underlying a PSA are commonly unknown.

Study objectives

The overarching goal of the research program is to identify the beneficial management practices that improve soil health vertically and temporally. We know that agricultural practices are one of the major disturbances for soil systems. Studies have reported the interaction between land use practices and soil physical, chemical, and biological properties to change soil health at one time point in the surface soil. However, the changes in soil health in deep soil layers and over time are still unclear. This research was designed to explore the interactions between soil formation and agricultural practices on soil health, particularly the fundamental changes in the

soil microbial properties, by detailed measurements of soil properties in different soil layers or at different crop development stages. The objectives of individual chapters are:

Chapter 2. Vertical changes in soil microbial properties in claypan soils. The objective of this study was to assess how management practices mediate changes in soil microbial properties with depth in a claypan soil.

Chapter 3. Temporal variability in soil microbial properties in claypan soils. The objective of this study was to assess the seasonal changes in soil microbial activity and community in a corn/winter-wheat/soybean rotation under conventional tillage (CT) and no-till (NT) practices.

Chapter 4. Long-term fertilization and tillage effects on soil microbial properties with depth. The objective of this study was to assess the vertical dynamics of soil microbial activity and community composition in response to tillage and fertilization management practices in the Central Great Plains in Kansas.

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Chapter 2 - Vertical changes of soil microbial properties in claypan soils¹

Abstract

Microbial activity within the soil is critical for plant growth and development, and a major determinant of crop performance and yield. Claypan soils are characterized by a dense, impermeable subsoil that impedes root system development. Little is known about soil microbial properties in claypan soils or how microbial activity changes with depth in the soil profile. We explored how management practices mediate changes in soil microbial composition and potential enzyme activities with depth in a claypan soil. The soil microbial biomass and composition were examined through phospholipid fatty acid (PLFA) assay. We found that the soil organic carbon (SOC), microbial biomass, and oxidase activities declined with depth, while hydrolase activity increased in the upper layer of the claypan. Changes in soil management practices affected the degree of increase in hydrolase activity in subsoils, especially for N-acetyl- β -D-glucosaminidase. No accumulation of SOC in the claypan layer was observed. Contrary to our expectation, soil microbes deeper within the soil profile were phosphorus- and nitrogen-limited rather than carbon-limited. Vertical stratification of measured soil properties was found with an upper layer from 0 to 15 cm, an intermediate layer between 15 cm and approximately 30 cm, and the lowest layer of soils in the claypan below 30 cm. The interaction between clay content and changes in soil factors with depth resulted in an increased potential activity but unaltered microbial composition in the claypan layer.

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Introduction

Claypan soils cover approximately four million hectares in the central US, including portions of Illinois, Iowa, Kansas, Missouri, and Oklahoma (USDA-NRCS, 2006). Claypan soils are characterized by a dense, impermeable clay layer in the subsoil covered by silt loam soil at the surface. The soils can be productive, but the productive capacity is often limited by shallow topsoil depth. There is no clear delineation of clay amount, but a typical description is a sharp increase in clay over an abrupt boundary (Buckley et al., 2008). It is not known how the textural changes in claypan soils impact microbial activity and communities, or the potential impact of the soil microbial activity on plant production in claypan soils.

The clayey subsoils of the Cherokee Prairies ecoregion in the tallgrass prairie in southeast Kansas are classified as smectite dominant or smectite and kaolinite mixed mineralogy (Hartley et al., 2014). They were formed by clay translocation and loess deposition on top of clayey alluvium or residuum weathered mainly from Pennsylvanian shale and limestone (USDA-NRCS, 2006; Hartley et al., 2014). The claypan layer stores water. In southeast Kansas, the volumetric moisture content in the claypan layer generally exceeds 25% even in the dry season, compared to around 10% in surface soils, although the plant-available water is low in the claypan layer due to the high water retention by the clay (Buckley et al., 2008; Hartley et al., 2014). The low hydraulic conductivity of the clayey layer creates saturated surface soils after rainfall events, impairing root growth and exacerbating soil erosion (Soil Survey Staff, 2012). Moreover, plant roots do not develop extensively in the clay layer (Myers et al., 2007). Soil responses to crop management practices including crop rotation, irrigation, and tillage may be different on claypan soils than on well-drained soils (Buckley et al., 2008).

Inherent soil properties, such as clay, silt or sand composition and parent material, impact microbial properties directly or indirectly through nutrient distribution and stabilization (Solly et al., 2015). Amino sugars and carbohydrates tend to concentrate in clays, while phenolic compounds and fatty acids are more abundant in silts (Paul, 2016). Soil C in the topsoil is mainly associated with macroaggregates as a mineralizable resource, and vegetation and root exudates strongly influence soil organic carbon (SOC) stability. Conversely, below 30 cm in the soil profile, soil C absorbed by clay or other minerals is more protected from mineralization and stabilized as a C sink. Aluminum complexes have been shown to contribute to C stability and microbial activity, and soil pH changes both the solubility of the metal-humus complex and microbial properties, as well as enzyme activities and community composition (Heckman et al., 2009; Paul, 2016). Previous studies reported that clay content and clay mineralogy influenced enzyme kinetics through a reduction in the substrate turnover (K_{cat}) but an increase in the half-life of enzymes; thus the impact of clay on soil enzymes was not consistent (Fuka et al., 2008; Burns, 2013; Burns et al., 2013; An et al., 2015). The clay content of soil modifies the microbial community structure by favoring bacteria over fungi (Wei et al., 2014).

Microbial properties are different in subsoils than in surface soils. Soil nutrients, microbial biomass, and hydrolase activities decreased exponentially with depth in several studies (Allison et al., 2007; Eilers et al., 2012; Stone et al., 2014), but oxidase activities in subsoils were reported to be stable or even higher than in topsoils in taiga ecosystems (Schnecker et al., 2015). The soil organic matter (SOM) chemistry and spatial separation, rather than SOM content, had a greater influence on enzymatic activities in the subsoil (Stone et al., 2014; Schnecker et al., 2015). Microbial community composition was also found to shift with depth, along with a decline in fungal:bacterial ratios, an increase in Gram-positive and sulfate-reducing bacteria, and

a decrease in Gram-negative bacteria (Allison et al., 2007; Stone et al., 2014). Microbial communities in deep soils were relatively similar regardless of landscape position or cropping systems (Allison et al., 2007; Eilers et al., 2012). A laboratory soil incubation study reported that the microbes in the subsoil had higher utilization of amino acids, whereas the microbes in topsoil showed higher C mineralization (Tian et al., 2017).

Soil management practices also drastically affect the soil environment. Tillage is known to have negative impacts on soil nutrients, pH, and biological properties (Roger-Estrade et al., 2010; Capelle et al., 2012; Mbutia et al., 2015). Soil C and pH can further change soil microbial communities (Allison et al., 2007; Kaiser et al., 2016). Fungi were found to be more abundant than bacteria in no-till agricultural systems because of less disruption of fungal and plant communities (Hendrix et al., 1986). Studies have found that nutrient concentration is the predominant factor determining enzyme activity and microbial composition where climate conditions are not limiting (Margalef et al., 2017).

Crop production on claypan soils requires careful management to maintain productive capacity. Understanding the impact of management practices on soil microbial properties can be useful to sustain soil health in claypan soils. However, microbial properties within claypan soils are poorly characterized. The objective of this study was to assess how management practices mediate changes in soil microbial properties with depth in a claypan soil. We examined three production systems: conventionally tilled crop production, no-till crop production, and long-term grass (hay meadow). Soil extracellular enzyme activity was used as an indicator of microbial functional diversity, and phospholipid fatty acid (PLFA) profile was used as an indicator of the microbial community structure and living microbial biomass. Soil characteristics were measured with depth, including texture, pH, soil water content, and nutrient contents.

Materials and Methods

Study sites and experimental design

A 3.8 ha long-term research field located in Cherokee County, Kansas (37.21 N, 94.87 W) was used in this study. The experiment was a complete randomized design with uneven replications. Seventeen test plots were used: six for long-term conventional tillage row crop production (CT; plot size 9.1 m x 21.3 m), eight for long-term no-till row crop production (NT; plot size 9.1 m x 36.6 m), and three for grass (hay meadow, HM; plot size, 22.9 x 61 m). The CT practice included chisel plowing and disking prior to planting corn; and disk harrow after corn harvest prior to wheat planting. Soybean was planted by no-till after wheat harvest. All crops are grown each year in the long-term rotation study. The hay meadow was mowed twice yearly. Nitrogen, phosphorus, and potassium fertilizers and herbicides were applied according to standard agricultural practices for each production system.

The mean annual temperature for the area was 14.4 °C, with average annual precipitation of 1157.3 mm. The predominant soil type in the field is a Parsons silt loam (Fine, mixed, active, thermic Mollic Albaqualfs) with 0.2% slope. It is an Alfisol that has an abrupt textural change between the mollic epipedon and the argillic horizon, with low saturated hydraulic conductivity, rich ferrous iron, and aquic moisture conditions (Soil Survey Staff, 2014). Parsons silt loam soils are characterized as fertile surface soils with poorly drained subsoils that formed in clayey old alluvium or residuum weathered from sandstones, shales, and limestones of Permian, Pennsylvanian, and Mississippian age. This soil is common to the claypan region of the Midwest.

Soil sample processing

Soil samples were collected near the end of June 2015 from corn and soybean plots and the hay meadow fields using a tractor-mounted hydraulic press (Giddings, Windsor, CO). Within each plot, two 75-cm deep soil cores (diameter 7.6 cm) were collected at random locations and partitioned into seven depth intervals. Samples were refrigerated at 4 °C and transported to the Soil Microbial Ecology lab in Manhattan, KS. Cores from each plot were composited by depth, homogenized, and subsampled for subsequent analyses. Subsamples for physical and chemical properties were air-dried, ground, and sieved through 2 mm mesh. Subsamples for microbial properties were stored at -20 °C.

Soil physical and chemical properties analysis

Soil gravimetric moisture content was determined after oven-drying samples for 24 h at 105 °C. Soil particle size analysis was completed using the standard pipette method (Kilmer and Alexander, 1949). Soil pH was determined in a 1:10 soil:water slurry. Total C and total N concentrations of soils were determined by dry combustion analysis using a Carlo-Erba C and N analyzer (Thermo Finnegan Flash EA1112, Milan, Italy). Soil properties were measured using the standard Mehlich-3 method (Frank et al., 1998) for extractable P and the standard ammonium acetate method for both extractable K (Warncke and Brown, 1998) and cation exchange content (CEC; Chapman, 1965) at the Soil Testing Lab at Kansas State University, Manhattan, KS.

Extracellular enzyme activities

The potential activities of hydrolases were measured following a modified fluorometric method using fluorometric substrates 4-methylumbelliferone (MUB), and the potential activities of oxidases were measured using colorimetric substrate L-3,4-dihydroxyphenylalanine (L-DOPA) (Zeglin et al., 2013). Hydrolase assays included a C-acquiring enzyme (β -glucosidase,

bG, EC 3.2.1.21), a phosphorus- (P) acquiring enzyme (acid phosphatase, AP, EC 3.1.3.2), and a nitrogen- (N) acquiring enzyme (N-acetyl- β -D-glucosaminidase, NAG, EC 3.2.1.30). The bG hydrolyzes β -d-glucoopyranosides in the degradation of cellulose. The NAG cleaves the amino sugar N-acetyl- β -D-galactosamine from chitin in soils. Preliminary data indicated that leucine-aminopeptidase (LAP) activity was relatively low compared to NAG, which is common in acid-to-neutral soils such as those in this study. Therefore, we examined only NAG activity levels in the soils. Acid phosphatase releases inorganic P from soil organic matter into biologically available forms. Oxidase assays included two main categories of lignin degradation enzymes: phenol oxidase (POX, EC 1.10.3.2) and peroxidase (PER, EC 1.11.1.7). All assays were run at room temperature in 50 mM pH 5 acetate buffer for 1 hour for bG, 2 hours for AP, 4 hours for NAG, or 18-20 hours for POX and PER. Buffer blank, soil blank, negative control, MUB reference standard, and quench control were measured for each sample. Fluorescent absorbance was determined by a Multi-Mode Microplate Reader (FilterMax F5, Molecular Devices, Sunnyvale, California) with 365/450 nm excitation/emission for hydrolases and 450 nm absorbance for oxidases. Potential enzyme activities were reported as nanomoles activity per gram of dry soil per hour. Enzyme activities were normalized relative to total PLFA microbial biomass as a proxy for specific activity.

Phospholipid fatty acid (PLFA) analysis

The PLFA procedure was modified from the White and Ringelberg method (White and Ringelberg, 1998; Zeglin et al., 2013). Total lipids were extracted using 10 mL of methanol, 5 mL of chloroform, and 4 mL of phosphate buffer (pH 7.4) on 5 g freeze-dried soil. Water and chloroform were added 3 h after extraction to separate the mixture into polar and nonpolar fractions, while total lipids remained in the nonpolar phase. Phospholipids were separated from

neutral lipids and glycolipids using silicic acid chromatography columns (Disposable BAKERBOND[®] SPE Columns, J.T. Baker[®]) and eluted with methanol. The phospholipids were then saponified by KOH, methylated to fatty acid methyl esters (FAME), and analyzed with a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts) equipped with a DB5-MS column (30 m x 250 µm in diameter x 0.25 µm film thickness; Agilent Technologies, Santa Clara, California). Helium was used as the carrier gas; FAME peaks were recognized by retention time in comparison with the bacterial acid methyl esters mix (BAME; Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania). Internal standards 19:0 FAME were used to determine concentrations. A total of 36 biomarkers were identified from all soil samples. Microbial groups were assigned based on characteristics of biomarkers: iso and ante-iso branched lipids often belong to Gram-positive bacteria; monosaturated and cyclopropyl lipids often belong to Gram-negative bacteria; actinobacteria have more methyl branched fatty acids; methyl linoleate (18:2 ω 9,12c) is typically found in fungi. Phospholipid fatty acid abundance was reported as nmol per gram of dry soil. The fungal:bacterial ratios were calculated by dividing the sum of fungal biomarkers by the sum of Gram-positive bacteria, Gram-negative bacteria, and actinomycetes. Microbial biomass was estimated as the sum of all PLFA biomarkers.

Data analysis

For analyzing depth and management practice effects on soil physio-chemical and microbial properties, a randomized, repeated-measures design with uneven replications was used that included management practice as a fixed factor, depth as a random factor with repeat measurements, and the variance of depth as covariance. Two-way analysis of variance (ANOVA) in SAS (University edition, SAS[®] Institute Inc., Cary, NC, USA) was used. All

errors are reported as standard error. Means separation was performed using LSMEANS statement and PDIFF option test at $P \leq 0.1$ for unbalanced designs. Pairwise comparisons were made between management practices and depth intervals for each soil property. The relation between the measured soil parameters and depth or management practice factors was summarized using principal component analysis (PCA) in R (R Development Core Team, 2013). The k-means clustering algorithm was incorporated with PCA to partition out observations into three clusters with the nearest mean. The composition of the soil microbial community was summarized using PCA on the relative abundance of PLFAs in each sample. Pearson's correlation coefficient (R^2) was used to determine the degree of association between the measured parameters and the depth or management factors.

Results

Soil physical properties

The depth to the clay layer and the percent clay content varied by location in the field (Fig. 2-1A; Fig. A-1). The clay content in the top 5 cm of soils was 10-20% and increased with depth to more than 50% at 20 to 40 cm depth. In general, the HM plots had the shallowest depth to the claypan.

The gravimetric soil water content was lowest in the top 5 cm of the soil and gradually increased with depth (data not shown). The gravimetric moisture content was significantly positively correlated with soil clay content with Pearson's R^2 of 0.81 (** $P < 0.01$; Fig. A-2A).

Soil chemical properties

Soils were moderately acidic (pH 6) in the top 5 cm, with soil pH in CT (6.3) slightly higher than NT (6.0), followed by HM (5.8) (Fig. 2-1B; ** $P = 0.0029$). Soil pH increased

slightly to a maximum near 20 cm and then declined to an average of 5.5 for all management practices.

Soil organic C content decreased rapidly with depth (Fig. 2-1C; *** $P < 0.001$). In the top 5 cm, the HM had more than 2% of SOC content on average but the cropped soils had only 1% SOC; carbon in the NT soil was greater than in the CT soil (* $P = 0.0027$). Below 30 cm, the SOC level decreased to less than 0.8% for all management systems, with HM and NT soils significant higher than CT only at 40 and 50 cm ($P_{HM} = 0.0763$, $P_{NT} = 0.0681$). The SOC was positively correlated to the change in clay content with Pearson's R^2 of 0.70 within the claypan layer (soils below 35cm; Fig. A-2B).

Soil total N content showed a similar decline as C with depth (Fig. 2-1D; *** $P < 0.001$), with nearly a two-fold greater soil N level at the surface of HM than in either of the cropping systems. A level of 0.1 to 0.15 % total N is considered low for agricultural soils in Kansas (Leikam et al., 2003). Soil total N was greatest in HM soils in the top 15 cm, followed by NT and then CT soils, though the difference was only significant for the HM. Below 30 cm, total N decreased to less than 0.9%, then decreased steadily to 0.05% at 65 cm depth.

Soil C:N ratio decreased with depth (Fig. 2-1E; *** $P < 0.001$). The C:N ratios in both HM and NT were greater than in CT in the top 5 cm (* $P_{HM} = 0.0034$, $P_{NT} = 0.0557$), while the difference between HM and NT was non-significant. No differences in C:N ratios were measured for either hay meadow or cropland soils below 5 cm.

Soil extractable P decreased sharply with depth (Fig. 2-1F; *** $P < 0.001$). The measured level of 10 to 20 mg kg⁻¹ extractable P in the top 5 cm of soil is considered low for agriculture fields in Kansas (Leikam et al., 2003). Extractable P content in NT was significantly greater than in both HM and CT at 10 cm (* $P_{CT} = 0.0426$). Below 30 cm, extractable P content stabilized

around 1.5 mg kg⁻¹, with virtually no detectable differences between management practices. Soil organic C and total N contents were closely correlated, and extractable P was moderately correlated to both SOC and total N contents (Fig. A-2A).

In contrast to changes in C, N and P with depth, soil extractable K content decreased slightly and then increased with depth to a maximum near 180 mg kg⁻¹ at 50 cm (Fig. 2-1G; ***P < 0.001). The extractable K content was significantly correlated to the change in the clay content (Pearson's R² = 0.78; ***P < 0.001). Soil extractable K in the top 5 cm was greater in HM, followed by NT and CT, though the difference was only significant for the HM. The soil extractable K was the highest around 40 to 50 cm depth in all management systems.

The CEC was correlated to soil clay content (Pearson's R² = 0.73; ***P < 0.001). The maximum CEC was measured at 30 cm depth in CT, and at 50 cm in both NT and HM.

Extracellular enzyme activities profiles

Soil bG activity decreased with depth in the soil profile (Fig. 2-2A; ***P < 0.001). The bG activity dropped sharply from an average of 375 nmol hr⁻¹ g⁻¹ soil in the top 5 cm to an average of 125 nmol hr⁻¹ g⁻¹ soil at 10 cm in the HM soil profile. The bG activity in the cropped soils also decreased between the upper two soil layers, and the overall activity was much less than that in the HM soils. There was a significant increase in bG activity at 40 cm in HM soils (P = 0.068). The difference between NT and CT soils was not significant.

Soil AP activity also decreased with depth in the soil profile but showed greater variability than for bG (Fig. 2-2B; ***P < 0.001). The AP activity in HM soils was significantly greater than cropland soils in the top 15 cm and at 40 cm. As with bG activity, AP activity dropped quickly from the maximum at the upper soil layer (5 cm) in all management systems. An increase in AP activity at depths of 40 and 50 cm was seen in the HM (P = 0.0871) and NT

($P = 0.0938$) systems. The AP activity in NT was not significantly greater than in the CT soils throughout the soil profile.

Changes in soil NAG activity with depth were more complex than bG or AP (Fig. 2-2C; $***P = 0.0008$). The NAG activity in the HM soil decreased slightly from 125 nmol hr⁻¹ g⁻¹ soil at the surface (5 cm) and then increased more than 3-fold to a maximum at 40-50 cm, then decreased at the greatest depth (65 cm) to an activity similar to that in the topsoil. The NAG activity in the cropping systems remained nearly constant throughout the soil profile, showing only a slight increase with depth in the NT system. The NAG activities in HM soils were significantly greater than in the cropped soils in the top 5 cm and below 35 cm. The difference in NAG between NT and CT was not significant throughout the soil profile.

Soil POX activity was greatest in the top 5 cm of the soil profile, declining rapidly with depth (Fig. 2-2D; $***P < 0.001$). Some locations in the cropland systems showed no detectable POX activity below 20 cm. There was no significant change in POX activity in subsoils for any of the management systems, and the difference between CT and NT was only significant at 10 and 20 cm ($P = 0.0647, 0.0007$).

Soil PER activity decreased with depth in all management systems (Fig. 2-2E; $***P < 0.001$). Although the enzyme activity levels were much greater than for the other enzymes measured, there was also greater variability, obscuring clear trends. The PER activities in HM soils were significantly greater than cropland soils throughout the soil profile except at 10 cm. The PER activities in NT soils were not significantly greater than CT soils throughout the soil profile.

Most soil extracellular enzyme activities decreased with depth in the soil profile. However, bG, AP, and especially NAG activity increased at around 40 cm depth. This increase

was particularly evident in the HM soils. The bG, AP, POX, and PER activities were significantly positively correlated with soil C with Pearson's R^2 of 0.87, 0.71, 0.71, and 0.59, respectively (Fig. A-2A; *** $P < 0.001$). Conversely, NAG activity was not correlated to either soil C or N in the entire soil profile. The NAG activity was only weakly correlated to clay content, soil moisture, and negatively correlated to pH (0.44, 0.37, and -0.56, respectively; Fig. A-2A; * $P < 0.05$). Within the claypan soils, all hydrolases had a positive correlation with clay content with R^2 of 0.57, 0.74, and 0.43 for bG, AP, and NAG, respectively (Fig. A-2B; * $P < 0.05$).

Specific enzyme activity and potential C:N:P acquisition ability

In contrast to the decline in hydrolase and oxidase activities with depth in the soil profile, the specific hydrolase (sum of bG, AP, and NAG) and oxidase (sum of POX and PER) activities per microbial biomass increased with depth in the soil profile (Fig. 2-3). Soil specific hydrolase activity increased at 30 cm in NT and CT soils and 40 cm in HM soils. There were no significant differences in specific hydrolase activities based on management system. The specific oxidase activity showed much greater variability than the specific hydrolase activity, and no difference with depth or management systems was observed except at 50 and 65 cm in CT.

Ratios of C-acquiring to P-acquiring enzyme activities ($\ln(\text{bG}):\ln(\text{AP})$) were less than 0.959 and decreased with depth (Fig. 2-4A). Conversely, ratios of C-acquiring to N-acquiring enzyme activities ($\ln(\text{bG}):\ln(\text{NAG})$) were greater than one in surface soils but decreased rapidly to 0.8 with depth in the HM and NT systems (Fig. 2-4B). They increased to just above 1.0 in the CT system below 30 cm. Ratios of P-acquiring to N-acquiring enzyme activities ($\ln(\text{AP}):\ln(\text{NAG})$) were always greater than one in all management systems and at all depths (Fig. 2-4C).

Phospholipid fatty acid (PLFA) profiles

Microbial biomass decreased rapidly with depth ($***P < 0.001$) in all systems, but changes were only significant in the HM soil (Fig. 2-5A). Unlike hydrolase activity profiles, there was no increase at 40 cm in HM soils. Microbial biomass in the NT and CT soils were not significantly different throughout the profile, though levels were elevated in the surface soils. The microbial biomass was much higher at the surface in the HM system, and remained above the biomass levels measured in CT and NT throughout the profile. The microbial biomass was significantly correlated to SOC with Pearson's R^2 of 0.8 (Fig. A-2A; $**P < 0.01$).

Microbial community composition did not change significantly with management practices or depth (Fig. A-3A and B). The fungal to bacterial ratios were similar for both cropping systems and decreased with depth in the soil profile (Fig. 2-5B). Compared to the cropping systems, HM supported greater fungal populations except in the top 5 cm of the soil. In contrast to the cropping systems, HM had an increase in fungal to bacterial ratio from 5 to 10 cm in the soil, followed by a gradual decline to levels similar to those observed in the cropping systems.

The textural changes with depth led to changes in soil properties. Clay content, oxidase activity, CEC, and soil C explained 46% of the variation in measured soil parameters in PCA axis 1 (Fig. 2-6). The PCA axis 2 explained an additional 24.2% of the variability, mainly from hydrolase activity, soil N, and microbial biomass. Soils at lower depths in the profile and those in the claypan fell to the left of PCA axis 1, and included clay content, CEC, K, and soil water. Soils above the claypan layer fell to the right of PCA axis 1 because of greater soil C, hydrolase activity, and microbial biomass (Fig. 2-6A). Results from the k-means clustering algorithm

suggested clay content, CEC, extractable K, and soil water had a similar PCA distribution, while hydrolase, microbial biomass, soil C and N were grouped in a second distribution (Fig. 2-6B).

Discussion

The textural changes with depth in claypan soils impact the physical and chemical characteristics of the soil profile. The presence of the clay layer was notable as an increase in clay content beginning at around 20 cm in the soil profiles (Fig. A-1). As in other studies (Jobbágy and Jackson, 2001; Stone et al., 2014), SOC, total N, and extractable P contents decreased rapidly from the highest levels measured at the soil surface to lowest levels within the profile. Conversely, extractable K increased with depth in parallel with the increase in clay content (Fig. 2-1; Fig. A-2A). This is expected in a claypan soil (Myers et al, 2007) and highlights the impact of the claypan on the nutrient profile.

Claypan soils have been found to restrict root development. Myers et al. (2007) found soybean root length density declined to a minimum in the soil layer above the claypan, and then increased to a secondary maximum below the clay layer as the increasing soil pH increased nutrient availability for plant roots. Although claypan soils restrict root development in general, Clark et al. (1998) found Eastern gamagrass roots can penetrate the claypan layer to obtain moisture and nutrients. However, no significant increase in SOC with depth was measured in any of the management practices in our study (Fig. 2-1C). The lack of a strong eluvial horizon above the claypan and a thicker claypan layer may explain the insignificant SOC change in subsoils in our study, even in the long-term HM. Although other studies indicated rooting activity in and below the clay layer, the high clay content soil did not appear to accumulate SOC in our study.

The textural changes with depth led to a vertical stratification of soil properties (Fig. 2-6A): topsoil from 0 to 15 cm, intermediate from 15 to 35 cm, and claypan layer below 35 cm. Soil properties in the top layer were primarily determined by SOC, total N, microbial biomass, and hydrolase activity. Soil properties in the claypan layer were affected by clay content and its correlated parameters including CEC, extractable K, and soil moisture content (Fig. 2-6B). A stratification of hydrolase activity was also found (Fig. 2-3; Fig. A-3C). The measured soil enzymes were closely correlated to SOC in the topsoil, which was affected by management practices (Fig. 2-C). Within the claypan layer, however, the hydrolases and PER activity increased substantially and were correlated to clay content (Fig. 2-2; Fig. A-2B). The impact of the clay content may account for the differences in soil enzyme profiles measured in the subsoils. Although the impact of clay content on enzyme activities was not consistent, all enzymes measured in this study except NAG were reported to have greater potential activities in the clay-enzyme complex forms (Sarkar et al., 1989; Allison and Jastrow, 2006). Conversely, although changes in actual microbial biomass concentration were observed with depth and production system (Fig. 2-5), no changes in microbial community assessed by PLFA relative abundance were observed with either depth or management practice (Fig. A-3A and B). The dissimilar profiles of potential enzyme activity and microbial PLFAs within the claypan layer are consistent with the idea that the microbial community structure and function are uncoupled in response to environment (Purahong et al., 2014).

The production system impacted the enzyme activity profiles in both the topsoil and the claypan layer. Soil bG, AP, and oxidase (POX, PER) activities decreased with depth, yet hydrolases showed some increase in activity at 40 and 50 cm especially in NT and HM soils (Fig. 2-2; Fig. A-2B). In contrast to annual cropping systems, a long-term perennial grass system

(such as the HM) has plants that occupy the land continuously, allowing development of substantial rooting systems with the potential to penetrate the clay layer (Clark et al., 1998). The micropore networks and channels created by previous perennial grass roots can assist root growth and SOC accumulation in claypan soil (Jassogne et al., 2009). Although we observed no SOC accumulation in the claypan, the long-term establishment of persistent plant roots in the HM may account for the increase in soil enzyme activities observed in this study, especially in the HM (Fig. 2-2A, B, C, and somewhat E). This may be similar to the increased oxidase activity observed in taiga ecosystems (Schnecker et al., 2015). The substantial increase in NAG activity in the claypan layer of the HM system is intriguing. It may indicate the potential of grass systems to utilize more of the soil profile by establishing roots within the clay layer. Additional research is needed to fully delineate the impact of clay on NAG activity and the interactions between perennial roots and NAG activity.

The fungal to bacterial ratio was greater in CT than NT in the top 5 cm in our study (Fig. 2-5B), contradicting the notion that no-till agricultural systems lead to fungal dominance because the hyphae of fungi are relatively more affected by tillage (Hendrix et al., 1986). However, consistent evidence in support of the impacts of tillage on fungal:bacterial ratios have not been established (Strickland and Rousk, 2010). As other studies have found, changes from NT to CT led to an overall increase in both bacterial and fungal biomass. The abundance of fungi and bacteria has also been shown to be affected by soil pH, moisture, texture, and so on. Although fungi have been reported to be more acid-tolerant than bacteria, the fungal:bacterial ratio does not always increase with acidity (Rousk et al., 2010; Strickland and Rousk, 2010). However, soils with high clay content favor bacteria over fungi (Stotzky, 1966a, 1966b; Wei et al., 2014). Thus, it is possible that lower pH did not really affect the fungal:bacterial ratio, but instead the

combination of higher clay content shifted the microbial community to bacterial dominance with depth in our study.

Compared to soils from global surveys, ratios of enzymatic activities in these soils indicated greater investment toward P acquisition relative to C acquisition (Fig. 2-4A) and N acquisition (Fig. 2-4C), implying a primary microbial P limitation (Sinsabaugh et al., 2008). Both HM and cropping systems were P-limited throughout the soil profile, but the P limitation was not as restrictive in the HM soils. Ratios of $\ln(\text{bG}):\ln(\text{NAG})$ were greater than one in surface soils but decreased rapidly with depth, indicating a relatively N-rich condition in the surface soil but N-limiting conditions to soil microbes below 20 cm in the HM and NT systems (Fig. 2-4B). Overall, P and N became more limiting to microbes with increasing depth in all management systems. Nitrogen was limiting in subsoils, but P was more restricted than N throughout the soil profile. Our data indicated that P was the most limiting nutrient for microbes at lower levels, followed by N and C. Fertilizer applications may have contributed to N being limiting only in the lower soil layers. It is reasonable that higher N availability relative to P is required for organisms to begin investing in the production of phosphatase (Margalef et al., 2017), and it also suggests that C is not the predominant factor limiting microbial activity. This is consistent with research in remnant prairie soils in Illinois that showed that soil C was not the primary factor controlling soil microbial community composition in lower soil layers. Instead, P, exchangeable calcium, and soil water had a greater influence (Allison et al., 2007).

We acknowledge that clay mineral stabilization of enzymes should increase through the soil profile. Our interpretations are based on the assumptions that activities within all categories of hydrolytic enzyme are equally affected by changing soil abiotic conditions and that vertical translocation of enzymes is minimal. Also note that despite using extracellular enzyme activity

as a convenient tool to represent the functional diversity of soil ecosystems, the assumption that the ratio of nutrient-acquiring enzyme activities is an indicator of nutrient dynamics in soil may over-simplify the many direct and indirect interactions between enzymes and other abiotic or biotic factors involved in soil microbial activity (Nannipieri et al., 2012).

The complex profiles in oxidases and specific oxidase activity are similar to the patterns observed by other researchers, and are likely due to control by both biological and chemical environmental factors (Allison and Jastrow, 2006; Sinsabaugh, 2010). The observed increase in specific hydrolase activity at 30-40 cm (Fig. 2-3) may arise because the clay minerals protect the extracellular enzymes from degradation. Alternatively, soil microorganisms may spend more energy on enzyme production in deep soils. This is consistent with the cellular economics research showing that microbes continuously secrete low levels of enzymes to maintain the capacity to rapidly respond to substrate availability changes even in a nutrient poor environment (Burns et al., 2013). The third possible explanation is that clay-stabilized AP could be released during Fe(III) reduction (Chacon et al. 2006). Therefore, the increased specific microbial activity could be due both to the clay-enzyme interaction and the greater amount of enzymatic production.

Phosphatase activity was correlated with SOC and total N content but not available P in our study (Fig. A-2A). This finding is in agreement with other research demonstrating that organic P, rather than available P, is a better predictor of phosphatase activity (Margalef et al., 2017), because phosphatase activity is related to the potential capacity to release phosphate from the soil organic material. Soil with higher SOC content is considered to contain more organic matter, which can be a good proxy of organic P (Margalef et al., 2017).

Conclusions

Vertical stratification of soil properties was found in the claypan soils in southeast Kansas, with an upper layer from 0 to 15 cm, a lower layer in the claypan (below 35 cm), and an intermediate soil layer between 15 to 35 cm. The microbial biomass and extracellular enzyme activities in the upper layer were high and primarily affected by management practices. Soils in the middle layer had rapidly increasing clay and gravimetric moisture contents, K, and CEC, while SOC, total N, and extractable P concentration gradually decreased in this layer. Microbial biomass, enzyme activities, and fungal to bacterial ratio decreased with depth. Soils in the claypan layer were P- and N-limited for microbes. Soil hydrolase activities increased in the upper part of the claypan layer and then decreased with depth, especially in HM systems. Carbon accumulation was not observed. The inherent soil properties, such as the particle size, determined the amount of potential hydrolase activity changes in the claypan layer. Our results indicate that agricultural practices primarily control soil biological activity in the topsoil, while inherent soil properties dictate the potential enzyme activities in the clay layer, with production system (HM versus crop) impacting enzyme activities. Additional research is required to delineate causative factors impacting enzyme activity in the clay layer.

Incorporating more grasses in the crop rotation or as cover crops may allow nutrients to be extracted from deeper within the soil profile, enhancing the utilization of the entire soil profile and providing additional nutrient resources to cash crops. By simultaneously examining soil physical, chemical, and biological property in different land use practices with depth, we were able to delineate the impact of human activity and inherent soil properties on soil microbial properties.

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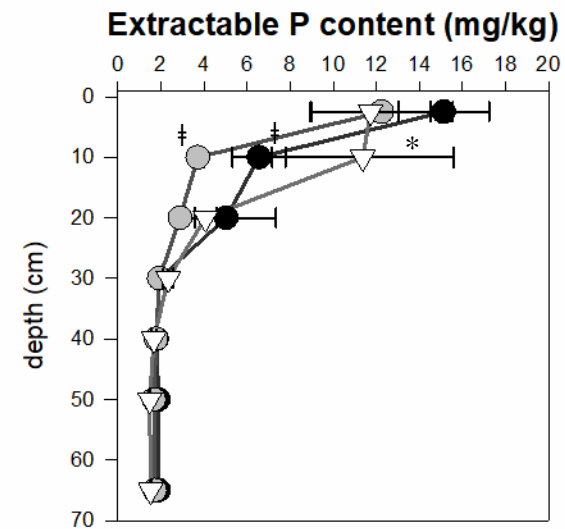
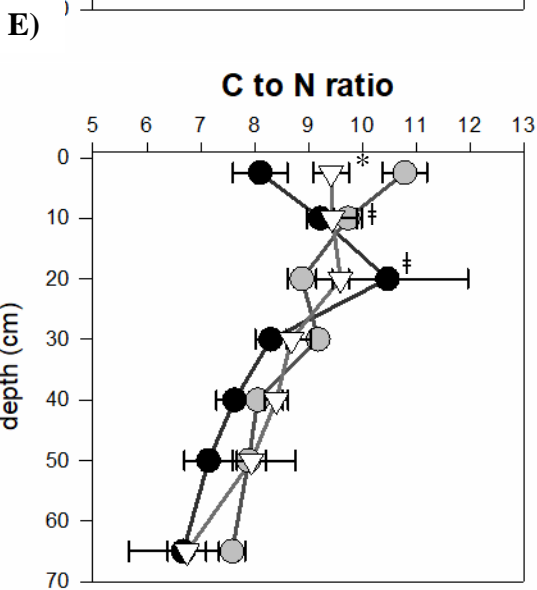
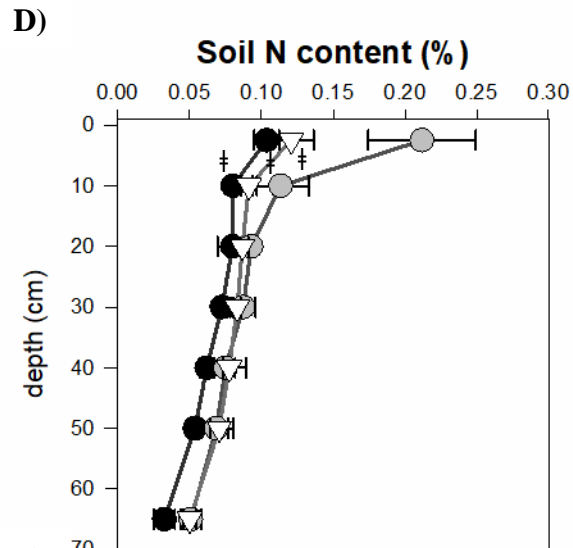
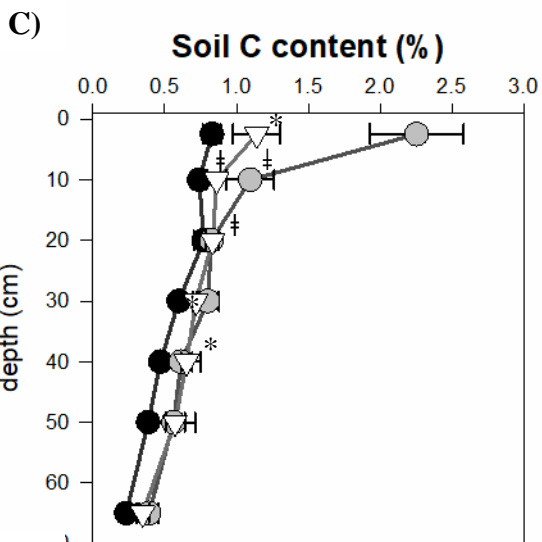
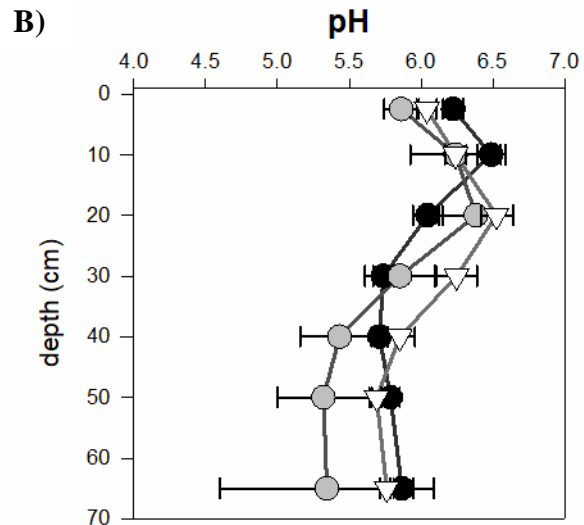
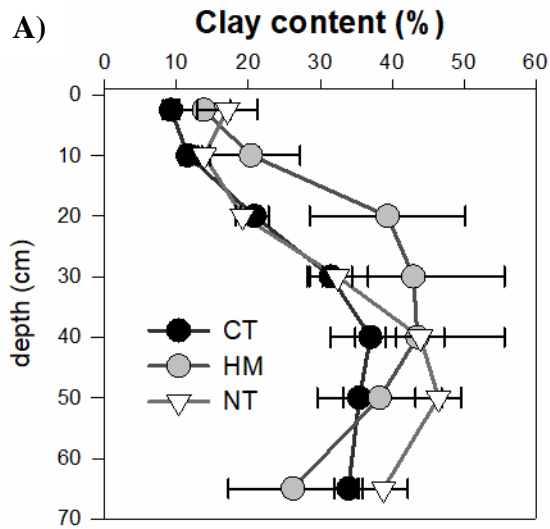
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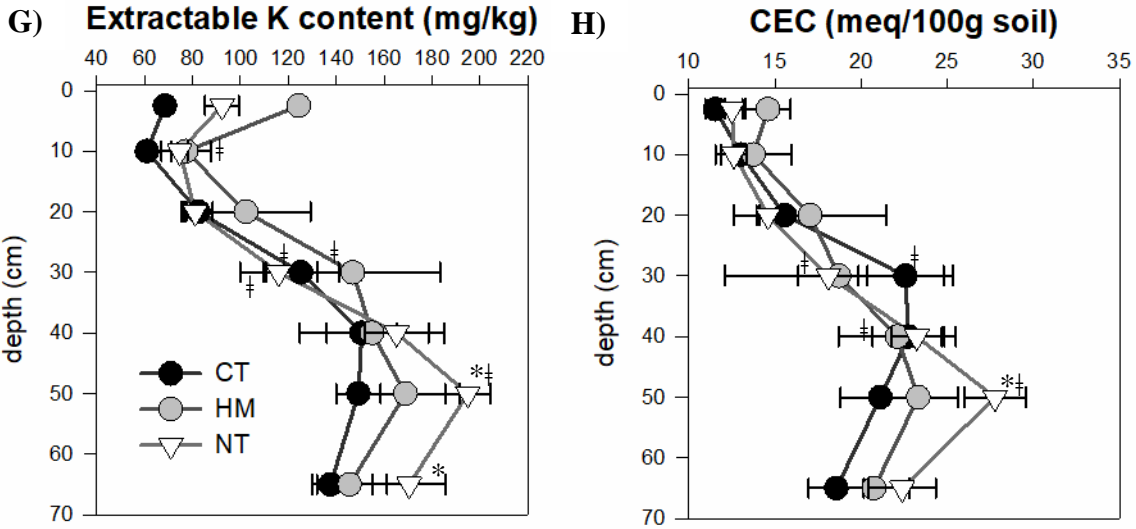


Figure 2-1. Change of selected environmental variables with depth for different management practices (mean \pm standard error of the mean (SE), $n_{CT} = 6$, $n_{NT} = 8$, $n_{HM} = 3$). * significant difference between NT and CT soils at the same depth at the 90% confidence level. † significant difference with upward depth intervals at the 90% confidence level.

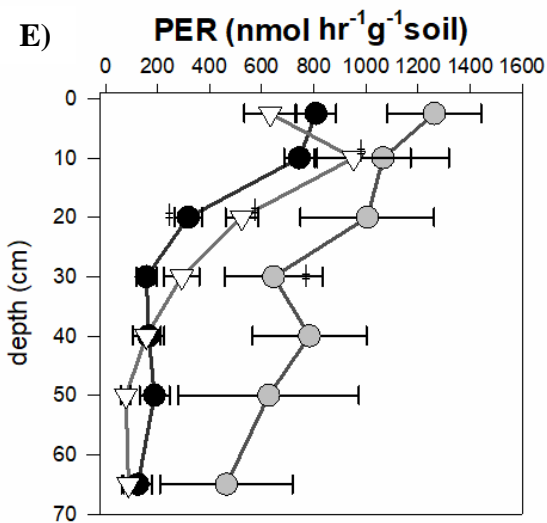
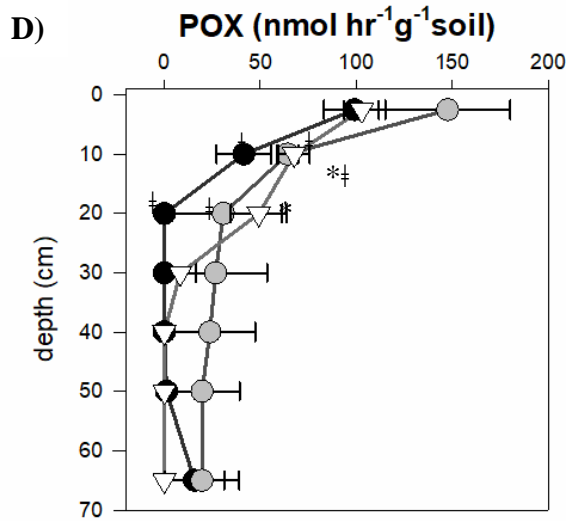
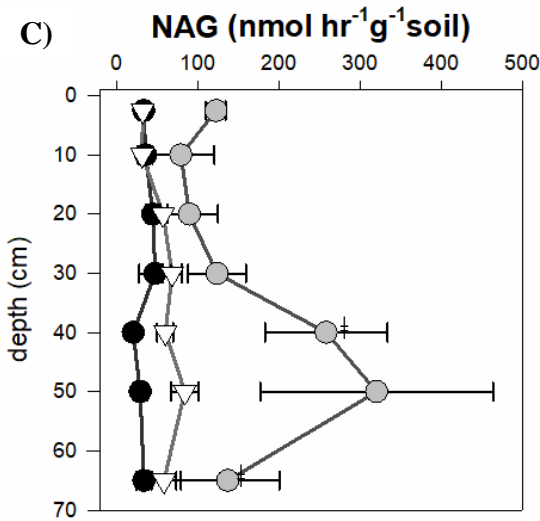
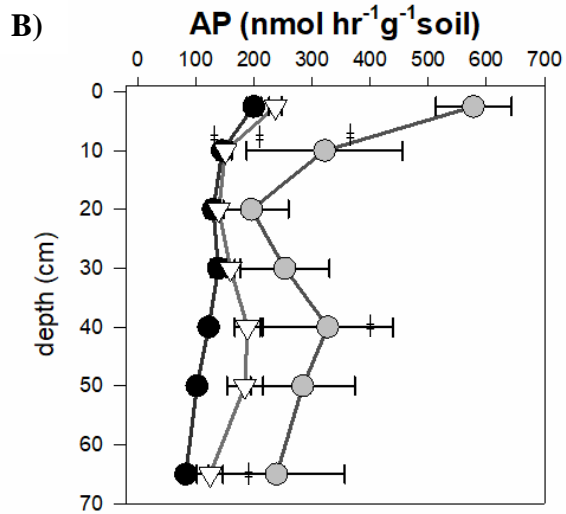
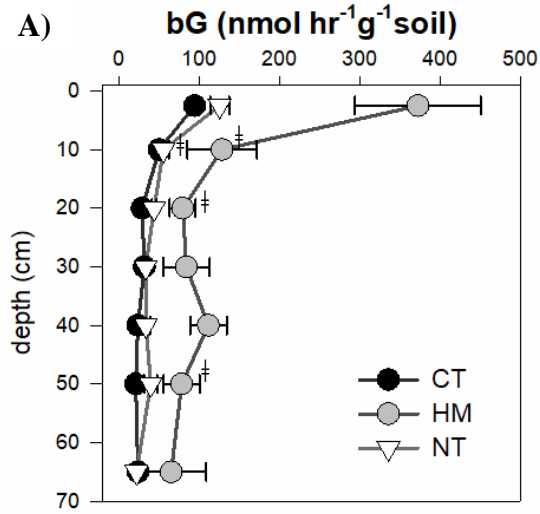
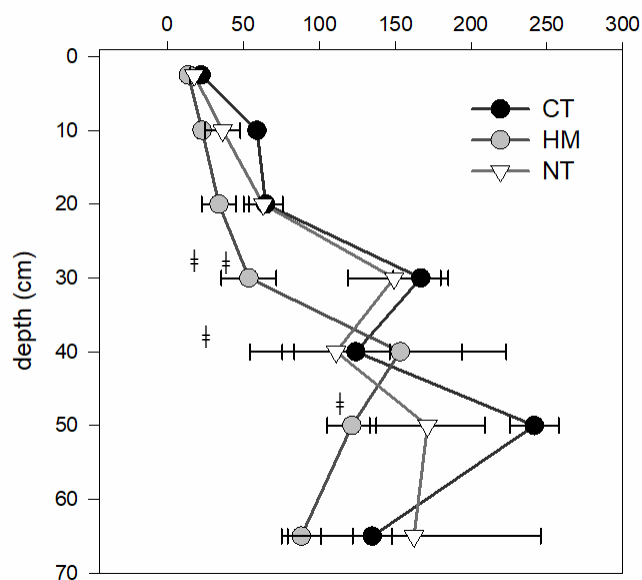


Figure 2-2. Change in soil extracellular enzyme activities with depth for different management practices (mean \pm 1 SE, $n_{CT} = 6$, $n_{NT} = 8$, $n_{HM} = 3$). bG: β -glucosidase, C-acquiring enzyme; AP: acid phosphatase, C & P-acquiring enzyme; NAG: N-acetyl glucosidase, C & N-acquiring enzyme; POX: phenol oxidase; PER: peroxidase. Both POX and PER are lignin degradation enzymes. The bG, AP, NAG are hydrolases, and POX, PER are oxidases. * significant difference between NT and CT soils at the same depth at the 90% confidence level. † significant difference with upward depth intervals at the 90% confidence level.

Specific hydrolase activity (per microbial biomass)



Specific oxidase activity (per microbial biomass)

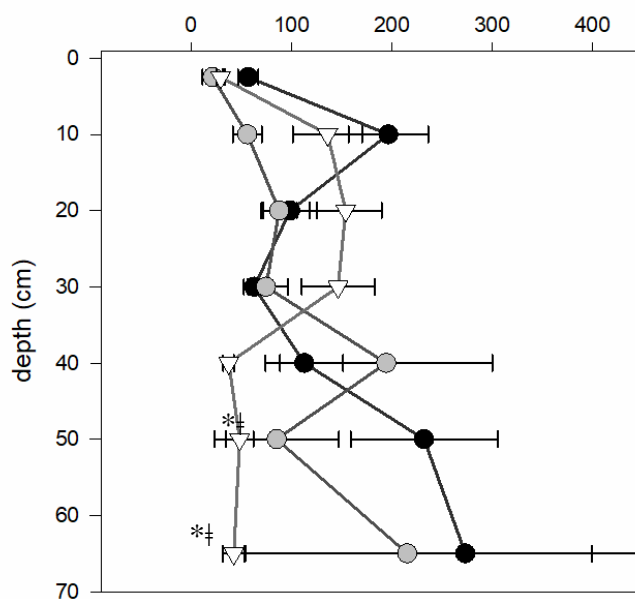


Figure 2-3. Change in soil specific hydrolase and oxidase activities per unit microbial biomass with depth for different management practices (mean \pm 1 SE, $n_{CT} = 6$, $n_{NT} = 8$, $n_{HM} = 3$). Hydrolase = bG + AP + NAG; Oxidase = POX + PER. * significant difference between NT and CT soils at the same depth at the 90% confidence level. † significant difference with upward depth intervals at the 90% confidence level.

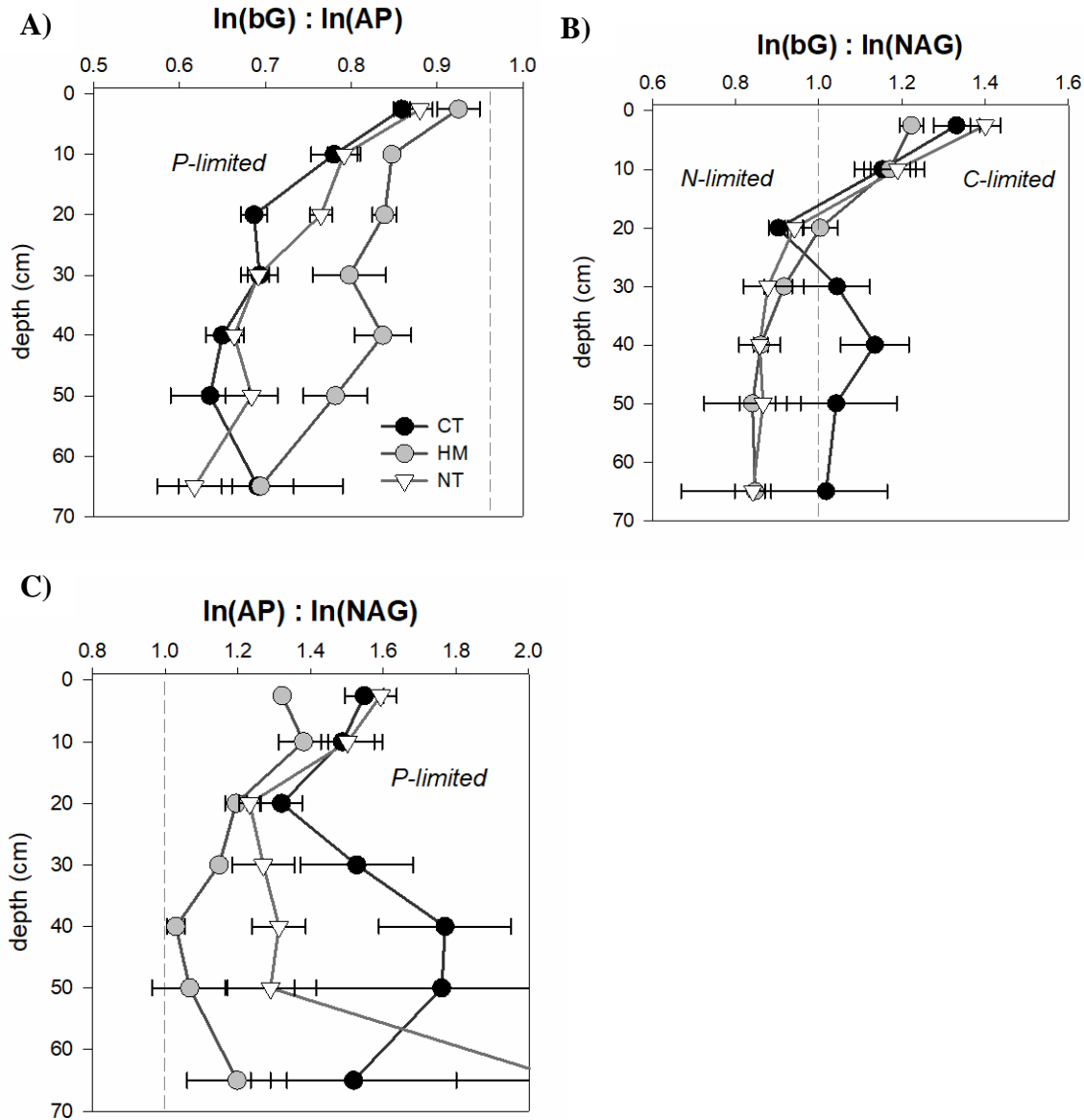
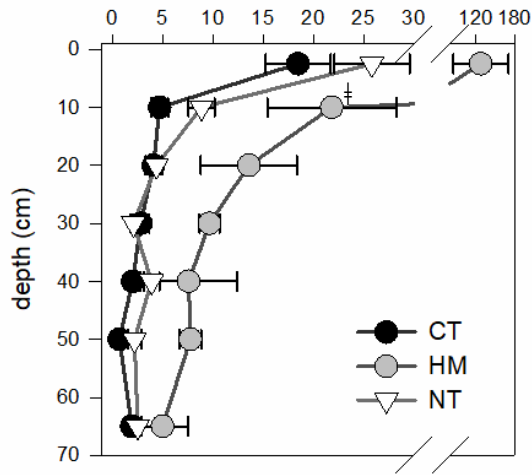


Figure 2-4. Change in enzyme activity ratios with depth for different management practices (mean \pm 1 SE, $n_{CT} = 6$, $n_{NT} = 8$, $n_{HM} = 3$). A) C-acquiring to P-acquiring enzyme activities. A ratio below 0.959 indicates a P-limited environment; B) C-acquiring to N-acquiring enzyme activities. A ratio greater than one indicates a C-limited environment; C) P-acquiring to N-acquiring enzyme activities. A ratio greater than one indicates a P-limited environment. * significant difference between NT and CT soils at the same depth at the 90% confidence level. † significant difference with upward depth intervals at the 90% confidence level.

A) Microbial biomass (nmol PLFA g⁻¹ soil)



B) Fungi : bacteria ratio

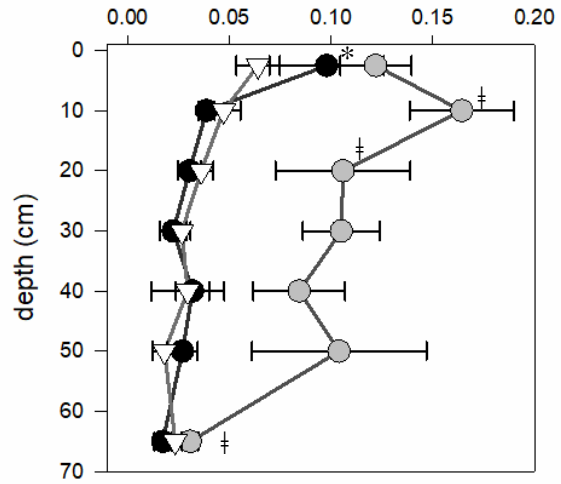
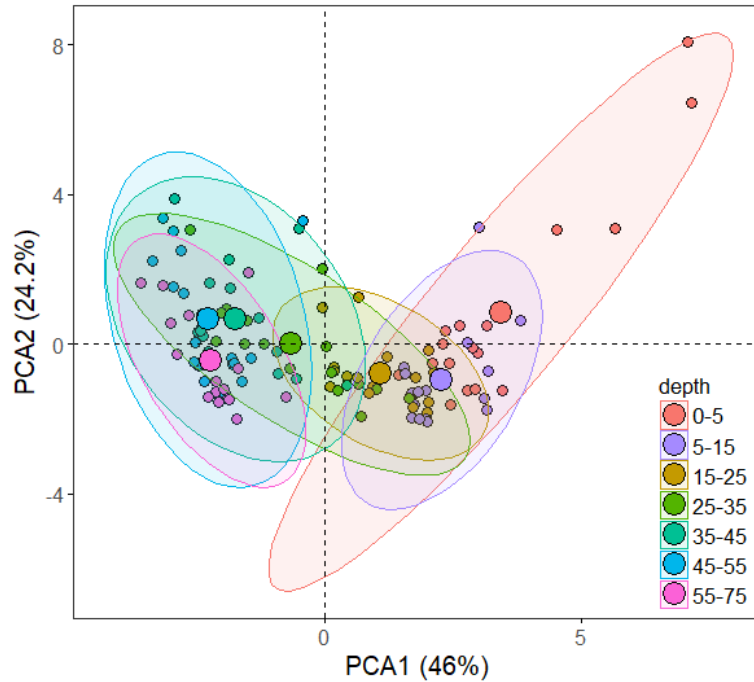


Figure 2-5. Change in soil microbial community compositions with depth for different management practices (mean \pm 1 SE, $n_{CT} = 6$, $n_{NT} = 8$, $n_{HM} = 3$). * significant difference between NT and CT soils at the same depth at the 90% confidence level. † significant difference with upward depth intervals at the 90% confidence level.

A)



B)

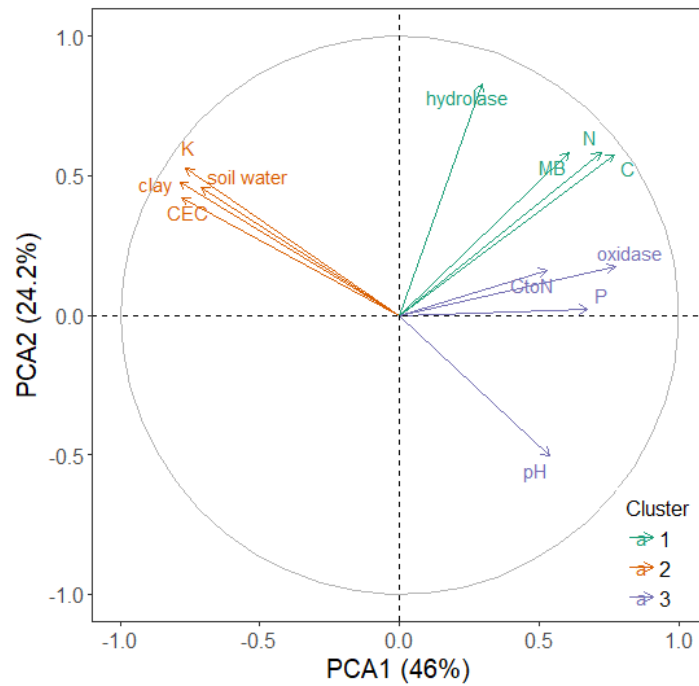


Figure 2-6. Impact of depth on measured soil properties, with each experimental unit summarized by a single point using principal component analysis (PCA). A) Change in soil parameters with depth. The centroid was marked as a big solid point, indicating the group mean point of the site scores on each axis. B) Soil properties cluster using K-means algorithm. MB: PLFA microbial biomass.

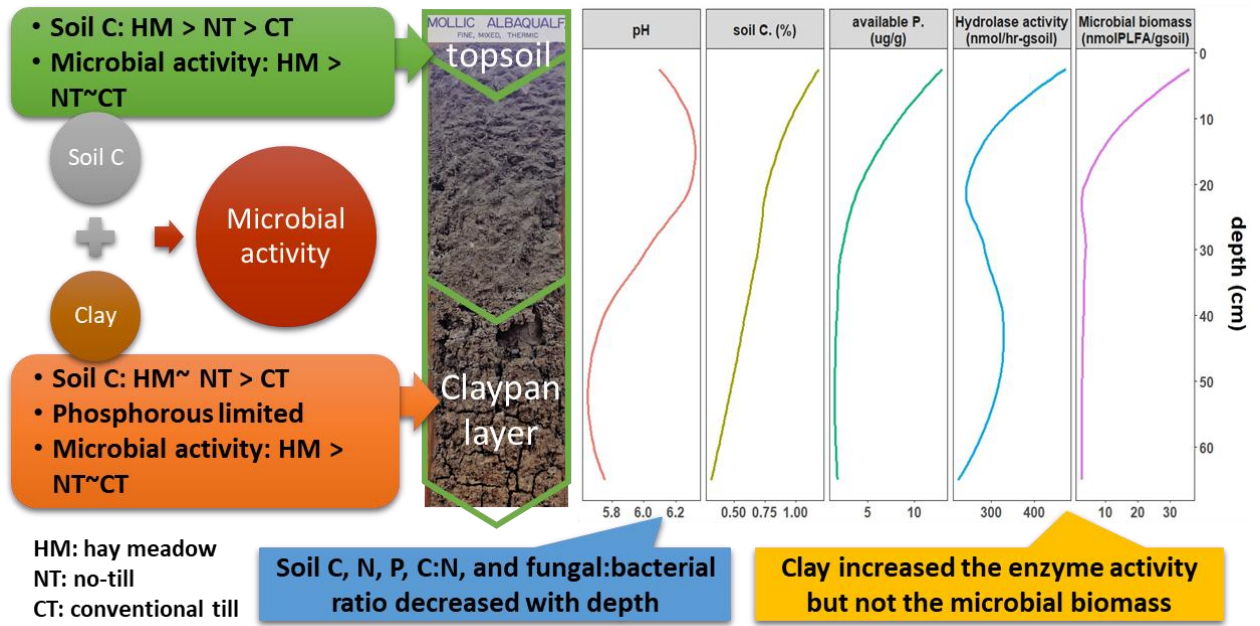


Figure 2-7. Graphical abstract.

Chapter 3 - Temporal variability in soil microbial properties in claypan soils

Abstract

Soil microbial properties may vary temporally with crop development and crop species, but this change is poorly characterized in claypan soils. The objective of the research was to assess how tillage and crop development mediate changes in soil microbial properties in a corn/winter wheat/soybean rotation system. We evaluated the microbial activity through extracellular hydrolase and oxidase activities, microbial communities through phospholipid fatty acid (PLFA) profile, and key soil chemical properties including pH, water content, and soil nutrients at different crop development stages. Tillage reduced microbial activity and microbial biomass but only in the upper 5 cm. While both hydrolase activities and microbial biomass exhibited temporal variation with crop growth, measured microbial properties were unresponsive to tillage or fertilizer application events. Wheat resulted in higher active C and greater soil organic matter quality as indicated by β -glucosidase:oxidase activity ratio than corn, potentially because a greater substrate availability after corn harvest stimulated the production of enzymes and higher bacterial and fungal biomass to decompose more recalcitrant C. The microbial lipid community was different in soybean than in either corn or wheat. These results suggested that in addition to tillage, crop development stage was an important driver of change in soil microbial properties. Wheat resulted in higher soil hydrolase activity and microbial biomass than corn. The lack of residue input from the preceding crop may explain the lower microbial biomass and activity during corn growth. Incorporating wheat in the crop rotation may increase microbial activity after corn harvest. Due to the significant temporal variation in soil microbial properties,

soil samples need to be collected more often. Alternatively, standardized sampling times should be considered to minimize the variability in soil health assessment.

Abbreviations: PLFA, phospholipid fatty acid; NT, no-till; CT, conventional tillage

Introduction

Soil is a dynamic ecosystem that responds to weather and plant growth. Distinct soil environments may be only micrometers or centimeters apart, and yet differ in their abiotic features, microbial activity, and microbial community composition (Baldrian, 2014; Fierer, 2017). Environmental conditions including soil temperature and water content (Castaño et al., 2017), the quality and quantity of organic matter (Fierer, 2017), soil pH, and macro- or micronutrients can vary at different timescales due to plant development (De-la-Peña et al., 2010) and human disturbances such as tillage operations or fertilizer application (Ashworth et al., 2017).

Crop rotation can affect microbial properties in agricultural systems (Ashworth et al., 2017; Lauber et al., 2013). Greater heterogeneity of crop residue inputs to soil organic matter (SOM) over time produce more habitable resource niches (McDaniel et al., 2014), and enhance microbial diversity (Eisenhauer et al., 2010; Venter et al., 2016), microbial biomass (McDaniel et al., 2014), microbial carbon (C)-use efficiency (McDaniel and Grandy, 2016), and can also enhance soil physical properties including water-use efficiency and the stability of soil temperature (Venter et al., 2016). Soil microbial properties are influenced by the amount and quality of plant residues from the previous crop as well as root exudates from the current crop (McDaniel et al., 2014; Sasse et al., 2018; Venter et al., 2016). Despite evidence of crop

rotational benefits, benefits to microbial properties from different crops are inconsistent between locations (Venter et al., 2016).

Differences in soil type and the associated physiochemical environment, and complicated climate-plant-microbe interactions may overshadow the direct effects of various crop residues on soil microbial properties in the first few years after beginning a rotation (Ashworth et al., 2017; Balota et al., 2004). A study of denitrifiers and nitrite oxidizers in microbial communities found that some microbial communities can recover quickly after disturbance events such as exposure to increased temperature (Shade et al., 2013). This is likely due to acclimation of microbial growth or nutrient use efficiency to the new conditions (Slaughter et al., 2015). Widespread dormancy of populations within a microbial community can also reduce the degree of changes in soil microbial properties after an event-based disturbance in a range of systems (Duncan et al., 2016). In addition to providing a nutrient-rich environment, plants can mediate positive or negative interactions in the rhizosphere, including stimulating symbiosis with rhizobia and mycorrhizal fungi that increase nutrient uptake (Haichar et al., 2014). These patterns highlighted a need to study the temporal variability of soil biological and geochemical changes over time (Ashworth et al., 2017). As soil microbes are responsible for organic matter degradation and nutrient cycling and respond more quickly to changing environmental conditions, an understanding of the temporal variability in soil microbial properties can establish a more comprehensive picture of soil function and how soil function changes with agricultural practices.

The chemical composition of crop residue may influence the decomposition rate of labile C in residue (Broder and Wagner, 1988; Beyaert and Paul Voroney, 2011), nutrient cycling, and microbial properties in soil (McDaniel et al., 2014). Residue decomposition rate is negatively correlated to the C:N ratio and hemicellulose content (Beyaert and Paul Voroney, 2011;

McDaniel et al., 2014). Both corn and wheat residues have high C:N ratios around 35 to 40. Conversely, the C:N ratio of soybean residue is less than 15. The hemicellulose content in corn is approximately 300 g hemicellulose kg⁻¹ dry weight plant residue, while the level is around 200 g hemicellulose kg⁻¹ plant residue in wheat and 100 g hemicellulose kg⁻¹ plant residue in soybean (Broder and Wagner, 1988; McDaniel et al., 2014). Of these three residues, soybean is expected to have the most rapid decay rate.

Tillage is known to have negative impacts on soil chemical and biological properties in claypan soil (Hsiao et al., 2018) and other soil types (Mbuthia et al., 2015; Roger-Estrade et al., 2010; vanCapelle et al., 2012). Claypan soils are identified as having a dense, impermeable claypan layer in the subsoil covered by silt loam soil at the surface. Claypan soils cover approximately four million hectares of land in the Midwest U.S. and make a significant contribution to food production (Conway et al., 2017; Myers et al., 2007). Hsiao et al. (2018) reported that management practices influence the microbial activity in both the surface soil and the claypan layer. No-till production led to an overall increase in enzyme activities, bacterial biomass, and fungal biomass, especially in the 0-5 cm layer of the soil profile, and differed from that measured in the conventional till plots. Both soil nutrient pools and microbial biomass declined with increasing soil depth for both production systems. After investigating the effects of production systems on soil microbial properties in claypan soils, the dynamics of these effects over the crop growing season are of primary interest.

Compared to spatial variability, fewer studies have specifically focused on temporal variability in soil microbial properties. The majority of temporal studies focused on either changes with seasons or after a long-term crop rotation. In this study, we investigated a fundamental and unanswered question of how crop development stage interacted with tillage

management to alter soil microbial properties. The objective of this study was to assess the seasonal changes in soil microbial activity and community composition at different crop development stages in a corn/winter-wheat/soybean rotation under conventional tillage (CT) and no-till (NT) practices. We hypothesized that given the difference in amount and composition of crop residues, soil microbial properties would vary with crop development stages. Crop residues dictate the microbial activity and community composition, but abiotic conditions, such as temperature and soil water, can restrict microbial activity and biomass. Soil extracellular enzyme activity was used as an indicator of microbial activity, and phospholipid fatty acid (PLFA) was used as an indicator of active microbial biomass (Paul, 2016). Soil chemical characteristics were also measured over time, including pH, soil water content, permanganate oxidizable C (also known as active C), plant available nitrogen (N), and available phosphorus (P).

Materials and Methods

Site and Experimental Design

The research was conducted at the Southeast Research and Extension Center located in Cherokee County, Kansas (37.21 N, 94.87 W). The field was located on the northern edge of the Humid Subtropical climate zone near the Humid Continental Zone in the Cherokee Lowlands ecoregion of the tallgrass prairie. Average daily temperatures in the region ranged from -0.7 °C in January to 20.5 °C in July. Average annual precipitation was 1050 mm, with most precipitation commonly received in the spring (March – June; Table 3-1). The predominant soil type in the field was a Parsons silt loam (fine, mixed, active, thermic Mollic Albaqualfs) with 0.2% slope. The Parsons soil had an abrupt textural change between the mollic epipedon and the argillic horizon, with low saturated hydraulic conductivity, rich ferrous iron, and aquic moisture

conditions (Soil Survey Staff, 2014). Parsons silt loam soils were characterized as fertile surface soils with poorly drained subsoils that formed in clayey old alluvium or residuum. This soil was common to the claypan region of the Midwest.

The experiment compared two tillage treatments over two years in a 3-crop rotation common to the region (corn/winter wheat/soybean/fallow over two years) in a crossed design with three replications and repeat measurements. The field had been in a corn/winter wheat/soybean rotation for more than 5 years, with all crops grown each year. The first treatment factor was tillage, including conventional tillage (CT; plot size 9.1 m x 21.3 m) and no-till (NT; plot size 9.1 m x 36.6 m). The CT practice included chisel plowing and disking prior to planting corn, and disk harrow after corn harvest prior to wheat planting. Soybean was planted by no-till after wheat harvest for both tillage treatments, as is the common practice in the region. All crops were grown each year and replicated over two years. Crop sequence was the second factor, including corn/winter wheat/soybean/fallow sequence (sequence 1) and soybean/fallow/corn/winter wheat sequence (sequence 2) as shown in Fig. 3-1. Sampling was conducted at roughly three development stages within each of the three crops: after planting, flowering, and harvest. Dormant wheat and fallow between soybean and corn planting in December and February were also included. Wheat harvest and soybean planting were coincident. Mineral fertilizers were applied. For corn, 179.3 kg N ha⁻¹, 51.6 kg P ha⁻¹, and 67.3 kg K ha⁻¹ were knifed in 12-15 cm deep each year as base fertilizer. For wheat, 33.6 kg N ha⁻¹, 10.1 kg P ha⁻¹, and 25.8 kg K ha⁻¹ were applied prior to wheat planting with a side-dress application of an additional 89.7 kg N ha⁻¹ broadcast after the dormant period. No fertilizer was applied to soybean.

Soil Sampling

Soils were sampled below the plant litter using a standard soil corer (2.5 cm diameter) at two depths from 0 to 5 and 5 to 15 cm in each plot every two months between April 2016 and February 2018. Soil samples were refrigerated at 4 °C and transported to the Soil Microbial Ecology lab at Kansas State University in Manhattan, KS. Soil from each plot was composited, homogenized, and subsampled for subsequent analyses. Subsamples for chemical properties were air-dried, ground, and sieved through 2 mm mesh. Subsamples for microbial properties were stored at -20 °C before further processing.

Soil Chemical and Environmental Measurements

Soil gravimetric water content was determined after oven-drying samples for 24 h at 105 °C. Soil pH was determined in a 1:10 soil:water slurry. Permanganate oxidizable C, also known as active C, was determined following a modified Weil method (Weil et al., 2003). Briefly, 2.5 g of air-dried soil was added to a 50 mL polypropylene centrifuge tube with 2 mL of 0.2 M KMnO_4 solution and 18 mL of distilled water. Tubes were shaken for exactly 2 min at 200 oscillations per minute and settled for exactly 5 min. After that, 0.5 mL of the upper 1 cm supernatant was transferred into a second 50 mL centrifuge tube and mixed with 49.5 mL of distilled water. A set of internal standards, blank, and 1 mL of the diluted solution was loaded individually into 1.5 mL glass cuvettes, and the absorbance read with a colorimeter at 550 nm.

Soil properties were measured using the standard 1 M KCl extraction for plant available N (Keeney, 1982), Mehlich-3 (Frank et al., 1988) for available P, and the standard ammonium acetate method for both extractable K (Warncke and Brown, 1988) and cation exchange capacity (Chapman, 1965) by the Soil Testing Lab at Kansas State University, Manhattan, KS. Air

temperature and precipitation data were downloaded from a weather station located at Columbus, KS through the Kansas Mesonet (Kansas Mesonet, 2018).

Extracellular Enzyme Activities

Soil hydrolytic and oxidative potential activities were measured using fluorometric substrates 4-methylumbelliferone (MUB) and colorimetric substrate L-3,4-dihydroxyphenylalanine (L-DOPA) following protocols presented by Zeglin et al. (2013). Hydrolase assays included a C-acquiring enzyme (β -glucosidase, bG, EC 3.2.1.21, 2h incubation), a P-acquiring enzyme (acid phosphatase, AP, EC 3.1.3.2, 2h incubation), and an N-acquiring enzyme (N-acetyl- β -D-glucosaminidase, NAG, EC 3.2.1.30, 4h incubation). Preliminary data indicated that leucine-aminopeptidase (LAP, EC 3.4.11.1) activity was relatively low compared to NAG, which is common in acid-to-neutral soils such as those in this study. Therefore, only NAG activity was examined and used as levels of N-acquiring enzyme in this study. The bG hydrolyzes β -d-glucopyranosides in the degradation of cellulose. The NAG cleaves the amino sugar N-acetyl- β -D-galactosamine from chitin in soils. Acid phosphatase releases inorganic P from soil organic matter into biologically available forms. Oxidase assays included two main categories of lignin degradation enzymes: phenol oxidase (PHX, EC 1.10.3.2, 18h assay) and peroxidase (POX, EC 1.11.1.7, 18h assay). These enzymes control the rate-limiting steps of nutrient cycling. All assays were run at room temperature in 50 mM pH 5 acetate buffer with blanks and quench controls for each sample. Potential enzyme activities were reported as nanomoles activity per gram of dry soil per hour. The bG:oxidase activity ratio was an indicator used of soil organic matter quality (McDaniel et al., 2014; Sinsabaugh and Follstad Shah, 2012), calculated by dividing the bG activity by the sum of PHX and POX activities.

Phospholipid Fatty Acid (PLFA)

Soil microbial biomass was estimated as the sum of all PLFA concentrations following the modified protocol from the White and Ringelberg method (Hsiao et al., 2018; White et al., 1997). Total lipids were extracted using a 2:1:0.8 solution of methanol, chloroform, and phosphate buffer on 5 g of freeze-dried soil. Phospholipids were isolated using silicic acid chromatography columns (Disposable BAKERBOND® SPE Columns, J.T. Baker®) and methylated to fatty acid methyl esters (FAME) using 0.2 M methanolic potassium hydroxide. Fatty acid methyl esters were dissolved in hexane and quantified with a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts) equipped with a DB5-MS column (30 m x 250 µm in diameter x 0.25 µm film thickness; Agilent Technologies, Santa Clara, California). Internal standard 19:0 FAME was used to determine concentrations. The PLFA concentration was reported as nmol PLFA per gram of dry soil. Microbial groups were assigned based on characteristics of biomarkers: iso and ante-iso branched lipids often belong to Gram-positive bacteria; monosaturated and cyclopropyl lipids often belong to Gram-negative bacteria; actinobacteria have more methyl-branched fatty acids; methyl linoleate (18:2 ω 9,12c) is typically found in fungi (Frostegård et al., 2011; White et al., 1997). The fungal:bacterial ratios were calculated by dividing the sum of fungal biomarkers by the sum of Gram-positive bacteria, Gram-negative bacteria, and actinomycetes. Microbial biomass was estimated as the sum of all PLFA biomarkers.

Phospholipid fatty acid was also used to assess microbial community structure (Duncan et al., 2016; Findlay and Dobbs, 1993; Slaughter et al., 2015). While the assignment of individual fatty acid biomarkers to a particular group of PLFA for detecting community change is controversial in some studies (Frostegård et al., 2011), we used fatty acid biomarkers to

identify a broad scale shift of soil microbial community to avoid potential errors in this study (Duncan et al., 2016; Slaughter et al., 2015). The relative abundance of individual fatty acid biomarker was calculated. Of those, 18 fatty acid biomarkers with >1 % abundance were used for the community structure analyses (10Me16, 10Me18, i15, a15, 16:0, i16, 16:1 ω 5c, 16:1 ω 7c, 16:1 ω 9c, 17:0, i17, a17, cy17, 18:0, 18:1 ω 7c, 18:1 ω 9c, 18:2 ω 9c, and 18:2 ω 6c).

Data analysis

The effect of tillage (NT and CT), crop sequence (sequence 1 and 2), crop development stage and their interactions on soil chemical and microbial properties were compared using repeated measures analysis of variance (rm-ANOVA) in linear mixed effects model by using R (R Core Team, 2013). Post-hoc multiple comparisons were made using the Tukey adjustment. Correlation between the measured soil parameters and factors was summarized using Pearson's correlation coefficient (Pearson r). Block and crop sequence within block were random factors. Dependent variables were tested for the assumption of normality using Shapiro-Wilk test and then log-transformed when necessary. The Bray-Curtis dissimilarity of the most abundant fatty acid biomarkers between different sampling points was assessed using nonmetric multidimensional scaling (NMDS) ordination. The significance of the experimental factors on the dissimilarity of PLFAs were analyzed by permutational multivariate ANOVA (Per-MANOVA). The significance of all tests was evaluated using an α value of 0.05. All errors were reported as standard errors.

Results

Climate and Soil Chemical Properties

Soil temperatures at 5 cm had the expected annual variation with warmer temperatures in the summer and cooler temperatures in the winter (Table 3-1). Soil temperatures on sampling dates did not vary considerably from the 5-year average soil temperatures. The only noticeable shift was an increase in soil temperatures during the fall of 2016 through winter 2017.

Cumulative precipitation in the region had two periods of high precipitation in the spring (May to June) and fall (Sep. to Oct.), with lowest precipitation received in the winter months (Dec. to Feb.). The precipitation patterns deviated from normal, with less rain in late spring in 2016 than normal (196 vs 260 mm in June), and more rain in fall than normal (374 vs. 215 in Dec.).

Conversely, late spring of 2017 (June) was much wetter than normal, with 445 mm rain compared to an average of 287 mm (Table 3-1). Overall, about 250 mm less rain was received from April to June 2016 than in 2017, indicating a drier season for corn growth in sequence 1 and wheat harvest in sequence 2.

Soil water content was significantly affected by crop stage-tillage-sequence interactions at the 0-5 cm depth (Table 3-2A). Crop stage-sequence and crop stage-tillage interactions were significant in the 5-15 cm depth (Table 3-2B). Soil water content decreased to less than 0.10 g H₂O g⁻¹ soil after corn flowering, increased after corn harvest and remained constant during the wheat growing period at both depths (Fig. 3-2). Soil water decreased after wheat harvest and increased after soybean harvest. The lowest water content after corn flowering in sequence 1 resulted from two potential reasons: the higher water consumption during corn growth and lower precipitation. In sequence 2, the higher water consumption during corn growth likely explained the lower soil water content after corn flowering, while less precipitation led to the lower water

content after the wheat harvest. Soil water content was higher in NT than CT after corn harvest and during wheat growth.

Soil pH was significantly affected by crop stage-sequence and crop stage-tillage interactions in the 0-5 cm depth (Table 3-2A). Soil pH decreased after corn flowering in June, potentially due to the lower soil water content. The pH increased after corn harvest, and further increased during the winter fallow period (Fig. 3-2A).

Active C was significantly affected by crop stage- tillage-sequence interaction at 0-5 cm depth (Table 3-2A) and crop stage-sequence interaction at 5-15 cm depth (Table 3-2B). Both NT and CT soils had similar temporal trends at both depths, with active C nearly level during corn and soybean growth, increasing during the winter, and highest in the fallow period in sequence 1 and wheat dormancy period in sequence 2 (Fig. 3-2). In the 0-5 cm layer depth, active C in NT was higher than CT after soybean flowering and fallow period in sequence 1, while active C in NT was higher after wheat dormancy and flowering in sequence 2.

Soil available N was significantly affected by crop stage-sequence and crop stage-tillage interactions in the 0-5 cm depth (Table 3-2A) and crop stage-tillage-sequence interactions in the 5-15 cm depth (Table 3-2B). Available N increased after fertilizer application prior to corn, decreased after corn harvest, and then increased during wheat dormancy in February (Fig. 3-2). Levels decreased after wheat flowering and continued a slow decrease during the fallow periods. The increases after fertilization were higher in NT than CT. The available N in sequence 1 was lower than sequence 2 after corn flowering and during wheat growth.

Soil available P was significantly affected by tillage and crop stage-sequence interactions in the 0-5 cm depth (Table 3-2A). Interactions between tillage, crop stage, and sequence were significant in the 5-15 cm depth (Table 3-2B). Available P also increased after fertilizer

application prior to corn, and decreased after corn harvest at both depths. Available P then increased during the wheat dormancy period at 0-5 cm depth. However, P levels remained higher for longer in the soil than for available N. Available P in NT was higher than CT at 0-5 cm depth and at most measurement times at 5-15 cm depth.

Soil available K was significantly affected by tillage-crop stage-sequence interactions at both depths (Table B-1). The patterns of temporal variability of available K were similar to available P, increasing during corn growth and wheat dormancy (Fig. B-1). Available K in NT was higher than CT at most sampling times.

Cation exchange capacity was significantly affected by tillage and crop stage-sequence interactions at both depths (Table 3-2). The CEC was higher in NT than in CT (Fig. 3-2). In sequence 1, CEC levels fluctuated during corn, wheat, and soybean growth periods, and then increased during the fallow periods in winter especially in the 5-15 cm depth. In sequence 2, CEC levels fluctuated during corn, soybean, and fallow periods, and increased in the wheat dormancy periods in winter.

Extracellular Enzyme Activity Profiles

Soil bG activity, an indicator of C mineralization potential, was significantly affected by tillage at the 0-5 cm depth (Table 3-2A). Higher bG activity was measured at the 0-5 cm depth of NT soils (Fig. 3-3A). The activity of bG was lower in the 5-15 cm soil depth, with no statistical difference between tillage treatments (Fig. 3-3B). Soil bG activity increased during the winter wheat or fallow periods, with higher activity during Dec. 2017 – Feb. 2018 (winter fallow in Sequence 1; wheat dormant period if Sequence 2). The activity was steady or declining during the summer growing seasons for corn and soybeans, with inconsistent patterns between sequences.

Soil AP activity, an indicator of P mineralization potential, was significantly affected by tillage-sequence and crop stage-sequence interactions at 0-5 cm depth (Table 3-2A). A significant interaction of crop stage and sequence occurred in the 5-15 cm depth (Table 3-2B). Temporal dynamics of AP activity were different between the two sequences. In sequence 1, levels of AP activity were constant during corn growth and wheat dormancy, decreased after soybean harvest, and declined further during the winter fallow in February (Fig. 3-3). In sequence 2, AP activities decreased from corn planting to flowering, increased after corn harvest, and increased further after wheat flowering. Activity then remained relatively constant during soybean growth and winter fallow. This pattern was more apparent in the top 0-5 cm and was similar in both NT and CT, with higher activities in NT in the 0-5 cm depth. Soil AP activity was positively correlated with bG activity with a Pearson r of 0.75 ($p < 0.001$; Fig. 3-6).

Soil NAG activity, an indicator of N mineralization potential, was significantly affected by tillage in the 0-5 cm depth (Table 3-2A) and by crop stage and sequence at 5-15 cm depth (Table 3-2B). The NAG activity levels were higher in NT compared to CT in the 0-5 cm depth (Fig. 3-3A). During the wheat growing season, NAG increased, and then declined during soybean growth (Fig. 3-3B). The pattern was similar at both depths, but only significant in the 5-15 cm depth. This pattern was clearer in sequence 1. Soil NAG activity was positively correlated with bG and AP activity with Pearson r of 0.67 and 0.62, respectively ($p < 0.001$ for both correlations; Fig. 3-6).

The ratio of bG activity to oxidative enzyme activity, reflecting the degree to which microbes degrade lignin (oxidases, PHX and POX) to access cellulose C (bG), can be used as an indicator of soil organic matter quality, with higher values suggesting greater lability (Sinsabaugh and Shah 2010). The bG:oxidase ratio was significantly affected by crop stage-

tillage-sequence interactions in the 0-5 cm depth (Table 3-2A). During the wheat growth and fallow periods, the ratio increased, while it decreased or remained relatively level during corn and soybean growth (Fig. 3-5A). The ratios were higher in NT compared to CT. There was no discernible temporal pattern of bG:oxidase ratio at 5-15 cm depth (Fig. 3-5B).

Both PHX and POX mediate lignin-degradation capacity. Temporal dynamics of PHX activity was affected by crop stage-sequence interactions at both depths (Table B-1). Levels of PHX activity were nearly constant throughout the cropping sequences, with a noticeable decrease during the winter for both wheat production in sequence 2 or fallow in sequence 1 (Fig. B-2).

Soil POX activity was significantly affected by crop stage-sequence interactions in the 0-5 cm depth (Table B-1A) and crop stage-tillage-sequence interactions in the 5-15 cm depth (Table B-1B). The highest activity occurred in the early spring after corn planting and wheat dormancy in sequence 1, while in sequence 2, soil POX was highest after corn planting and wheat flowering in both soil layers (Fig. B-2). Activity of POX was lowest during the fallow period in sequence 1 and wheat dormant periods in sequence 2, but was relatively stable during the other growing seasons. The POX activity was higher in NT at 5-15 cm depth.

Phospholipid Fatty Acid Profiles

Microbial biomass was significantly affected by tillage and crop stage-sequence interactions at 0-5 cm depth (Table 3-2A). In the 5-15 cm depth, crop stage-sequence interaction was significant (Table 3-2B). Microbial biomass was significantly higher in NT in the 0-5 cm depth. Microbial biomass decreased during corn growth, increased to the highest level after soybean flowering, then remained level during the winter fallow at both depths in sequence 1 (Fig. 3-4). In sequence 2, microbial biomass levels decreased during corn growth, increased during wheat

dormancy, decreased after wheat flowering, and increased during winter fallow. Microbial biomass was positively correlated with active C and bG with a Pearson r of 0.48 and 0.69, respectively ($p < 0.001$ for both correlations; Fig. 3-6).

Fungal biomass was significantly affected by the crop stage-sequence interaction at both depths (Table 3-2) and by crop stage-tillage-sequence interactions at 5-15 cm. In the 0-5 cm depth, fungal biomass decreased after corn flowering, increased during winter wheat dormancy, decreased with soybean growth, and increased during the winter fallow for both crop sequences (Fig. 3-4A). In the 5-15 cm depth, the fungal biomass fluctuated without a discernible temporal pattern (Fig. 3-4B). Fungal biomass in sequence 1 was higher than sequence 2 during soybean growth, while no difference was measured at other sampling times.

Fungal to bacterial ratio varied with the crop stage-sequence interaction in the 0-5 cm depth (Table 3-2A) and crop stage at 5-15 cm depth (Table 3-2B). The ratios were highest during winter wheat dormancy in sequence 1 or the fallow period in sequence 2, with no difference between NT and CT in the 0-5 cm depth (Fig. 3-5A). In the 5-15 cm depth, the ratios increased during wheat growth and decreased during soybean growth (Fig. 3-5B).

The crop stage-sequence interaction, crop stage, sequence, and tillage significantly explained the variability in the most abundant PLFA community composition at both depths in PerMANOVA (Table 3-3). For the 0-5 and 5-15 cm depths, 23.8% and 34.9% respectively of variability in the PLFA composition was explained by crop stage-sequence interactions, 11.6% and 11.2% respectively was explained by crop stage, and 19.8% and 2.6% respectively was explained by tillage. The differences in soil microbial PLFA composition among sites throughout sampling were represented by the first two NMDS axes. The NMDS ordination models summarized the observed distances with a stress of 0.052 in the 0-5 cm depth (Fig. 3-7A) and

0.049 in the 5-15 cm depth (Fig. 3-7B). At the 0-5 cm depth, the abundant PLFA biomarkers in soybean were less different temporally than biomarkers in corn or wheat (Fig. 3-7A). The same effect was not observed at the 5-15 cm depth.

Discussion

This study documented significant temporal variability in soil microbiological properties under different cropping systems in a claypan soil, and this variability was strongly linked to the timing and development of different crop rotations, as we expected. Complexity in the results was related to interactions between crop stage, preceding conditions (likely detected both as a sequence effect, and as influence from precipitation and temperature), and tillage practice. Tillage practice and depth had some overarching effects, in that microbial biomass and activity tended to be higher in 0-5 cm depth soils than 5-15 cm depth soils, particularly under no-tillage management. Beyond these differences, temporal patterns in our data followed similar trajectories in both CT and NT soils, and at 0-5 and 5-15 cm soil depths, indicated a consistent influence of crop stage on soil microbial properties. This suggests a combined influence of root exudate inputs from the active crop and residual litter inputs from the previous crop.

The temporal variability of soil microbial properties was stronger in the 0-5 cm depth in both tillage systems, which likely reflects the more direct interactions between soil microbes, plant inputs, and surface abiotic conditions that take place in shallow soils. These findings were similar to those observed in other studies (Bell et al., 2010; Kandeler et al., 2006; Sun et al., 2016). Soil microbes in the 0-5 cm depth received greater amounts of crop residues and were more responsive to changes in weather conditions compared to the 5-15 cm depth. Our results supported the conclusion that most plant-derived substrates were immobilized in the topsoil and

were not transported in large amounts to subsequent depths (Kramer et al., 2013). Differences in soil properties between the 0-5 cm and the 5-15 cm depths were most evident in NT. This was likely due to reduced physical disturbance and greater root density in the surface soil. Greater water storage capacity, bulk density, penetration resistance, soil nutrients, and a greater aggregation are typically expected in the surface soil layer in NT due to reduced soil mixing (Baker et al., 2007; Chassot et al., 2001; Osunbitan et al., 2005; Wendt and Hauser, 2013). These characteristics encouraged greater root density in the surface soil of NT soils (Baker et al., 2007; Chassot et al., 2001), resulting in greater microbial biomass, enzyme activity, and soil nutrients (Figs. 3-2 to 3-4).

The interaction between crop development stage and cropping sequence was the most significant factor in this study (Table 3-2). Similar to other studies, yearly variability driven by weather variables created the differences between crop sequences (McDaniel et al., 2014; Venter et al., 2016; Yin et al., 2010).

We found some support for the prediction that soil microbial properties would respond to both quality and quantity of crop residues. Generally, both microbial biomass and enzyme activity increased during fallow periods. Plant residues from the previous crop can occupy up to 40% of the total root biomass in cultivated soils (Hirte et al., 2017). Here, soybean preceded fallow. Soybean produces only one-third the amount of residue as corn resulting in lower soil organic matter and enzyme production after soybean harvest compared to after corn harvest (Ashworth et al., 2017). Soybean residue decomposes rapidly in soils due to low lignin content and C:N ratio, losing 68% of its plant residue after 32 days compared with 42% and 47% for corn and wheat residue, respectively (Beyaert and Paul Voroney, 2011; Broder and Wagner, 1988). Therefore, soybean residue input increased microbial biomass as well as bG and NAG

activities during the fallow period after soybean harvest. In contrast, there was minimal new plant residue input to the soil over the fallow period, which supported stable or decreasing microbial biomass and activity during the following period of corn growth.

Soil microbial biomass and most extracellular enzyme activities increased during wheat growing periods, which may be a consequence of the preceding buildup of corn residues plus wheat residues and root inputs. Similar findings were reported in previous studies in both corn-wheat rotation systems (Samuel et al., 2008) and monocultural wheat and corn (Kramer et al., 2013). The combination of greater substrate availability and low-quality of corn residues resulted in production and activation of enzymes that decompose more recalcitrant C for an extended period (Kramer et al., 2013). Following the wheat crop, during the summer soybean growth period, soil microbial activity and biomass at 0-5 cm depth stabilized. This is likely due to the slower decomposition rate of wheat residue and the lower fungal biomass during soybean growth. McDaniel et al. (2014) also reported no effect on soil active C and available N after addition of lignin-rich and high C:N ratio wheat residue. As the major decomposer of recalcitrant plant substrate in the topsoil (Kramer et al., 2013), the fungal community and the fungal to bacterial biomass ratio were higher in winter but lower in summer (Figs. 3-4 and 3-5). The restricted enzyme activity during soybean growth also corresponded to a lack of turnover in the microbial PLFA community compared to corn and wheat at 0-5 cm depth (Fig. 3-7A), suggesting both microbial community composition and activity were affected by crop development stage.

Active C was expected to increase after residue decomposition as it is indicative of the labile fractions of C. Although a characterization of the active C pool is still needed, the active C represents the most readily oxidizable forms of soil C pool capable of converting Mn(VII) in diluted, slightly alkaline KMnO_4 to Mn(II), and generally includes microbial biomass C,

particulate organic matter, and carbohydrate C as measured as anthrone-reactive C (Weil et al., 2003). Higher extractable organic C was reported in wheat compared to corn in previous studies (Kramer et al., 2013). In the research presented here, active C was higher during the wheat growth in sequence 1 and the fallow in sequence 2 (Fig. 3-2), both of which occurred during the wetter winter of Dec, 2016 – Feb, 2017. This corresponds to the increases in microbial biomass, as more microorganisms grow during a warm period as well as more crop residues are input. Extracellular enzyme activities then increased for substrate decomposition.

During the same winter wheat growth and fallow periods, bG activities also increased (Fig. 3-2A), while PHX and POX activity either decreased or remained relatively constant (Figs. 3-2D and E), thereby increasing the bG:(PHX+POX) ratio. The activity ratio of bG to lignin-degrading enzymes (PHX and POX) is another indicator of organic C quality (McDaniel et al., 2014; Sinsabaugh and Follstad Shah, 2012). Thus, the increment of labile substrates during wheat growth and winter fallow was clearly indicated by the increase in active C as well as the changes in enzyme activities.

Although studies have reported that plants influence the microbial community in the rhizosphere through root exudates (Houlden et al., 2008; Cavaglieri et al., 2009; Li et al., 2014; McDaniel and Grandy, 2016; Degruno et al., 2017), more evidence is needed to justify root exudates as significant drivers of temporal dynamics of bulk soil microbial properties (Dennis et al., 2010; Hu et al., 2018). First, the direct impact of root exudates on the rhizosphere is limited to small spatial and temporal scales (Dennis et al., 2010). Second, the metabolization of exudates is rapid: Ryan et al. (2001) reported that most sugars, organic acids, and amino acids are mineralized with a half-life of less than 120 mins in the rhizosphere. Third, it is still challenging to differentiate the root exudates from sloughed-off root cells, microbial exudates, or microbial

lysates (Dennis et al., 2010; Uren, 2000, Sasse et al., 2018, Li et al., 2016). Further, the amount and quality of root exudates change with plant development stage, presence of microbes or other plants, and under nutrient deficiency (Carvalhais et al., 2011; Somers et al., 2004). In a tallgrass prairie soil, which has high perennial root biomass and a large extent of root exudate impact, bulk soil microbial biomass can grow substantially through the growing season, with an order of magnitude increase in bacterial populations, then drop again after grass flowering and senescence (Garcia and Rice 1994, Carson and Zeglin 2008). However, the degree and the dynamics of such a plant effect on the bulk soil microbial properties in cropped soils needs more study.

Of note was the significant crop stage-sequence effects on CEC at both depths (Table 3-2). Temporal variability in CEC has been reported in other studies (Docherty et al., 2015; Fokam et al., 2015). Clay content and soil organic matter were shown to be positively correlated with CEC (Parfitt et al., 1995; Soares and Alleoni, 2008). Our study indicated that CEC levels increased during the fallow periods in sequence 1 and the wheat dormancy in sequence 2 (Fig. 3-2), a pattern similar to that measured for active C, and occurring during the Dec, 2017 and Feb. 2018 winter. The limitation of CEC analytical methodology might explain the changes in CEC. Considerable amounts of organic matter may dissolve in the ammonium acetate solution during the CEC determination, releasing cations, and resulting in an overestimation of CEC (Butler et al., 2001; Harada and Inoko, 1980; Yagi and Ferreira, 2003). The power of the ammonium acetate extraction method on CEC should be further studied in claypan soils in the future to remove any discrepancies in analysis.

In contrast to the long-term tillage effects, the lack of ephemeral responses after tillage and fertilizer application was not expected, given that C- and P-acquiring enzyme activities often

increased in response to N inputs (Gallo et al., 2004; Saiya-Cork et al., 2002). Event-based pulses of nutrient availability can modify soil pH and stimulate temporary changes in microbial community composition (Kuzyakov and Blagodatskaya, 2015; Armstrong et al., 2016). However, our study agrees with other research that suggests the ephemeral microbial responses to changing environmental conditions may be different from or weaker than responses to decadal-scale management changes (Regan et al., 2014, Carson and Zeglin, 2018). Similarly, while soil water has been reported to influence enzyme activities (Burns et al., 2013; Henry, 2013), lower soil water content in June for both years did not always lead to lower enzyme activities and microbial biomass (Figs. 3-3 and 3-4). A potential explanation is that roots exuded more C or allocated more C to beneficial soil microbes under water stress, thus maintaining the microbial biomass and activities (Naylor and Coleman-Derr, 2018; Preece et al., 2018; Song et al., 2012).

The PLFA profile changes beyond shifts in fungal to bacterial ratio were also minimal, suggesting that microbial community change happens within a narrow range set by pretreatment conditions (Bardgett and Van DerPutten, 2014), or that changes in microbial community composition occurred at a finer taxonomic level than detectable by PLFA (Degruene et al., 2017).

In addition to the tillage regime, the growing season of the crop is a major driver of the soil microbial activity and biomass in agricultural systems. We have suggested that incorporating grasses in the crop rotation may provide additional nutrient resources to cash crops in the same field (Hsiao et al., 2018). The greater microbial activity during wheat growth in this study further extends our suggestion that incorporating wheat, a species in grass family, in the crop rotation may improve microbial properties after corn harvest. No-till practices resulted in greater microbial biomass and activity associated with an increase in soil nutrient cycling with time in

claypan soils. Our study also highlighted that soil samples need to be collected more often due to the significant temporal variability of soil microbial properties. Alternatively, standardized sampling time and laboratory measurement should be considered in an attempt to minimize the variability in soil health assessment. In the corn/winter wheat/soybean/fallow rotation in the Midwest United States, sample collection in fall after soybean harvest would be an appropriate time for long-term field experiments.

Conclusion

Soil microbial activity and biomass regulate soil organic matter decomposition and nutrient cycling. They are important indicators of soil ecosystem functioning. Our study highlighted the dynamic nature of soil microbial properties and the need to understand the timescales over which the greatest microbial responses occur. Wheat crop supported higher soil microbial biomass and extracellular hydrolase activity compared to corn, potentially because of the limited plant residue input during the fallow period that preceded corn. Incorporating winter wheat in the crop rotation may increase the microbial activity and biomass, providing rhizosphere C inputs that can support greater microbial biomass, and consequent faster nutrient cycling and improved soil functionality. Soybean cultivation stabilized microbial activity compared to corn and wheat, which is likely due to the slower decomposition rate of wheat residue and the lower biomass during soybean growth. Temporal variation in soil chemical and microbial properties was stronger in the 0-5 cm of the soil profile in NT. Although changes in soil nutrients after fertilization may influence crop growth and microbial activity in the long-term, very few soil microbial properties displayed ephemeral responses to tillage and fertilization. In the future, the contribution from living root exudates on temporal dynamics in

soil microbial properties should be determined. Also, additional research with greater resolution of soil microbial communities and soil C composition will be able to generate a more complete picture of the relationships between changes in microbial properties and crop development in agroecosystems. Furthermore, examining the changes in microbial properties directly due to changes in climate is critical; however, factors such as temperature and precipitation are always confounded with crop growth effects and are currently difficult to differentiate.

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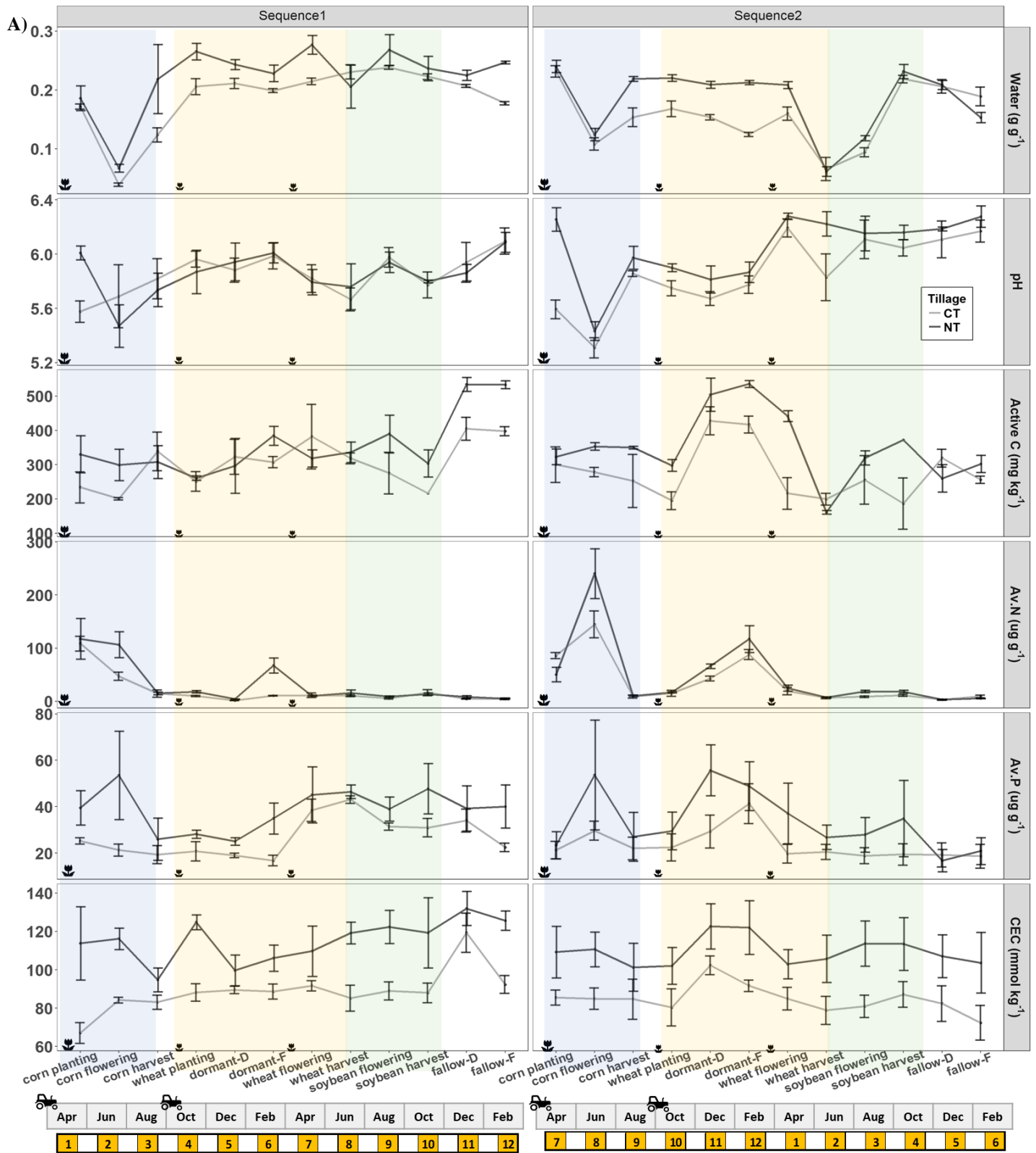
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Year	2016												2017												2018	
Month	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2		
Sample Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
Sequence1	Corn				Wheat								Soybean				Fallow									
Sequence2	Wheat		Soybean				Fallow		Corn				Wheat													

Figure 3-1. Crop growing sequence and sampling times during the study period. Sampling time and crop stages are indicated by shaded areas: sample number (orange), corn (blue), wheat (yellow), soybean (green), and fallow (white).



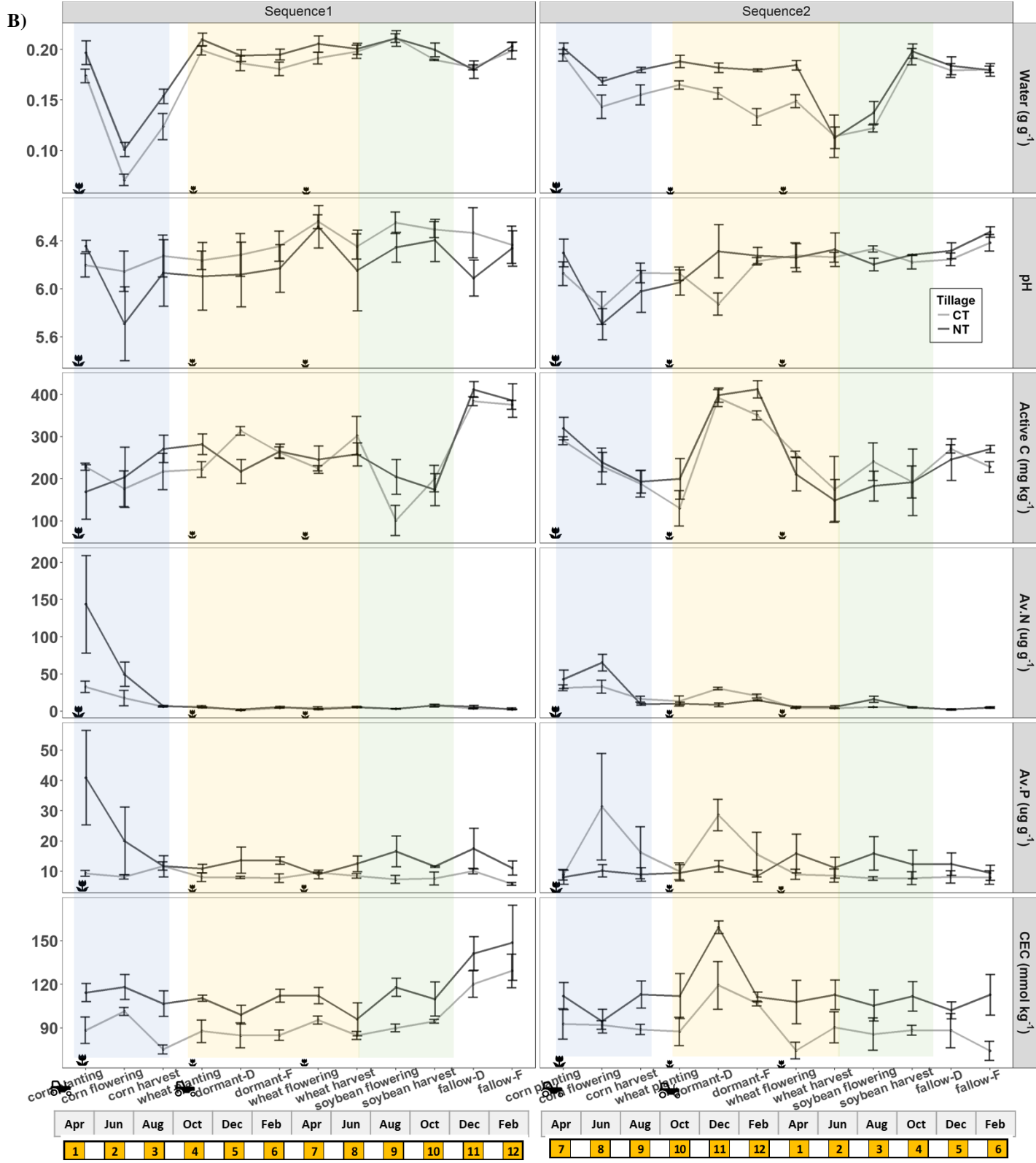
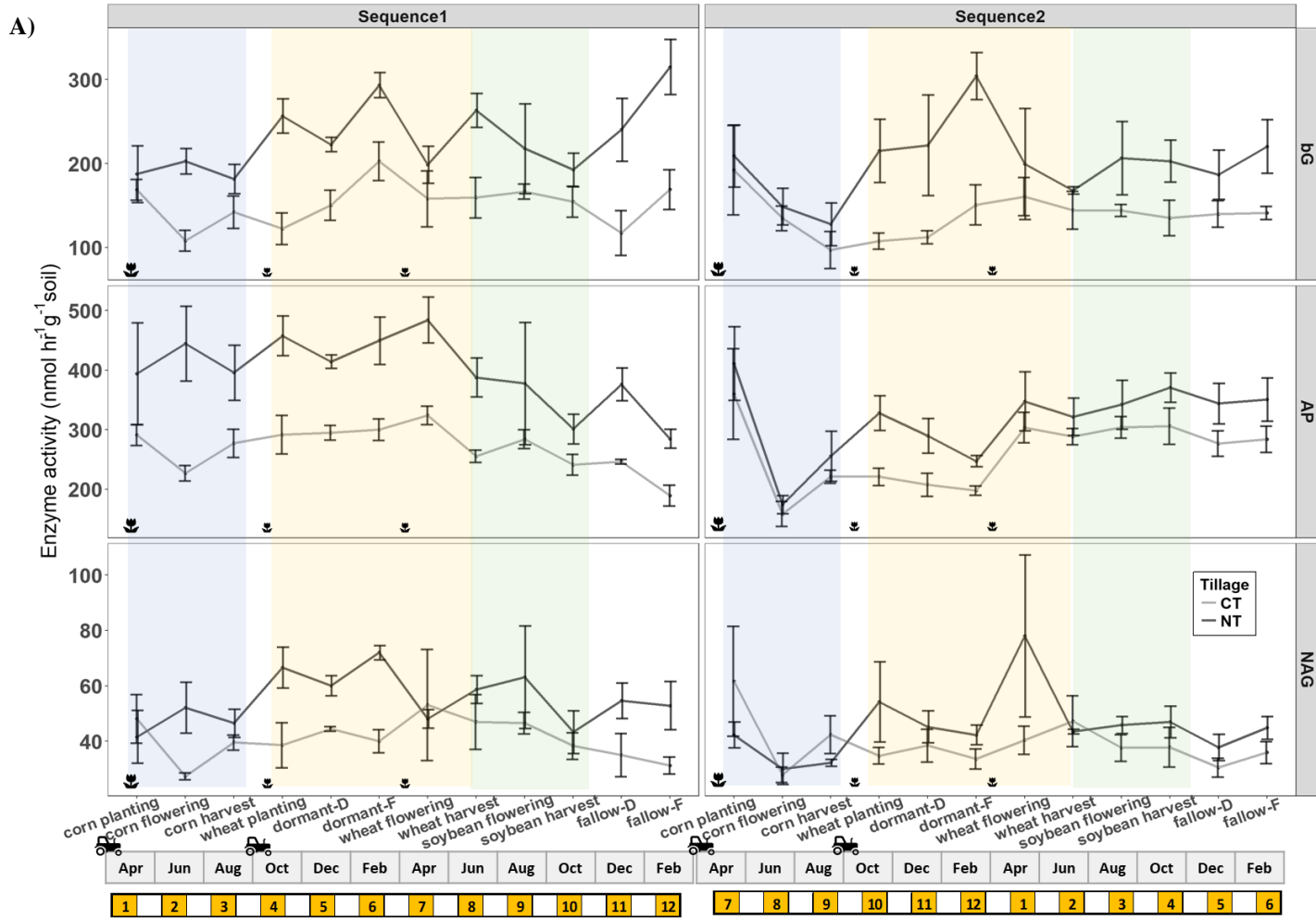


Figure 3-2. Temporal changes in soil chemical properties for conventional tillage (CT) and no-till (NT) during the crop development stages at A) 0-5 and B) 5-15 cm. Sequence 1 is corn/wheat/soybean/fallow crop sequence in two years, while sequence 2 is soybean/fallow/corn/wheat sequence. Crop stages are indicated by shaded areas: corn (blue), wheat (yellow), soybean (green), and fallow (white). Sample numbers are given in the bar at the bottom (1-12). Av. N, plant available nitrogen; Av. P, plant available phosphorus; CEC, cation exchange capacity; Active C is also known as permanganate oxidizable C. Flowers (♣) indicate mineral fertilizer application; tractors (♣) indicate tillage event. Results are given as means + standard error (n=8).



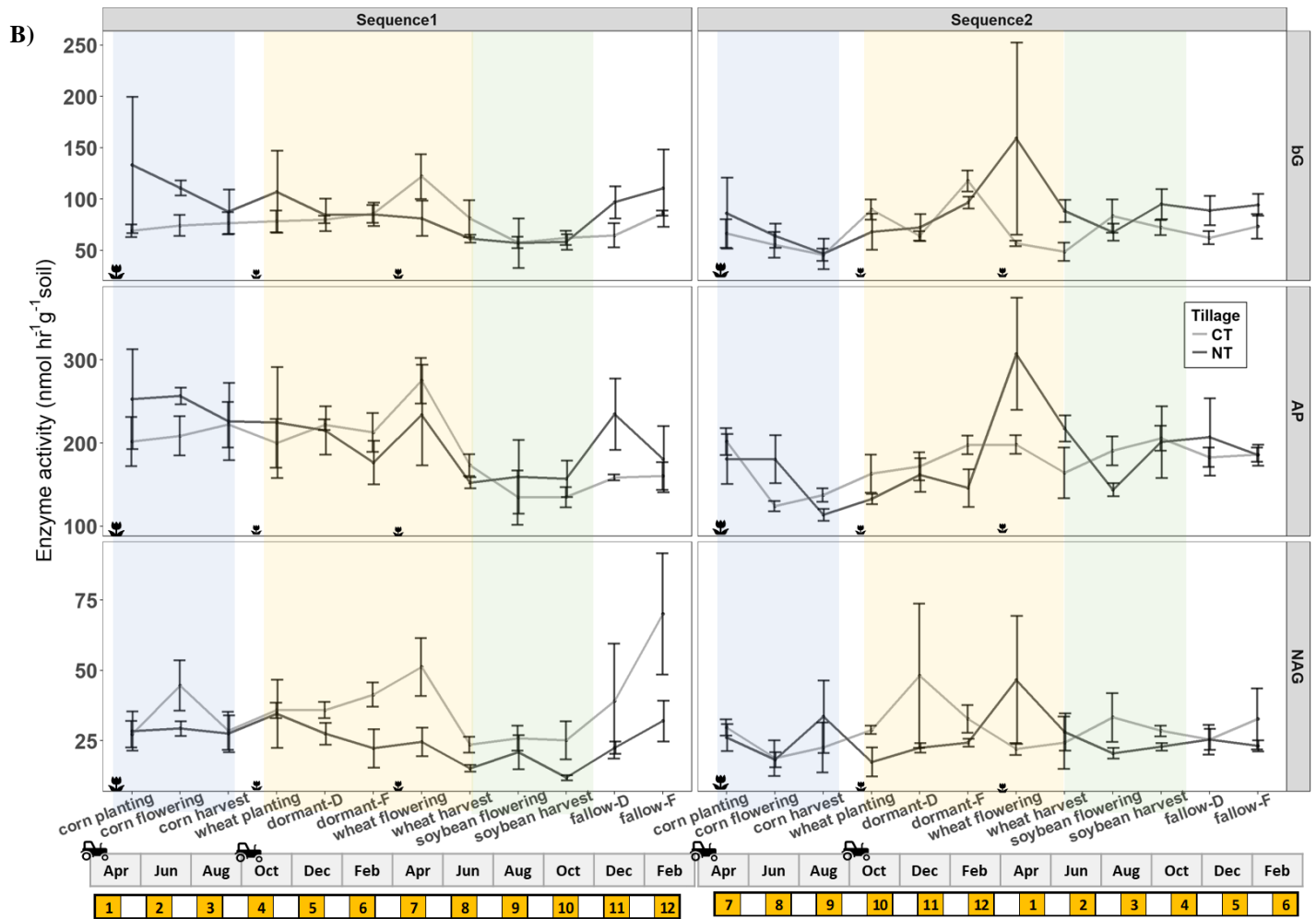


Figure 3-3. Temporal changes in soil extracellular enzyme activities for conventional tillage (CT) and no-till (NT) during the crop development stages at A) 0-5 and B) 5-15 cm. Dormant-D, wheat dormant stage in December; dormant-F, wheat dormant stage in February; fallow-D, fallow in December; fallow-F, fallow in February. Sequence 1 is corn/wheat/soybean/fallow crop sequence in two years, while sequence 2 is soybean/fallow/corn/wheat sequence. Crop stages are indicated by shaded areas: corn (blue), wheat (yellow), soybean (green), and fallow (white). Sample numbers are given in the bar at the bottom (1-12). Hydrolase abbreviations are β -glucosidase (bG), acid phosphatase (AP), and N-acetyl glucosaminidase (NAG). Flowers (🌻) indicate mineral fertilizer application; tractors (🚜) indicate tillage event. Results are given as means \pm standard error ($n=8$).

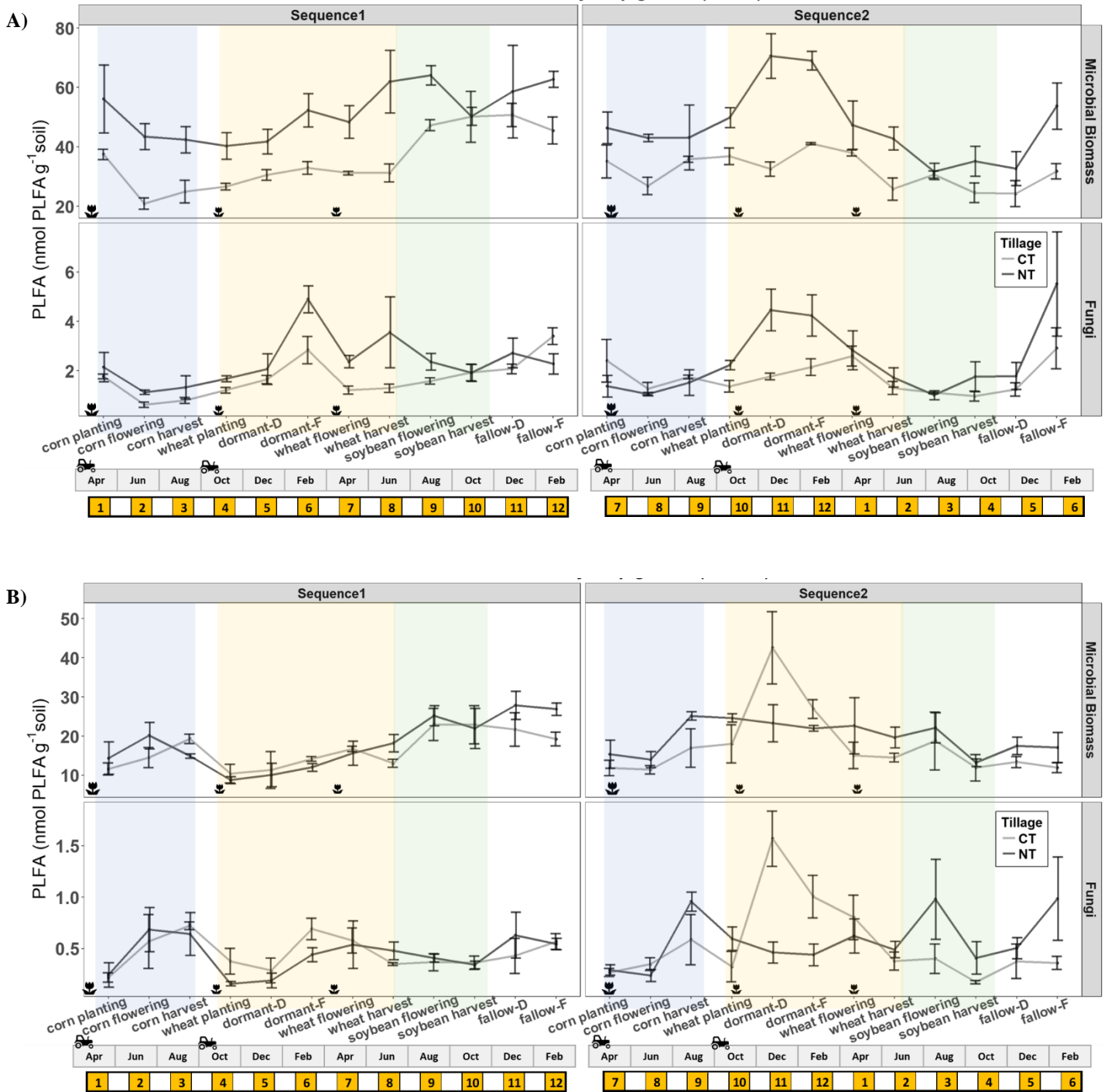


Figure 3-4. Temporal changes in soil PLFAs for conventional tillage (CT) and no-till (NT) during the crop growth periods at A) 0-5 and B) 5-15 cm. Dormant-D, wheat dormant stage in December; dormant-F, wheat dormant stage in February; fallow-D, fallow in December; fallow-F, fallow in February. Crop stages are indicated by shaded areas: corn (blue), wheat (yellow), soybean (green), and fallow (white). Sample numbers are given in the bar at the bottom (1-12). Flowers (🌻) indicate mineral fertilizer application; tractors (🚜) indicate tillage event. Results are given as means \pm standard error (n=8).

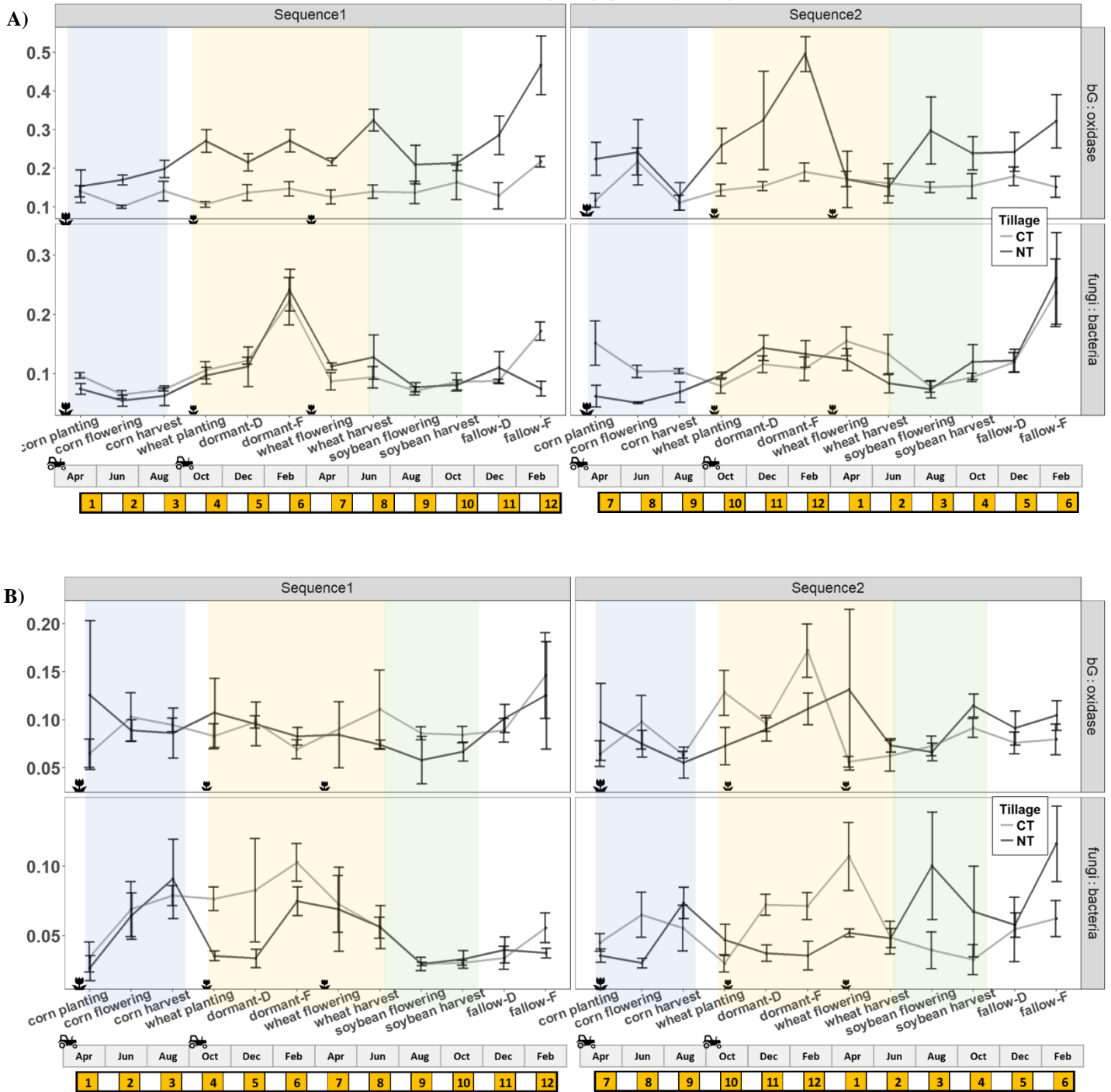


Figure 3-5. Temporal changes in soil fungal to bacterial ratio and β -glucosidase to oxidases (phenol oxidase, PHX, and peroxidase, POX) for conventional tillage (CT) and no-till (NT) during the crop growth periods at A) 0-5 and B) 5-15 cm. Dormant-D, wheat dormant stage in December; dormant-F, wheat dormant stage in February; fallow-D, fallow in December; fallow-F, fallow in February. Crop stages are indicated by shaded areas: corn (blue), wheat (yellow), soybean (green), and fallow (white). Sample numbers are given in the bar at the bottom (1-12).

Flowers (🌻) indicate mineral fertilizer application; tractors (🚜) indicate tillage event. Results are given as means \pm standard error (n=8).

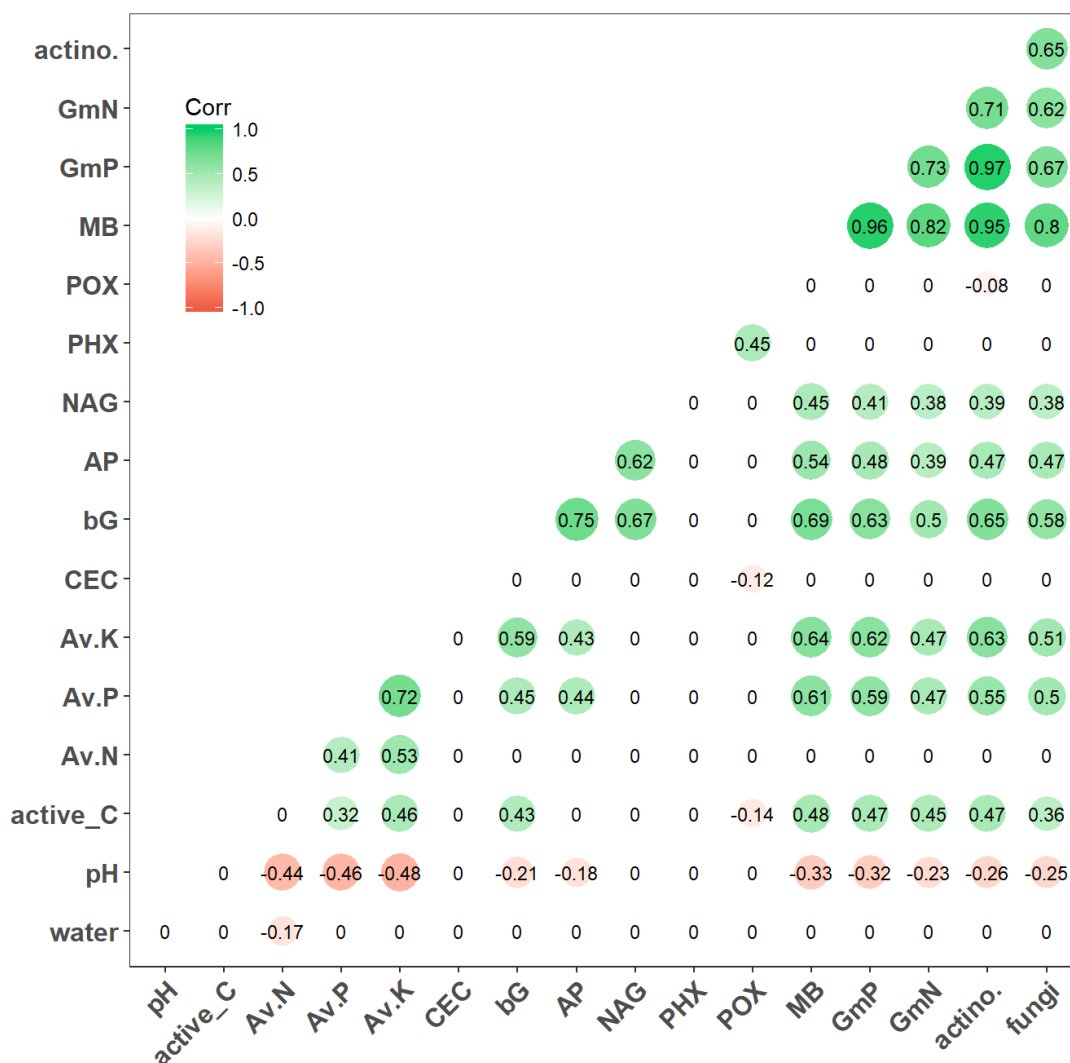


Figure 3-6. The correlation between all measured soil properties throughout the crop growth periods. The Pearson correlation coefficient is given in each circle; insignificant levels are 0. Dependent variables were log-transformed when necessary to meet the assumption of normality. pH; active C, mg kg⁻¹; av. N: available N, av. P: available P, av. K: available K, (μg g⁻¹); CEC: cation exchange capacity, meq g⁻¹ soil; bG: β-glucosidase, AP: acid phosphatase, NAG: N-acetyl glucosidase, PHX: phenol oxidase, POX: peroxidase, (nmol hr⁻¹ g⁻¹ soil); MB: PLFA microbial biomass, GmP: Gram-positive bacteria, GmN: Gram-negative bacteria, actino.: actinomycete, fungi, (nmol PLFA g⁻¹ soil).

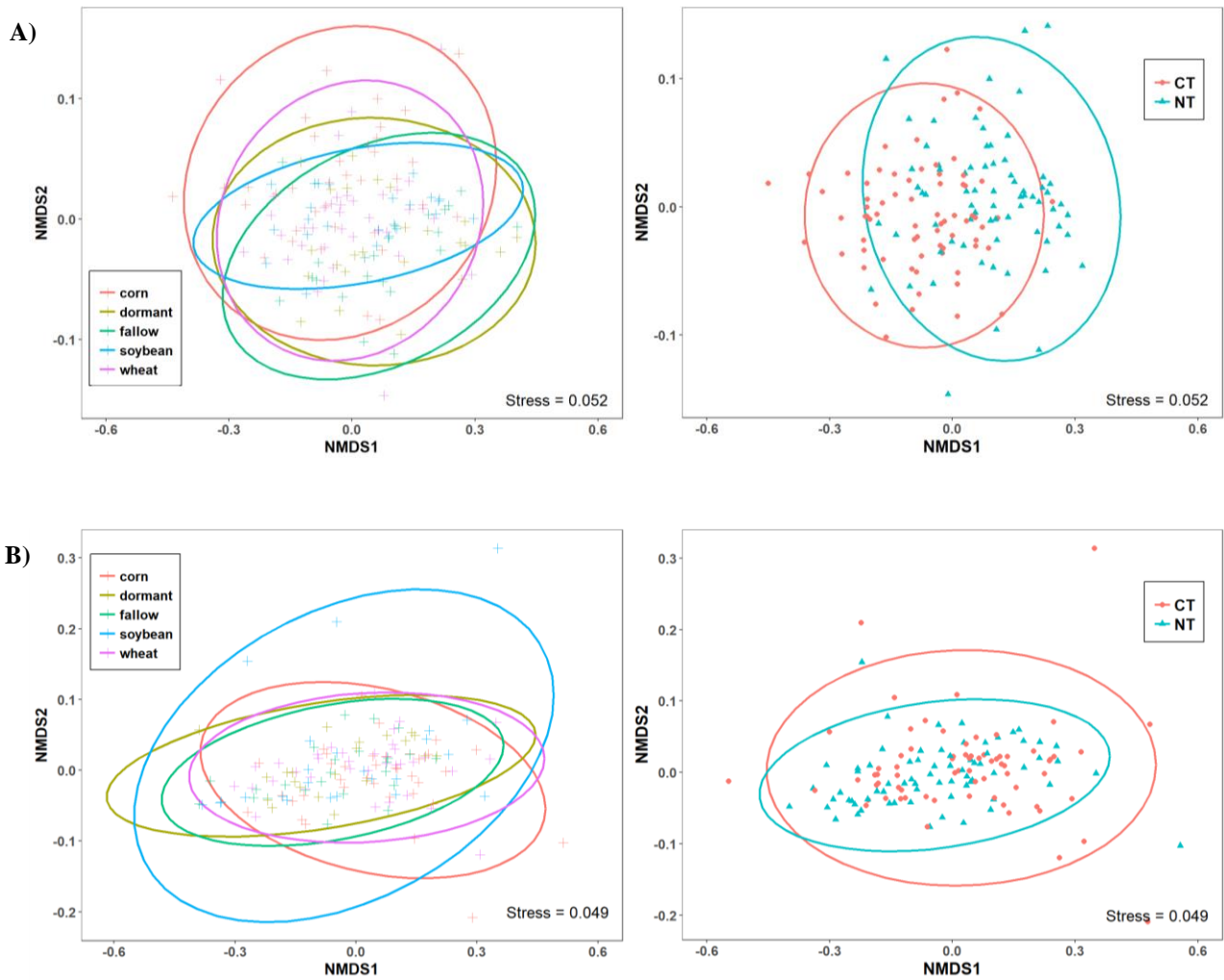


Figure 3-7. Impact of crop stage and tillage on Bray-Curtis dissimilarity in most abundant phospholipid biomarkers for all soil samples at A) 0-5 and B) 5-15 cm, with each experimental unit summarized by a single point using nonmetric multidimensional scaling (NMDS).

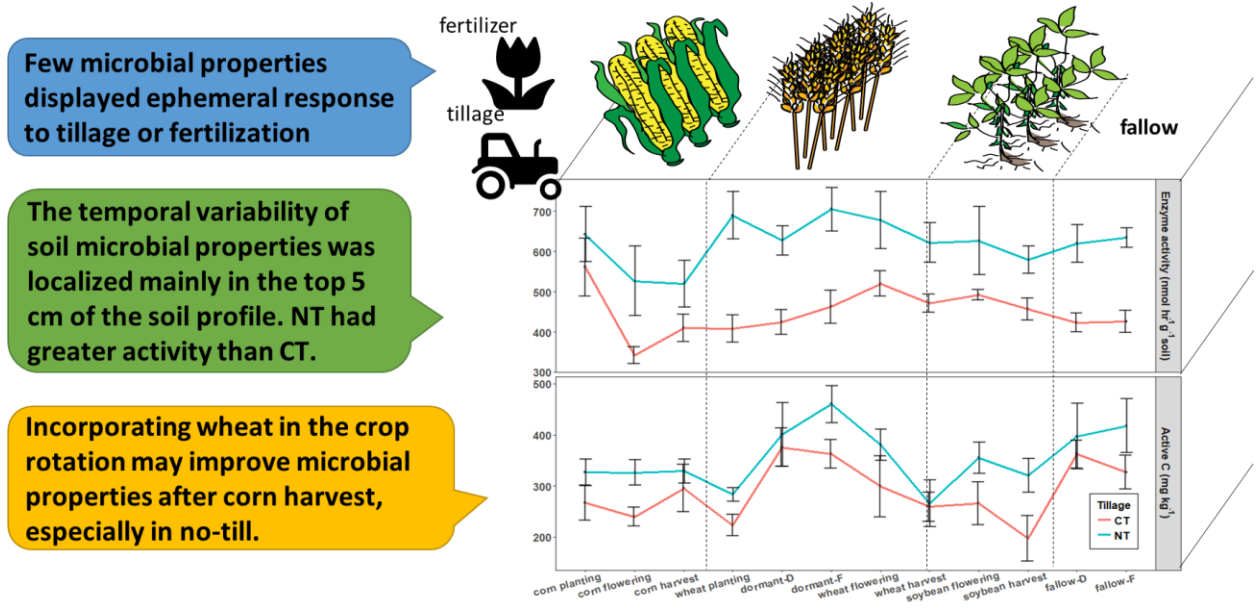


Figure 3-8. Graphical abstract.

Table 3-1. Soil average temperature at 5 cm and annually cumulative rainfall during the study period. All data were gathered from Kansas Mesonet (Kansas Mesonet, 2018).

Sample Number	Sampling Date (M/D/Y)	5-year average soil temperature at 5 cm (°C)	Average soil temperature at 5 cm (°C) on sampling date	Average cumulative 10-year rainfall between sampling dates	Cumulative rainfall between sampling dates (mm)
1	04/26/2016	15.6	17.8	180.0	149.9
2	06/21/2016	25.3	25.8	260.0	195.6
3	08/24/2016	23.2	23.0	172.5	220.5
4	10/21/2016	16.2	18.5	215.0	374.7
5	12/29/2016	4.0	7.8	125.0	45.0
6	02/18/2017	6.4	8.8	52.5	88.1
7	04/19/2017	15.6	19.6	145.0	133.9
8	06/14/2017	24.8	24.6	287.5	445.0
9	08/20/2017	23.9	24.2	180.0	262.6
10	10/28/2017	14.8	13.2	232.5	205.5
11	12/20/2017	6.2	8.0	87.5	32.3
12	02/14/2018	5.7	3.0	75.0	37.6

Table 3-2. Summary of the p-values from ANOVA for effects of treatment and crop stages on soil properties. Dependent variables were log-transformed when necessary to meet the assumption of normality. Av. N: available nitrogen; Av. P: available phosphorus; bG: β -glucosidase; AP: acid phosphatase; NAG: N-acetyl glucosaminidase; b:Oxi, bG to oxidase (phenol oxidase and peroxidase) activities ratio; F:B, fungal to bacterial ratio.

A) 0-5 cm	Water	pH	Active C	ln(Av. N)	ln(Av. P)	CEC
Tillage	0.247	0.196	0.084	0.014*	0.004**	0.005**
Sequence	0.004**	0.021*	0.148	0.002**	0.307	0.969
Crop stage	<0.001***	<0.001***	0.001**	<0.001***	<0.001***	<0.001***
Tillage* Sequence	0.693	0.120	0.755	0.548	0.393	0.759
Crop stage* Sequence	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	0.001**
Tillage*Crop stage	<0.001***	0.022*	0.087	0.001**	0.214	0.068
Tillage*Crop stage* Sequence	0.009**	0.958	0.002**	0.090	0.297	0.704

	ln(bG)	ln(AP)	ln(NAG)	b:Oxi	Microbial biomass	ln(Fungi)	F:B
Tillage	0.003**	<0.001***	0.009**	0.192	0.007**	0.053	0.746
Sequence	0.330	0.026*	0.956	0.058	0.446	0.024*	0.211
Crop stage	0.097	0.015*	0.313	0.779	<0.001***	<0.001***	<0.001***
Tillage* Sequence	0.090	0.020*	0.102	0.590	0.565	0.082	0.334
Crop stage* Sequence	0.491	<0.001***	0.981	0.590	<0.001***	0.004**	0.006**
Tillage*Crop stage	0.244	0.667	0.291	0.084	0.301	0.257	0.291
Tillage*Crop stage* Sequence	0.478	0.835	0.712	0.031*	0.136	0.031*	0.097

B) 5-15 cm	Water	pH	Active C	ln(Av. N)	ln(Av. P)	CEC
Tillage	0.009**	0.142	0.585	<0.001***	0.054	0.150
Sequence	<0.001***	0.145	0.289	0.011*	0.026*	0.395
Crop stage	<0.001***	0.052	<0.001***	<0.001***	0.657**	<0.001***
Tillage* Sequence	0.738	0.296	0.802	0.255	0.031*	0.430
Crop stage* Sequence	<0.001***	0.590	<0.001***	<0.001***	0.007**	<0.001***
Tillage*Crop stage	0.023*	0.179	0.262	0.036*	0.120	0.760
Tillage*Crop stage* Sequence	0.052	0.360	0.363	0.019*	0.019*	0.197

	ln(bG)	ln(AP)	ln(NAG)	b:Oxi	Microbial biomass	ln(fungi)	F:B
Tillage	0.231	0.349	0.243	0.738	0.239	0.535	0.824
Sequence	0.328	0.010**	0.016*	0.891	0.521	0.477	0.853
Crop stage	0.497	<0.001***	0.014*	0.369	0.044*	0.085	0.008**
Tillage* Sequence	0.577	0.626	0.390	0.875	0.660	0.268	0.330
Crop stage* Sequence	0.144	<0.001***	0.077	0.055	<0.001***	0.007**	0.371
Tillage*Crop stage	0.497	0.395	0.595	0.442	0.725	0.735	0.645
Tillage*Crop stage* Sequence	0.203	0.088	0.262	0.576	0.202	0.148	0.145

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

*** Significant at the 0.001 probability level

Table 3-3. Permutational MANOVA results for soil PLFA.

A) 0-5 cm	Sum of Squares	F	R ²	P	
Tillage	0.3030	53.30	0.1978	0.001	***
Sequence	0.0313	5.51	0.0204	0.009	**
Crop stage	0.1776	2.84	0.1159	0.002	**
Tillage*Sequence	0.0025	0.45	0.0017	0.601	
Sequence*Crop stage	0.3644	5.83	0.2378	0.001	***
Tillage*Crop stage	0.0668	1.07	0.0436	0.376	
Tillage*Sequence*Crop stage	0.0410	0.66	0.0268	0.849	
Residuals	0.5457	-	0.3561	-	

B) 5-15 cm	Sum of Squares	F	R ²	P	
Tillage	0.0502	6.10	0.0263	0.010	**
Sequence	0.0102	1.24	0.0053	0.261	
Crop stage	0.2097	2.32	0.1102	0.008	**
Tillage*Sequence	0.0180	2.19	0.0095	0.126	
Sequence*Crop stage	0.6648	7.35	0.3491	0.001	***
Tillage*Crop stage	0.0891	0.99	0.0468	0.484	
Tillage* Sequence*Crop stage	0.0727	0.80	0.0382	0.701	
Residuals	0.7895	-	0.4146	-	

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

*** Significant at the 0.001 probability level

Chapter 4 - Long-term fertilization and tillage effects on soil microbial properties with depth

Abstract

Tillage and fertilization impact crop growth, development, and yield. Soil microbial properties can be managed to improve productivity. However, vertical changes of soil microbial properties within the soil profile as a result of long-term management have not been delineated. The buried A horizon is a darker, nutrient-enriched horizon covered by a layer of alluvium during erosion on many valley floodplain surfaces. Little is known about how management practices and pedogenetic processes for a buried A horizon changes soil microbial properties. The objective of this research was to assess vertical changes of soil microbial properties in response to long-term (28 yrs) tillage and mineral or organic fertilization at a site with a buried A horizon. Selected microbial properties included extracellular enzyme activity, microbial community structure as measured by phospholipid fatty acids (PLFA), as well as key soil chemical properties including pH, soil C, N, P, and CEC. Tillage regimes included conventional tillage and no-till. The fertilization treatments were 168 kg N ha⁻¹ as mineral fertilizer or organic fertilizer, with unfertilized soil as a control. Long-term organic fertilization increased soil C, enzyme activities, and PLFAs but decreased arbuscular mycorrhizal fungi throughout the 90 cm soil profile. The increase in C-acquiring enzyme activity and microbial PLFAs in a buried A horizon was a result of root growth under no-till practice and mineral fertilization. Surprisingly, long-term mineral fertilizer applications had little effect on enzyme activities and microbial biomass. Our study indicated that the interaction between pedogenesis and management practices changed microbial structure and function in the soil profile.

Keywords: Buried A horizon; Phospholipid fatty acid (PLFA); Soil microbial biomass; Soil extracellular enzyme activity

Introduction

Soil microbial activity alters the surrounding chemical and physical environment, yet the physiochemical environment affects microbial activity (Burns et al., 2013; Gianfreda and Rao, 2004; Lugtenberg and Kamilova, 2009). In addition, human activity through land management can alter the soil bio-physiochemical environment (Alvarez and Steinbach, 2009; Busari et al., 2015; Karlen et al., 2011; McLaughlin et al., 2011; Mehra et al., 2018). Therefore, soil microbial properties are responsive to the soil matrix and indicative of productivity and provisioning ecosystem services (Andrews et al., 2004; Chaparro et al., 2012; Sinsabaugh and Shah, 2011). No-till (NT) crop management affects the soil environment through changes in nutrient availability, cation exchange capacity, soil structure, soil water dynamics, and gas exchange (Franzluebbers, 2005; Mikha and Rice, 2004; Roger-Estrade et al., 2010; Smith et al., 2016; vanCapelle et al., 2012). No-till conserves and promotes the accumulation of soil organic matter C (de Oliveira Ferreira et al., 2016; Nicolos et al., 2018), thereby increasing resources for soil microbes. Soil fertility can further change soil microbial communities (Allison et al., 2007; Kaiser et al., 2016). A meta-analysis from 62 studies with 139 observations indicated that overall, microbial biomass and most extracellular enzyme activities were higher under NT compared to CT (Zuber and Villamil, 2016). White and Rice (2009) found higher biomass of Gram-positive and -negative bacteria and fungi resulting in overall higher biological activity under NT than CT. Mbuthia et al. (2015) also demonstrated that Gram-positive bacteria,

arbuscular mycorrhizal fungi (AMF), and activities of C-, nitrogen (N)-, and phosphorus (P)-acquiring enzymes were increased in NT systems.

Fertilization can significantly change soil properties, microbial activity, and microbial community composition (Gu et al., 2017; Li et al., 2014; Mandal et al., 2007). Organic amendments add C directly to the soil and lead to increased microbial biomass, soil respiration, and microbial diversity (Francioli et al., 2016; Marschner et al., 2003; Zhong et al., 2010). Mineral fertilization indirectly increased soil microbial properties by enhancing root growth and crop productivity, resulting in a higher input of organic material in the form of root exudates and decaying crop residues (Costa et al., 2002). A meta-analysis reported that mineral fertilization enhanced microbial biomass C by 15% compared to unfertilized soils due to the increased soil organic C (Geisseler and Scow, 2014). The response of the microbial community to fertilizer varies with fertilizer sources (Marschner et al., 2003). Organic fertilization simulated copiotrophic microorganisms (those defined as populations that grow faster in nutrient-rich environments), while more oligotrophic microorganisms (populations that maintain lower densities in nutrient-poor environments) were found in soils under mineral fertilizer application (Francioli et al., 2016). Extracellular enzyme activities including β -glucosidase and N-acetyl- β -D-glucosaminidase were also enhanced with organic amendments (Francioli et al., 2016; Lupwayi et al., 2018). Eukaryotic and fungal growth appeared to be less sensitive to organic fertilization, however, and resulted in a lower fungal to bacterial ratio in fertilized soil (Marschner et al., 2003).

Many tillage and fertilization studies focus on soil dynamics near the soil surface. There is a lack of information on how crop and soil management practices interact with inherent soil properties deeper in the soil profiles that would affect microbial composition and activity. One

important deeper soil feature is post-settlement alluvium, a layer of alluvium found underlying many valley floodplain surfaces. During the process of erosion, upland topsoil travels downstream and buries riparian land surfaces, filling wetlands and oxbows. Though post-settlement alluvium alters the original landscape by reducing the original relief, this alluvium and the buried soil are not a well-researched topic (Portenga et al., 2016). When measured, carbon in deep alluvial soil layers may account for more than 50% of the total C pools (Bernal et al., 2016; Lawrence et al., 2015). Previous studies indicated that the supply of fresh plant-derived C to the subsoil could stimulate the microbial mineralization of old soil C (Bernal et al., 2016; Fontaine et al., 2007). Soluble nutrients could translocate into the subsoil, or soil organic matter chemistry and spatial distribution, rather than total concentration, could significantly influence subsoil microbial biomass and activity (Schnecker et al., 2015; Stone et al., 2014). Therefore, fertilizer application can influence soil C and associated microbial properties deeper in the soil profile (Li et al., 2014; Nicoloso et al., 2018; Stowe et al., 2010). Pedogenesis can interact with crop and soil management practices to drive vertical changes in microbial diversity and community composition (Bernal et al., 2016). Hsiao et al. (2018) found that β -glucosidase, acid phosphatase, and N-acetyl- β -D-glucosaminidase activities increased in the upper part of a claypan layer in the subsoil, while the degree of the increase was affected by soil management.

Nicoloso et al. (2018) elaborated the mechanism of soil C saturation and translocation in NT under organic fertilizer amendment in a post-settlement alluvium soil. New C moved from a saturated surface layer to a C-depleted deeper layer, but the changes in microbial properties in relation to the translocated C were not quantified. To fill this knowledge gap, the objective of the current study was to assess the vertical changes in soil microbial activity and community composition in response to long-term tillage and fertilization management practices. We

hypothesized that soil microbial biomass and activity would display analogous profiles of soil C due to shifting microbial properties tied to the resource environment. We also predicted vertical stratification of soil properties, with different physiochemical properties and microbial community composition between the upper layer and the deeper soil horizon. Soil extracellular enzyme activity was used as an indicator of microbial activity, and the phospholipid fatty acid (PLFA) profile was used as an indicator of active microbial biomass. Genomic DNA content was also analyzed. Soil chemical characteristics were also measured at different depth intervals, including total C, total N, pH, soil water content, Mehlich-3 extractable phosphorus (P) and cation exchange capacity (CEC).

Materials and methods

Site and Experimental Design

Soil was sampled from a long-term tillage N source study in continuous corn established in 1990 at the North Farm of Kansas State University in Manhattan, Kansas (39° 12' 42" N, 96° 35' 39" W). The mean annual temperature was 11.4 °C with an average annual precipitation of 800 mm. The predominant soil type in the field was a moderately well-drained Kennebec silt loam (fine-silty, mixed, superactive, mesic Cumulic Hapludoll). This site had an original grassland vegetation, then was planted with wheat and other small grains using conventional tillage from 1930 to 1990 (Nicoloso et al., 2018). It is a Mollisol on floodplains, where a layer of developed pre-settlement soil was buried by post-European settlement alluvium (Soil Survey Staff, 1999). Depth to the buried soil varies by location in the landscape. Soil Survey Staff (1999) described a blackish buried A (Ab) horizon at 29 to 66 cm depth in the soil profile and an underlying brownish weathered B (Bwb) horizon in this field (Appendix C). Harris (1993)

reported a buried A horizon located at 41 to 59 cm, followed by a buried A/E horizon at 59 to 71 cm and another buried A horizon at 71 to 85 cm soil layer (Appendix C).

The experiment was a split-plot randomized block design with four replications. The tillage systems were the whole-plot treatment with two levels: conventional tillage (CT) and no-till (NT). Conventional tillage included chisel plowing in fall and offset disking in spring to the depth of 15 and 10 cm, respectively. For NT, corn (*Zea mays* L.) was planted directly through the crop residues with minimal soil disturbance. The fertilization with different N sources was the subplot treatment with three levels: a control with no N addition (CO), 168 kg N ha⁻¹ either as mineral fertilizer (ammonium nitrate or urea; MF) or organic fertilizer (composted organic waste; OF). The subplot size was 7.5 m × 6 m. Further details were described by Nicoloso et al. (2018). The composted organic waste was collected from the North Farm's composting facility at Kansas State University. Fertilizers were broadcasted before planting. For CT, the fertilizer was disked into the soil prior to planting. The application amount was calculated assuming that 100% of mineral N and 30% of organic N were available during the crop growing season (Mikha and Rice, 2004).

The fractions of soil C derived from C4 plants in NT and CT in the 30-45 cm soil layer of the areas used in this study were presented in Table 4-1. Loss of C4-derived soil C was observed in CT-CO, CT-MF, NT-MF, and CT-OF (-5.2, -9, -0.8, and -2.8 Mg C ha⁻¹, respectively) from 1992 to 2014. For NT-CO and NT-OF, C4-derived soil C increased (+4.8 and +1.3 Mg C ha⁻¹, respectively) from 1992 to 2014.

Soil Sampling

The soil profile was sampled below the plant litter using a tractor-mounted hydraulic press (Giddings, Windsor, Colorado) in September 2017. Within each plot, two 90 cm soil cores

(diameter 2.54 cm) were collected at random locations and partitioned into six depth intervals: 0-5, 5-15, 15-30, 30-45, 45-60, and 60-90 cm. Soil samples were stored at 4 °C. Cores from each plot were composited by depth, homogenized, and subsampled for subsequent analyses. Subsamples for physiochemical properties were air-dried, ground, and sieved through 2-mm mesh sieve. Subsamples for microbial properties were stored at -20 °C before further processing.

Soil Chemical Measurements

Soil pH was determined in a 1:10 soil:water slurry. Soil organic C and total N concentrations were determined by dry combustion analysis using a Carlo-Erba C and N analyzer (Thermo Finnegan Flash EA1112, Milan, Italy). Additional soil chemical properties were measured using Mehlich-3 for extractable P (Frank et al., 1988) and ammonium acetate method for carbon exchange capacity (CEC; Chapman, 1965) by the Soil Testing Lab at Kansas State University, Manhattan, KS.

Extracellular Enzyme Activities

Soil hydrolytic and oxidative potential activities were measured using fluorometric substrates 4-methylumbelliferone (MUB) and colorimetric substrate L-3,4-dihydroxyphenylalanine (L-DOPA) following protocols presented by Zeglin et al (2013). Hydrolase assays included a C-acquiring enzyme (β -glucosidase, bG, EC 3.2.1.21, 2h incubation), a P-acquiring enzyme (acid phosphatase, AP, EC 3.1.3.2, 2h incubation), and two N-acquiring enzymes (N-acetyl- β -D-glucosaminidase, NAG, EC 3.2.1.30, 4h incubation; leucyl aminopeptidase, LAP, EC 3.4.11.1, 18h incubation). The bG hydrolyzes β -d-glucopyranosides in the degradation of cellulose. The NAG cleaves the amino sugar N-acetyl- β -D-galactosamine from chitin in soils. The LAP hydrolyzes leucine and other hydrophobic amino acids from polypeptides. Acid phosphatase releases inorganic P from soil organic matter into biologically

available forms. Oxidase assays included two main categories of lignin degradation enzymes: phenol oxidase (PHX, EC 1.10.3.2, 18h incubation) and peroxidase (POX, EC 1.11.1.7, 18h incubation). These enzymes control the rate-limiting steps of soil C, N, and P cycling. All assays were run at room temperature in 50 mM pH 5 acetate buffer with blank sets and quench controls for each sample. Potential enzyme activities were reported as nanomoles activity per gram of dry soil per hour.

Phospholipid Fatty Acid (PLFA) and genomic DNA measurements

Soil microbial biomass was estimated as the sum of all PLFA concentrations following the modified protocol from the White and Ringelberg method (Hsiao et al., 2018; White et al., 1997). Total lipids were extracted using 2:1:0.8 of methanol, chloroform, and phosphate buffer on 5 g of freeze-dried soil. Phospholipids were isolated using silicic acid chromatography columns (Disposable BAKERBOND® SPE Columns, J.T. Baker®) and methylated to fatty acid methyl esters (FAME) using 0.2 M methanolic potassium hydroxide. Fatty acid methyl esters were dissolved in hexane and quantified with a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts) equipped with a DB5-MS column (30 m x 250 µm in diameter x 0.25 µm film thickness; Agilent Technologies, Santa Clara, California). Internal standards 19:0 FAME were used to determine concentrations. The PLFA concentration was reported as nmol PLFA per gram of dry soil. The neutral lipid fatty acid (NLFA) 16:1 ω 5c was used to represent biomass of arbuscular mycorrhizal fungi (AMF; Frostegård et al., 2011). Microbial groups were assigned based on characteristics of biomarkers: iso and ante-iso branched lipids often belong to Gram-positive bacteria; monosaturated and cyclopropyl lipids often belong to Gram-negative bacteria; actinobacteria have more methyl-branched fatty acids; and methyl linoleate (18:2 ω 9,12c) is typically found in fungi (Williams and

Hedlund, 2013). The fungal to bacterial ratios were calculated by dividing the sum of fungi and AMF biomarkers by the sum of Gram-positive bacteria, Gram-negative bacteria, and actinomycetes.

Phospholipid fatty acid analysis was also used to assess microbial community structure (Duncan et al., 2016; Findlay and Dobbs, 1993; Slaughter et al., 2015). While the assignment of individual fatty acid biomarkers to a particular group of PLFA for detecting community change is controversial in some cases (Frostegård et al., 2011), we used fatty acid biomarkers to identify a broad scale shift of soil microbial community to avoid potential errors in this study (Duncan et al., 2016; Slaughter et al., 2015). The relative abundance of individual fatty acid biomarker was calculated. Of those, 19 FAMES with >1 % abundance were used for the community structure analyses (10Me16, 10Me18, 14:0, i15, a15, 16:0, i16, 16:1 ω 5c, 16:1 ω 5c NLFA, 16:1 ω 7c, 17:0, i17, a17, cy17, 18:0, 18:1 ω 7c, 18:1 ω 9c, 18:2 ω 9c, and 18:2 ω 6c).

Total genomic DNA (gDNA) was extracted from approximately 0.5 g of soil per sample using DNeasy Power Soil Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol. The amount of gDNA was measured by NanoDrop 100 spectrophotometer (Thermo Scientific, Waltham, USA).

Data analysis

The effects of tillage, depth, and fertilizer on soil physiochemical and microbial properties were analyzed separately using two-way analysis of variance (ANOVA) with repeated measures in a linear mixed effects model with restricted maximum likelihood estimations. Depth was treated as a random factor with repeat measurements. Post hoc multiple comparisons were made using the Tukey adjustment. Dependent variables were tested for the assumption of normality using Shapiro-Wilk test and then log-transformed when necessary to meet assumptions

of normality before analysis (PLFAs and enzyme activities except LAP and PHX). The Bray-Curtis dissimilarity of the fatty acid biomarkers between different sampling points was visualized using a two-dimensional nonmetric multidimensional scaling (NMDS) ordination model. The significance of treatments on the dissimilarity of PLFAs was analyzed by permutational multivariate ANOVA (PerMANOVA). The relation between the measured soil parameters and depth was summarized using principal components analysis (PCA). Correlation between the measured soil parameters and factors was summarized using Pearson's correlation coefficient (Pearson r). The significance of all tests was evaluated using α value of 0.05. All errors were reported as standard errors. All statistical analyses were performed in R (R Core Team, 2013), using package nlme (Pinheiro et al., 2012) for ANOVA and vegan (Oksanen et al., 2011) for PerMANOVA and NMDS.

Results

Soil Chemical Properties

Soil C, N, and extractable P were significantly affected by a 3-way interaction between tillage, fertilizer source, and depth (Table 4-1). Soil C decreased with depth in all treatments. Organic fertilizer enhanced C to a depth of 30 cm with significantly higher levels of soil C observed in NT (Fig. 4-1A.). Soil C was twice as high in the OF treatment to a depth of 15 cm compared to the CO and MF treatments. For the 0-5 cm depth, soil C was 46 g C kg⁻¹ in NT-OF and 28 g C kg⁻¹ in CT-OF, compared to 16 g C kg⁻¹ for the control and 14 C kg⁻¹ for the MF treatments (Table 4-3). Soil C stabilized around 12 g C kg⁻¹ in the buried A horizon. There were no significant effects of fertilizer source or tillage on soil C in the buried A horizon.

The vertical profile for total N and extractable P were similar to the C profile, decreasing with depth and higher in OF than MF and CO (Fig. 4-1B & C). Total N was significantly higher in NT than CT with OF for the top 15 cm (Fig. 4-1B). Tillage had no significant effect with depth, but total N was higher in NT under MF and CO treatments for the top 5 cm (Table 4-2). Soil extractable P was about ten-fold higher with OF than MF and CO (Fig. 4-1C). The differences in CT and NT under MF and CO for the top 15 cm were not significant (Table 4-2). Below 30 cm, extractable P stabilized around 20 mg P kg⁻¹, with no detectable differences between fertilizer sources. Soil C, N, and extractable P were highly correlated with each other with Pearson r greater than 0.8 (p < 0.001; Fig. 4-6).

Soil pH was significantly affected by the tillage-fertilization interaction (Table 4-1). Soil pH decreased with mineral fertilizer in NT from 7.3 to 6.5 in the top 15 cm, due to nitrification of the ammonium-based fertilizer and the lack of soil mixing (Fig. 4-1D). There were no detectable differences between tillage and fertilizer sources below 15 cm (Table 4-2).

Soil CEC was significantly affected by depth and the interaction between tillage and fertilizer source (Table C-1). Soil CEC increased with depth especially in the buried A horizon (Fig. C-1). Long-term organic fertilizer enhanced CEC in the top 5 cm with higher levels in NT.

Extracellular Enzyme Activity Profiles

Soil bG was significantly affected by depth as bG activity decreased from an average of 270.4 nmol h⁻¹ g⁻¹ in the 0-5 cm depth to 60.3 nmol h⁻¹ g⁻¹ soil at 60-90 cm depth (Table 4-1; Fig. 4-2A). Soil bG activity was significantly greater in OF than the CO and MF amendment in the top 30 cm of the soil, although the difference was only significant for NT-OF at 5 to 15 cm depth (Table 4-2). Soil bG activity under NT-MF treatment was higher than CT-MF at 30 to 45 cm depth (Tukey's comparisons, p = 0.02). The bG activity was positively correlated with soil C

content with Pearson r of 0.7 ($p < 0.001$; Fig. 4-4), probably because bG is a C-acquiring enzyme.

Soil AP was significantly affected by tillage (Table 4-1). In general, AP was higher in NT with added fertilizer relative to CT throughout the soil profile (Fig. 4-2B; Table 4-2). Soil AP was significantly higher in NT than CT for the 0 to 5 and 30 to 45 cm depths (Tukey's comparisons, $p < 0.04$). Soil AP activity was negatively correlated with soil pH with Pearson r of -0.7 ($P < 0.001$; Fig. 4-7). Interestingly, soil AP activity was not correlated with extractable P.

Soil NAG activity was only significantly affected by depth (Table 4-1). In general, NAG decreased until 30 cm but increased with depth especially in the buried A horizon (Fig. 4-2C). The increase at 45 to 60 cm was significantly greater than at 5 to 15 cm (Tukey's comparisons, $p < 0.05$). Tillage and fertilizer source did not significantly affect NAG activity. Although NAG is considered a C- and N-acquiring enzyme, soil NAG activity was not correlated with either soil C or N (Fig. 4-7).

Soil LAP activity was significantly affected by the 3-way interaction with tillage, fertilizer source, and depth (Table 4-1). Soil LAP activity was greater in OF than CO and MF, and greater in NT than CT, to a depth of 15 cm (Fig. 4-2D). Below 30 cm, soil LAP activity was low and constant except for an increase at the 45 to 60 cm depth with the NT-MF treatment (Table 4-2).

Soil PHX was highly variable and was not affected by any of the treatments or depths (Table C-1; Fig. C-2). Soil POX activity was significantly affected by the interaction between tillage and fertilizer source (Table 4-1). Soil POX activity was depressed in the NT-OF treatment followed by CT-OF, with no difference in the other treatments in the surface 15 cm (Fig. 4-2E;

Table 4-2). Soil POX activity increased with depth and reached the maximum near 445.9 nmol h⁻¹ g⁻¹ soil at 30 to 45 cm depth which was the location of the buried A horizon.

Phospholipid Fatty Acid and gDNA Profiles

In general, PFLA biomarkers were affected by depth except for AMF (Table 4-1). Microbial biomass decreased with depth, from an average of 60.3 nmol PLFA g⁻¹ soil in the top 5 cm to an average of 16.4 nmol PLFA g⁻¹ soil at 15 to 30 cm depth and 8.6 nmol PLFA g⁻¹ soil at 60 to 90 cm depth (Fig. 4-3A). Microbial biomass in OF was greater than CO and MF in the top 15 cm in CT and in the top 30 cm in NT (Table 4-3). Microbial biomass was higher in NT-MF than CT-MF at 45 to 60 cm depth (Tukey's comparisons, $p = 0.03$), and higher in NT-OF than CT-OF at 15 to 45 cm depth (Tukey's comparisons, $p = 0.02/0.01$ at depth 15 to 30/30 to 45 cm). Microbial biomass was positively correlated with soil C with Pearson r of 0.74 ($p < 0.001$; Fig. 4-4B), indicating C was one of the factors influencing microbial biomass in the soil profile.

Bacterial groups, including Gram-positive bacteria, Gram-negative bacteria, and actinomycete, and fungi biomass had similar vertical patterns as microbial biomass (Fig. C-3). Both bacterial groups and fungal PLFAs decreased with depth. Gram-positive bacteria and actinomycete had significantly higher biomass in NT-MF than CT-MF at 45 to 60 cm. Fungi had significantly higher biomass in NT-MF at 30 to 60 cm, while Gram-negative bacteria was also higher in NT-MF but the difference was not significant. Gram-positive bacteria, Gram-negative bacteria, actinomycete, and fungi were positively correlated with microbial biomass with Pearson r of 0.96, 0.89, 0.93, and 0.92, respectively (Fig. 4-7).

The AMF biomass was significantly affected by fertilizer source (Table 4-2). Organic fertilizer significantly decreased AMF, and the control treatment had higher AMF (Fig. 4-3B;

Table 4-3). Tillage did not affect AMF, but it was slightly higher in the NT-OF treatment compared to CT-OF in the top 15 cm.

The fungal to bacterial ratios significantly increased with depth because AMF did not decrease with depth as bacterial biomass decreased with depth (Table C-1; Fig. C-4). Interestingly, fungal to bacterial ratio was positively correlated with NAG activity with Pearson r of 0.55 ($P < 0.001$; Fig. 4-7).

Genomic DNA was significantly affected by the interaction between fertilizer and tillage (Table 4-1). The gDNA was significantly enhanced with OF and to a greater extent in NT to a depth of 15 cm, similar to the soil C and N profiles (Fig. 4-3C). There were no differences between tillage and fertilizer source below 15 cm (Table 4-2). There was a slight increase in gDNA at 45-60 cm in the NT-MF treatment in the buried A horizon. Genomic DNA content was positively correlated with soil C, bG activity, and microbial biomass with Pearson r of 0.83, 0.74 and 0.73, respectively ($p < 0.001$ for all three; Fig. 4-7).

Tillage, fertilizer source, and depth significantly changed the overall soil microbial PLFA community composition (Table 4-3). The PerMANOVA results indicated that 56.1%, 11.7%, and 1.5% of the variability of the PLFA community composition was explained by depth, fertilizer source, and tillage, respectively. The distribution of the most abundant phospholipid biomarkers among all samples were represented by the first two NMDS axes with a stress level of 0.058 (Fig. 4-5). Microbial PLFAs from the 0-5 cm and 5-15 cm clustered to the left of NMDS axis 1, separating from the other depths, and variability among the soil samples was higher at depths below 15 cm (Fig. 4-5A). Microbial PLFAs with the OF treatment clustered to the top part of NMDS axis 2, while soils with the CO treatment clustered to the bottom part (Fig.

4-5B). Tillage had a minor effect on the microbial PLFA community distribution compared to depth and fertilization (Fig. 4-5C).

The impacts of depth on all measured soil properties were summarized using PCA (Fig. 4-6). The first two principal components captured 58.2 % of the variability, with PC1 explaining 45.8%. PC1 summarizes soil properties that covaried with depth. In the surface 5 cm, higher PC1 levels were associated with the greater levels of soil C, N, extractable P, and gDNA. Below 15 cm, lower PC1 levels were associated with lower levels of bG activity, microbial biomass, and PLFA biomass (data not shown).

Discussion

Long-term NT-OF treatment significantly increased soil C approximately by three times that of the control soil, supporting observations made in 2014 at the same site (Nicoloso et al., 2018). Microbial activity and PLFA contents were also enhanced with increased soil C, a result confirmed by our study and observed in other studies (Francioli et al., 2016; Lupwayi et al., 2018; Muthia et al., 2015). However, that enhancement was not linear. Microbial biomass and bG activity plateaued at high soil C content, indicating that soil C substrate was non-limiting above a maximum biomass/activity level, or reached saturation similar to Michaelis-Menten kinetics (Fig 4-4). As noted by Nicoloso et al. (2016; 2018), C saturation occurred in the top 5 cm with 28 yr of organic fertilizer inputs at this site. In the same study, soil C within water-stable aggregates reached saturation levels in the NT-OF treatment (Nicoloso et al., 2016; 2018). This saturation suggests that above 30-40 g C kg⁻¹ soil, substrate C was non-limiting, or some other factor was limiting increases in microbial biomass and activity. Similar quadratic responses have been observed with microbial properties that increase less with each added increment of soil C.

Soil respiration had a quadratic response to available C and N (Eberwein et al., 2017). Lupwayi et al. (2018) reported quadratic responses for total PLFAs and bG activity with increasing manure application, and organic matter-specific microbial densities and activities did not increase between 1 and 10% soil C in a young Andisol (Zeglin et al. 2016). After soil C saturation, Nicolso et al. (2018) observed significant C translocation to the underlying soil layer, presumably as dissolved soil organic C. The bG activity measured in this study was significantly greater for NT-OF in the 5-15 cm depth, and PLFA biomass was greater at 15-45 cm depth, providing further support for the mechanism of labile C translocation from the overlying 0-5 cm depth.

Pedogenetic processes can change the responses of soil microbial properties to different tillage practices and fertilizer sources (He et al., 2009; Hsiao et al., 2018; Li et al., 2018). In a claypan soil study in southeast Kansas, increased hydrolase activity was observed in the upper part of claypan layer in long-term NT (Hsiao et al., 2018). The potential reasons for this increased activity were the clay-enzyme interactions and the development of substantial rooting systems from plants penetrating the claypan layers in NT (Allison and Jastrow, 2006; Grecu et al., 1988; Hsiao et al., 2018). In the current study, results demonstrated higher levels of soil bG and AP activity, microbial biomass, Gram-positive bacteria, and actinomycete PLFAs in NT-MF compared to CT-MF in the buried A horizon (Fig. 4-2A/B, Fig. 4-3A, Fig. C-3A/C). Other studies have found that deeper parts of the soil profile can provide beneficial fertility conditions to support deep roots (Gocke et al., 2016). Therefore, effects on corn root distribution might be expected in the buried A horizon of these post-alluvial soils.

While long-term OF treatment increased microbial activity and biomass, MF treatment had little impact on soil C, N, and P, or microbiological properties compared to the unfertilized

treatment in this study (Table 4-2). Except for AMF, soil C, enzyme activities, and PLFAs were not significantly different between CO and MF treatments, with either NT and CT, in our study. The lack of MF effect was surprising. Mineral fertilizer is expected to increase crop productivity, resulting in greater inputs of plant residues. For this study field, corn grain yields were significantly higher in MF than CO (Rivera-Zayas and Rice, 2017) and the resulting residue inputs were 2.6 Mg C ha⁻¹ yr⁻¹ for MF compared with 1.6 Mg C ha⁻¹ yr⁻¹ for the control (Nicoloso et al., 2018). This addition of plant residue might be expected to increase microbial activity and biomass (Geisseler and Scow, 2014; Gu et al., 2017; Lupwayi et al., 2018; Marschner et al., 2003; Muthia et al., 2015). No-till is also generally expected to increase soil C and microbial activity, as well as shift the microbial community structure, as characterized by greater amounts of Gram-positive bacteria, actinomycetes, AMF, and overall microbial biomass (White and Rice, 2009; Muthia et al., 2015; Zuber and Villamil, 2016; Hsiao et al., 2018). Soil C was stable in the CT for both CO and MF treatments over the length of the study; however, soil C under NT-CO and NT-MF increased by 42 and 45% (Nicoloso et al., 2018). A meta-analysis from 64 long-term studies around the world indicated that overall, MF application increased soil C by an average of 12.8% compared to an unfertilized control (Geisseler and Scow, 2014). In the study field, soil C mass and macroaggregates were also higher in the top 15 cm soil under NT (Mikha and Rice, 2004; Nicoloso et al., 2018). The temporal variability of surface soil microbial properties was also greater in NT compared to CT (Sun et al., 2016; Hsiao et al. 2018). Still, the minimal soil microbial response to NT treatment with no fertilizer or mineral fertilizer, or the mineral fertilizer inputs in general, despite changes in soil C remains unresolved. Time of soil sampling might contribute to the lack of detected change in microbial properties. Residue inputs from MF might be small compared to the C inputs from organic

fertilizer. The lower microbial activity and biomass in NT may also indicate that the underlying mechanisms controlling soil microbial properties and C cycling activity are associated with a broader set of factors.

To the best of our knowledge, few studies have specifically examined the vertical dynamics of soil NAG activity. The increased NAG activity with depth in both fertilized and unfertilized soils in CT and NT was distinct from other enzyme activity profiles (Fig. 4-2C). A study in claypan soil also reported increased NAG activity with depth, partially due to the clay-enzyme interactions (Hsiao et al., 2018). While the vertical changes in soil texture were not measured in our study, Soil Survey Staff (1999) reported that clay content increased with depth from 19% in the top 13 cm to 24% in the buried A horizon at this site (Table C-1). At the same site, Harris (1993) reported 22% of clay in the top 5 cm, 26% in the 30 to 45 cm, and 29% of clay in soil layers in below 45 cm (Table C-2). The gradual increase in clay content with depth may be related to the observed increase in NAG with depth. Another potential explanation is that changes in NAG activity were associated with fungal biomass (Miller et al., 1998), since fungi use NAG to recycle fungal necromass as a C and N source (Zeglin and Myrold 2013). The NAG activity was positively correlated with fungal to bacterial ratio with Pearson r of 0.55 and 0.76 ($p < 0.001$ for both correlations) in our study and the claypan soil study, respectively. A higher fungal to bacterial ratio indicated more N-acetylglucosamine and chitin, the main component of fungi cell wall as well as the substrate of NAG in the deep soil.

Fertilizer negatively affected the abundance of mycorrhizal fungi. Our study indicated that unfertilized soil had the highest AMF followed by MF and then the OF treatment (Fig. 4-3B). Higher levels of key nutrients, especially P, can decrease mycorrhizal colonization (Liu et al., 2016; Mbuthia et al., 2015). When soil nutrients are readily available, plant aboveground

biomass had preferential utilization of photosynthetic C, followed by roots and mycorrhizae symbionts (Fageria and Moreira, 2011; Mbutia et al., 2015). No-till soils had significantly higher AMF compared to CT in the top 45 cm in CO and in the top 15 cm in OF in our study. This may result from the reduced soil disturbance in NT that keeps the hyphal network intact (Sun et al., 2016). An intact hyphal network was generally expected to improve aggregate stability and promote microbial growth (Sun et al., 2016; Vos et al., 2013). Given that our OF treatments had the lowest AMF but the greatest microbial biomass and macroaggregates (Nicoloso et al., 2018), the negative effect of fertilizer was stronger than the beneficial effect of NT on AMF.

The use of a soil health indicator is intended to assess the response of soil properties to management practices. Soil microbial properties are often treated as major soil health indicators because microorganisms in soil connect the changes in management practices to their related functions in nutrient cycling (Adhikari and Hartemink, 2016; Bünemann et al., 2018; Lagomarsino et al., 2009). Organic fertilizer application, coupled with NT practices, increased the C sequestration (Nicoloso et al., 2018) and the formation of macroaggregates (Mikha and Rice, 2004) in the topsoil. Our results confirmed that bG activity and microbial biomass through PLFA were sensitive to changes in soil C content, and thus are good indicators of soil health. While the AP (C- and P-acquiring enzyme) activity also responded to changes in soil C, a greater AP activity was found in NT-MF compared to other treatments in the top 15 cm (Fig. 4-2B). This was likely due to the decrease in soil pH with MF application in NT (Fig. 4-1D; Ding et al., 2016; Francioli et al., 2016). It has been correctly noted that measuring the activity of specific enzymes might over-simplify many direct and indirect interactions between numerous enzymes, microorganisms, and soil factors in soil nutrient cycling. Our interpretations of enzyme activity

profiles were based on the assumptions that all measured enzyme activities were equally affected by changing soil abiotic conditions and that vertical translocation of enzymes was minimal. Despite these possible limitations, enzyme activities were useful in comparing the biochemical properties of soils with different treatments (Hsiao et al., 2018; Nannipieri et al., 2012). Our study established linkages between the shifts in extracellular enzyme activity and PLFAs due to NT and fertilization, which also changed soil C, N, available P, and pH with depth. We demonstrated that NT-OF significantly enhanced soil microbial properties, and that management practices can impact the microbial properties in both the topsoil and the buried soil (Hsiao et al., 2018). Further studies concerning the use of microbial activity and microbial PLFAs to assess soil health are needed to better understand the effects of management practices and pedogenesis on soil properties.

Conclusion

Long-term NT-OF treatment increased the bG activity, microbial biomass, and gDNA content in the top 15 cm of the soil. This was likely due to the increases in soil C. The responses of bG activity and microbial biomass to soil C were quadratic, indicating the bG activity and microbial biomass reached saturation when soil C was saturated. Higher levels of bG and AP activity, microbial biomass, Gram-positive bacteria, actinomycete, and fungi PLFAs were found in NT-MF than in CT-MF treatment in the buried A horizon. Both OF and MF changed the microbial community structure. Fertilization decreased arbuscular mycorrhizal fungi throughout the soil profiles in both NT and CT. Long-term MF application had little effect on enzyme activities and microbial biomass. The temporal variability of soil microbial properties may have masked the MF effects on soil microbial properties.

Evaluating microbial properties across different treatments and soil depths help delineate the effects of management practices and pedogenesis on soil health. The indefinite MF and NT effects on microbial properties indicate that a broader set of factors (e.g. soil type, weather, spatial and temporal variation) may need to be considered to delineate the underlying mechanism of changes in soil health.

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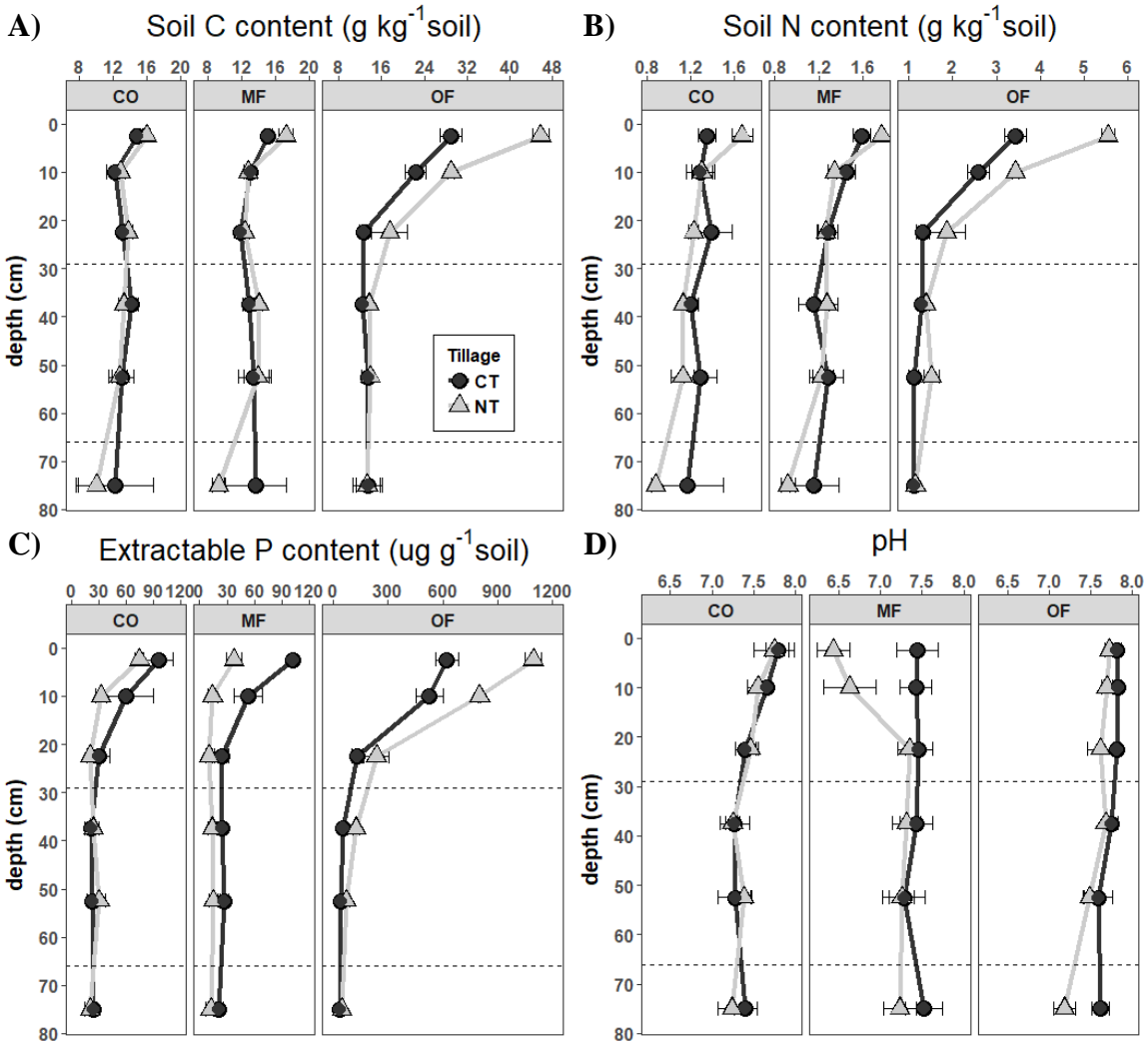


Figure 4-1. Vertical changes in soil chemical properties for conventional tillage (CT) and no-till (NT) under organic fertilizer (OF), mineral fertilizer (MF), and unfertilized control (CO). Buried A horizon was located at 29 to 66 cm and marked by dash lines. Results are given as means \pm standard error of the mean.

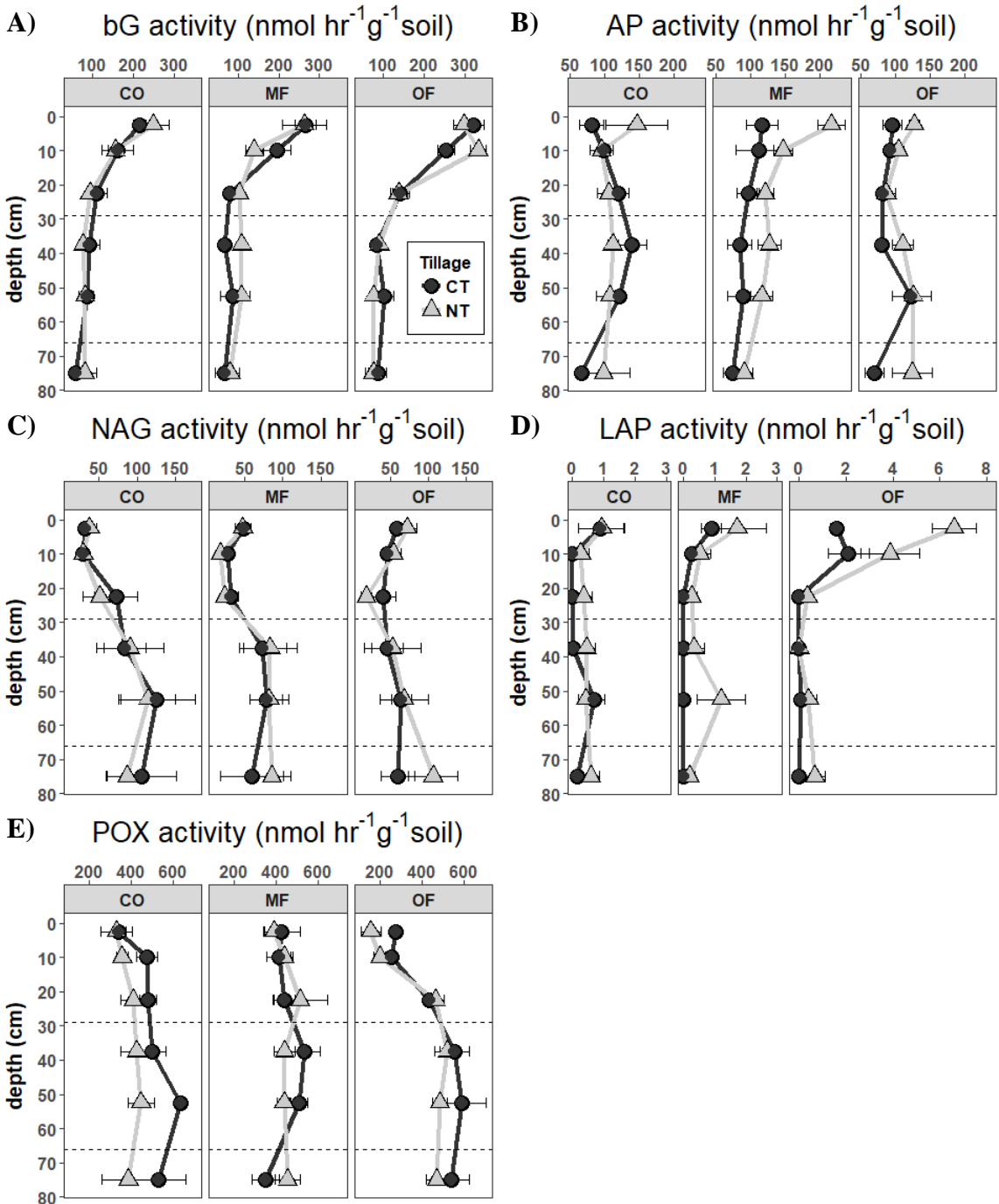


Figure 4-2. Vertical changes in soil extracellular enzyme activities for conventional tillage (CT) and no-till (NT) under organic fertilizer (OF), mineral fertilizer (MF), and unfertilized control (CO). Hydrolase abbreviations are β -glucosidase (bG), acid phosphatase (AP), N-acetyl glucosaminidase (NAG), and leucyl aminopeptidase (LAP). Oxidase abbreviation is peroxidase (POX). Buried A horizon was located at 29 to 66 cm and marked by dash lines. Results are given as means \pm standard error of the mean.

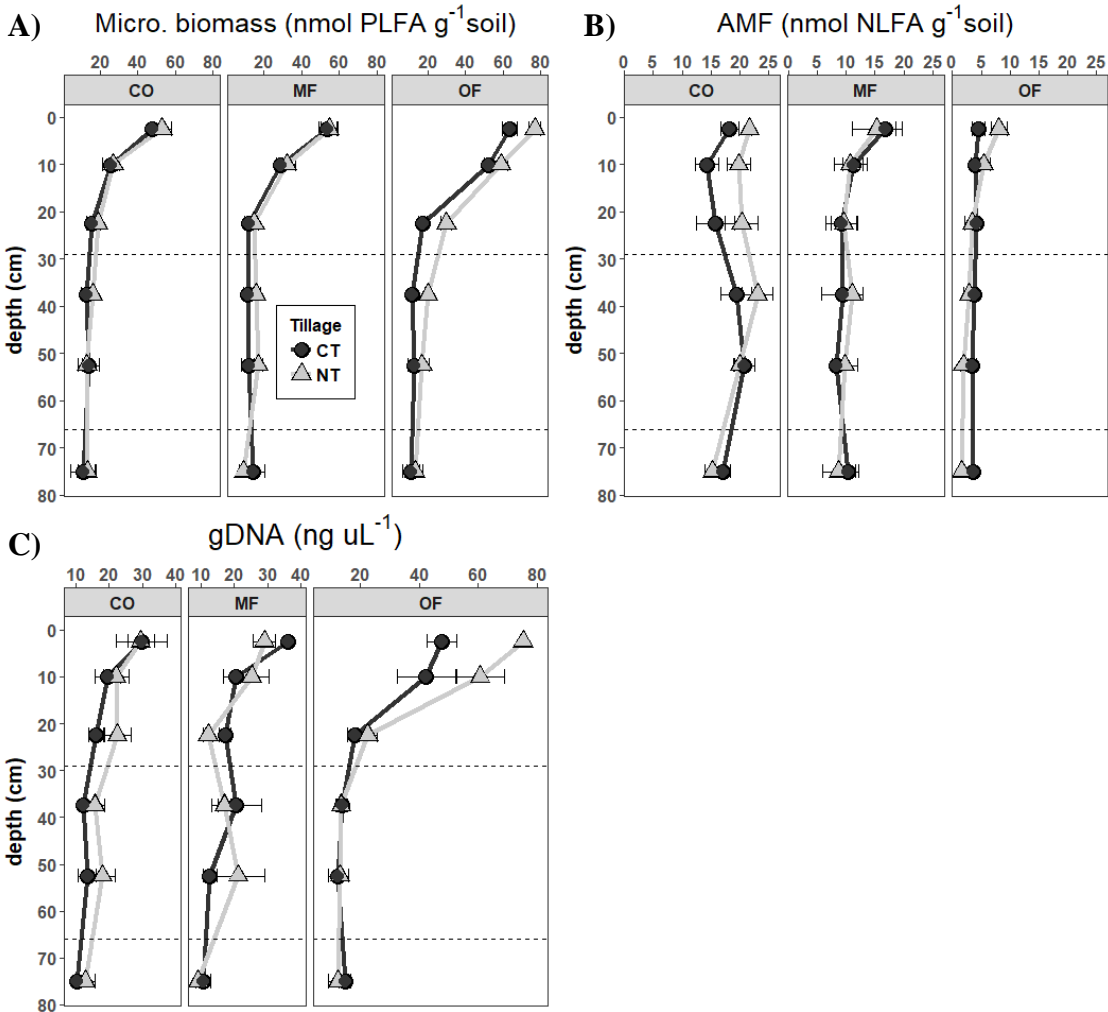


Figure 4-3. Vertical changes in (A) soil microbial biomass, (B) AMF biomass, and (C) genomic DNA content for conventional tillage (CT) and no-till (NT) under organic fertilizer (OF), mineral fertilizer (MF), and unfertilized control (CO). AMF, arbuscular mycorrhizal fungi. Buried A horizon was located at 29 to 66 cm and marked by dash lines. Results are given as means \pm standard error of the mean.

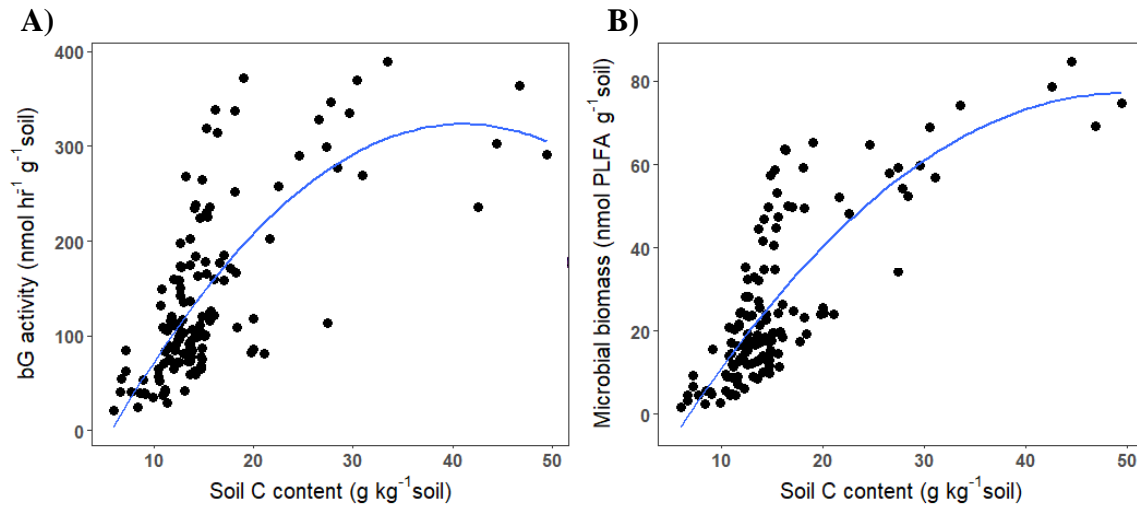


Figure 4-4. Relationship between soil C and A) β -glucosidase and B) microbial biomass. The blue lines represent the best-fit regressions ($p < 0.001$).

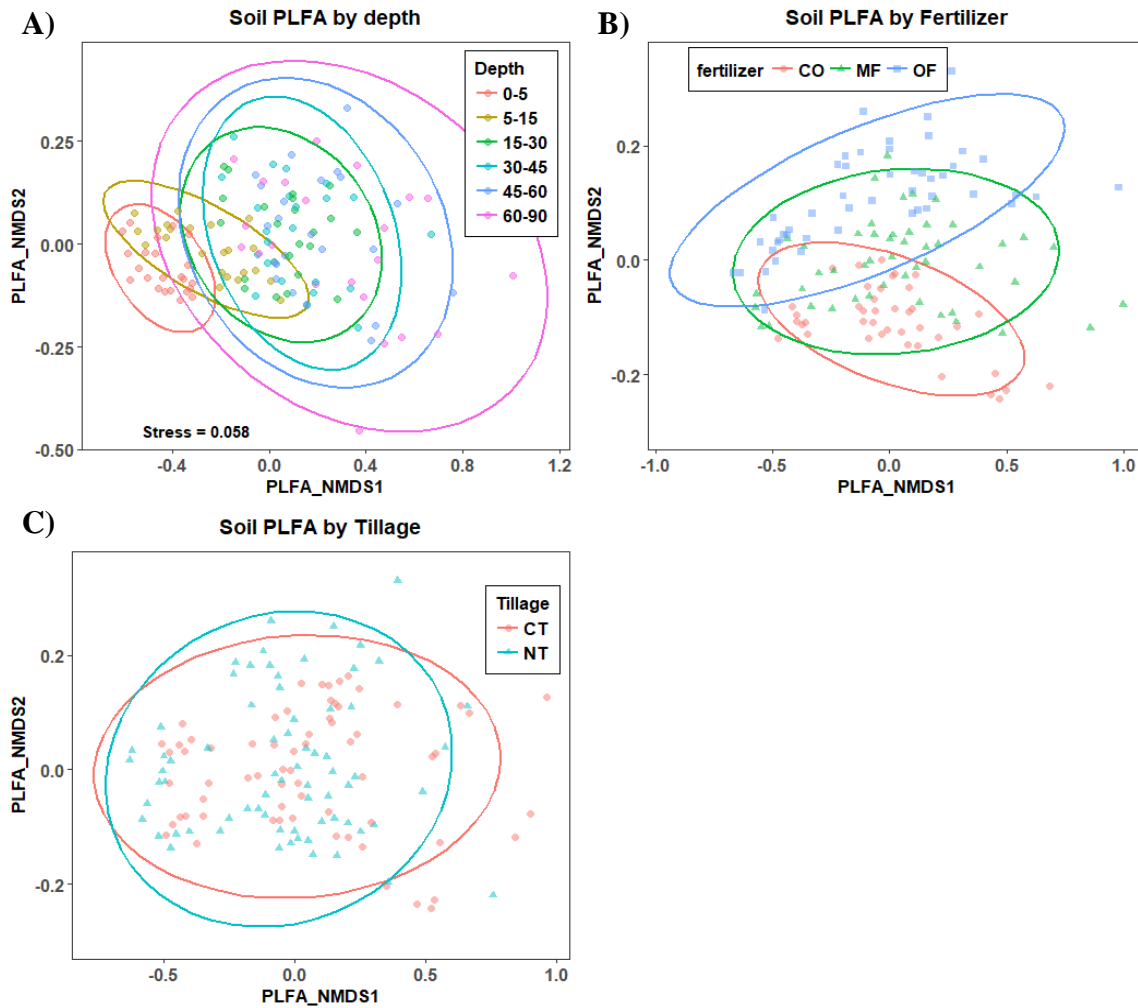


Figure 4-5. Impact of (A) depth, (B) tillage regimes, and (C) fertilizer amendment on Bray-Curtis dissimilarity in most abundant phospholipid biomarkers for all samples, with each experimental unit summarized by a single point using nonmetric multidimensional scaling (NMDS).

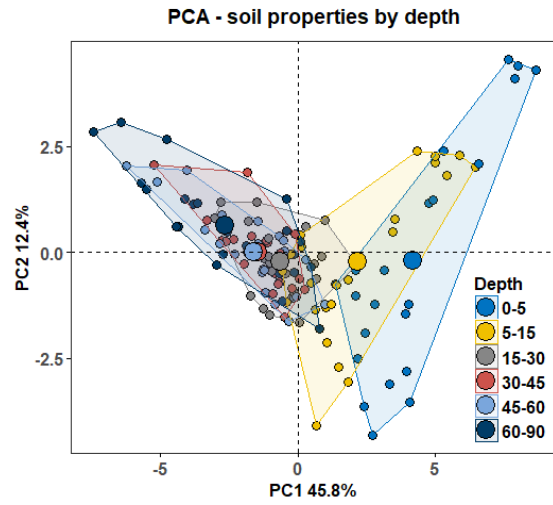


Figure 4-6. Impact of depth on all measured soil properties with each experimental unit summarized by a single point by using principal component analysis (PCA).

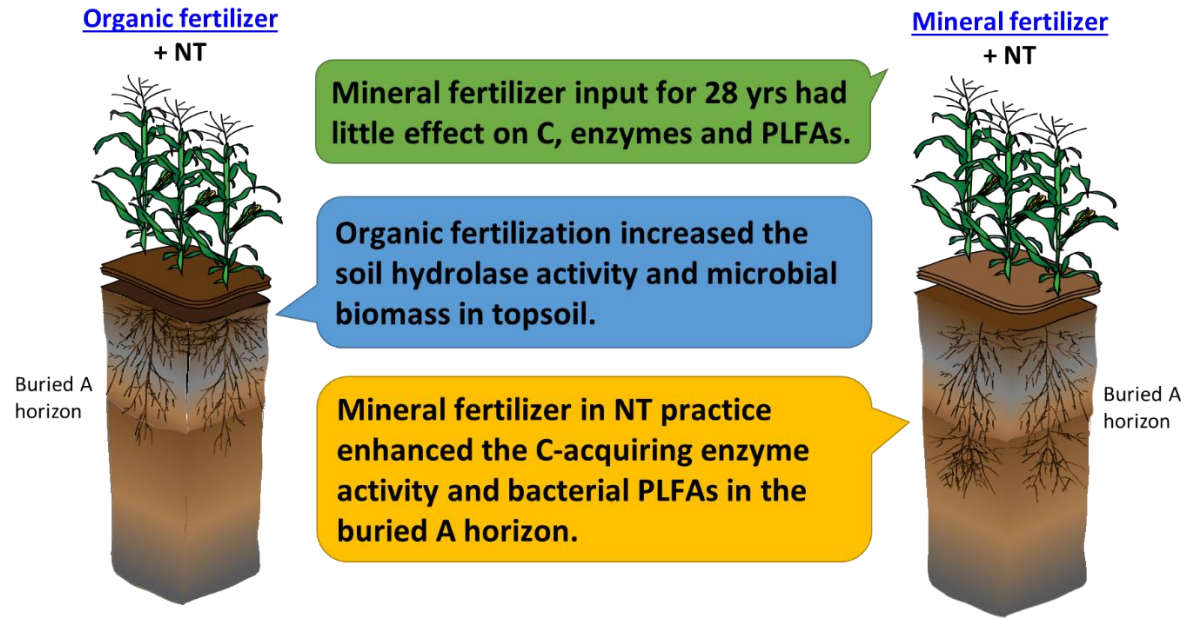


Figure 4-8. Graphical abstract

Table 4-1. Summary of the p-value from ANOVA for effects of tillage, depth, and fertilizer amendment regimes on soil properties. Ex. P, extractable phosphorous; bG, β -glucosidase; AP, acid phosphatase; NAG, N-acetyl glucosaminidase; LAP, leucyl aminopeptidase; PHX, phenol oxidase; POX, peroxidase; MB, microbial biomass; Gram (+), gram-positive bacteria; Gram (-), gram-negative bacteria; Acti., actinomycete; AMF, arbuscular mycorrhizal fungi.

	Total C	Total N	Ex. P	pH
Tillage	0.238	0.093	0.571	0.695
Fertilizer	<0.001	<0.001	<0.001	0.197
Depth	0.715	0.903	0.404	0.269
Tillage*fertilizer	<0.001	<0.001	<0.001	0.004
Fertilizer*depth	<0.001	<0.001	<0.001	0.862
Tillage*depth	0.674	0.503	0.983	0.972
Tillage*fertilizer*depth	<0.001	<0.001	<0.001	0.184

	bG	AP	NAG	LAP	POX
Tillage	0.604	0.055	0.828	0.821	0.847
Fertilizer	0.227	0.364	0.257	0.320	0.305
Depth	0.005	0.153	0.065	0.635	0.301
Tillage*fertilizer	0.797	0.621	0.843	<0.001	0.017
Fertilizer*depth	0.777	0.349	0.104	0.198	0.219
Tillage*depth	0.892	0.423	0.982	0.934	0.799
Tillage*fertilizer*depth	0.302	0.867	0.374	0.006	0.306

	MB	AMF	gDNA
Tillage	0.778	0.536	0.877
Fertilizer	0.588	<0.001	0.065
Depth	<0.001	0.788	0.198
Tillage*fertilizer	0.839	0.209	0.001
Fertilizer*depth	0.561	0.674	0.207
Tillage*depth	0.912	0.877	0.998
Tillage*fertilizer*depth	0.729	0.279	0.173

Table 4-2. Effect of tillage and fertilizer source on measured soil properties by depth. CT, conventional tillage; NT, no-till. OF, organic fertilizer; MF, mineral fertilizer; CO, unfertilized control. Ex. P, extractable phosphorous; bG, β -glucosidase; AP, acid phosphatase; NAG, N-acetyl glucosaminidase; LAP, leucyl aminopeptidase; POX, peroxidase; AMF, Arbuscular mycorrhizal fungi

Depth (cm)	Fertilizer	Total C (g kg ⁻¹)		Total N (g kg ⁻¹)		Ex. P (μ g g ⁻¹)		pH	
		NT	CT	NT	CT	NT	CT	NT	CT
0-5	CO	16.2a	13.9a	1.7a	1.3a	72.9a	93.9a	7.7b	7.8b
	MF	17.0a	15.8a	1.8a	1.6a	35.8a	103a	6.5a	7.5b
	OF	46.4c	28.3b	5.6c	3.4b	1096c	622b	7.7b	7.8b
5-15	CO	12.9a	11.4a	1.3a	1.3a	31.2a	57.4a	7.6b	7.7b
	MF	12.6a	13.4a	1.3a	1.5a	14.5a	54.8a	6.6a	7.4b
	OF	29.5c	22.1b	2.6b	3.4c	802c	525b	7.7b	7.8b
15-30	CO	14.6ab	12.3ab	1.3a	1.4ab	18.8a	29.1ab	7.5a	7.4a
	MF	11.7a	12.2a	1.2a	1.3a	9.1a	25.2a	7.4a	7.4a
	OF	17.8b	11.4a	1.9b	1.2a	234c	134b	7.6a	7.8a
30-45	CO	12.7a	13.2a	1.2a	1.1a	24.3a	21.3ab	7.2a	7.3a
	MF	13.5a	12.9a	1.3a	1.2a	13.6a	24.5a	7.3a	7.4a
	OF	13.9a	13.7a	1.4a	1.3a	124b	54.8ab	7.7a	7.8a
45-60	CO	12.7a	13.2a	1.1a	1.3a	30.8a	22.1a	7.3a	7.3a
	MF	13.5a	12.9a	1.2a	1.2a	15.0a	27.4a	7.3a	7.3a
	OF	13.9a	13.7a	1.5a	1.1a	70.6a	37.4a	7.5a	7.6a
60-90	CO	10.3ab	11.1ab	0.9a	1.1a	20.9a	23.4a	7.2a	7.4a
	MF	7.2a	13.5b	0.8a	1.1a	11.9a	20.9a	7.3a	7.5a
	OF	13.9b	13.3b	1.2a	1.1a	45.6a	31.2a	7.2a	7.6a

Depth (cm)	Fertilizer	bG		AP		NAG		LAP		POX	
		(ln nmol hr ⁻¹ g ⁻¹ soil)									
		NT	CT	NT	CT	NT	CT	NT	CT	NT	CT
0-5	CO	5.5a	5.3a	4.8ab	4.3a	3.5a	3.4a	0.9a	1.1a	5.8b	5.8b
	MF	5.5a	5.6a	5.4b	4.7ab	3.8a	3.8a	1.7a	0.6a	5.9b	5.9b
	OF	5.7a	5.8a	4.9ab	4.5a	4.3a	4.0a	6.6b	1.5a	4.9a	5.6b
5-15	CO	5.0a	5.0a	4.5a	4.5a	3.4ab	3.3ab	0a	0.2ab	5.9b	6.1b
	MF	4.9a	5.2ab	4.9a	4.6a	2.8a	3.3ab	0.8ab	0a	6.1b	6.0b
	OF	5.9b	5.5ab	4.7a	4.5a	4.0b	3.8ab	3.9c	2.3bc	5.3a	5.5ab
15-30	CO	4.5a	4.6a	4.6a	4.7a	3.7b	4.0b	0.4a	0a	6.0a	6.1a
	MF	4.7a	4.3a	4.8a	4.5a	3.2ab	3.3ab	0.3a	0a	6.1a	6.1a
	OF	4.9a	4.9a	4.4a	4.4a	2.6a	3.4ab	0.4a	0a	6.1a	6.1a
30-45	CO	4.3a	4.4a	4.7a	4.9a	4.2a	4.3a	0.4a	0.1a	6.0a	6.2a
	MF	4.7a	4.1a	4.8a	4.3a	4.3a	3.8a	0.4a	0a	6.1a	6.3a
	OF	4.5a	4.4a	4.7a	4.3a	3.3a	3.5a	0a	0a	6.2a	6.3a
45-60	CO	4.3a	4.4a	4.6a	4.8a	4.5a	4.6a	0.3a	0.7a	6.1a	6.4a
	MF	4.6a	4.2a	4.7a	4.4a	4.4a	4.2a	1.2a	0a	6.1a	6.3a
	OF	4.4a	4.5a	4.9a	4.7a	4.1a	4.1a	0.5a	0a	6.1a	6.3a
60-90	CO	4.3a	4.0a	4.4a	4.2a	4.3ab	4.4ab	0.6a	0.2a	5.8a	6.2a
	MF	4.2a	3.9a	4.4a	4.3a	4.5b	3.4a	0.2a	0a	6.1a	5.8a
	OF	4.2a	4.3a	4.7a	4.2a	4.5b	3.8ab	0.7a	0a	6.1a	6.2a

Depth (cm)	Fertilizer	Microbial biomass (ln nmol PLFA g ⁻¹ soil)		AMF		gDNA (µg g ⁻¹ soil)	
		NT	CT	NT	CT	NT	CT
0-5	CO	4.0a	3.9a	3.1c	2.9bc	6.2ab	6.0ab
	MF	4.0a	4.0a	2.6bc	2.8bc	5.6a	7.4ab
	OF	4.4a	4.1a	2.0ab	1.4a	14.9c	9.5b
5-15	CO	3.3ab	3.1a	3.0c	2.6c	4.5a	3.9a
	MF	3.4abc	3.4ab	2.3bc	2.4bc	5.2ab	4.2a
	OF	4.1c	3.9bc	1.6ab	1.3a	12.0c	8.5bc
15-30	CO	2.9ab	2.6ab	3.0c	2.7c	4.3a	3.2a
	MF	2.7ab	2.5a	2.2bc	2.1bc	2.6a	3.4a
	OF	3.4b	2.8ab	1.0a	1.4ab	4.7a	3.7a
30-45	CO	2.8a	2.4a	3.1d	2.9cd	3.0a	2.7a
	MF	2.7a	2.3a	2.3cd	2.0bc	3.5a	4.2a
	OF	3.0a	2.4a	0.9a	1.3ab	2.7a	2.8a
45-60	CO	2.5a	2.4a	3.0c	3.0c	3.4a	2.6a
	MF	2.8a	2.2a	2.1bc	2.1b	4.3a	2.6a
	OF	2.8a	2.4a	0.5a	1.2a	2.9a	2.5a
60-90	CO	2.4a	1.9a	2.7c	2.8c	2.6a	2.1a
	MF	1.8a	2.2a	2.0bc	2.3c	1.9a	2.2a
	OF	2.4a	2.1a	0.3a	1.3b	2.6a	2.9a

Table 4-3. Permutational MANOVA results for PLFA in the top 5 cm soil depth.

Factor	Sum of Squares	F	R ²	P
Tillage	0.0742	5.9858	0.0151	0.006
Fertilizer	0.5772	23.2730	0.1174	0.001
Depth	2.7610	44.5334	0.5614	0.001
Tillage*Fertilizer	0.0111	0.4458	0.0023	0.751
Fertilizer*Depth	0.0970	0.7819	0.0197	0.725
Tillage*Depth	0.0854	1.3782	0.0174	0.209
Tillage*Fertilizer*Depth	0.0478	0.3855	0.0097	0.987
Residuals	1.2648	-	0.2572	-

Chapter 5 - Summary

Healthy soil creates a better habitat for plants and microorganisms, and consequently provides greater crop productivity and environmental functionality. Soil microbial properties connect the changes in management practices to soil biogeochemical features, and hence are often used as soil health indicators. Agricultural practices, including tillage, fertilizer application, crop selection and rotation, can modify soil health. Inherent soil properties, such as texture and parent material, impact microbial properties directly or indirectly through nutrient distribution and stabilization. Interactions between agricultural practices and soil physical, chemical, and biological properties could have an impact on soil health in the surface soil, but there is uncertainty regarding how these factors impact soil health status in the deeper soil layers and over time.

In claypan soil, soil organic carbon (C), extracellular enzyme activities, and microbial biomass were greatest in a long-term grass production system (hay meadow, HM), followed by no-till (NT) and then conventional tillage (CT) crop production systems. The NT effects occurred mostly in the top 15 cm and the upper part of claypan layer, while the effects in the HM occurred throughout the soil profile. As clay content increased with depth in the claypan soils, soil properties including gravimetric moisture contents, available potassium, and cation exchange capacity also increased. Soil C, nitrogen (N), available phosphorus (P), and microbial biomass generally decreased with depth. Hydrolase activities increased in the upper part of the claypan layer in HM and NT, then decreased with depth. This is likely due to both the clay-enzyme interactions and management practices. Enzymatic stoichiometric data indicated that soils in the claypan layer were P- and N-limited for microbes.

Production systems can impact the enzyme activity profiles in both the surface soil and the claypan layer. Contrary to annual cropping systems, perennial grasslands have continuous vegetative coverage, allowing the development of substantial rooting systems with the potential to penetrate the claypan layer. This accounts for the increase in soil enzyme activity. Incorporating grass in the crop rotation or as cover crops may allow nutrients to be extracted from the entire soil profile.

In both chapters 2 and 3, root deposits (rhizodepositions) were one of the major drivers of microbial dynamics. Root development with crop rotation altered the total amount and the vertical distribution of organic compounds, further shaping the microbial ecology. Interactions between crop development stage and crop sequence were significant in most measured soil properties in our study. Yearly variability driven by weather variables created the differences between crop sequences. Wheat resulted in higher hydrolase activity and microbial biomass compared to corn. The lack of preceding residue input may explain the lower microbial biomass and activity during corn growth. The greater substrate availability from corn residue resulted in production and activation of enzymes that decomposed more recalcitrant C during wheat growth. Soybean cultivation stabilized microbial activity compared to corn and wheat, which may be attributed to the slower decomposition rate of the wheat residue from the preceding crop as well as the lower fungal biomass during soybean growth. These findings suggest that incorporating winter wheat in the crop rotation may increase the microbial activity and biomass, and hence increase nutrient cycling and soil functionality. Future studies should examine the contribution from living root exudates on temporal dynamics in soil microbial properties.

The temporal variation in soil chemical and microbial properties were localized mainly in the top 5 cm of the soil profile under no-till management. Soil in the 0-5 cm layer received

more crop residues and was more exposed to weather variability compared to the 5-15 cm layer. Although root biomass was not measured here, other studies demonstrated that soils under NT generally had a higher root length density in the topsoil, potentially resulting in greater microbial biomass, enzymatic activity, and soil nutrients. Very few soil microbial properties displayed ephemeral responses to tillage or fertilizer application, even though changes in soil pH and plant available nutrients after mineral fertilizer application may influence plant growth and microbial activities in the long-term. This suggested soil microbial communities either maintained their functionality or had recovered from the disturbances before sample collection. These findings demonstrated the importance of understanding the temporal variability of soil microbial properties. Soil samples need to be collected more often or the sampling time should be carefully selected based on the study and resources to minimize the variability in soil health assessment.

In the post-alluvium settlement soil studied in Chapter 4, the nutrient-enriched buried A horizon interacted with tillage and fertilizer application to change soil microbial properties. Higher levels of β -glucosidase and acid phosphatase activities, microbial biomass, Gram-positive bacteria, actinomycete, and fungi PLFAs were found in NT compared to CT under mineral fertilization treatment. This is likely due to the beneficial fertility conditions provided in the lower parts of the soil profile that support deep roots.

Long-term organic fertilizer application, coupled with NT practices, increased the β -glucosidase activity, microbial biomass, and gDNA content in the top 15 cm soil. This is likely caused by associated increases in soil C. The correlative relationship between soil C and both β -glucosidase activity and microbial biomass was quadratic, showing a typical Michaelis-Menten saturation curve. When the soil C was saturated and physically protected by aggregates in the top 5 cm soil layer in NT and organic fertilizer application, the β -glucosidase (C-acquiring enzyme)

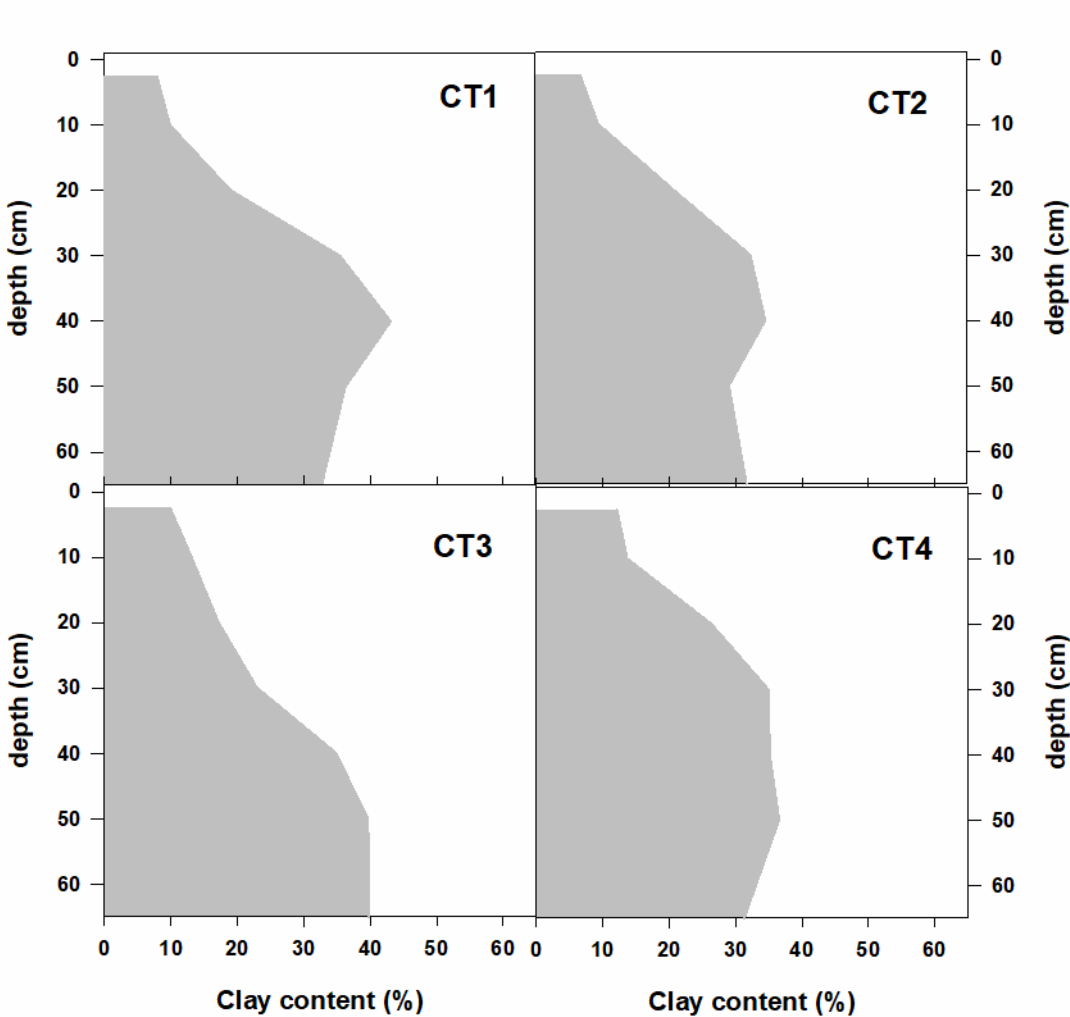
activity and microbial biomass were also saturated. This saturation suggests that while substrate C was non-limiting, some other factor was limiting for microbial biomass and activity.

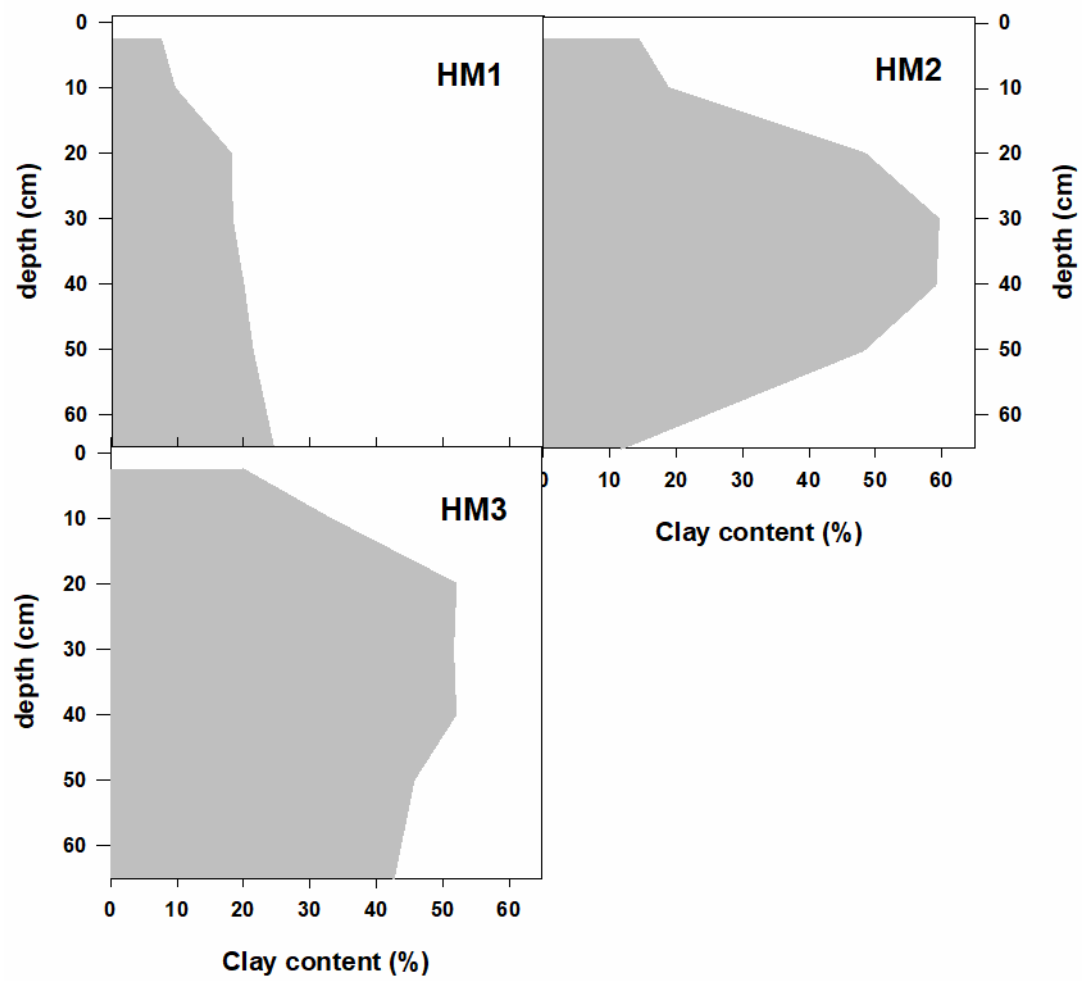
While long-term organic fertilizer treatment increased microbial activity and biomass, mineral fertilizer treatment had little impact on soil biogeochemical components and microbial properties compared to the unfertilized treatment. The lack of mineral fertilizer effects was surprising because corn grain yield was observed to be significantly higher in the mineral fertilizer treatment than in the unfertilized treatment in this field. Several potential explanations included the time of sampling and the lower soil organic matter decomposition rate under long-term N application. Time of soil sampling, including any limiting effect of crop residue removal, might contribute to the lack of detected changes in microbial properties. Residue inputs from MF might be small compared to the C inputs from organic fertilizer. The lower microbial activity and biomass in NT may also indicate that the underlying mechanisms controlling soil microbial properties and C cycling activity are associated with a broader set of factors. Additional research is required to delineate causative factors impacting enzyme activity under higher rates of mineral N fertilizer application in post-settlement alluvium soils.

The results indicated that management practices and pedogenesis can have large impacts on both the soil physiochemical and soil microbial properties in agroecosystems. While pedogenesis primarily dictates the soil enzyme activity and microbial PLFA in the subsurface soil, management practices control soil health through the entire soil profile. Soil health assessment largely depends on the selection of indicators that can promptly respond to changes in soil conditions. Additionally, soil health indicators should be used in relation to specific soil functions. Based on the findings of this study, potential hydrolase activities were useful in comparing the biochemical properties of soils with different treatments. Microbial biomass

through PLFA also appear to be an ideal soil health indicator. Optimal management practices are necessary to maintain or restore soil health. No-till practices, organic fertilizer application, and including wheat in the crop rotation had relatively higher microbial activity, biomass, and soil C, making them effective management practices to improve soil health.

Appendix A - Chapter 2





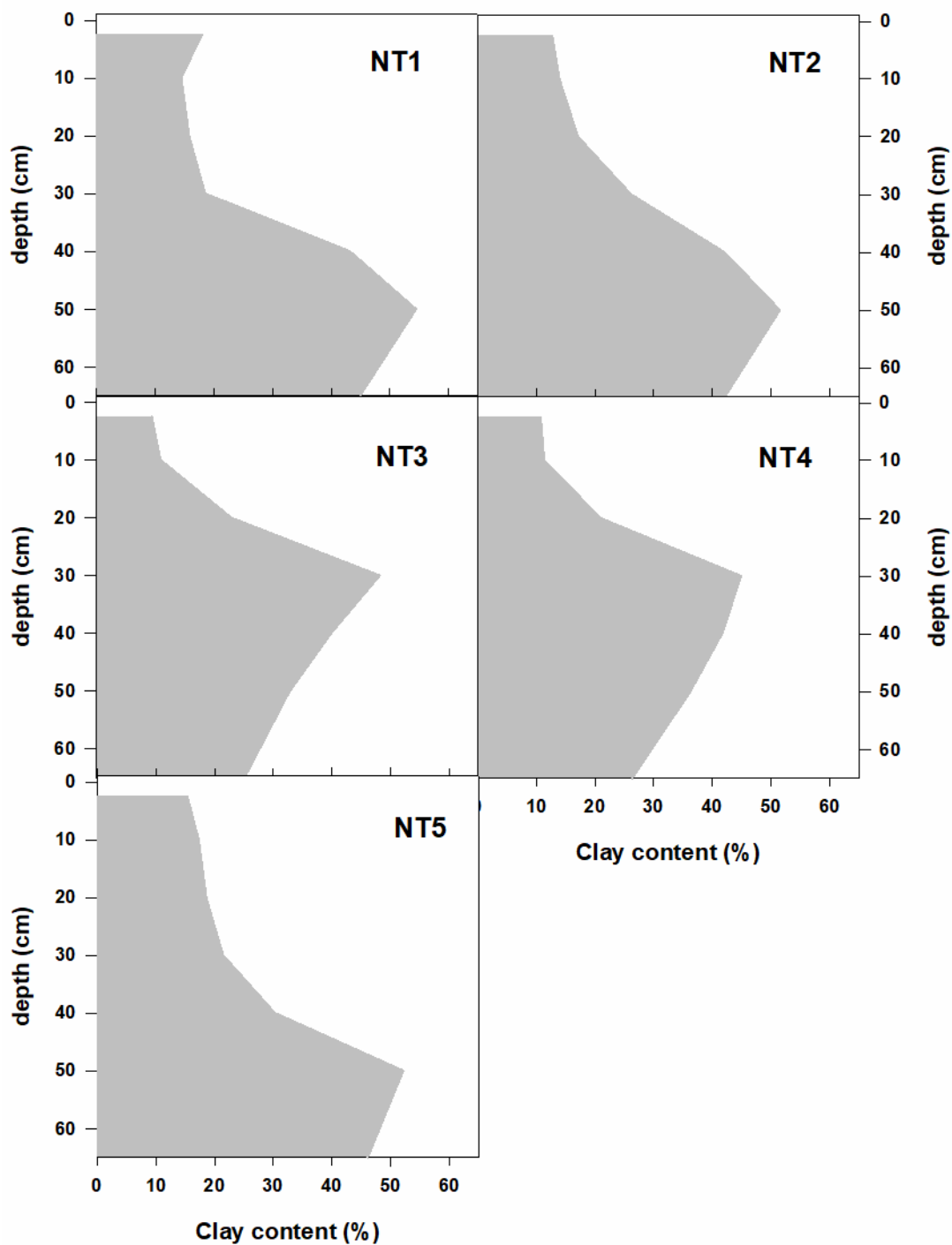
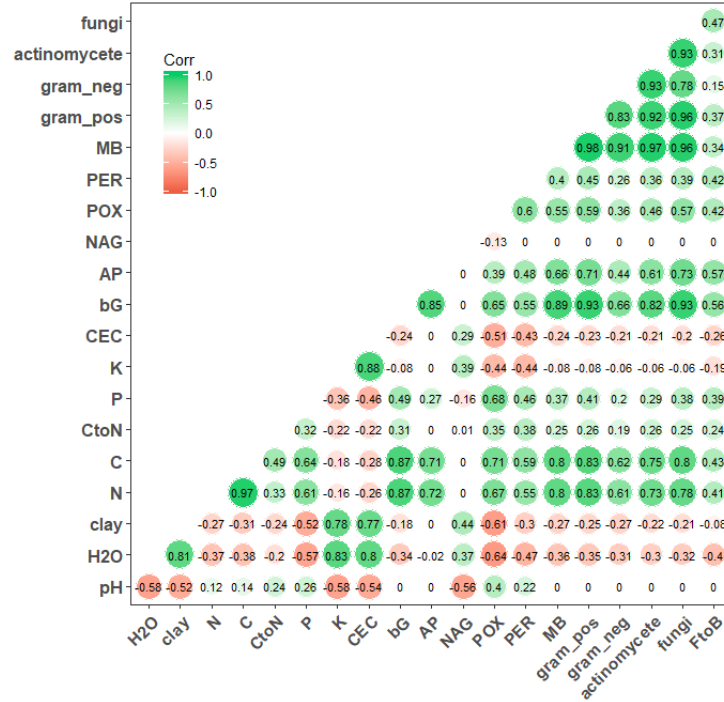


Figure A-1. Changes in soil clay content with depth for individual sampling sites. CT1~4: Sites under conventional tillage practice; HM1~3: sites under hay meadow practice; NT1~5: sites under no-till practice. The HM1 site was close to a transition to a Dennis silt loam soil, rather than the Parsons silt loam.

A)



B)

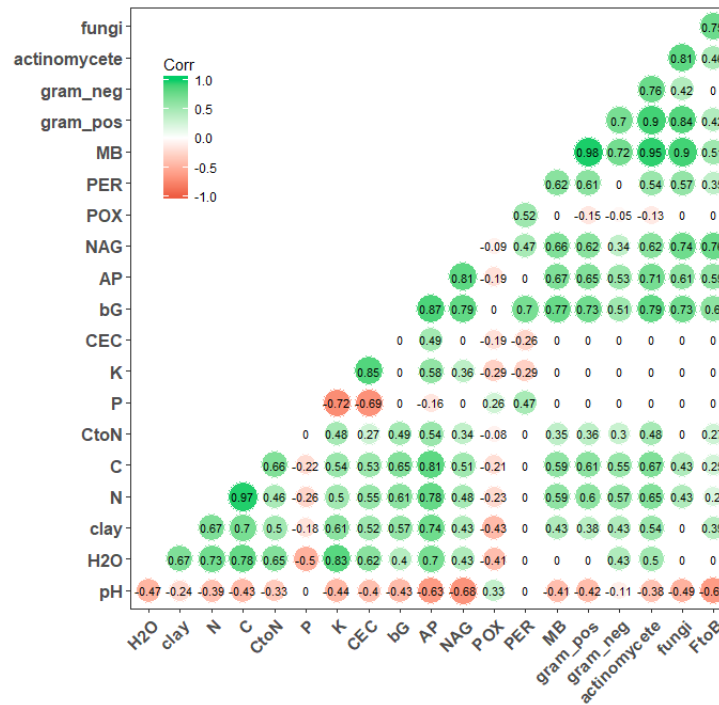


Figure A-2. The correlation between all measured soil properties A) throughout the soil profile; B) within the claypan layer (below 35 cm depth). The Pearson correlation coefficient is given in each circle; insignificant levels are 0. pH; H2O, g g⁻¹; Clay, g g⁻¹; N, g kg⁻¹; C, g kg⁻¹; CtoN: C:N ratio; P, ppm; K, ppm; bG: β -glucosidase; AP: acid phosphatase; NAG: N-acetyl glucosidase; POX: phenol oxidase; PER: peroxidase, nmol hr⁻¹ g⁻¹ soil; MB: PLFA microbial biomass; gram_pos: gram-positive bacteria; gram_neg: gram-negative bacteria; actinomycete; fungi, nmol PLFA g⁻¹ soil; FtoB: fungal:bacterial ratio.

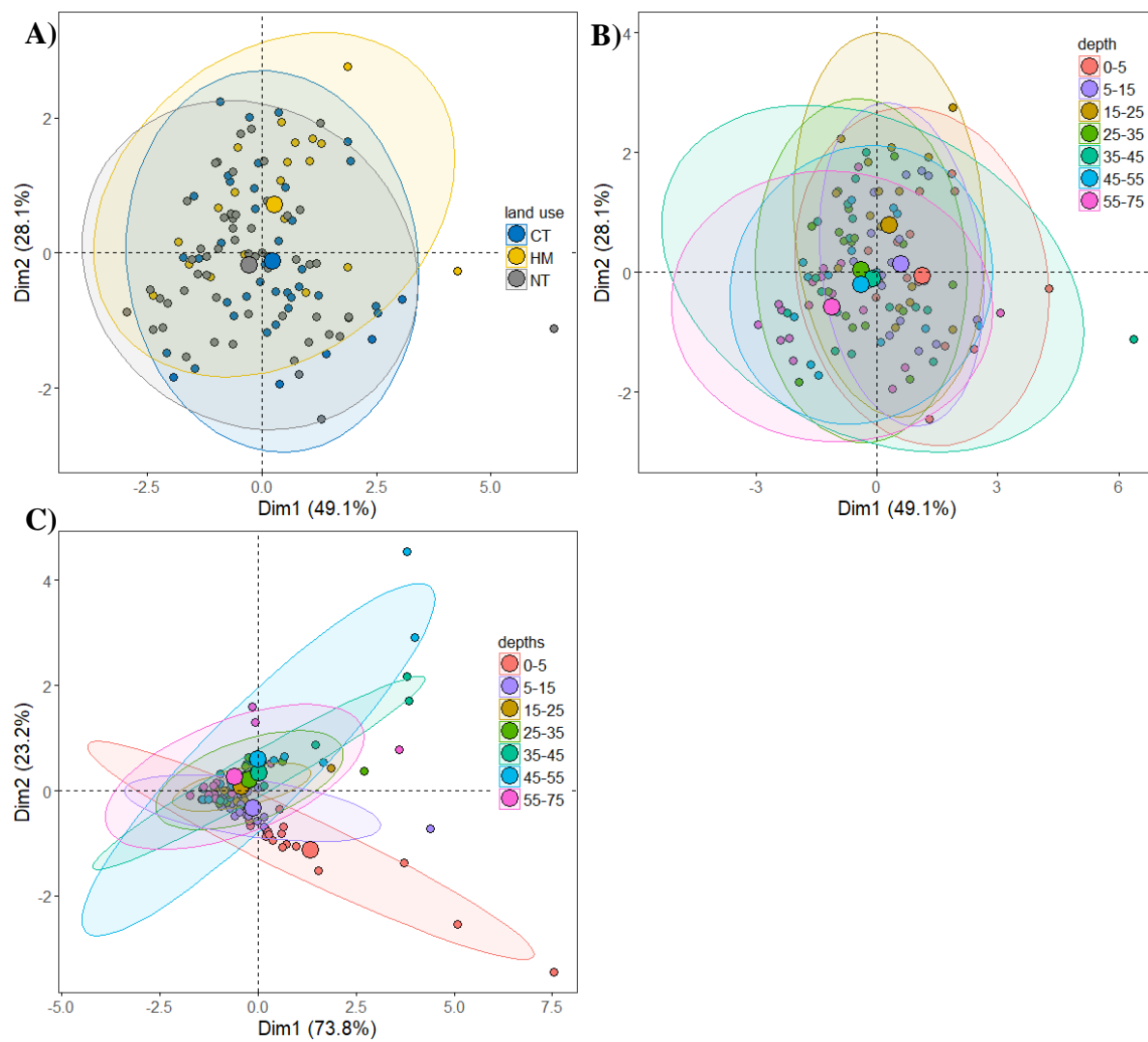


Figure A-3. The correlation between A) soil microbial community composition and management practices; B) soil microbial community composition and depth; C) hydrolase activity and depth. Soil microbial community composition assessed as PLFA relative abundance by using principal component analysis (PCA). The centroid was marked as a big solid point, indicating the group mean point of the site scores on each axis.

Table A-1. The ANOVA table of soil properties.

Type III Tests of Fixed Effects										
pH										
Effect	Num DF	Den DF	F value	Pr > F	Den DF	F value	Pr > F	Den DF	F value	Pr > F
Trt	2	91.9	6.22	0.0029	56	17.06	<.0001	26.94	29.4	<.0001
Depth	6	93.09	15.76	<.0001	56	1.52	0.1438	20.71	16.87	<.0001
Trt*depth	12	93	2.47		56	0.72	0.7254	21.97	5.47	0.0003
bG activity										
Effect	Num DF	Den DF	F value	Pr > F	Den DF	F value	Pr > F	Den DF	F value	Pr > F
Trt	2	97.07	26.86	<.0001	4.962	5.29	0.0589	42.84	46.48	<.0001
Depth	6	95.32	51.36	<.0001	40.22	20.09	<.0001	23.2	27.28	<.0001
Trt*depth	12	95.32	7.31	<.0001	37.49	4.27	0.0003	23.23	7.34	<.0001
Microbial biomass										
Effect	Num DF	Den DF	F value	Pr > F	Den DF	F value	Pr > F	Den DF	F value	Pr > F
Trt	2	97.07	26.86	<.0001	4.962	5.29	0.0589	42.84	46.48	<.0001
Depth	6	95.32	51.36	<.0001	40.22	20.09	<.0001	23.2	27.28	<.0001
Trt*depth	12	95.32	7.31	<.0001	37.49	4.27	0.0003	23.23	7.34	<.0001
Gram-pos bacteria										
Effect	Num DF	Den DF	F value	Pr > F	Den DF	F value	Pr > F	Den DF	F value	Pr > F
Trt	2	97	16.74	<.0001	7.386	9.68	0.0086	7.449	3.78	0.0736
Depth	6	95.35	35.73	<.0001	27.95	5.43	0.0008	16.31	3.91	0.0131
Trt*depth	12	95.35	3.63	0.0002	25.98	3.73	0.0024	17.74	1.66	0.1625
Gram-neg bacteria										
Effect	Num DF	Den DF	F value	Pr > F	Den DF	F value	Pr > F	Den DF	F value	Pr > F
Trt	2	98	0.63	0.534	96.26	6.53	0.0022	15	16	0.0002
Depth	6	98	13.92	<.0001	95.54	34.84	<.0001	19.5	7.45	0.0003
Trt*depth	12	98	0.82	0.628	95.54	1.51	0.1321	21.3	3.46	0.006
Actinomycete										
Effect	Num DF	Den DF	F value	Pr > F	Den DF	F value	Pr > F	Den DF	F value	Pr > F
Trt	2	98	0.63	0.534	96.26	6.53	0.0022	15	16	0.0002
Depth	6	98	13.92	<.0001	95.54	34.84	<.0001	19.5	7.45	0.0003
Trt*depth	12	98	0.82	0.628	95.54	1.51	0.1321	21.3	3.46	0.006
16:1ω5										
Effect	Num DF	Den DF	F value	Pr > F	Den DF	F value	Pr > F	Den DF	F value	Pr > F
Trt	2	5.69	1.57	0.2856	96.96	27.06	<.0001	91.43	38.46	<.0001
Depth	6	63.07	26.18	<.0001	95.39	17.96	<.0001	91.73	71.84	<.0001
Trt*depth	12	59.9	1.11	0.3717	95.39	1	0.4524	91.76	13.38	<.0001
PER activity										
Effect	Num DF	Den DF	F value	Pr > F	Den DF	F value	Pr > F	Den DF	F value	Pr > F
Trt	2	3.696	0.12	0.8937	18.73	1.5	0.2491	3.674	21.73	0.0092
Depth	6	16.49	16.71	<.0001	48.39	4.05	0.0023	17.88	14.43	<.0001
Trt*depth	12	13.32	1.34	0.3021	45.07	0.35	0.9746	18.59	7.21	<.0001
Specific hydrolase activity										
Effect	Num DF	Den DF	F value	Pr > F	Den DF	F value	Pr > F	Den DF	F value	Pr > F
Trt	2	3.696	0.12	0.8937	18.73	1.5	0.2491	3.674	21.73	0.0092
Depth	6	16.49	16.71	<.0001	48.39	4.05	0.0023	17.88	14.43	<.0001
Trt*depth	12	13.32	1.34	0.3021	45.07	0.35	0.9746	18.59	7.21	<.0001
Fungi										
Effect	Num DF	Den DF	F value	Pr > F	Den DF	F value	Pr > F	Den DF	F value	Pr > F
Trt	2	94.34	2.09	0.1288	91.98	1.73	0.1824	102	42.51	<.0001
Depth	6	94.58	9.3	<.0001	91.58	1.11	0.3607	102	9.73	<.0001
Trt*depth	12	94.59	1.17	0.3183	91.61	1.32	0.2204	102	2.25	0.0144
Fungal:Bacterial										
Effect	Num DF	Den DF	F value	Pr > F	Den DF	F value	Pr > F	Den DF	F value	Pr > F
Trt	2	94.34	2.09	0.1288	91.98	1.73	0.1824	102	42.51	<.0001
Depth	6	94.58	9.3	<.0001	91.58	1.11	0.3607	102	9.73	<.0001
Trt*depth	12	94.59	1.17	0.3183	91.61	1.32	0.2204	102	2.25	0.0144

Equation A-1. SAS code for analysis in Chapter 2

```
proc means data=work.import;
class Trt dcode; *dcode = depth code, included 2.5, 10, 20, 30, 40, 50, 65 cm deep
Var
Microbial_biomass
group_total
gram_positive
gram_negative
actinomycete
AMF
fungi
N C C_N P K CEC
;
output out=PLFA_Trtddepth mean= stderr= /autoname;
run;
proc print data=PLFA_Trtddepth;
run;
-----
*PLFA;
proc glimmix data=work.import plots=studentpanel;
class Trt rep depth;
model microbial_biomass=Trt|depth /ddfm=kr;
*model gram_positive=Trt|depth /ddfm=kr;
*model gram_negative=Trt|depth /ddfm=kr;
*model actinomycete=Trt|depth /ddfm=kr;
*model AMF=Trt|depth /ddfm=kr;
*model fungi=Trt|depth /ddfm=kr;
random rep ;
*random depth/subject=rep(Trt) type=ar(1);
*random depth/subject=rep(TrtVar) type=cs;
*random depth/subject=rep(TrtVar) type=un;
*random depth/subject=rep(TrtVar) type=TOEP;
random depth/subject=rep(TrtVar) type=vc;
*random depth/subject=rep(TrtVar) type=arh(1);
*random depth/subject=rep(TrtVar) type=csh;
*random depth/subject=rep(TrtVar) type=toeph;
lsmeans Trt*depth/slicediff=depth slicediff=Trt pdiff plot=meanplot(sliceby=Trt cl);
nloptions tech=nrridg;
run;
-----
*EEA (extracellular enzyme activity);
proc glimmix data=work.import plots=studentpanel;
class Trt rep depth;
model BG=Trt|depth /ddfm=kr;
*model Phos=Trt|depth /ddfm=kr;
```

```

*model NAG=Trt|depth /ddfm=kr;
*model POX=Trt|depth /ddfm=kr;
*model PER=Trt|depth /ddfm=kr;
*model hydrolase=Trt|depth /ddfm=kr;
*model oxidase=Trt|depth /ddfm=kr;
random rep ;
random depth/subject=rep(Trt) type= vc;
lsmeans Trt*depth/slicediff=depth slicediff=Trt pdiff plot=meanplot(sliceby=Trt cl);
nloptions tech=nrridg;
run;
*****;
*Chemical properties;
proc glimmix data=work.import plots=studentpanel;
class Trt rep depth;
model clay=Trt|depth /ddfm=kr;
*model moisture=Trt|depth /ddfm=kr;
*model ph=Trt|depth /ddfm=kr;
*model N=Trt|depth /ddfm=kr;
*model C=Trt|depth /ddfm=kr;
*model P=Trt|depth /ddfm=kr;
*model K=Trt|depth /ddfm=kr;
random rep ;
random depth/subject=rep(Trt) type= vc;
lsmeans Trt*depth/slicediff=depth slicediff=Trt pdiff plot=meanplot(sliceby=Trt cl);
*lsmeans Trt*depth/pdiff lines plot=meanplot(sliceby=Trt|depth join)lines;
nloptions tech=nrridg;
output out=giad student=student;
run;

```

Appendix B - Chapter 3

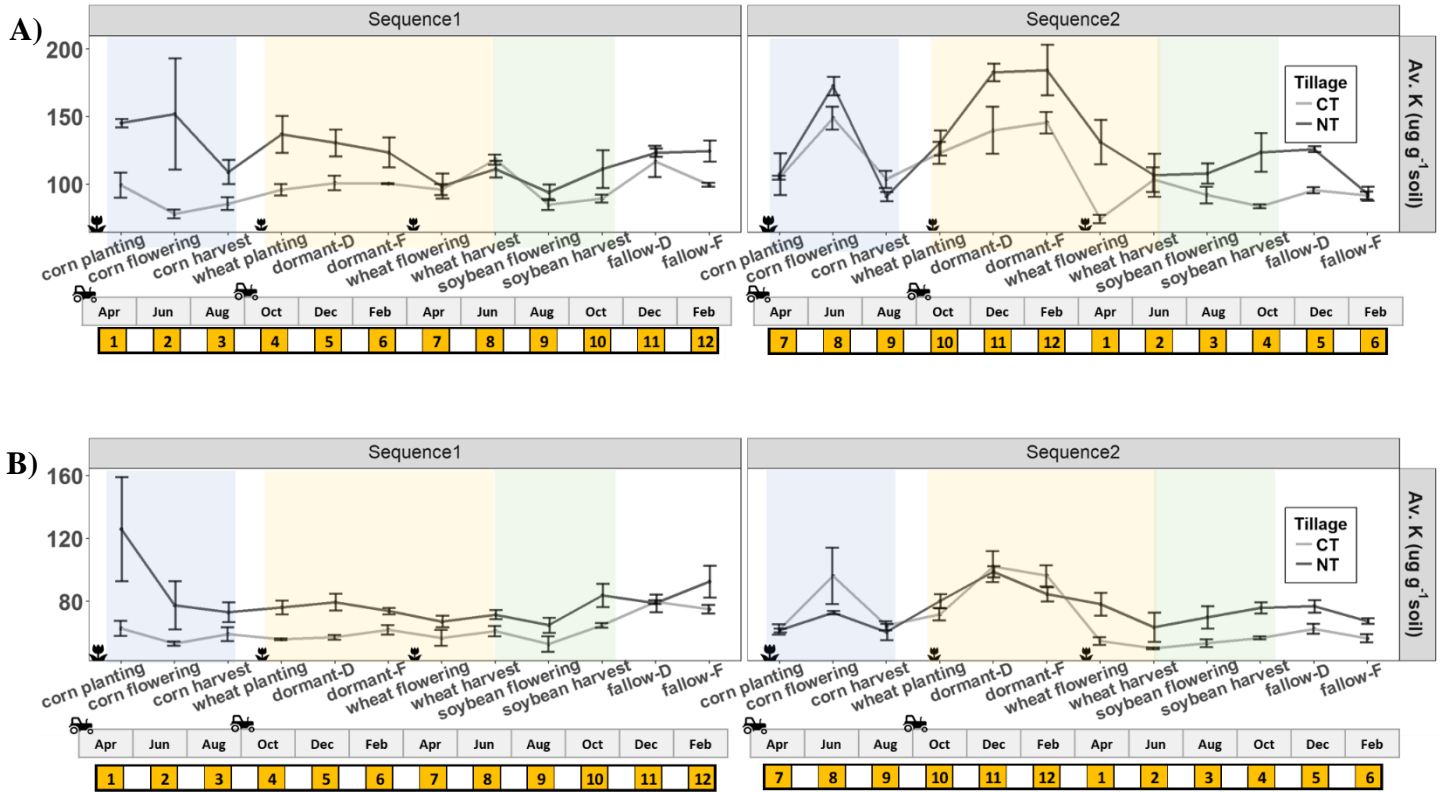


Figure B-1. Temporal changes in plant available potassium for conventional tillage (CT) and no-till (NT) during the crop development stages at A) 0-5 and B) 5-15 cm. Dormant-D, wheat dormant stage in December; dormant-F, wheat dormant stage in February; fallow-D, fallow in December; fallow-F, fallow in February. Crop stages are indicated by shaded areas: corn (blue), wheat (yellow), soybean (green), and fallow (white). Sample numbers are given in the bar at the bottom (1-12). Flowers (🌻) indicate mineral fertilizer application; tractors (🚜) indicate tillage event. Results are given as means \pm standard error (n=8).

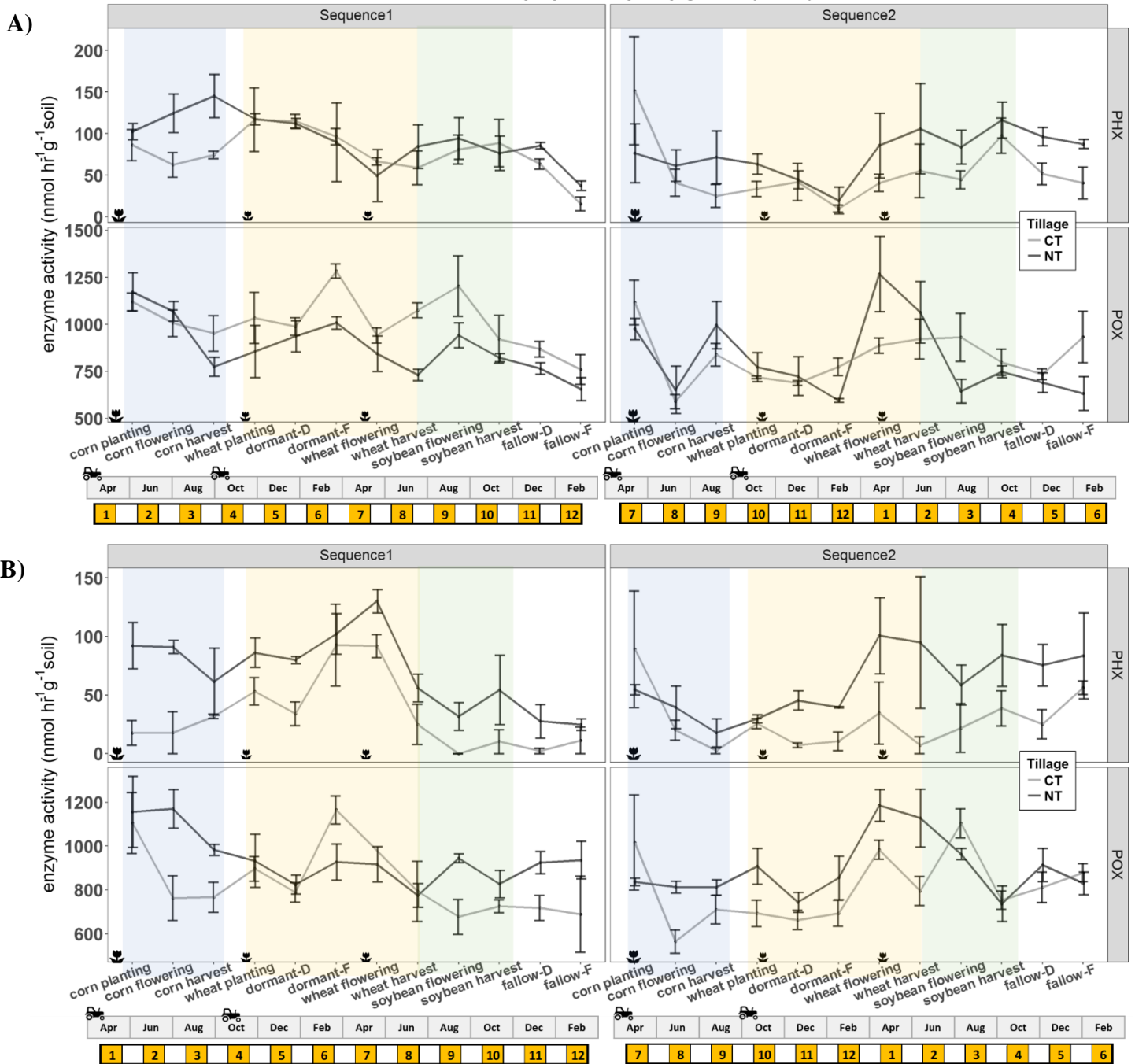
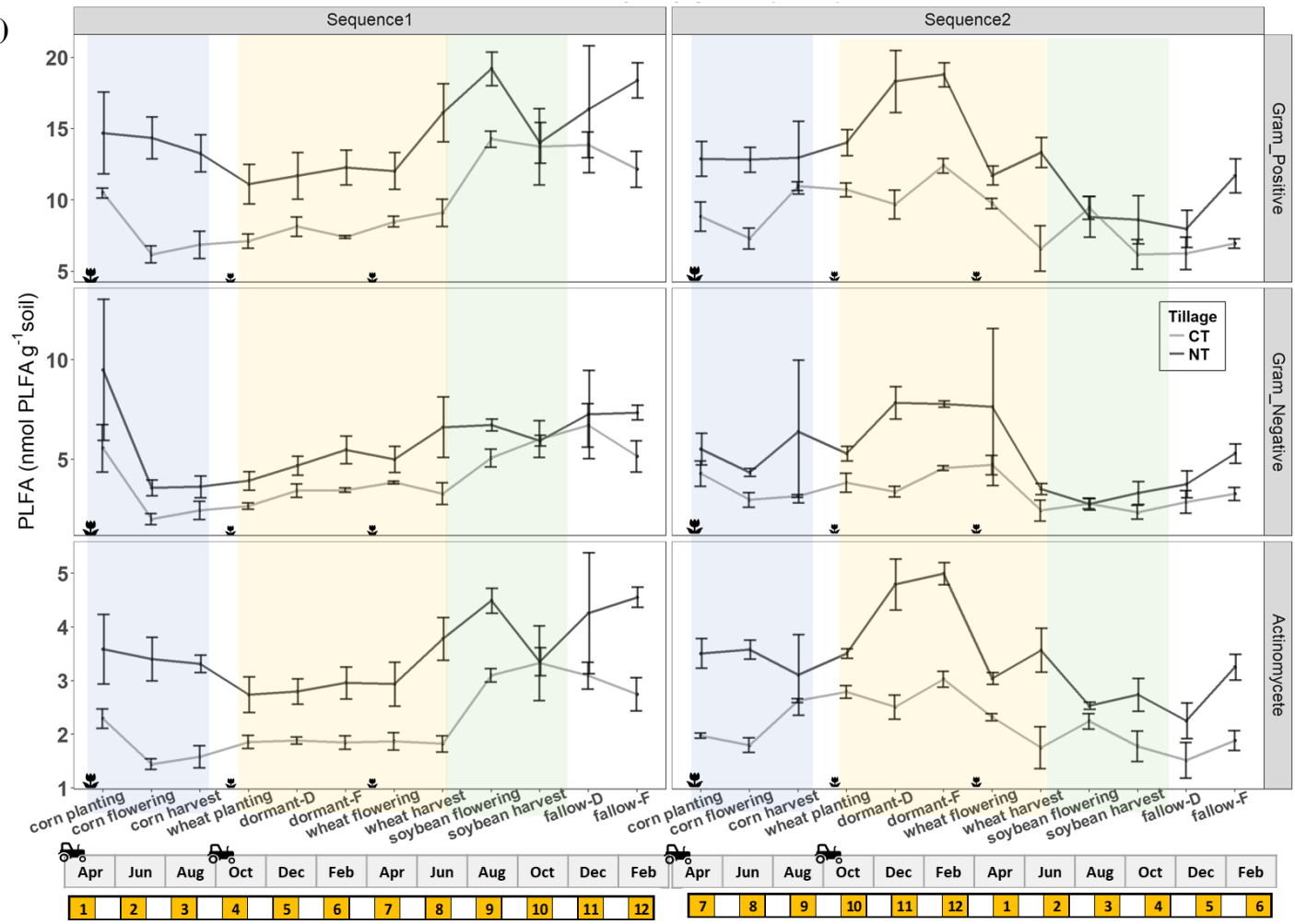


Figure B-2. Temporal changes in soil extracellular enzyme activities for conventional tillage (CT) and no-till (NT) during the crop development stages at A) 0-5 and B) 5-15 cm. Dormant-D, wheat dormant stage in December; dormant-F, wheat dormant stage in February; fallow-D, fallow in December; fallow-F, fallow in February. Crop stages are indicated by shaded areas: corn (blue), wheat (yellow), soybean (green), and fallow (white). Sample numbers are given in the bar at the bottom (1-12). PHX, phenol oxidase; POX, peroxidase. Flowers (☘) indicate mineral fertilizer application; tractors (🚜) indicate tillage event. Results are given as means \pm standard error (n=8).

A)



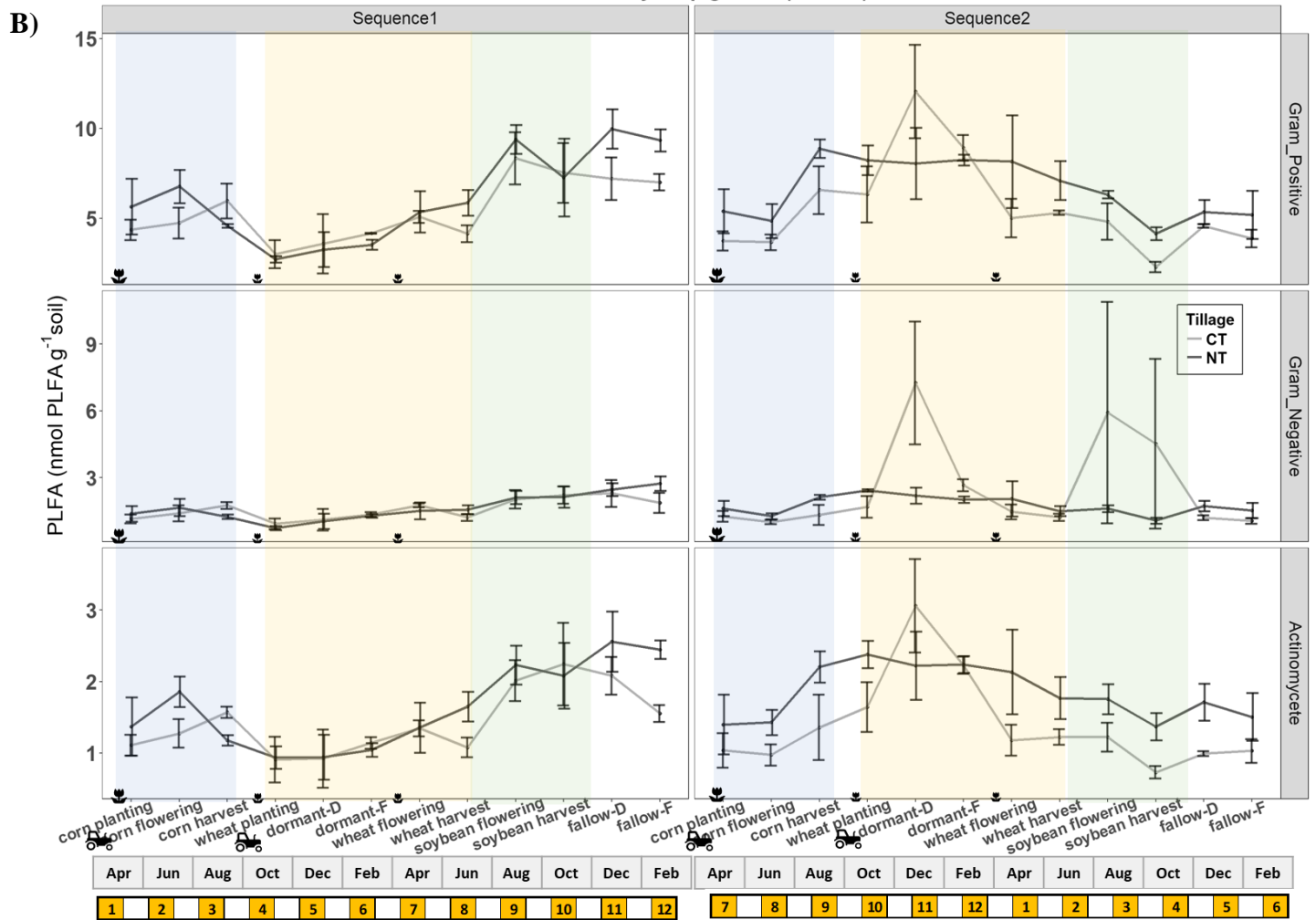


Figure B-3. Temporal changes in soil PLFAs for conventional tillage (CT) and no-till (NT) during the crop growth periods at A) 0-5 and B) 5-15 cm. Dormant-D, wheat dormant stage in December; dormant-F, wheat dormant stage in February; fallow-D, fallow in December; fallow-F, fallow in February. Crop stages are indicated by shaded areas: corn (blue), wheat (yellow), soybean (green), and fallow (white). Sample numbers are given in the bar at the bottom (1-12). Flowers (♣) indicate mineral fertilizer application; tractors (🚛) indicate tillage event. Results are given as means \pm standard error (n=8).

Table B-1. Summary of the p-values from ANOVA for effects of treatment and crop stages on soil properties. Dependent variables were log-transformed when necessary to meet the assumption of normality. Av. K: available potassium; PHX, phenol oxidase; POX, peroxidase.

A) 0-5 cm	ln(Av.K)	ln(PHX)	POX
Tillage	<0.001***	0.126	0.608
Sequence	<0.001***	0.268	0.002**
Crop stage	0.002**	0.278	0.002**
Tillage* Sequence	0.006**	0.676	0.997
Crop stage* Sequence	<0.001***	0.002**	0.008**
Tillage*Crop stage	0.001**	0.945	0.338
Tillage*Crop stage* Sequence	<0.001***	0.756	0.075

	Gram_positive	Gram_negative	Actinomycete
Tillage	<0.001***	0.038*	<0.001***
Sequence	0.596	0.123	0.472
Crop stage	<0.001***	<0.001***	<0.001***
Tillage* Sequence	0.373	0.598	0.795
Crop stage* Sequence	<0.001***	<0.001***	<0.001***
Tillage*Crop stage	0.189	0.765	0.086
Tillage*Crop stage* Sequence	0.278	0.878	0.078

B) 5-15 cm	ln(Av.K)	ln(PHX)	POX
Tillage	0.003**	0.516	0.002**
Sequence	<0.001***	0.141	0.098
Crop stage	0.002**	0.009**	<0.001***
Tillage* Sequence	0.001**	0.879	0.346
Crop stage* Sequence	<0.001***	<0.001***	<0.001***
Tillage*Crop stage	0.020*	0.571	0.023*
Tillage*Crop stage* Sequence	<0.001***	0.290	0.008**

	Gram_positive	Gram_negative	Actinomycete
Tillage	0.178	0.679	0.163
Sequence	0.478	0.505	0.443
Crop stage	0.002**	0.179	0.002**
Tillage* Sequence	0.687	0.931	0.820
Crop stage* Sequence	<0.001***	<0.001***	<0.001***
Tillage*Crop stage	0.579	0.988	0.452
Tillage*Crop stage* Sequence	0.380	0.470	0.245

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

*** Significant at the 0.001 probability level

Equation B-1. R code for analysis in Chapter 3

```
cat("active.C..mg.kg.soil.")
model2_c <- lmer(active.C..mg.kg.soil. ~ Trt + depth + depth * Trt + growing.
phase * depth + Rotation * Trt + growing.phase + growing.phase * Rotation + R
otation * depth + Trt * growing.phase * Rotation * depth + (1 | BLK) + (Rotat
ion | BLK), data = soilTA, na.action = na.omit)
shapiro.test(resid(model2_c))
anova(model2_c, type = "marginal", adjustSigma = F)
marginal_c <- lsmeans(model2_c, specs = "Trt", by = c("growing.phase", "Rotat
ion", "depth"))
marginal_c1 <- lsmeans(model2_c, specs = "growing.phase", by = c("Trt", "Rota
tion", "depth"))
marginal_c2 <- lsmeans(model2_c, specs = "Rotation", by = c("growing.phase",
"Trt", "depth"))
marginal_c3 <- lsmeans(model2_c, specs = "depth", by = c("growing.phase", "Tr
t", "Rotation"))
cld(marginal_c, alpha = 0.05, sort = T, Letters = letters, adjust = "tukey")
cld(marginal_c1, alpha = 0.05, sort = T, Letters = letters, adjust = "tukey")
cld(marginal_c2, alpha = 0.05, sort = T, Letters = letters, adjust = "tukey")
cld(marginal_c3, alpha = 0.05, sort = T, Letters = letters, adjust = "tukey")
```

The complete R codes and row dataset are available at:

[https://drive.google.com/drive/folders/1xOkK8HOKqWAOumaZ3j7ua3baInaQhKP7?usp=shari
ng](https://drive.google.com/drive/folders/1xOkK8HOKqWAOumaZ3j7ua3baInaQhKP7?usp=sharing)

Appendix C - Chapter 4

Pedon description and soil texture data

1. Soil Survey Staff (1999).

Ap - 0 to 13 cm; very dark brown (10YR 2/2) exterior and very dark grayish brown (10YR 3/2) crushed silt loam; 26 percent clay; moderate medium granular structure; friable; many fine roots throughout; abrupt smooth boundary.

Ap – 13 to 29 cm; very dark brown (10YR 2/2) exterior silt loam; 28 percent clay; moderate coarse angular blocky structure; firm; common fine roots between peds; clear smooth boundary.

The structure of this horizon is related to compaction

Ab – 29 to 66 cm; very dark brown (10YR 2/2) exterior and very dark grayish brown (10YR 3/2) crushed silty clay loam; 30 percent clay; moderate medium granular structure; friable; common fine roots between peds; gradual smooth boundary.

Bwb – 66 to 113 cm; very dark grayish brown (10YR 3/2) exterior and dark brown (10YR 3/3) crushed silty clay loam; 33 percent clay; weak medium prismatic parts to moderate medium subangular blocky structure; friable; common fine roots between peds; many fine high continuity tubular pores; 1 percent faint 10YR 3/2), moist, clay films on faces of peds; iron manganese concretions; 1 percent fine irregular; gradual smooth boundary. There was much discussion about the few patchy clay films observed in this horizon, the conclusion was that the Ap1 and Ap2 are post settlement alluvium and that the original A starts at 29 cm. There is not a big enough clay increase from the Ab to the underlying horizons to qualify for an argillic horizon.

Bwb2 – 113 to 145 cm; very dark grayish brown (10YR 3/2) exterior and dark brown (10YR 3/3) crushed silty clay loam; 34 percent clay; 15 percent fine and medium distinct (10YR 5/2) mottles; weak medium prismatic parts to moderate medium subangular blocky structure; friable; common fine roots between peds; many fine high continuity tubular pores; 1 percent faint 10YR 3/2), moist, clay films on faces of peds; iron manganese concretions; 5 percent fine spherical between peds. Clay films in this horizon are similar to the horizon above and have a similar interpretation

Table C-1. Particle size analysis from Soil Survey Staff (1999). SiL, silty loam.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Texture
0-13	6.8	74.6	18.6	SiL
13-29	6.7	71.6	21.7	SiL
29-66	5.0	70.8	24.2	SiL
66-113	4.2	74.1	21.7	SiL
113-145	5.5	72.9	21.6	SiL

2. Harris (1993).

Soil series: Kennebec

Classification: fine-silty, mixed, mesic Cumulic Hapludoll

Physiography: floodplain

Parent materials: recent alluvium over old alluvium (post settlement alluvium)

Vegetation: plowed field

Hydraulic conductivity: moderately low

Drainage class: moderately well drained

Described by: Ransom, M. D., W. A. Wehmueller

Date: July 10, 1991

Ap - 0 to 17 cm, very dark brown (10YR 2/2) silt loam; dark grayish brown (10YR 4/2) dry; hard, friable; many fine roots throughout; few very fine and fine tubular pores; the structure is large clods that parts to small granular, vertical cracks 0.5 to 1 mm wide are between the large clods; the layer from 15 to 17 cm has thin platy structure from compaction; abrupt smooth boundary.

A - 17 to 32 cm; very dark brown (10YR 2/2) silt loam; 70% dark grayish brown (10YR 4/2), and 30% dark grayish brown (10YR 3/2) dry; weak fine subangular blocky structure; hard, friable; many fine roots throughout; common very fine and fine tubular pores; few horizontal cracks 0.5 mm wide; clear wavy boundary.

C - 32 to 41 cm; stratified very dark brown (10YR 2/2), and dark brown (10YR 3/3) silt loam; dark grayish brown (10YR 4/2), and brown to dark brown (10YR 4/3) dry; weak thin platy structure; hard, friable; many fine roots throughout; few very fine and fine tubular pores; abrupt wavy boundary.

Ab - 41 to 59 cm; black (10YR 2/1) silty clay loam; very dark gray (10YR 3/1) dry, weak medium subangular blocky structure parting to moderate medium granular; slightly hard, friable; many fine roots throughout; common very fine and fine tubular, and few medium tubular pores; clear wavy boundary.

A/Eb - 59 to 71 cm; very dark grey (10YR 3/1) silt loam; dark grayish brown (10YR 4/2), and grayish brown (10YR 5/2) exterior dry; moderate fine subangular blocky structure parting to

moderate medium granular; hard, friable; common fine roots throughout; common very fine and fine tubular, and few medium tubular pores; common distinct light brownish gray (10YR 6/2) continuous skeletal (sand or silt) on vertical and horizontal faces of peds; abrupt wavy boundary.

A'b - 71 to 85 cm; very dark grey (10YR 3/1) silty clay loam; dark grayish brown (10YR 4/2) dry; few very distinct brown to dark brown (10YR 4/3) mottles; moderate medium angular blocky structure; very hard, firm; common fine roots throughout; few very fine and fine tubular pores; few vertical cracks less than 0.5 mm wide; clear wavy boundary.

ABb - 85 to 121 cm; dark grayish brown (10YR 4/2) silty clay loam; grayish brown (10YR 5/2) dry; common fine distinct dark yellowish brown (10YR 4/4) mottles; moderate medium prismatic structure; very hard, firm; common fine roots throughout; few very fine and fine tubular pores; many distinct very dark gray (10YR 3/1) continuous organic coats on vertical and horizontal faces of peds, and very few very dark gray (10YR 3/1) discontinuous clay films (cutans) on faces of peds and in pores; few very fine gypsum crystals on faces of peds; gradual wavy boundary.

Btb1 - 121 to 155 cm; dark grayish brown (10YR 4/2) silty clay loam; few fine distinct dark yellowish brown (10YR 4/4) mottles; weak medium prismatic structure parting to moderate medium subangular blocky; hard, firm; few very fine roots throughout; few very fine and fine tubular pores; common distinct very dark gray (10YR 3/1) discontinuous organic coats on vertical and horizontal faces of peds, and very few very dark gray (10YR 3/1) clay films (cutans) on faces of peds and in pores; few very fine gypsum crystals on faces of peds; clear wavy boundary.

Btb2 - 155 to 193 cm; dark grayish brown (10YR 4/2) silty clay loam; common medium distinct brown (10YR 5/3), and few medium distinct yellowish brown (10YR 5/4) mottles; weak medium prismatic structure parting to moderate medium subangular blocky; hard, firm; few very fine roots throughout; common very fine and fine tubular pores; few distinct very dark gray (10YR 3/1) discontinuous organic coats on vertical and horizontal faces of peds, and many very dark gray (10YR 3/1) continuous clay films (cutans) on faces of peds and in pores; common very fine gypsum crystals on faces of peds; gradual wavy boundary.

Btb3 - 193 to 233 cm; dark grayish brown (10YR 4/2) clay loam; common medium distinct brown (10YR 5/3), and few medium distinct yellowish brown (10YR 5/4) mottles; weak medium

prismatic structure parting to moderate medium subangular blocky; very hard, firm; few very fine roots throughout; common very fine and fine tubular pores; many very dark gray (10YR 3/1) continuous clay films (cutans) on faces of peds and in pores; common very fine gypsum crystals on faces of peds.

Table C-2. Particle size analysis from Harris (1993). SiL, silty loam; SiCL, silty clay loam.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Texture
0-5	8.78 (5.57)	69.20 (4.49)	22.02 (2.15)	SiL
5-15	7.80 (3.23)	71.69 (0.82)	20.51 (3.60)	SiL
15-30	5.39 (1.08)	71.57 (3.04)	23.05 (1.96)	SiL
30-45	3.70 (1.63)	70.22 (0.68)	26.07 (1.84)	SiL
45-60	4.36 (1.85)	66.50 (0.49)	29.14 (1.38)	SiCL
60-90	9.01 (4.48)	64.76 (3.04)	29.70 (2.33)	SiCL
90-120	15.28 (1.46)	62.75 (8.42)	28.24 (3.93)	SiCL

Standard deviations below percentage.

Reference

Soil Survey Staff, 1999. Official Soil Series Descriptions. USDA-Natural Resources Conservation Service. URL

<https://ncsslslabdatamart.sc.egov.usda.gov/rptExecute.aspx?p=52437&r=1&r=2&r=3&r=4&r=6&r=9&r=10&>

Harris, J.G., 1993. Source and fate of N under no-tillage and conventional tillage corn production. Kansas State University.

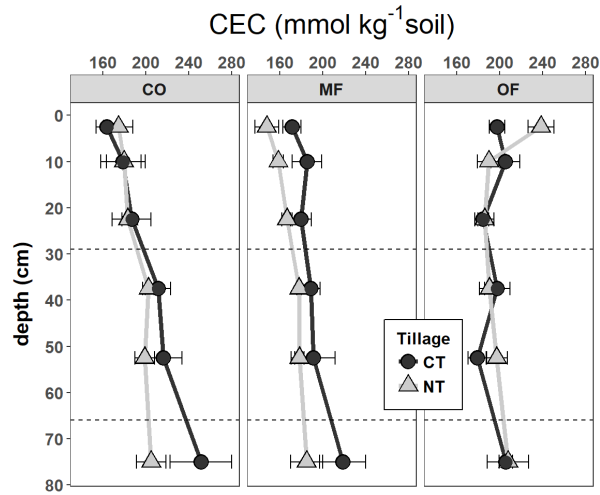


Figure C-1. Vertical changes in soil cation exchange capacity for conventional tillage (CT) and no-till (NT) under organic fertilizer (OF), mineral fertilizer (MF), and unfertilized control (CO). Buried A horizon was located at 29 to 66 cm and marked by dash lines. Results are given as means \pm standard error of the mean.

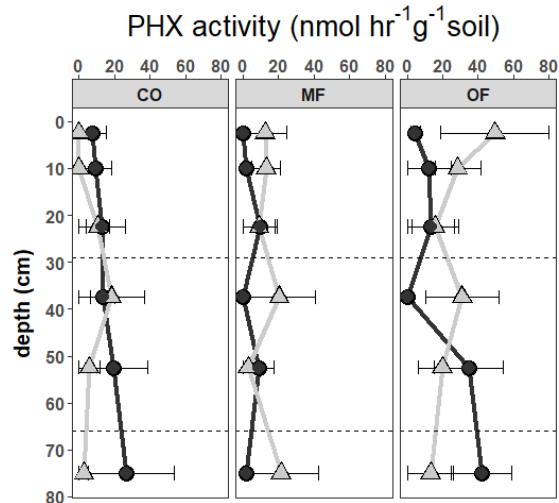


Figure C-2. Vertical changes in soil peroxidase activity with for conventional tillage (CT) and no-till (NT) under organic fertilizer (OF), mineral fertilizer (MF), and unfertilized control (CO). Buried A horizon was located at 29 to 66 cm and marked by dash lines. Results are given as means \pm standard error of the mean.

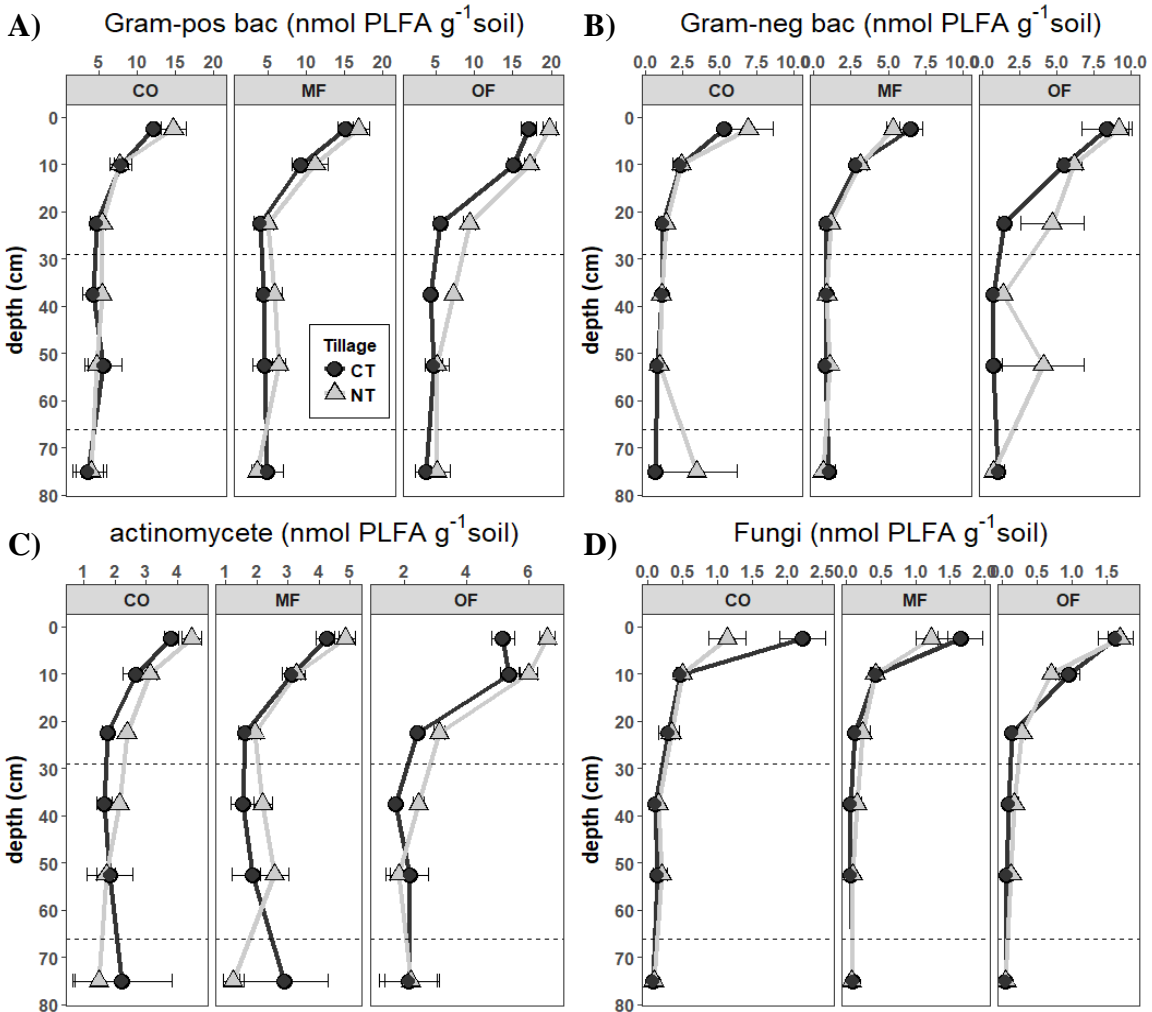


Figure C-3. Changes in soil microbial PLFAs with depth for conventional tillage (CT) and no-till (NT) under organic fertilizer (OF), mineral fertilizer (MF), and unfertilized control (CO). Buried A horizon was located at 29 to 66 cm and marked by dash lines. Results are given as means \pm standard error of the mean. Buried A horizon was located from 29 to 66 cm; Gram-pos, Gram-positive bacteria; Gram-neg, gram-negative bacteria

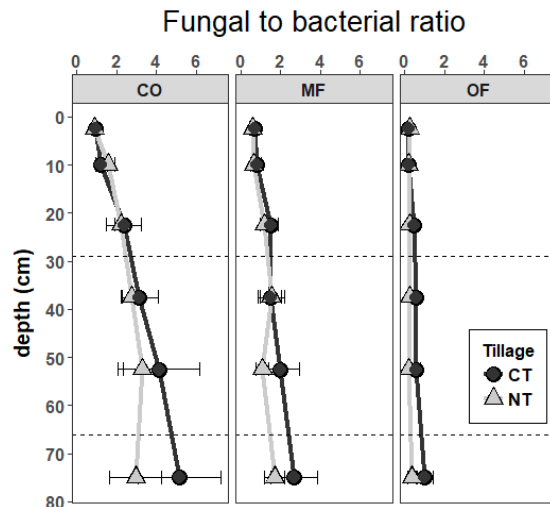


Figure C-4. Changes in soil fungal to bacterial ratio with depth for different fertilizer amendments and tillage regimes. Buried A horizon was located at 29 to 66 cm and marked by dash lines. Results are given as means \pm standard error of the mean. Buried A horizon was located from 29 to 66 cm.

Table C-3. Summary of the p-value from ANOVA for effects of tillage, depth, and fertilizer amendment regimes on soil properties. CEC, cation exchange capacity; PHX, phenol oxidase; Gram (+), gram-positive bacteria; Gram (-), gram-negative bacteria; Acti., actinomycete; F:B, fungal to bacterial ratio.

	CEC	PHX	Gram(+)	Gram(-)	Acti.	Fungi	F:B
Tillage	0.409	0.767	0.505	0.653	0.655	0.059	0.974
Fertilizer	0.108	0.983	0.375	0.681	0.564	0.587	0.601
Depth	0.005	0.895	0.003	0.002	0.042	<0.001	0.002
Tillage*fertilizer	0.012	0.101	0.959	0.786	0.915	0.327	0.991
Fertilizer*depth	0.073	0.858	0.535	0.801	0.896	0.232	0.201
Tillage*depth	0.264	0.854	0.961	0.761	0.976	0.280	0.235
Tillage*fertilizer*depth	0.441	0.581	0.714	0.714	0.478	0.984	0.993

Equation C-1. R code for analysis in Chapter 4

```
model_C <- lme(totalC ~ Tillage + fertilizer + depth + fertilizer * Tillage +
depth * fertilizer + depth * Tillage + depth * Tillage * fertilizer, random =
  list(~1 | BLK, ~1 | depth), correlation = corAR1(), data = soilN, na.action
= na.exclude)
shapiro.test(resid(model_C))
anova.lme(model_C, type = "marginal", adjustSigma = F) #Type III
model_C.rg1 <- ref.grid(model_C)
marginal_C1 <- lsmeans(model_C, specs = c("Tillage", "fertilizer"), by = "dep
th")
marginal_C2 <- lsmeans(model_C, specs = "depth", by = c("Tillage", "fertilize
r"))
cld(marginal_C1, alpha = 0.05, sort = T, Letters = letters, adjust = "tukey")
cld(marginal_C2, alpha = 0.05, sort = T, Letters = letters, adjust = "tukey")
cat("using contrast function to compare means")
### using 'contrast' function to compare means marginal_C1
contrast(marginal_C2, list(Thirtyvstwenty = c(0, 1, -1, 0, 0, 0)))
cat("----> P=0.3, no significant increase from 20 to 30 in HF")
contrast(marginal_C1, list(NTvsCTforHF = c(0, 0, 1, -1, 0, 0)))
cat("----> p=0.08, no sig difference btw CT/NT under HF @0-5")
contrast(marginal_C1, list(NTvsCTforHM = c(0, 0, 0, 0, 1, -1)))
cat("----> p=0.02, sig difference btw CT/NT in HM @0-30")
contrast(marginal_C1, list(CvsMfatCT = c(1, 0, -1, 0, 0, 0)))
contrast(marginal_C1, list(CvsMfatNT = c(0, 1, 0, -1, 0, 0)))
cat("----> p>0.3, no sig difference btw Control/mineral fertilizer in NT/CT @0
-5 or all depth")
```

The complete R codes and row dataset are available at:

<https://drive.google.com/drive/folders/1xOkK8HOKqWAOumaZ3j7ua3baInaQhKP7?usp=sharing>