Cover crop and P fertilizer management impacts on nutrient cycling capacity and the soil microbial community in a no-till corn-soybean rotation

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Abstract

Balancing crop nutrient requirements with environmental sustainability remains a critical challenge in phosphorus (P) management for grain crop production. Phosphorus is commonly a limited nutrient in crop production, requiring the application of P fertilizer in attempts to increase plant available P in order to meet crop requirements. However, excess fertilizer applications can often result in environmental disasters for soil and water quality. Soil health is a concept founded in the idea of preserving soil as a vital living resource to support plants. Optimizing soil health helps to preserve soil as a natural resource and allow for sustained crop production. The implementation of conservation management practices can enhance chemical, physical, and biological soil health metrics. The impacts that cover crops and phosphorus fertilizer management have on nutrient cycling and soil health metrics are complex and need further research.

A study was conducted in Manhattan, KS, at the Kansas agricultural watershed field laboratory during the years of 2014-2025. The study contained two levels of cover crop management: no cover (NC) and cover crop (CC) and three P fertilizer management scenarios: 2014-2019 included no phosphorus (NP), spring injected (SI), and fall surface broadcast (FB) applications with P fertilizer rates in SI and FB determined with a "build" recommendation. The SI and FB plots received 61 kg P_2O_5 ha^{-1} annually. The P management goal changed in 2020-2022 and aimed to maintain P in the SI plots and begin the drawdown of P in the FB plots. The maintenance P management approach included 55 kg P_2O_5 ha^{-1} applied to the SI plots, while both the drawdown P approach in the former FB plots and the NP control plots received no P fertilizer during these years. At this point, P fertilizer treatment labels shifted to no phosphorus (NP), sufficiency (SUF), and build and maintain (BAM). This shift in treatment labels better

reflects the goals of each P management scenario from 2023 to 2025. During the years of 2023-2025, the P managements goals were to follow the Kansas State University build and maintain or sufficiency recommendations. In 2023, the SI plots (BAM) received 53 kg P_2O_5 ha^{-1}, SUF plots received 23 kg P_2O_5 ha^{-1}, and the NP plots continued to remain a no P control. The experiment site was managed in a no-till, corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) crop rotation with soybeans planted on even years and corn planted on odd years. Soil samples were collected at 0-5 cm four times during the growing season, and the times were labelled as follows: SP/T0, T1, T2, and FL. The SP/T0 sampling was taken after cover crop termination and before planting, T1 and T2 were taken during the crop growth period and fall (FL) sampling took place after grain crop harvest. The soil health indicators analyzed included: soil respiration, autoclaved citrate extractable (ACE) protein, permanganate oxidizable carbon (POXC), dissolved total N (DTN), inorganic N (NO_3-N and NH_4-N), dissolved organic N (DON), dissolved organic C (DOC), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass P (MBP), water extractable P (WEP), and soil enzyme activities (β-D-glucosaminidase, β-glucosidase, alkaline phosphatase, and acid phosphatase). The soil health indicators were analyzed for the 2024 soybean and 2025 corn growing seasons. Phospholipid fatty acid (PLFA) analysis was also analyzed for soybean years 2018, 2020, 2022, and 2024. This study aimed to assess how cover crops and P fertilizer management impacts soil health indicators that assess different nutrient pools as well as the size, activity, diversity, and community abundance of the soil biological community in a no-till, corn-soybean rotation.

Sampling time had a notable effect on many soil health indicators evaluated, which is expected as the soil microbial community is susceptible to environmental conditions which can change within a growing season and between different years. Additionally, nutrient cycling can

be impacted by the present grain crop species and the presence or absence of a cover crop. The negative effects of CC were seen in NO_3-N in 2024 and are likely due to N uptake in the CC. Autoclavable citrate extractable protein, POXC, MBC, and acid phosphatase were all increased by the presence of a CC in 2024. A P fertilizer treatment by CC interaction was observed for alkaline phosphatase, β-D-glucosaminidase, β-glucosidase, and soil respiration in 2024. β-Glucosidase and alkaline phosphatase showed an increase in NP and SUF treatments due to the CC while soil respiration and β-D-glucosaminidase increased regardless of P fertilizer treatment. The response from β-glucosidase and alkaline phosphatase can potentially be attributed to the system having no nutrient limitations. Phosphorus fertilizer treatment had a minimal effect on soil health indicators overall. Phosphorus fertilizer treatment by time interactions were present in MBC, MBP, and NH_4-N in 2024 as well as in MBN, the ratio of MBN:MBP, and NH_4-N in 2025. Nitrogen as MBN, NH_4-N, and inorganic N had variable responses depending on year and sampling time within year. Microbial biomass P and MBC:MBP were also influenced by P fertilizer treatment alone in 2025 and responded, as expected, with MBP highest in BAM, then SUF, finally NP having the lowest MBP concentrations.

Microbial abundance quantified by PLFA showed CC had a strong influence. All microbial groups and total microbial biomass were increased in the CC treatment compared to the NC treatment in both the SP and FL sampling times. Sampling year influenced the abundance of microbial groups and total microbial biomass in the SP and FL sampling times. These results agree with what we would generally expect, because environment largely impacts the microbial community and the environment tends to be variable from year to year. Phosphorus fertilizer management had an impact in a 3-way interaction between CC, P fertilizer treatment, and year in fungi, AMF, and total microbial biomass in the FL. These interactions had variable responses,

but showed increases with the CC treatment compared to NC in different P fertilizer treatments in different soybean years. Gram-positive bacteria, gram-negative bacteria and eukaryote abundance were affected by a P fertilizer management by year interaction in the SP. The control treatment had significantly lower microbial abundance than the BAM treatment that has received annual P fertilizer applications through time. The DD/SUF sufficiency or drawdown treatment was not different from either the BAM or NP.

Cover crops have the potential to provide immediate benefits as shown in the SP and early in-season sampling times as well as legacy effects throughout the growing season as shown in the FL sampling time. Agreeing with the data previous research has shown, this research highlights the impact that cover crops can have on the soil microbial community especially when used in addition to no-till conservation management. Phosphorus fertilizer management provided variable results, and no generalizations can be made about P fertilizer management decisions in regard to the soil microbial community.

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List of Abbreviations

ACE: autoclavable citrate extractble protein
AMF: arbuscular mycorrhizal fungi
BAM: build and maintain
C: carbon
CC: cover crop
DD/SUF: drawdown/sufficiency
DOC: dissolved organic carbon
DON: dissolved organic nitrogen
DTN: dissolved total nitrogen
FL: fall
GLU: β-glucosidase
MBC: microbial biomass carbon
MBN: microbial biomass nitrogen
MBP: microbial biomass phosphorus
N: nitrogen
NAG: β-D-glucosaminidase
NC: no cover
NP: no phosphorus
P: phosphorus
PLFA: phospholipid fatty acid
POXC: permanganate oxidizable carbon
SOM: soil organic matter
SP: spring
SUF: sufficiency
WEP: water extractable phosphorus

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Dedication

To my fiancé, Jacoby Kerr – for always believing in me.

Chapter 1 - Introduction

Today the agricultural community seeks a more sustainable way to produce crops while also maintaining yields and being conscious of the environment. Increased focus on soil health and conservation management are efforts towards preserving and improving soils. A key component of the objective of maintaining both production agriculture and soil health is assuring that the essential nutrients required to nourish crops are available. Nitrogen (N), phosphorus (P), and potassium (K) make up the three primary nutrients required by plants (Acosta-Martínez & Tabatabai, 2011). Phosphorus is essential to all living organisms (Correll, 1998) as they require P for energy transfer in cellular reactions (Acosta-Martínez & Tabatabai, 2011). Phosphorus is an important nutrient in plant growth and, more specifically, in crop production. Of special concern is making sure that is available to plants. Phosphorus is one of the most restricting nutrients to crops in production agriculture and is often needed in large quantities, resulting in the need to apply P to soil in the form of chemical fertilizers (Acosta-Martínez & Tabatabai, 2011). The global demand for P has been continually growing and is expected to keep growing in the future. Global consumption of P_2O_5 in P fertilizers grew 700,000 tons between 2024 and 2025 and is expected to grow an additional 3.7 million tons by 2029 (U.S. Geological Survey, 2026). The increasing demand for P fertilizer could pose a potential issue as the distribution of P throughout the world is variable. China and Morocco are the leaders in global phosphate rock mining with the United States following behind them in production (U.S. Geological Survey, 2026). This global distribution of phosphate rock could potentially have an influence on availability and pricing of P fertilizers for crop producers. Phosphorus resources are increasingly rising in value (Lehman et al., 2015). The need for P, challenged with availability and cost, creates an issue for crop producers. However, these issues are not the only problems surrounding P fertilizer use.

The widespread application of P fertilizer results in nutrient runoff from agricultural fields, which pollutes natural water resources and can result in a significant environmental issue (Balaban et al., 2017). This phenomenon, known as eutrophication, occurs from an excess of nutrients in a water source (Correll, 1998). Freshwater environments exposed to extreme amounts of P are subject to eutrophication (Correll, 1998). Eutrophication transforms aquatic environments by depleting oxygen, changing pH levels, promoting increased growth of aquatic algae and plants, and altering the quality of available plant species, which, in turn, affects the food chain (Sharpley et al., 1994). The environmental consequences of eutrophication inhibit local areas from using water sources for nearly every use from recreational purposes to drinking water (Sharpley et al., 1994). These conditions emphasize the need for sustainable P management in agriculture to balance environmental conservation with continued crop production. The implementation of intentional nutrient management in agricultural systems is pivotal in preventing environmental disasters, such as eutrophication. Understanding nutrient cycling and soil health is essential for improving nutrient management, protecting soil resources, and sustaining crop production. Recognizing the role of soil microorganisms in linking these processes may provide a pathway toward more sustainable and effective nutrient management strategies in the future.

Conservation Management Practices

The soil health movement is largely focused on the implementation of conservation management practices that help to improve soil health. Karlen et al. (2021) explain that the four main pillars of soil health management include diversification, minimizing soil disturbance, soil coverage, and a continuous living root system. Conservation management practices often fall under one or more of these pillars of soil health. These management practices all help to protect

soils by preventing wind and water erosion and promoting functional biological and chemical processes (Karlen et al., 2021). Implementing management practices that positively influence soil is vital, as soil health becomes a priority.

Conservation management practices are largely put in place by the United States Department of Agriculture (USDA) as a way to promote soil health and prevent degradation of soils. The USDA conservation initiative is made up of different programs, such as the conservation reserve program (CRP), the environmental quality incentives program (EQIP), and the conservation stewardship program (CSP) (Fuller et. al, 2026). These programs are carried out by the USDA-Natural Resource Conservation Service (NRCS), which is funded by the Commodity Credit Corporation (CCC) that is integrated into each legislative Farm Bill (Fuller et. al, 2026). The three main programs, CRP, EQIP, and CSP, make up over 90% of the total conservation funding that is put into effect with each Farm Bill (Fuller et. al, 2026). This funding assists producers in implementing management practices through incentives, technical assistance, and risk-management tools helping to overcome economic challenges and knowledge limitations (Agnew & Zheng, 2025). The USDA-NRCS can also assist financially by offering cost-share payments, which essentially means they will assume part of the cost of setting the conservation practice in action (USDA NRCS, 2025). Individual states also help to provide support in the form of conservation districts that align with USDA-NRCS to reach conservation goals and carry out the conservation mission. Since 1940, soil and water conservation districts across the United States have been integral to the preventative and control measures of soil erosion and any damages due to sediment and floodwater, as well as financial assistance to carry out these measures (Hexem et al., 1979).

The use of excessive tillage in agriculture has proven to be destructive to soil. The practice of eliminating soil disturbance from the use of tillage equipment in agriculture and planting directly into the residue of the previous crop is known as no-till farming (Bezboruah et al., 2024). No-till farming is consistently one of the most widely adopted conservation management practices. In 2022, across the United States there were more acres of no-till adoption compared to acres of cover crop adoption (Pastina et al., 2024). Tillage has historically been used as a method of soil preparation and weed control. Now, it is well understood that tillage can be extremely destructive to the soil and no-till adoption has become more popular as a way of preventing soil erosion (Bezboruah et al., 2024). Research studies have proven there are many benefits of implementing no-till as a conservation management practice, such as reducing the potential for soil erosion, improving soil structure, increasing water infiltration, decreasing fuel and labor cost, and building soil organic matter (SOM) (Bezboruah et al., 2024).

Cover crops are widely adopted and studied across the United States as they are a valuable conservation management practice contributing to soil health. Cover crops are becoming a favorable management tool for implementing sustainable agriculture (Hallama et al., 2021) (Chavarría et al., 2016). Mutch and Martin (1998) define cover crops as crops that are not harvested but planted for the benefits that they bring to the soil and other crops. Living vegetation provides many benefits to the soil, including, but not limited to, increased root growth, a supply of root exudates for soil microorganisms, reduced risk of runoff, and a system to move water through the soil-plant-atmosphere continuum (Karlen et al., 2021). The influence that CC's have on biological, chemical, and physical properties within the soil are the driver behind these meaningful impacts on soil health.

Cover crops have many benefits that positively impact soil health. Cover crops have the ability to help the soil recover from some of the negative impacts of tillage, including the return of microbial biomass distribution with depth (Schmidt et al., 2018). Cover crops contribute to subsurface biomass, which increases labile organic matter and stimulates microbial abundance (Blanco, 2023). Schmidt et al. (2018) found that additional nutrients supplied by cover crops expanded microbial abundance and diversity as well as increased the metabolic activity of individual microorganisms. Some cover crops are better able to utilize soil P than cash crops by using adaptive root systems, the ability to drive P transformations, such as desorption, and mineralization of organic P (Hallama et al., 2021). Some cover crops have a greater ability to utilize soil P of limited availability compared to cash crops (Hallama et al., 2021). The inclusion of has the potential to enhance soil chemical properties resulting in meaningful effects on soil health (Blanco, 2023). Management practices, such as cover crops, greatly impact soil health as they affect soil microbial communities and processes (Lehman et al., 2015; Schmidt et al., 2018). Cover crops prove to be especially key to enhancing soil health and biology in areas challenged with degraded soils and soils with low organic matter in combination with limited water (Blanco, 2023). These examples mention notable benefits but are not a comprehensive list of all benefits that have been observed from the implementation of cover crops.

Although the benefits from cover crops are remarkable, it is important to note that their effects on soil health may be inconsistent. Blanco (2023) states that the benefits of adding cover crops can be variable as there is variability between different soils. The changes observed in soils from cover crops are specific to each environment, which is important to consider when interpreting the effects on soil health (Karlen et al., 2021). The effects of cover crops on soil

properties may not be seen over short periods of time (Blanco, 2023). Cover crops still have considerable benefits with these considerations in mind.

Increased nutrient cycling by cover crops can have positive impacts on soil health. Cover crops utilize residual nitrate and plant available phosphorus as they grow, resulting in the decrease of these nutrients in the soil (Blanco, 2023). However, after cover crops are terminated, the nitrogen and phosphorus held in the biomass is restored back to the soil and made available by mineralization (Blanco, 2023). The influence of cover crops on nutrient cycling can be advantageous, especially for nutrient management in crop production.

The impact of conservation management practices, such as cover crops and no-till farming, can be evaluated by examining different soil health indicators. Soil health, cover crops, and sustainable nutrient cycling are closely tied to the functions of soil microbial communities. As we learn more about promoting soil microorganisms, we get a more holistic view of nutrient management.

Nutrient Cycling

Nutrient cycling and availability are concepts vital to production agriculture. Phosphorus management is a key piece of achieving successful crop yields (Sharpley et al., 1994). Understanding P cycling and availability is crucial for successful nutrient management in crop production. The low levels of available inorganic P in the soil result in a major limiting component for agricultural yields (Balaban et al., 2017). Available P needs to be continually present in the soil solution in order to meet plant requirements (Pierzynski et al., 2005). Soil microorganisms impact nutrient cycling and, in some cases, are integral in the process of making P available to plants. For P to become plant available, it may need to undergo hydrolysis by means of enzymes to transform it from organic P to inorganic P (Hallama et al., 2018).

Phosphorus primarily comes from apatite, and it is weathered and preserved mainly in soils and sediments (Pierzynski et al., 2005). Phosphorus has the potential to go through many processes as it enters the soil solution. Phosphorus in the soil solution can go through both adsorption and desorption processes, which are in an equilibrium (Pierzynski et al., 2005). Diffusion is the main method of movement for P in soil solution (Pierzynski et al., 2005). Phosphorus is not guaranteed to stay in the soil and is susceptible to losses. Although erosion and surface runoff contribute to P losses, the majority of P is lost through crop removal (Pierzynski et al., 2005). Applications of fertilizers and by-products that contain P result in an instant addition of P in the soil solution that is subject to adsorption and precipitation (Pierzynski et al., 2005). These processes can be reversed by dissolution allowing for P to be available for plant use or lost by surface runoff (Pierzynski et al., 2005).

Many other factors affect P availability within the soil including soil pH and soil moisture content (Pierzynski et al., 2005). Soil pH is a determinant on whether P is available to plants. Phosphorus becomes limiting below pH 5.5 and between 7.5 and 8.5 (Alori et al., 2017). The ideal soil pH for P availability is between 6 and 7.5 (Alori et al., 2017). Soil pH values outside of this range restrict P from binding to aluminum, iron, or calcium resulting in P that is less available to plants (Alori et al., 2017). Climate can also impact P availability as climates that are warmer and more humid are able to solubilize P more rapidly compared to climates that are cool and dry (Alori et al., 2017).

The P cycle is an intricate system driven by the organic and inorganic forms of P present as well as other biological, chemical, and environmental factors (Pierzynski et al., 2005). Organic P often makes up a large percentage of total P in the soil solution ranging anywhere from 30-50% (Alori et al., 2017). The ability of plants to utilize P depends on whether P is

available in an inorganic or organic form. Soil microorganisms propel mineralization via enzyme production, influencing the conversion of organic P to inorganic P. The increase of plant available P in the soil is largely done by applying P fertilizers and the microbial processes of solubilization and mineralization of organic P (Alori et al., 2017). A large percentage of organic P in soils is found in the form of inositol phosphate also known as soil phytate (Alori et al., 2017; Hallama et al., 2018). The soil enzyme phytase is responsible for transformation of phytate from an organic form of P to an inorganic form that is plant available (Alori et al., 2017). Many microbial groups including bacteria, fungi, and micromycetes can promote plant growth by synthesizing phytases (Balaban et al., 2017). Soil microorganisms play an important role in P cycling though their ability to produce enzymes that drive the transformation of P, such as the relationship between soil phytase and soil phytate. Soil phytase is just one of many enzymes to influence nutrient cycling. Another soil enzyme group, the phosphomonoesterases, are highly important for the enzymatic hydrolysis of organic P (Hallama et al., 2021). The enzymes that control the dephosphorylation of organic compounds are acid and alkaline phosphomonoesterases (Alori et al., 2017; Tabatabai, 1994). Across all phosphatase enzymes, acid and alkaline phosphomoesterases are the most commonly investigated to assess P cycling and impacts from management effects (Acosta-Martínez & Tabatabai, 2011). These soil enzymes are commonly known as acid and alkaline phosphatase and are important for transforming P to become plant available through mineralization (Hallama et al., 2021). Soil microorganisms are responsible for enzyme production that then influences soil P and directly affects crop nutrition.

Soil Microorganisms

Understanding the relationship between soil microorganisms and P is vital to the P cycle and, therefore, vital to crop production. Soil biology is considered a principal determinant in soil

health and, in soils, as it deals with living systems (Blanco, 2023). This important component of soil health remains to be fully studied. The biological properties of soil and their relationship to soil health are heavily studied; however, there remains a lot to be discovered in the effects of management on this relationship. There is still much to understand about soil microorganisms, which presents a great obstacle for the implementation of soil health (Lehman et al., 2015). Increasing knowledge about soil microbial function will establish the future of intentional management of soil microorganisms and enhance agricultural practices (Schmidt et al., 2018). Beyond a general understanding of soil microbiology, it is important to investigate how microbial community composition and specifically microbial abundance, activity, and function respond to different management practices. The connection between soil microbial activity and community composition may be the key to clarifying the soil biological processes that control plant growth (Chavarría et al., 2016). A complete understanding of soil microorganisms within an ecosystem remains a major part of improved nutrient cycling and the future of soil health and crop production.

Soil microorganisms are an integral part of nutrient cycling in soils, especially for P. Soil microorganisms are required for P availability to plants. Many microbial groups have the ability to solubilize and mineralize P, including bacteria, fungi, actinomycetes, and algae (Alori et al., 2017). Soil microorganisms and plant roots are necessary for the solubilization of inorganic and organic P forms and the mineralization of organic P (Hallama et al., 2021). Soils contain high levels of P in unusable form that could become plant available with the correct soil microorganisms and suitable activity levels (Lehman et al., 2015). Further understanding is needed to uncover the different factors that play into the relationship between soil microorganisms and P availability. A plant's ability to solubilize and mineralize organic P is

perhaps connected to the soil microorganisms that inhabit the rhizosphere (Hallama et al., 2021). Soil microorganisms have some control over soil nutrient speciation, abundance, and availability to plants (Lehman et al., 2015). These interactions are complex and not fully understood at this point in time.

General soil microbial activity is a major part of nutrient cycling. Regular activity of soil microbes allows for the production of extracellular phosphate enzymes, such as alkaline phosphatase, which can mineralize organic phosphorus to become plant available (Lehman et al., 2015). Phosphatase activities convert many forms of phosphorus within the soil and the P from fertilizers and manure into free phosphates that are bioavailable in the soil (Acosta-Martínez & Tabatabai, 2011). These processes are essential and prove the importance of the relationship between soil microorganisms and nutrient cycling. Promoting microbial activity then influences nutrient systems, which is a key part of sustainable nutrient cycling in agriculture.

Soil Health

Soil health is a complex idea that is vital to global health and issues such as changes in climate, increasing food scarcity, and depleting environmental resources (Blanco, 2023). The foundation of soil health is built around sustainable agriculture while also prioritizing soil preservation over time. The terms soil quality and soil health are used commonly, but there can be confusion defining and assessing what these terms mean. Doran and Parkin (1994) define soil quality 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 health has been widely studied for many years with the origin dating back to 1910 to Henry Wallace when efforts were focused mainly on soil fertility (Brevik, 2019). The term soil health has been around for over 100 years but became popular after the establishment of the soil conservations

service in 1935 (Brevik, 2019). Soil health is defined by the USDA – NRCS soil health division as, “The continued capacity of the soil to function as a vital living ecosystem that sustains plants, animals, and humans.” (Stott and Moebius-Clune, 2017). Soil health started to gain more popularity as a conservation movement for protecting natural resources and the environment as a reaction to the Dust Bowl and other natural disasters (Karlen et al., 2021). The soil health movement is also driven by the need to protect soil as a natural resource for environmental quality, biodiversity, and a natural habitat for wildlife (Karlen et al., 2021). As the soil health movement has developed over time, the concept has focused on the ability of soil to continually support life and increase productivity. Soil health emphasizes the importance of soil to thrive as a dynamic living system. This communicates the importance of addressing the knowledge gaps in research regarding biological soil properties.

The importance of soil health is recognized as the demand for agricultural products increases and the vital role that soil plays in the success of agricultural crops is recognized (Karlen et al., 2021). Management of agricultural soils and soil health are closely tied together (USDA, NRCS, 2025). Improved management of agricultural crops begins with understanding soil health concepts and methods of evaluation for different soil systems. Soil health indicators are chemical, biological, or physical properties within a soil that are used to gauge how different soil processes are influenced by management (USDA, NRCS, 2025). Soil physical, chemical, and biological properties are all interrelated to one another and changes between these properties affect both soil health and environment (Blanco, 2023) (Karlen et al., 2021).

A few key soil health indicators include SOM, soil pH, soil respiration, enzyme activities, readily available carbon, available organic nitrogen, and measures of community structure, and diversity of soil microorganisms (USDA, NRCS, 2025). Soil health indicators can be measured

and used to interpret different soil functions such as microbial activity and diversity, carbon food sources for microorganisms, and nutrient cycling, which can have a profound effect on crop production.

Soil organic matter is considered to be the most significant indicator of soil health for soil worldwide (Lehman et al., 2015). Many important soil characteristics such as structure and physical stability, water drainage, nutrient cycling capabilities, and biological activity are heavily influenced by SOM (USDA, NRCS, 2025). Increases in SOM can have many benefits, from shifts in the microbial community and improved nutrient cycling to greater water-holding capacity and strengthened aggregate stability (Lehman et al., 2015). Soil organic matter and microbial communities have an interconnected relationship. Soil organic matter is an important soil health indicator and resource for soil microorganisms. Inversely, soil microorganisms play an important role in the contents of SOM as they influence nutrient systems. Soils high in SOM produce a conducive environment for soil microorganisms to be able to solubilize P (Alori et al., 2017). Microbial activity is a key soil health indicator as soil microorganisms promote SOM cycling, nutrient cycling and availability, and soil aggregate formation (USDA, NRCS, 2025).

Although SOM is one of the principal indicators of soil health, evaluating multiple indicators can improve our understanding of nutrient cycling capabilities within the soil. Additional soil health indicators include, but are not limited to, measuring the labile carbon pool, enzyme activity, soil respiration, available organic nitrogen, as well as microbial biomass, activity, and community structure. Utilizing many different soil health indicators can provide more insight into understanding more about different soil processes, especially nutrient cycling.

Soil Enzymes

Soil enzymes, including β-glucosidase, β-D-glucosaminidase, and acid and alkaline phosphatases, are measures that help estimate microbial activity. These enzymes influence chemical reactions as they are catalyst that allow reactions to move much faster (Tabatabai, 1994). Soil enzymes are able to break down complex organic compounds (Semenov et al., 2025). The soil enzymes β-glucosidase, β-D-glucosaminidase, acid and alkaline phosphatase, and arylsulfatase have been found to be instrumental for nutrient cycling and, therefore, their activity levels can be used in predicting soil health.

The soil enzyme β-glucosidase is a group of enzymes that impact hydrolysis reactions regarding many different glycosides (Tabatabai, 1994). Glycosides are mixed acetals that have different chemical formulations, in which different functional groups exchange hydrogen atoms (Tabatabai, 1994). The product of this hydrolysis reaction by β-glucosidase is generally considered to be an energy food source for soil microorganisms (Eivazi & Tabatabai, 1988). β-Glucosidase enzymes contribute to the breakdown of organic C (USDA, NRCS, 2025), while β-D-glucosaminidase enzymes contribute to the breakdown of organic C and N (Parham, 2000; USDA, NRCS, 2025). β-Glucosidase and β-D-glucosaminidase activity can be estimated colormetrically by measuring the p-nitrophenol released from soil enzymes after incubation and exposure to a buffer solution (Eivazi & Tabatabai, 1988).

The phosphomonoesterase enzymes, including acid and alkaline phosphatase, are widely recognized for hydrolyzing phosphomonoesters (Acosta-Martínez & Tabatabai, 2011; Tabatabai, 1994), such as, β-glycerophosphate, phenylphosphate, β-naphthyl phosphate, and p-nitrophenyl phosphate (Acosta-Martínez & Tabatabai, 2011). The phosphomonoesterases are commonly used to learn more about how P cycling is affected by management impacts (Acosta-Martínez &

Tabatabai, 2011). The phosphatase enzymes are involved in the bioavailability of P, making phosphatase activity a valuable soil health indicator (Chavarría et al., 2016). The function of phosphatase enzyme activity is often observed as a response to a lack of P in plant and soil systems and a process to drive mineralization and increase P availability for both plants and soil microorganisms (Janes-Bassett et al., 2022). The phosphatase enzymes are produced by both plants and soil microorganisms and play a role in availability of inorganic phosphorus (USDA, NRCS, 2025). Plants and soil microorganisms both contribute to acid phosphatase synthesis while only soil microorganisms contribute to alkaline phosphatase synthesis (Hallama et al., 2018). Acid phosphatase is generally active in acid soils while alkaline phosphatase is generally active in alkaline soils (Eivazi and Tabatabai, 2011; Tabatabai, 1994). Soil enzymes can originate from microbial biomass as well as plant and animal residue; however, soil enzymes predominantly originate from microbial biomass (Tabatabai, 1994). Acid and alkaline phosphatase activities are determined colormetrically by evaluating the amount of p-nitrophenol produced from enzyme activities after exposure to p-nitrophenol phosphate buffer solution and an incubation period (Tabatabai, 1994).

Soil enzyme activity can be used as an indicator of soil health and the effects of different management practices; however, it is unable to determine direct productivity of crops. Attempts to link soil enzyme activity to plant success are unclear and have contradicting results (Karlen et al., 2021). Although soil enzymes cannot provide details on their direct effects, they are still useful in interpreting the influence of different management practices. Soil enzyme activity can be influenced by many different things such as changes to the soil, cropping systems, and environmental conditions (Tabatabai, 1994).

Microbial Biomass

Microbial biomass makes an appropriate biological soil health indicator as it combines chemical, physical, and biological properties as well as being sensitive to management (Rice et al., 1997). The living portion of SOM is microbial biomass (Rice et al., 1997). Microbial biomass is involved in SOM and an integral part of nutrient cycling (Rice et al., 1997). Measuring C sources in the soil is important for assessing the available energy sources for soil microbial groups which can affect microbial community size, diversity, abundance, and activity (USDA, NRCS, 2025). Microbial biomass is measured to determine the concentration of nutrients in soil microbial communities that constitute SOM. Microbial biomass is comprised of different nutrients that can change over time (Rice et al., 1997). Microbial biomass C, N, and P (MBC, MBN, and MBP) are all determined using the same fumigation methods. Multiple different laboratory methods exist to determine microbial biomass. Common methodologies include fumigation-extraction and fumigation-incubation-extraction following the Jenkinson and Powlson (1976) procedure. Vance et al. (1987) further went on to investigate whether the fumigation-incubation methodology, described by Jenkinson and Powlson (1976), extracted C that could be associated as MBC. Brookes et al. (1985) sought out a new method for determining MBN. This method is considered a direct extraction and eliminates the aerobic incubation step. Brookes et al. (1985) found that soils from the fumigation-extraction method undergoing a one-day chloroform fumigation showed a strong correlation between MBN and total N. Many methods have been used to determine microbial biomass over time, although most use soil fumigation methods (Rice et al., 1997). Microbial biomass C can produce differing results depending on the analysis method (Semenov et al., 2025). Microbial Biomass C can be used to observe the impact that different management practices have on microbial abundance and

activity (Semenov et al., 2025). Microbial biomass C is energy that can be used by soil microorganisms (Rice et al., 1997).

Hedley & Stewart (1982) modified the procedure for measuring MBC to more precisely measure MBP. The chloroform fumigation and extraction method commonly used for MBC was used by Chauhan et al. (1979, 1981) to quantify MBP. However, this method may provide a poor estimate of MBP if held in the same regards as MBC. The diversity of P compounds within the soil and the cells of soil microorganisms, as well as the extraction of these P compounds, can provide a challenge (Hedley & Stewart, 1982). Additionally, issues with the extractant used in this method are cited in literature as the extractant does not align well with conditions that would be well represented in soil. Microbial biomass P is measured colormetrically by the molybdate blue method for P determination (Murphey & Riley, 1962). Microbial biomass P analysis is relevant to soil health as a measurement that is representative of part of the active organic P pool (Bünemann et al., 2018). Microbial biomass P is crucial to P cycling and P supply to plants and can be evaluated by measuring the P biomass (Brookes et al., 1982).

Autoclavable Citrate Extractible (ACE) Protein

Bioavailable nitrogen can be used as a soil health indicator as it measures the amount of organic nitrogen that can be utilized by soil microorganisms (USDA, NRCS, 2025). One method used to measure available organic nitrogen is autoclaved citrate-extractable (ACE) protein. Autoclavable Citrate Extractable protein is a measure of protein which makes up a pool of N that is organically bound but can be easily mineralized by soil microorganisms to be made available to plants (USDA, NRCS, 2025).

ACE protein has been used to determine the labile nitrogen that is available to soil microorganisms. Soil protein plays a crucial role in many N processes such as plant nutrition, N

release to plants, and N storage, which all prove to be influential to soil health (Karlen et al., 2021). Autoclavable citrate extractable protein is a method in which soils get autoclaved in a high temperature and pressure environment, which then allow for clarification and quantification (Karlen et al., 2021). Many researchers have concluded that ACE is a valuable soil health indicator in measuring soil protein as a part of soil N that is responsive to management practices (Hurisso et al., 2018).

Permanganate-Oxidizable Carbon (POXC)

Many plants, soil microbes, and other living organisms rely on labile soil C as a source of nutrition (Karlen et al., 2021). Permanganate-Oxidizable Carbon (POXC) analysis follows methods from Weil et al. (2003), which is a simpler method allowing for more precise measurement of readily oxidizable carbon. This method is used to estimate organic matter that is readily oxidizable, which is determined from the quantity of permanganate reduction observed in a soil sample (Christy et al., 2023). Originally, it was thought that POXC was a measurement of labile carbon that could be easily accessible to soil microorganisms (Weil et al., 2003). Christy et al. (2023) systematically determined that POXC more accurately describes the measurement of phenolic and polyphenolic structures, which are not seen as a favorable microbial food source. However, many studies have shown that POXC is a measure of readily oxidizable C as well as more stable forms of C that were not previously considered (Karlen et al., 2021).

Although the exact measured C pool has been questioned over time, Culman et al. (2012) concluded that POXC is a suitable indicator for assessing soil quality, especially when evaluating practices that could encourage carbon sequestration. POXC is a sensitive indicator that is favorable for observing contrasting management practices in comparison to other SOC indicators that are available (Karlen et al., 2021). The portion of ‘Active C’ can be used as a measure of soil

health and to observe changes in soil organic C as it is more responsive to management impacts (Karlen et al., 2021). The term 'Active C' is used to describe the pool of labile C and to differentiate it from the recalcitrant C in the bulk soil pool that is not as easily altered (Weil et al., 2003). Permanganate oxidizable carbon has been determined to be a sensitive method, making it an excellent tool in evaluating the portion of soil C that is biologically active (Culman et al., 2012).

Water Extractable Phosphorus (WEP)

Water extractable P (WEP) is a soil analysis often used to test for the potential of P loss in runoff, especially when regarding manure applications. Preventing dissolved P loss is mainly a function of maintaining soil P levels at an acceptable range so as not to cause environmental harm (Sharpley, 1995). Many studies have documented strong positive relationships between dissolved reactive P (DRP) and a variety of soil extraction methods. Pote et al., (1996), determined that distilled water extraction had one of the strongest correlations with DRP in runoff because it dissolves less mineral P than the other extractants. Wang et al. (2010) evaluated 5 different P extraction methods and concluded that, of the 5 methods assessed, WEP had the strongest correlation to DRP in runoff. These results are consistent with other studies found in the literature (Pote et al. 1999; Penn et al. 2006).

Although these studies emphasize the potential of estimating DRP by measuring WEP, they also express that this relationship is contingent on the amount of runoff and different soil properties, especially the soil to solution ratio in the amount of P extracted (Kovar & Claassen, 2005). Measuring WEP provides insight into the risk of P loss, which proves to be an appropriate indicator for assessing P management in addition to other P measurements.

There is a strong correlation between WEP and DRP, which presents the opportunity to estimate DRP from WEP. Water extractable P is a P extraction method that can be used to estimate dissolved reactive P (DRP) in runoff water. Water extractable P is measured using methods developed by Luscombe et al. (1979) and determined colormetrically by the molybdate blue method for P determination (Murphey & Riley, 1962).

Soil Respiration

Soil respiration measures microbial activity in soils by measuring the amount of carbon dioxide respired after a set incubation period (USDA, NRCS, 2025). This activity is important in the global C cycle as soil respiration by heterotrophs stabilizes photosynthesis done by autotrophs (Karlen et al., 2021). Soil respiration is an accurate estimate of soil microbial activity and decomposition in soils that provide insight into nutrient cycling (Wollum & Gomez, 1970; Zibilske, 1994). Soil respiration can indicate the productivity of soil in regard to nutrient cycling, decomposition of organic materials, and influence on ecosystem functions that are controlled by soil microorganisms (Karlen et al., 2021). The significance of microbial respiration to nutrient cycling and soil health proves soil respiration to be a valuable soil health indicator.

There are many different variations in methods that can be used to measure soil respiration. Soil respiration procedures may have variable incubation methods and timing, temperatures, soil water content, soil preparations, detection of carbon dioxide, and amount of soil analyzed (Karlen et al., 2021). A common method used to determine soil respiration involves the use of an alkali trap to measure the adsorbed carbon dioxide and analysis done by titrimetric, gravimetric, or conductimetric methods (Zibilske, 1994). The alkali trap method is able to be determined by conductimetric analysis due to the mobility differences of carbonates and hydroxides (Zibilske, 1994). Wollum & Gomez (1970) developed a procedure suitable for

measuring electrical conductivity of soil samples in large quantities as an assessment of soil microbial respiration. Carbonates have less mobility compared to hydroxides and an increase in carbonates will increase electrical resistance in the trap solution (Zibilske, 1994). The amount of carbon dioxide trapped is relative to the electrical conductivity of the trap solution. An increase in the carbon dioxide in the trap will result in an increase in electrical resistance, meaning that electrical conductivity of the trap solution will decrease (Zibilske, 1994).

Phospholipid Fatty Acid (PLFA) Analysis

Microbial community activity, abundance, and diversity are all important to soil health (Karlen et al., 2021; Blanco, 2023). There are many methods available to measure microbial communities. Phospholipid fatty acid analysis helps to determine microbial community size and composition of different soil microbial functional groups (USDA, NRCS, 2025).

Phospholipid fatty acid profiles use biomarkers to classify microorganisms and determine microbial community composition. Phospholipid fatty acid biomarkers are membrane lipids and the fatty acids that they are comprised of (Zelles, 1999). The use of PLFA biomarkers has generally been successful in determining microbial groups (Zelles, 1999). However, issues can arise as some fatty acids are shared between different soil microorganisms or between plants and soil microorganisms (Zelles, 1999). Although there is some overlap in biomarkers for different species, the biomarkers used to determine specific microbial groups are still in use today (Willers et al., 2015). This can become an issue in identifying individual species, but does not apply to different microbial groups, such as gram-negative bacteria, arbuscular mycorrhizal fungi (AMF), and eukaryotes, etc.

Arbuscular mycorrhizal fungi are an important group of soil microorganisms that have been known to form symbiotic relationships with plants that can aid in nutrient uptake (Frąc et

al., 2018). Performing PLFA analysis through time will allow for the monitoring of AMF abundance in response to different management practices. This research is relevant as it is still unclear about the exact drivers affecting AMF and their influence in soybean production (Higo et al., 2018).

Phospholipid fatty acids can be analyzed to assess many elements of microbial communities at any given point of sampling collection (Willers et al., 2015). Phospholipid fatty acid analysis can provide an estimate of microbial biomass, information on biomarkers that can be used to assess community structure, and details on the functioning of the microbial community overall (Willers et al., 2015). Measuring PLFA over time can provide information on how the dynamics of the soil microbial community are changing and individual groups, such as AMF.

The history of PLFA began when Bligh & Dyer (1959) developed a method of extracting lipids from marine life. The Bligh-Dyer extractant utilizes chloroform and methanol to create distinct layers separating lipids from non-lipids (Bligh & Dyer, 1959). Although the developed method has changed throughout time, the extractant used in Bligh & Dyer (1959) is still in use today (Li et al., 2020). The original method from Bligh & Dyer (1959) was modified by White et al (1979) to be used in the first PLFA analysis which was used to evaluate microbial biomass in marine samples (Willers et al., 2015). It is well known that this method used to analyze PLFA is time consuming and costly (Buyer & Sasser, 2012; Karlen et al., 2021; Li et al., 2020). Buyer and Sasser (2012) introduced a modified method that would allow for high-throughput PLFA analysis. This high-throughput method is widely accepted and commonly used today. Phospholipid fatty acid analysis methods involve using appropriate solvents to extract and separate lipids in different categories (Zelles, 1999). The Buyer and Sasser (2012) method

contains four steps: drying and extraction, lipid separation, transesterification, and then analysis via gas chromatography.

Phospholipid fatty acid analysis has been in use for decades because of its value in characterizing aspects of soil microbial communities. The quantitative data from PLFA analysis can effectively be used to measure the current state of the microbial community from a sample at any point in time (Kaur et al., 2005). Measuring PLFA will provide insight into how management decisions like P fertilizer management, cover crops, and no-till farming, impact the soil microbial community.

Hypothesis/objectives

The goal of this study was to gain a better understanding of the impact that cover crops and phosphorus fertilizer management have on soil microbial communities. The following objectives describe what this research expects to address.

Objective 1: Determine the impacts of a cereal rye cover crop and sufficiency and build and maintain P fertilizer management approaches on dynamic biological soil health indicators that assess the size and activity of the soil biological community and nutrient cycling capacities.

Hypothesis 1: Soil health will be improved by the presence of cover crops seen with increased nutrient pools as well as increased microbial activity observed with soil respiration and soil enzyme activities.

Hypothesis 2: Enhanced nutrient cycling will be observed as the level of P fertilizer management decreases as observed by different inorganic, organic, and biological nutrient pools.

Hypothesis 3: Soil health indicators will change throughout the growing season due to climatic factors.

Objective 2: Determine cover crop and P fertilizer management impacts on microbial community abundance and structure.

Hypothesis 1: The addition of cover crops will increase the abundance of all microbial groups and total microbial biomass.

Hypothesis 2: Reducing P fertilizer inputs will drive an increase in the abundance of all microbial groups.

Hypothesis 3: Cover crops and P fertilizer management will improve soil microbial community structure.

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Chapter 2 - Evaluating the effects of cover crops and phosphorus fertilizer management on various soil health indicators

Abstract

The impacts that cover crops and phosphorus (P) fertilizer management have on nutrient cycling and soil health metrics are complex and need further research to fully understand their influence. A study was conducted in Manhattan, KS, during the 2024-soybean (*Glycine max* L.) and 2025-corn (*Zea mays* L.) growing seasons. The study contained two levels of cover crop management: no cover (NC) and cover crop (CC) as well as three levels of P fertilizer management: a control (NP), sufficiency (SUF), and build and maintain (BAM). This study aimed to assess how cover crops and P fertilizer management impacts soil health indicators that assess different nutrient pools as well as the size and activity of the soil biological community. Soil samples were collected after CC termination (T0), 30-days after the initial sampling (T1), 60-days after the initial sampling (T2), and after grain crop harvest (FL). Sampling time had a widespread effect on many soil health indicators evaluated in both 2024 and 2025. Temperature and moisture effects on the soil microbial community and nutrient cycling are present with the sampling time effects observed. In 2025, microbial biomass nitrogen (MBN) was the only soil health indicator impacted by cover crops in which the presence of a cover crop resulted in increased MBN in the T0, T1, and T2 sample times. Phosphorus fertilizer treatment mainly influenced inorganic and biological nutrient pools. Phosphorus fertilizer treatment by time interactions were present in microbial biomass carbon (MBC), microbial biomass P (MBP), and NH_4-N in 2024, as well as NH_4-N and the ratio of microbial biomass N:P in 2025. The influence of P fertilizer treatment rarely interacted with the CC treatment, aside from β-glucosaminidase, β-glucosidase, alkaline phosphatase, and soil respiration in 2024. In general, the CC treatment increased enzyme activities and soil respiration. This research continues to support that CC are known to be beneficial to soil health and soil microorganisms.

Introduction

Soil health has a widespread impact in crop production, especially in the nutrient cycling capacities of C, N, and P in the soil and crop system. Phosphorus is an essential nutrient for all life (Pierzynski et al., 2005) and is the second most limiting nutrient in crop production (Ali et al., 2019). This presents challenges as plants require P from the soil. Soils generally contain sufficient amounts of P; however, the P in the soil is not always available in a form that plants can use (Ali et al., 2019). As a result, P fertilizer often gets applied to the soil in many crop production systems (Martinez and Tabatabai, 2011). Excess P applications to soil can result from attempts to increase plant available P resulting in environmental concerns with eutrophication (Balaban et al., 2017).

Soil health is a concept that has been relevant for decades. Original soil health efforts were focused on soil fertility. Presently, the key premise of soil health lies in preserving soil as a natural resource from degradation and loss (Brevik, 2019). Management of agricultural soils and soil health are closely tied together (USDA, NRCS, 2025). Enhancing soil health can provide benefits such as increased SOM, increased water holding capacity, enhanced nutrient cycling, and much more (USDA, NRCS, 2025). Improving soil health has become of great interest to crop producers as these many benefits to soil have become well known. Conservation management practices are implemented to promote and improve soil health. The implementation of CC and no-till farming are the two most widely adopted conservation management practices. Both CC and no-till farming have been known to be beneficial to soil health and soil microorganisms. No-till farming is the practice of eliminating soil disturbance from the use of tillage equipment and planting directly into the residue of the previous crop (Bezboruah et al., 2024). Research studies have proven there are many benefits of implementing no-till as a conservation management practice, such as reducing potential of soil erosion, improving soil structure, increasing water infiltration, decreasing fuel and labor cost, and building of SOM (Bezboruah et al., 2024). No-till farming is consistently one of the most widely adopted conservation management practices. In 2022, across the

United States there were more acres of no-till adoption compared to acres of CC adoption (Pastina et al., 2024).

Cover crops are widely adopted across the United States as they are a valuable conservation management practice contributing to soil health. Cover cropping is the practice of growing plants not for the purpose of harvesting, but to leave their biomass on the field with the intention of gaining agroecosystem benefits, such as erosion control, increased SOM, prevention of pathogen and weed problems, and improved nutrient resources (Hallama et al., 2021). According to the United States Department of Agriculture census in 2022, almost 18 million acres of CC were planted across the US representing 4.7% of total crop land (Bowman & Morales, 2024). The total CC acreage planted in the US in 2022 was a 17% increase from the total acreage recorded in 2017 (Bowman & Morales, 2024). Cover crops are rising as a favorable management tool for implementing sustainable agriculture (Chavarría et al., 2016; Hallama et al., 2021). Cover crops have the ability to help the soil recover from some of the negative impacts of tillage, including the return of microbial biomass distribution with depth (Schmidt et al., 2018). They also contribute to nutrient input in the soil with increasing subsurface root biomass, which increases labile organic matter and stimulates microbial abundance (Blanco, 2023). Some CC are better able to utilize soil P than cash crops by using adaptive root systems, the ability to drive P transformations, such as desorption, and mineralization of organic P (Hallama et al., 2021). Additionally, some CC species have a greater ability to utilize soil P of limited availability compared to cash crops (Hallama et al., 2021). The inclusion of a CC has the potential to enhance soil chemical properties resulting in meaningful effects on soil health (Blanco, 2023). Management practices, such as CC, greatly impact soil health as they affect soil microbial communities and processes (Lehman et al., 2015; Schmidt et al., 2018). Cover crops prove to be especially key to enhancing soil health and biology in areas challenged with degraded soils and soils with low organic matter in combination with limited water (Blanco, 2023). These examples show some of the benefits of implementing conservation management practices, but are not a comprehensive list.

Soil health indicators are metrics used to assess soil health and monitor how different management practices affect soil over time. These soil health indicators are chemical, biological, or physical properties within soil that are measurable (USDA, NRCS, 2025). Better understanding how management practices influence soil health will allow agriculture producers to be able to make more intentional decisions regarding crop production. Researchers consider SOM to be the greatest soil health indicator (Lehman et al., 2015), but there are many other soil health indicators commonly used to assess soil health such as, measuring soil enzyme activities, soil respiration, available organic N, the labile C pool, and microbial biomass (USDA, NRCS, 2025).

Soil enzyme activities are a widely used soil health indicator as they influence nutrient cycling. Soil enzymes are involved in the speed of nutrient transformations and the decomposition of SOM (Ali et al., 2019). The soil enzyme activities of β-glucosidase, β-D-glucosaminidase, acid and alkaline phosphatase, and arylsulfatase are commonly measured as they are instrumental in nutrient cycling (Tabatabai, 1994). The phosphomonoesterases, including acid and alkaline phosphatase, are commonly used to learn more about the effect of management impacts on P cycling (Martinez and Tabatabai, 2011). The soil enzyme β-glucosidase is a group of enzymes that impact hydrolysis reactions associated with different glycosides (Tabatabai, 1994). Glycosides are mixed acetals that have different chemical formulations, in which different functional groups exchange hydrogen atoms (Tabatabai, 1994). The product of this hydrolysis reaction by β-glucosidase is generally considered to be an energy food source for soil microorganisms (Eivazi & Tabatabai, 1988). β-Glucosidase enzymes contribute to the breakdown of organic C (USDA, NRCS, 2025), while β-D-glucosaminidase enzymes contribute to the breakdown of organic C and N (Parham, 2000; USDA, NRCS, 2025). The phosphomonoesterase enzymes, including acid and alkaline phosphatase, are widely recognized for hydrolyzing phosphomonoesters (Acosta-Martínez & Tabatabai, 2011; Tabatabai, 1994). Examples of phosphomonoesters include, β-glycerophosphate, phenylphosphate, β-naphthyl phosphate, and p-nitrophenyl phosphate (Acosta-Martínez & Tabatabai, 2011). The phosphomonoesterases are commonly used to learn more about how P cycling is affected by management impacts (Acosta-Martínez & Tabatabai, 2011). The phosphatase

enzymes are involved in the bioavailability of P, making phosphatase activity a valuable soil health indicator (Chavarría et al., 2016). The function of phosphatase enzyme activity is often observed as a response to a lack of P in plant and soil systems and a process to drive mineralization and increase P availability for both plants and soil microorganisms (Janes-Bassett et al., 2022). The phosphatase enzymes are produced by both plants and soil microorganisms and play a role in availability of inorganic phosphorus (USDA, NRCS, 2025). Plants and soil microorganisms both contribute to acid phosphatase synthesis while only soil microorganisms contribute to alkaline phosphatase synthesis (Hallama et al., 2018). Acid phosphatase is generally active in acid soils while alkaline phosphatase is generally active in alkaline soils (Eivazi and Tabatabai, 2011; Tabatabai, 1994). The phosphatase enzymes are generally impacted by soil pH levels, excluding SOM and soil disturbance, more so than other soil enzymes (Martinez and Tabatabai, 2011). In P deficient systems, plants and microbes typically produce more phosphatase enzymes (Martinez and Tabatabai, 2011). Soil enzyme activity can be influenced by many different things such as changes to the soil, cropping systems, and environmental conditions (Tabatabai, 1994).

Permanganate-oxidizable carbon (POXC) is used to estimate organic matter that is readily oxidizable, which is determined from the quantity of permanganate reduction observed in a soil sample (Christy et al., 2023). Many studies have shown that POXC is a measure of readily oxidizable C as well as more stable forms of C that were not previously considered (Karlen et al., 2021). Although the exact measured C pool has been questioned over time, Culman et al. (2012) concluded that POXC is a suitable indicator for assessing soil quality, especially when evaluating practices that could encourage carbon sequestration. A study done by French et al., (2026), found across many study sites and crop rotations POXC was greater under CC treatments compared to no cover crop treatments across all P and potassium fertilizer trials. Permanganate-Oxidizable Carbon has been determined to be a sensitive method, making it an excellent tool in evaluating the portion of soil C that is biologically active (Culman et al., 2012). Some studies argue that POXC could be useful in determining crop productivity (Svedin et al., 2023); however,

others have concluded that POXC is better suited for soil health needs and is not a strong predictor of crop yield (French et al., 2026).

Soil respiration measures microbial activity in soils by measuring the amount of carbon dioxide respired after a set incubation period (USDA, NRCS, 2025). Soil respiration is an accurate estimate of soil microbial activity and decomposition in soils that provide insight into nutrient cycling (Wollum & Gomez, 1970; Zibilske, 1994). Measuring soil respiration as a way to relate to biological activity. The majority of laboratory methods quantify this by measuring the carbon dioxide burst from microbial activity following the rewetting of dried soil under set incubation conditions (Karlen et al., 2021). Carbon dioxide respiration methods have variable results under different agriculture managements. French et al., (2026) found that carbon dioxide respiration methods were positively influenced by the presence of a CC, but had variability overall.

Autoclavable citrate extractable (ACE) protein is a measure of protein, which makes up a pool of N that is organically bound but can be easily mineralized by soil microorganisms to be made available to plants (USDA, NRCS, 2025). Bioavailable nitrogen can be used as a soil health indicator as it measures the amount of organic nitrogen that can be utilized by soil microorganisms (USDA, NRCS, 2025). Many researchers have concluded that ACE is a valuable soil health indicator in measuring soil protein as a part of soil N that is responsive to management practices (Hurisso et al., 2018).

Microbial biomass is the living portion of SOM that is comprised of different nutrients making microbial biomass integral to nutrient cycling (Rice et al., 1997). Many studies have included MBC and MBN as a way to assess nutrient concentrations. Measuring carbon sources in the soil is important for assessing the available energy sources for soil microbial groups which can affect microbial community size, diversity, abundance, and activity (USDA, NRCS, 2025). Some studies also include MBP, as a way to evaluate P cycling and P supply to plants (Brookes et al., 1982).

The impacts of conservation management practices on soil health indicators are well documented in literature. In a study done by Chavarría et al. (2016), it was observed that the presence of a CC increased MBC values compared to the no cover treatment. In the same study, an increase in enzyme

activity, specifically acid phosphatase activity, was prevalent in the CC treatment compared to the no cover treatment. The increase in phosphatase activity under a CC can be linked to SOM decomposition and enhanced nutrient cycling over time (Chavarría et al., 2016). A study by Schmidt et al. (2018) found increased microbial abundance and increased metabolic capacities of soil microbes with the presence of a CC which they attributed to the increase in nutrients supplied from the CC. Many can agree that long term use of CC's in conservation management is key to improving the soil microbial community and furthering soil health (Chavarría et al., 2016; Mendis et al., 2025).

Application of P fertilizer is often necessary in crop production, but can lead to environmental issues if not intentionally managed. This idea highlights the importance of increasing P nutrient efficiency in agriculture. Schneider et al. (2019) describe in a review how plants and soil microorganisms have the potential to lead to sustained crop yields at lower soil test P concentrations through enhanced P cycling and arbuscular mycorrhizal fungi. Maintaining crop production at a lower soil test P with increased focus on plant and soil functions could mean decreased rates of P fertilizer applications in agriculture. Phosphorus cycling in the soil is complex and involves many chemical, physical, and biological processes (Schneider et al., 2019). The concept of decreasing P fertilizer rate while continuing plant productivity is not fully understood.

The main objective of this study was to determine the impacts of a cereal rye CC and sufficiency and build and maintain P fertilizer management approaches on dynamic biological soil health indicators that assess the size and activity of the soil biological community and nutrient cycling capacities in a no-till corn-soybean rotation in Kansas. The three hypotheses tested were: (1) Soil health will be improved by the presence of cover crops seen with increased nutrient pools and increased microbial activity observed with soil respiration and soil enzyme activities, (2) Enhanced nutrient cycling will be observed as the level of P fertilizer management decreases as observed by different inorganic, organic, and biological nutrient pools, and (3) Soil health indicators will change throughout the growing season due to climatic factors.

Materials and Methods

Experimental Design and Site Characteristics

The study was conducted from 2024-2025 at the Kansas Agricultural Watershed (KAW) field laboratory located in Manhattan, KS. The site has a soil type of primarily Smolan silty clay loam (fine, smectitic, mesic Pachic Argiustoll) and a slope of 6-8% with soil pH ranging between 6 and 7. This site has a mean annual precipitation of 951 mm and a mean annual temperature of 13.6° C and is classified as having a hot, humid continental climate. The site consisted of 18 total plots arranged in 3 blocks with each block containing a 2x3 complete factorial all arranged in a randomized complete block design (Figure 2.1). Each plot serves as a small, terraced watershed with surface areas of approximately 0.5 ha. The site was managed in a no-till, corn (*Zea mays* L.) and soybean (*Glycine max* L.) crop rotation since 2014, with even years planted to soybeans and odd years planted to corn. The plot layout includes three levels of P fertilizer treatments and two levels of cover crop treatments. The phosphorus treatments in this study include: no phosphorus (NP), sufficiency (SUF), and build and maintain (BAM). The cover crop treatments include: no cover crop (NC) and cover crop (CC). Cover crop treatments from 2024-2025 consisted of cereal rye (*Secale cereale* L.), which was established after harvest of the main crop.

The KAW field laboratory project began in 2014 focusing research efforts on assessing different P fertilizer managements and their effects on P loss in an agricultural system. Many studies have been conducted at this site to evaluate P fertilizer management and CC effects on crop productivity, water quality, P cycling, and economic impact (Bourns et al., 2026; Nelson et al., 2023). Phosphorus fertilizer management and CC effects on soil health indicators and the soil microbial community have also been evaluated at this site (Pasket, 2023.; Starr et al., 2025; Stewart, 2021).

Phosphorus fertilizer management treatments changed throughout the course of the KAW project. From 2014-2019, the treatments included no phosphorus (NP), spring injected (SI), and fall surface broadcast (FB) applications with P fertilizer rates in SI and FB determined with a “build” recommendation. The SI and FB plots received 61 kg P_2O_5 ha^{-1} annually. The CC treatment from 2018-

2019 was a CC mix, which contained wheat (*Triticum aestivum* L.), triticale (*Secale X Triticum* L.), and rapeseed (*Brassica napus*). The P management goal changed in 2020-2022 and aimed to maintain P in the SI plots and begin the drawdown of P in the FB plots. The maintenance P management approach included 55 kg P_2O_5 ha^{-1} applied to the SI plots, while both the drawdown P approach in the former FB plots and the NP control plots received no P fertilizer during these years. At this point, P fertilizer treatment labels shifted to no phosphorus (NP), sufficiency (SUF), and build and maintain (BAM). This shift in treatment labels better reflects the goals of each P management scenario from 2023 to 2025. During the years of 2023-2025, the P managements goals were to follow the Kansas State University build and maintain or sufficiency recommendations. In 2023, the SI plots (BAM) received 53 kg P_2O_5 ha^{-1}, SUF plots received 23 kg P_2O_5 ha^{-1}, and the NP plots continued to remain a no P control. Nitrogen fertilizer, in the form of UAN, was applied at planting for corn years, in order to meet crop nutrient requirements. This application was based on Kansas State University recommendations to meet N requirements. The N fertilizer applications were balanced so the applied N rate was equal across all P fertilizer treatments (Nelson et al., 2023).

Soil Sampling

Composite soil samples were collected 4 times (T0, T1, T2 and FL) during the soybean and corn growing seasons of 2024 and 2025, respectively. The T0 sampling was taken in the spring after cover crop termination and before planting. Subsequently, T1 was taken 30 days after the initial sampling and T2 was taken 60 days after the initial sampling. Fall (FL) sample collection took place after grain crop harvest and included samples taken from 3 depths, 0-5 cm, 5-10 cm, and 10-15 cm. In season samplings T0, T1, and T2 were sampled at 0-5 cm depth only. Soil samples were collected along established line transects in each plot. The line transects were comprised of two-line segments connecting three GPS points. The outer two points were approximately in the mid slope region of the plot, and the middle point was at the top of the terrace channel forming a V shape. Ten soil samples were randomly collected along each line segment using a soil probe resulting in a composite sample of 20 soil cores of field-moist soil in

each plot. A second set of ten soil samples was randomly collected along each line segment to make a composite sample of 20 soil cores for air-dried soil in each plot. Field-moist soil was passed through a 2 mm sieve and stored at 4 °C until analysis. Field-moist analysis included microbial biomass C, N, and P, dissolved total N, extractable inorganic N, and dissolved organic C and N. The air-dried soil samples were dried at room temperature, ground, and passed through a 2 mm sieve. Air-dried analysis included soil enzymes, soil respiration, ACE, and POXC.

Microbial Biomass

Microbial biomass C and N were extracted at the same time using the direct extraction method outlined by Brookes et al. (1985). Microbial biomass P was also extracted at the same time, but used a different extraction method (Brookes et al., 1982; Hedley & Stewart, 1982). Samples for MBC, MBN, and MBP were weighed and analyzed using field-moist soil within 3 weeks of the sampling date. Each sample weight was determined based on the gravimetric water content to correct to 8 g dry weight equivalent and no other moisture corrections were used. Unfumigated and fumigated samples were run for each soil sample. Microbial biomass C and MBN were extracted using 0.5 M potassium sulfate (K_2SO_4) while MBP samples were extracted using 2 mM citric acid at a pH of 5.0. The extraction was done with a soil to solution ratio of 1:5 resulting in 40 mL of extractant being added to each sample. Extraction of the unfumigated samples began first by adding 40 mL of the respective reagent to each sample, then shaking for a duration of 30 minutes on an oscillating shaker. The fumigated soils were fumigated before extraction using alcohol free chloroform ($CHCl_3$) at 25 °C for 24 hours. After the 24-hour incubation, the extraction for both the fumigated and unfumigated samples could take place. After shaking for 30 minutes, MBP samples were centrifuged at 10,000 rpm for 10 mins. All extracts were filtered following shaking through an Ahlstrom 74 filter paper into 40 mL glass vials and nutrient concentrations determined. Once the extraction was finished the C-N samples were analyzed for non-purgeable organic C on a Total Organic C analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). Once this was finished, the samples were then digested using the potassium persulfate digestion

method to determine total N (Cabrera & Beare, 1993). To determine total N, a $K_2S_2O_8$ reagent was added to samples, then autoclaved for 30 min at 120 °C. The oxidization allowed for the conversion of all N forms to NO_3-N and the digested extracts were then analyzed colormetrically using a Rapid Flow Analyzer Model RFA-300 (Alpkem Corporation, Clackamas, OR, USA). Inorganic N (NO_3-N and NH_4-N) was measured by the Kansas State Soil Testing Lab. The MBP samples, which are a different set of soil, were determined using the Murphy-Riley phosphorus determination method (Murphy & Riley, 1962). Microbial biomass C, N, and P were calculated by subtracting the nutrient content extracted from the non-fumigated samples from the fumigated samples. Dissolved organic N was determined by calculating the difference between dissolved total N and inorganic N.

Soil Enzymes

Soil enzymes analyzed included β-D-glucosaminidase, β-glucosidase, alkaline phosphatase, and acid phosphatase. All enzymes were analyzed using the ρ-nitrophenol calibration curve modified from Dick (2011). These four enzymes follow similar methodology to measure the amount of p-nitrophenol compound that was released during the incubation period and then determined colormetrically to estimate enzyme activity. Parham & Deng (2000) describe a method to accurately quantify β-D-glucosaminidase in soils. Eivazi & Tabatabai (1988) provide details for a method to determine β-glucosidase activity in soils. Alkaline and acid phosphatase were determined based on the procedure designed by Tabatabai & Bremner (1969). Specific soil enzyme buffers and substrates are listed in Table 2.1.

These three methods followed the same general procedure. Enzyme activity was measured by weighing out 0.5 g ± 0.05 g of air-dried soil into 20 mL scintillation vials with 3 replicates for each sample (A, B, and C). Starting with the control vials (C), 2 mL of start buffer was added to each vial. They were gently swirled and then incubated at 37 °C for 1 hour. This same process was done with the A and B vials only; 0.5 mL of substrate was also added before the incubation. After 1 hour, the stop procedure was completed by adding 2 mL of stop buffer and 0.5 mL of calcium chloride to each vial. After the reaction was stopped in the control set (C), 0.5 mL of substrate was added. The contents of each

vial were then filtered through Whatman no. 2 filter paper (11 cm) and enzyme activity was determined colormetrically at 400 nm using a Shimadzu UV-1900 i UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA).

ACE Protein

Autoclavable citrate extractable protein was quantified using a method developed to measure bioavailable N (Hurisso et al., 2018; Wright & Upadhyaya, 1996, 1998) The procedure to measure ACE started by weighing out 3 g ± 0.03 g of air-dried soil in Thermo Scientific Nunc 50 mL autoclavable plastic tubes. Once samples were weighed out, 24 mL of 0.2 M sodium citrate pH 7 was added to each tube. The tubes were then shaken at 180 rpm for 5 minutes. After 5 minutes, the tubes were swirled to remove any soil from the sides of the tube. The tubes were autoclaved for 30 minutes at 121 °C and cooled to room temperature for approximately 1 hour. The clarification procedure began by pipetting 1.75 mL of liquid into 2.2 mL microcentrifuge tubes which were then centrifuged for 3 minutes at 10,000 xg. Then 1 mL of liquid was extracted from the 2.2 mL centrifuge tubes and pipetted into 1.5 mL centrifuge tubes and centrifuged again at 10,000 xg for 3 minutes. The quantification of protein was done by pipetting 200 µL of each sample into a corresponding well in a 96-well plate. Bicinchoninic acid (BCA) standards were also prepared the same way as samples. A new 96-well plate was then readied for the reaction, 10 µL of each sample was placed into corresponding wells using an 8-channel, multichannel pipettor. The same was done with the standards. Next 200 µL of the BCA working reagent was mixed into wells containing samples using an 8-channel, multichannel pipettor. Once this was complete, a tape seal was added to the top of the reaction plate and placed on the heat block (VWR heat block, 97043-610, Radnor, PA, USA) at 61.5 °C for 1 hour. After 1 hour, the plate was removed from the heat block, the tape seal discarded, and set on the bench top to cool before reading absorbance. Absorbance was read with an Absorbance 96 plate reader (BioTek Synergy H1, Santa Clara, CA, USA) at 562 nm.

POXC

The methods used for POXC follow the procedure outlined in Weil et al., (2003). First 2.5 g ± 0.05 g of air-dried soil was weighed out in duplicates into 50 mL Falcon centrifuge tubes allowing for two tubes for each sample (A and B). An identical set of empty tubes were labeled with corresponding labels on the tubes containing soil. Next 20 mL of deionized water was added, and then the tubes were set aside. Dilutions were then prepared to measure the standard curve by diluting 1.25 mL of 0.2 M KMnO4 with 3.75 mL of deionized water, 2.5 mL of 0.2 M $KMnO_4$ with 2.5 mL of deionized water, and 5 mL of 0.2 M $KMnO_4$ that did not receive any deionized water. The end dilution concentrations were 0.005, 0.01, and 0.02 M $KMnO_4$, respectively. The tubes containing soil then had 18 mL of deionized water added to each. After adding the water to the soil, 2 mL of 0.2 M $KMnO_4$ was also added to begin the reaction. Next the tubes were placed on the end-to-end shaker at 120 rpm, and a timer was set for 10 minutes. The tubes were then removed from the shaker at 2 minutes and allowed to sit for the remaining 8 minutes. A 0.2 mL aliquot was taken from the reaction tubes and transferred into the set of tubes containing 20 mL of water using a pipette. Finally, the tubes were capped and shaken by hand for 10 seconds. Each of the diluted samples were read at 550 nanometers using a spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA).

Soil Respiration

The procedure used to measure soil respiration in this study follows the carbon dioxide alkali trap method defined in Zibilske (1994) in which soil respiration was quantified by measuring the amount of carbon dioxide trapped in the KOH after a 96-hour incubation. This procedure began by preparing the mason jars for incubation by labelling two jars for each sample allowing for a replicate (A and B). Two filter papers (Fisher Scientific 5.5 cm) were then placed at the bottom of each mason jar making sure to overlap them to cover most of the bottom of the jar. Then an aluminum weigh boat (diameter 51 mm) was perforated by poking 9 holes in a 3x3 square pattern on the bottom of the boat. Next, 20 g ± 0.05 g air-dried soil was weighed into each aluminum weigh boat using a sheet of weigh paper underneath to catch

any soil that may have fallen through the holes. The soil from the weigh sheet was added back to the weigh boat. Then the aluminum weigh boat was placed into the mason jar on top of the filter papers using forceps. Once the weigh boat was in the jar, an Americare Royal pizza saver stand was placed over the weigh boat making sure all of the legs were within the boat. The trap jar was then set on top of the stand, and the mason jar lid was closed tight. The incubation began by opening the jars and adding the water and KOH and closing the lid again on each jar, one by one. The 7.5 mL of deionized water was carefully dispensed from a pipettor to the side of the jar to soak the filter papers at the bottom of the mason jar while avoiding the soil in the weigh boat. Then 9 mL of KOH were added into the trap jar that was placed on top of the pizza stand. The lid was then closed to have an air-tight seal and left to incubate undisturbed for 96-hours. After the incubation period was over, the trap jar was removed from the mason jar, and the KOH was poured into a plastic tube. The electrical conductivity of the KOH was then read and the amount of carbon dioxide absorbed to the KOH could be calculated. The amount of CO2 trapped was calculated from the change in conductivity from the trap jars and a clean KOH sample, which was used to estimate the amount of CO2 evolved from the samples.

Water Extractable Phosphorus (WEP)

The procedure for measuring WEP began by weighing 2 g of air-dried soil into 20 mL centrifuge tubes to prepare replicates of each sample (A and B). The procedure started by adding 20 mL of deionized water to each tube and then placing the tubes on the end-to-end shaker on low speed for 1 hour. Once the tubes were done shaking, they were then placed in the centrifuge for 10 minutes at 10,000 rpm. Next the tubes were taken from the centrifuge, and the supernatant was filtered through a 0.45 μm syringe filter. The filtrate was dispensed into 20 mL scintillation vials and 2 drops of HCl were added to acidify the sample to keep the P in solution. The samples were then analyzed colormetrically at 800 nanometers using a spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) (Murphy & Riley, 1962; Watanabe & Olsen, 1965).

Statistical Analysis

The effects of P fertilizer treatment, CC, sampling time, and all interactions were analyzed using SAS version 9.4 (Cary, NC, U.S.A). Phosphorus fertilizer treatment, cover crop, time, and block were all fixed effects. Data were analyzed using PROC MIXED with the Kenward-Roger approximation for denominator degrees of freedom (DDFM = KR). Time was treated as a repeated measure with covariance structures determined by fit statistics. The covariance structure was chosen based on the best fit statistics for Akaike information criterion (AIC) and Bayesian information criterion (BIC). Covariance structures selected for this study included compound symmetry (CS), heterogenous compound symmetry (CSH), first-order autoregressive (AR (1)), heterogenous first-order autoregressive (ARH (1)), and unstructured (UN). Least-squares means estimates and standard errors were used to create all figures and tables. The years 2024 and 2025 were analyzed separately and not compared statistically. Significant differences were detected using $p < 0.05$.

Results

The soil health indicators measured in this study can be divided into four categories: inorganic nutrient pool, organic nutrient pool, microbial biomass nutrient pool, and biological activities. Inorganic nutrient pool measures include, water extractable P (WEP), inorganic N as ammonium (NH_4-N) and nitrate (NO_3-N) as well as dissolved total N (DTN). All inorganic nutrient pool measures were analyzed, aside from DTN in 2024 and WEP in 2024, and 2025. Complete WEP data for 2024 and 2025 were not able to be included in this thesis, however, data from the T0 sampling time in 2024 is provided for reference (Table 2.2). Organic nutrient pool measures include dissolved organic N (DON), dissolved organic C (DOC), ACE, and POXC. Microbial biomass nutrient pool measures include, MBC, MBN, MBC, MBC:MBN, MBN:MBP, and MBC:MBP. Biological activity measures include the enzyme activities of β-glucosidase, β-D-glucosaminidase, and acid and alkaline phosphatase, as well as soil respiration. ANOVA tables were produced for each of the four categories: inorganic nutrient pool

measures (Table 2.3), organic nutrient pool measures (Table 2.4), microbial biomass nutrient pool measures (Table 2.5), and biological activities (Table 2.6).

Inorganic Nutrient Pools

There were no significant three-way interactions for inorganic nutrient pool measures in 2024 or 2025. In 2024, NH_4-N had a P fertilizer treatment (TRT)*TIME interaction (p=0.0032) (Figure 2.2). The NH_4-N values in all P fertilizer treatments continued to decline from T0 to FL. There were no effects from P fertilizer treatment on NH_4-N for the T1 and T2 sampling times. At T0, the BAM treatment was greater than both NP and SUF treatments and for FL, the BAM treatment was greater than the NP treatment. In 2025, a TRT*TIME interaction was also observed for NH_4-N (p=0.023) (Figure 2.3). Phosphorus fertilizer treatments had no effects on NH_4-N for T0, T2, or FL, but had a significant effect for T1, where NP and SUF were both greater than BAM. The NH_4-N in all P fertilizer treatments continued to decline from T1 through FL.

Nitrogen as NO_3-N had a CC*TIME interaction in 2024 (p=0.0142) (Figure 2.4). The NO_3-N value in the NC treatment at the T0 sample time was much higher than the NO_3-N in the CC treatment at T0. There was no CC treatment effect on NO_3-N values for T1, T2, or FL sampling times. The NO_3-N values in the NC treatment continued to decrease from T0 to T2 while the NO_3-N values in the CC treatment remained consistent throughout the 2024 sampling times. There was no CC*TIME interaction for NO_3-N in 2025, and there were no additional significant interactions for inorganic nutrient pool measures.

In 2024, inorganic N (p=0.0016) had a TIME effect where inorganic N increased from T0 to T1 and then decreased for the rest of the growing season from T1 to FL. In 2025, a TIME effect was observed in NO_3-N (p=0.0001), inorganic N (p=0.0001), and DTN (p=0.0001). Inorganic N, NO_3-N, and DTN, all followed similar trends throughout the growing season, with an increase from T0 to T1 and T2 sample times followed by a decrease in the FL sample time.

Organic Nutrient Pools

All organic nutrient pool measures were analyzed for 2024 aside from DON. There were no significant two- or three-way interactions for any of the organic nutrient pool measures in 2024 or 2025. TIME was a main effect for measures, DOC (p=0.0097), autoclavable citrate extractable (ACE) protein (p=0.0136), and permanganate oxidizable C (POXC) (p=0.0001), in 2024. Autoclavable citrate extractable (ACE) protein had the highest values for T0 and FL and decreased slightly during the in-season samplings in 2024. Dissolved organic C followed a similar trend with an increase in the in-season sampling times from T0 to T1 and T2, followed by a decrease in the FL sampling time. Permanganate oxidizable C also had a TRT main effect in 2024 (p=0.017), where the SUF P fertilizer treatment had the greatest value but were not different from the value in the BAM treatments. The BAM treatment was also not different from the NP treatment, which had the lowest of the values. In 2024, the main effect of CC was observed in ACE (p=0.023) and POXC (p=0.0001). POXC values in the CC treatment were 117% higher compared to the NC treatment. ACE values in the CC treatment were 111% higher compared to the NC treatment.

TIME was a main effect for all measures, DON (p=0.0001), ACE protein (p=0.0001), and POXC (p=0.0001) in 2025, aside from DOC. Dissolved organic N increased from T0 to T1, remained steady in-season from T1 to T2, followed by a decrease from T2 to the FL sampling time. In ACE, there was a decrease from T0 to T1, followed by an increase from T1 to T2, and remained steady the rest of the growing season. Permanganate oxidizable C was more variable with a decrease from T0 to T1, followed by an increase in T2, and finally decreasing to the lowest value in the FL sampling time.

Microbial Biomass Nutrient Pools

There were no significant three-way interactions for any microbial biomass nutrient pools in 2024 or 2025. In 2024, MBC had two interactions: CC*TIME (p=0.004) (Figure 2.5) and TRT*TIME (p=0.0074) (Figure 2.6). For the TRT*TIME interaction, the MBC for the NP and SUF followed the same pattern over time, with the greatest values at T0 and FL and lesser MBC for T1 and T2. For the BAM

treatment, the MBC remained high at T1, declined for T2, and then increased again for the FL sampling. There were not any P treatment effects on MBC for T0. In T1, MBC was greatest in BAM. In T2 the BAM and SUF had greater MBC than NP. However, for FL sampling, SUF had greater MBC than BAM. The CC increased MBC for every sampling, though the increase was not significant for T2 (Figure 2.5). Also, the CC increased MBC more for the FL sampling than for samples collected at other times. In 2025, there were no significant interactions or main effects for MBC.

In 2024, MBN had a TIME main effect (p=0.0001). The FL sampling time had the highest MBN value while all other sample times had lower values and were not different from each other. In 2025, MBN had a CC*TIME interaction (p=0.0182) (Figure 2.7). Microbial biomass N values in the CC*TIME interaction showed values in the T2 sample time were greater than all other sampling times for both CC and NC treatments. Cover crop treatments in T0 and T1 were greater than NC treatments at the same sampling times. Microbial biomass N was not affected by the CC treatment in the T2 and FL sampling times. Also in 2025, MBN had a TRT*TIME interaction, where all three P fertilizer treatments at the T2 sample time had the highest MBN values and all three P fertilizer treatments at the FL sample time had the lowest (p=0.0345) (Figure 2.8). For the T0 and T1 sampling time, the NP had the greatest MBN and the BAM had the least, whereas there were no significant treatment effects for T2 and FL sampling times.

In 2024, MBP had a TRT*TIME interaction (p=0.045) (Figure 2.9). The SUF and BAM P fertilizer treatments at the T1 and T2 timing were greater than the same P fertilizer treatments at T0 and FL sample times. The SUF and BAM treatments were also greater than the NP treatment at both the T1 and T2 sample times. In the T0 and FL sample times, there was no difference between the three P fertilizer treatments. In 2025, Microbial biomass P had a TRT main effect (p=0.0001) (Figure 2.10) as well as a TIME main effect (p=0.0004). Microbial biomass P was highest in the BAM treatment, decreased in the SUF treatment, and was the lowest in the NP treatment (Figure 2.10). Microbial biomass P was also highest in the T0 sample time. The T1 sample time was not different from the FL sample time, and MBP was greater in T1 than T2. The FL sampling time was also not different from the T2 sample time.

The MBC:MBN ratio had a time effect in 2024 (p=0.0001) and 2025 (p=0.0002). In 2024, the T0, T1, and T2 sampling times all have higher ratios than the FL sampling time. In 2025, the ratio of MBC:MBN was higher in the fall compared to the T0, T1, and T2 sampling times. The ratio of MBC:MBN was not influenced by CC or P fertilizer treatment in 2024 or 2025.

There were no significant main effects or interactions for MBN:MBP in 2024, but there was a TRT*TIME interaction observed for the MBN:MBP ratio in 2025 (p=0.0002) (Figure 2.11). The MBN:MBP ratio was highest in the T2 sampling time for all three P fertilizer treatments compared to other sampling times. The NP P fertilizer treatment was greater than the SUF and BAM treatments at T0, T2, and FL sampling times. Additionally, there was no difference in the MBN:MBP ratio between the SUF and BAM P fertilizer treatments at all sampling times.

There were no significant main effects or interactions for MBC:MBP in 2024, but the main effects of time and treatment affected the MBC:MBP ratio (p=0.0059) in 2025. The MBC:MBP ratio was higher in the NP P fertilizer treatment compared to the SUF and BAM treatments. There was no difference between the SUF and BAM treatments.

Biological Activity

There were no significant three-way interactions for any microbial biomass nutrient pools in 2024 or 2025. β-Glucosidase (GLU) activity had a TRT*CC interaction in 2024 (p=0.0101) (Figure 2.12). The presence of a CC in SUF and NP resulted in higher GLU activity in the CC treatment compared to the NC treatment. There was no change in GLU activity between the CC and NC treatments in the BAM P fertilizer treatment. Also, in 2024, GLU activity had a main effect of TIME (p=0.0001). Activity levels for GLU decreased gradually from T0 to T2 followed by an increase in FL sampling time. Activity levels in T0 and FL were not different from each other. There were no significant two-way interactions for GLU in 2025, however GLU had a main effect of TIME (p=0.0001). In GLU, activity levels were lowest at the T0 sampling time and increased throughout the growing season, with FL having the highest activity levels.

β-D-Glucosaminidase (NAG) also had a TRT*CC interaction in 2024 (p=0.0373) (Figure 2.13). The NP and SUF P fertilizer treatments had higher NAG activity in the CC treatments compared to the NC treatments. There was no difference in activity between the CC and NC treatments in the BAM P fertilizer treatment. There were no significant two-way interactions for NAG in 2025, however NAG had a main effect of TIME (p=0.0001). Activity levels in NAG increased from T0 to T1, followed by a decrease from T1 to T2, and finally increasing again to the highest activity in the FL sampling time.

Alkaline phosphatase had a TRT*CC interaction in 2024 as well (p=0.0045) (Figure 2.14). Following a similar trend, the NP and SUF P fertilizer treatments had higher alkaline phosphatase activity in the CC treatments compared to the NC treatments. There was no difference in activity between the CC and NC treatments in the BAM P fertilizer treatment. Alkaline phosphatase also had a main effect of TIME in 2024 (p=0.0002). Activity levels for alkaline phosphatase gradually increased throughout the growing season from T0 to FL, with the lowest activity level at the T0 sampling time and the highest at the FL sampling time. Alkaline phosphatase activity levels at T1 were not different than activity levels at T2 or at T0. Alkaline phosphatase had no significant two-way interactions in 2025, but had a main effect of TIME (p=0.0001). Alkaline phosphatase followed a trend similar to GLU, with the lowest activity levels at the T0 sampling time followed by an increase in activity throughout the growing season, resulting in FL having the highest activity levels.

Acid phosphatase had no significant two-way interactions, but had main effects of CC (p=0.0006) and TIME (p=0.0001) in 2024. The acid phosphatase activity levels in the CC treatment were 125% greater than levels at the NC treatment. Acid phosphatase activity levels also varied with sampling time as T2 was the timing with the highest levels and FL being the lowest. Sample times T0 and T1 fell in between T2 and FL and were also not different from each other. Acid phosphatase had a main effect of TIME in 2025 (p=0.0001). Acid phosphatase followed a trend similar to alkaline phosphatase and GLU, with the lowest activity levels at the T0 and T1 sampling times followed by an increase in activity throughout the growing season, resulting in FL having the highest activity levels.

Soil respiration had a TRT*CC interaction (p=0.0056) (Figure 2.15) as well as a main effect of TIME (p=0.0002) in 2024. In all three P fertilizer treatments, BAM, SUF, and NP, the CC treatment had higher soil respiration levels compared to the NC treatment at the same level of P fertilizer treatment. However, the effect of CC on respiration was greatest in the SUF treatment. The sampling time FL had the highest soil respiration levels but were not different from T2. Soil respiration levels at T2 were also not different from levels at T1. Sampling time T1 had soil respiration levels lower than FL but also not different from T0, which was notably lower than levels at T2 and FL. In 2024, soil respiration was lowest at the T0 sampling time and then progressively increased with the highest soil respiration rates in the FL. Soil respiration had a main effect of TIME in 2025 (p=0.0001). A trend similar to 2024 was observed in 2025, with the lowest soil respiration rate at T0, followed by increasing rate throughout the season with the highest soil respiration rate in FL.

Discussion

Inorganic Nutrient Pools

Inorganic nutrient pools, including total inorganic N, NH_4-N, and NO_3-N as well as DTN, were impacted by time in 2024 and 2025. The in-season temporal variability in inorganic N and DTN has been documented in a previous study at this experiment site (Pasket, 2023; Stewart, 2021) and in the literature (Adhikari et al., 2026; Gonzalez-Maldonado et al., 2025; Siwach et al., 2026). The influence of precipitation has been found to have substantial effects on soil nutrients (Singh et al., 2018), which could explain some of the in-season and year-to-year variability. As inorganic N is a fraction of the total N pool, it is more susceptible to seasonal variability (Gonzalez-Maldonado et al., 2025); even so, in season variability was still observed for DTN. Changes in available forms of N during different times of the crop growing season are logical as this available N can undergo loss pathways, be used by plants and soil microorganisms, or undergo other transformation in the soil. There is also the potential for available N to be added to the system via soil microorganisms solubilizing organic matter.

A P fertilizer treatment by time interaction was influential to NH_4-N in both 2024 (Figure 2.2) and 2025 (Figure 2.3). In 2025, NH_4-N values were noticeably higher in the T1 and T2 sampling times. While in 2024, NH_4-N values were fairly steady across all four sampling times. Total inorganic N was also influenced by P fertilizer treatment in 2024. The BAM P fertilizer treatment had higher inorganic N compared to the NP treatment, while inorganic N in the SUF treatment was not different from either BAM or NP treatments. Similar results were observed previously at this study site (Pasket, 2023).

In 2024, NO_3-N was influenced by a CC*TIME interaction resulting in the highest NO_3-N values in the NC treatment at the T0 sampling time taken shortly after CC termination. This observation was seen shortly after CC termination and can be logically explained as the use of a CC, especially cereal rye, can be known to immobilize N (Dabney et al., 2011). This interaction also occurred before crop planting, meaning there was no other living plant to utilize the N. Cover crops utilize residual nitrate and plant available phosphorus as they grow, resulting in the decrease of these nutrients in the soil (Blanco, 2023). However, after cover crops are terminated, the nitrogen and phosphorus held in the biomass is restored back to the soil and made available by mineralization (Blanco, 2023). The timeliness of N mineralization after CC termination is affected by many different factors, especially cover crop biomass quality (Dabney et al., 2011). Cover crop decomposition times are variable, but Adhikari et al., (2026) reported observations of a cereal rye cover crop with high C:N ratios having increased residual NO_3-N and NH_4-N in the fall meaning CC decomposition likely took the entire grain crop growing season.

Organic Nutrient Pools

Organic nutrient pools, DON, DOC, ACE, and POXC, all had a time effect in both 2024 and 2025. In 2024, ACE gradually increased as time progressed through the growing season with the lowest ACE values in T0 and the highest being in FL. This was not the case in 2025 as there was no difference in ACE values at the T0, T2, and FL sampling times and ACE decreases at the T1 sampling time only. The in-season variability in organic nutrient pools is well documented in the literature (Gonzalez-Maldonado et al., 2025). Similar to inorganic N, Gonzalez-Maldonado et al. (2025) reported that indicators POXC

and ACE, were more susceptible to seasonal variability compared to indicators that measure total nutrient pools. Pasket (2023) determined that seasonal variability greatly influence soil health indicators and this was likely due to soil moisture and temperature. Similarly, in a study done by Kundert et al., (2026), it was determined that annual changes in soil health indicators were strongly correlated to growing season weather patterns.

Autoclavable citrate extractable protein and POXC were both positively influenced by the presence of a CC in the 2024 soybean growing season. This increase in ACE and POXC due to the CC has been observed previously in the 2021-corn and 2022-soybean years at this study site (Pasket, 2023). A study evaluating soil health indicators in a no-till, corn-soybean rotation reported increased POXC under the presence of a CC (French et al., 2026). An increase in residue in the CC treatments is likely the reason behind increased ACE and POXC under the CC treatments. The increased amount of residue in the CC treatment supplements the soil with additional nutrients resulting in increased C and N.

Permanganate oxidizable C also had a P fertilizer treatment effect in 2024. The SUF treatment had greater C than the NP treatment, but interestingly the BAM treatment was not different from either the SUF or NP treatments. A study done by Ni et al., (2025) determined that interactions between microbial driven soil organic C and P can be complicated and treatments that had 25% and 50% reductions in P fertilizer application were able to maintain particulate organic C. They also determined that soil organic C fractions significantly changed over four years of P fertilizer applications.

Biological Nutrient Pools

In the CC*TIME interaction in MBC in 2024, the CC treatment had greater MBC compared to the NC treatment at T0, T1, and FL sampling times (Figure 2.5). This observation aligns with what has been presented in the literature (Dutter et al., 2025; Siwach et al., 2026). The increase of MBC in the CC treatment is likely due to the increase in plant residues, which provide organic inputs to the soil (He et al., 1997). In a corn-soybean rotation, the CC planted after corn typically has greater biomass than the CC planted after soybeans (Table 2.7). This is due to corn harvested typically occurring earlier than soybean

harvest, allowing for earlier planting of the CC and additional time for CC growth. This would result in the positive effects of the CC to be more prevalent in the soybeans rather than the corn. Dutter et al., (2025) found that a winter CC, such as cereal rye, had positive effects on microbial biomass, as well as potentially mineralizable C and N. They also established the effects of the cereal rye cover crop were more positive in soybeans than in corn (Dutter et al., 2025), which aligns with the results from this study. The increase in MBC at the FL sampling time in 2024 is likely due to the increased availability of nutrient inputs supplied by the CC. After CC termination, nutrients are released in the soil from decomposition of CC residue (Blanco, 2023). Microbial biomass C concentrations are strongly linked to availability of organic inputs, such as plant residue (He et al., 1997).

Microbial biomass C had a TRT*TIME interaction in 2024 (Figure 2.6). The MBC values in the SUF and NP treatments had more variability throughout the growing season, decreasing from T0 to T2, and then increasing again in the FL. The BAM treatment had steadier MBC values throughout the growing season. More consistent MBC throughout the growing season may potentially be due to greater nutrient inputs into the system allowing for a more stable pool of MBC. Tang et al. (2016) discovered that increases in MBC were observed only in P fertilizer treatments, and determined that P was the limiting nutrient in the rhizosphere in unfertilized treatments, directly impacting soil microbial biomass. Microbial biomass C decreased in all three P fertilizer treatments at the T2 sampling time. A study done by He et al. (1997) evaluated the effects of mineral P fertilizer on MBC and found that MBC significantly decreases in summer months. The authors attributed this decrease in MBC to the decrease in soil moisture during the summer months. Microbial biomass P had a TRT*TIME interaction in 2024 (Figure 2.9). In this interaction, the spread between P fertilizer treatments was present during the in-season sample times as BAM, clearly was the highest, then SUF, followed by the NP treatment. Microbial biomass P had notably lower values in the T0 and FL sampling times, with no effects from P fertilizer treatments. Phosphorus fertilizer was applied at planting, which would have occurred after the T0 sampling time, and would explain the increase in MBP observed in the T1 and T2 sampling times. Microbial biomass P decreased from T2 to FL in all P fertilizer treatments, and observed no effect from P fertilizer treatment. He et al.

(1997) determined that MBP varied greatly with soil moisture. A study done by Shi et al., (2013) found increased MBP with P fertilization likely due to soil moisture effects and SOM. The soil moisture at the time of sampling could have influenced the decreased MBP values at the FL sampling time. The soybean crop utilizing soil P may another driving factor in the decreasing MBP in the FL.

Phosphorus fertilizer treatments influenced MBP and the ratio of MBC:MBP in 2025. We observed the highest MBP values in the BAM treatment and the lowest in NP. Microbial biomass P increased as the level of P fertilizer treatment increased, which agrees with results from a study done by Shi et al. (2013) who showed increased MBP with P fertilization. With the ratio of MBC:MBP, we saw the highest ratio in the NP treatment while BAM and SUF P fertilizer treatments had the lowest ratios. The lower MBP in the NP treatment would cause this ratio to increase, while the BAM and SUF treatments are receiving P fertilizer and observed higher MBP resulting in lower MBC:MBP ratios. This observation agrees with a study done by He et al. (1997) in which they saw treatments that received P fertilizer had enriched MBP values resulting in a lower MBC:MBP ratio.

A CC*TIME interaction was observed for MBN in 2025 (Figure 2.7). The T2 timing had notably greater MBN values in both the CC and NC treatments, compared to other sample times. The MBN value at T2 in the CC treatment was 1,230% higher than the value in the CC treatment at FL. Shi et al. (2013) found that MBN was highly variable under no-till due to environmental conditions, specifically soil moisture. Another potential explanation for this increase in MBN at the T2 sample time, could be the additions of N supplied by the CC.

There was a TRT*TIME interaction observed for both the ratio of MBN:MBP and NH_4-N in 2025. The NP treatment at T2 had a higher MBN:MBP ratio than all other treatments and timings. This result is logical as we would expect to see lower P values in the NP treatment where we are not applying P. The lower P values would increase this ratio. This effect in T2 at the NP treatments is likely because, as the growing season progresses, we would expect plant uptake of P to increase in corn. The treatment by time interaction in NH_4-N values shows that the NP and SUF treatments in T1 were the highest while all P treatments at T0 and FL were the lowest.

Biological Activity

The TRT*CC interaction in 2024-soybeans was observed for alkaline phosphatase, NAG, GLU, and soil respiration. For NAG and soil respiration, we see that regardless of P fertilizer treatment the CC treatment was greater compared to the NC treatment. The increase in soil enzyme activity due to integrating a CC is likely due to the additional nutrients added into the system. French et al. (2026) reported P fertilizer rate and CC interactions influencing GLU under a corn-cotton-soybean rotation. They observed increased enzyme activity with the presence of a CC compared to fallow under the optimum P fertilization recommendation. Cover crops serve as an added substrate to the system influencing soil enzyme activities and soil respiration allowing for increased C and N storage (Mbuthia et al., 2015). For alkaline phosphatase and GLU activities, the NP and SUF treatments have increased activity in the presence of a CC compared to activity levels in the NC plots, but there was no difference in the CC or NC treatments within the BAM P fertilizer management. A P fertilizer treatment and CC interaction was observed in soil enzymes at this study site in 2022-soybeans as well (Pasket, 2023). The result of this interaction in alkaline phosphatase and GLU replicates the exact results from 2022. Previous results have been attributed to the lack of response to a CC in the BAM treatments to the system having no nutrient limitations. As enzyme production was not increased by a CC, resource allocation for enzyme production was not necessary at the highest level of P fertilizer management. Enzyme production is a costly cellular process requiring nutrients essential for energy (Burns et al., 2013). The cellular requirements for enzyme production are often weighed with the need for increased nutrient availability in the soil (Burns et al., 2013). Increased phosphatase enzyme activity generally occurs in P deficient systems (Janes-Bassett et al., 2022; Martinez and Tabatabai, 2011). Enzyme synthesis can be tied to C, P, and N nutrient concentrations in the soil (Grobelak et al., 2026; Sainju et al., 2022). In P deficient systems, plants and microbes typically produce more phosphatase enzymes (Martinez and Tabatabai, 2011). Phosphorus fertilizer applications result in a P rich environment that can inhibit phosphatase enzyme activities. We

see in the highest P fertilizer treatment, P was not deficient and the positive benefits from the CC on alkaline phosphatase activity are no longer present.

Acid phosphatase was also influenced by the CC treatment in 2024 with the CC treatment being greater than the NC treatment. Siwach et al. (2026) reported similar findings with acid phosphatase response to cover crop in a no-till, corn-soybean rotation with a cereal rye cover crop. Investigations in previous years found similar results, and acid phosphatase was significantly increased in the presence of a CC in soybeans (Pasket, 2023).

Soil enzymes were all affected by in-season sampling time throughout the year in 2024-soybean and 2025-corn aside from NAG in 2024. Soil respiration was also influenced by sampling time in both 2024 and 2025. Soil enzyme activity levels increased as time progressed with the T0 timing being the lowest and FL being the highest. Similarly, soil respiration was lowest in the T0 sampling and increased as the season progressed in both 2024 and 2025. The in-season variability of soil enzyme activities and soil respiration aligns with what has been previously found in 2021-corn and 2022-soybeans at this study site (Pasket, 2023).

Conclusion

Evaluating different soil health indicators in this study showed the influence of CC, P fertilizer treatment, and sampling time within a corn-soybean system. Cover crops appear to have had the greatest influence during the soybean crop year in 2024. Several soil health indicators reinforced the potential benefits provided by the presence of a CC, such as soil enzyme activities, MBN, MBC, soil respiration, ACE, POXC, and NO_3-N. Cover crops provide C and N inputs to the system increasing available substrate for soil microorganisms to utilize, which drives many of these effects. We can confirm that soil health was improved by the presence of cover crops seen with increased nutrient pools and increased microbial activity observed with soil microbial activity.

Phosphorus fertilizer treatment influenced β-D-glucosaminidase, β-glucosidase, alkaline phosphatase, and soil respiration in 2024. The hypothesis that enhanced nutrient cycling was observed as

the level of P fertilizer management decreases, as observed by different inorganic, organic, and biological nutrient pools, can be confirmed.

Sample timing greatly affected soil health indicators in both 2024 and 2025. As temperature and moisture influence the soil microbial community and nutrient cycling processes, time can affect soil health indictors because of variability within a season and from year to year. We determined that soil health indicators changed throughout the growing season due to climatic factors. Soil health indicators appear to be influenced more under the soybean crop year in 2024 compared to the corn crop year in 2025. The research done from a previous study in soybeans in 2022 appears to have similar results to this research completed in soybeans in 2024.

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Table 2.1 Soil enzyme substrates and start and stop buffers specific to each enzyme analysis.

Soil enzyme	Substrate	Start Buffer	Stop Buffer
β-Glucosaminidase	0.01 M ρ-Nitrophenyl-N-acetyl-β-D-glucosaminide	0.1 M Acetate buffer	0.5 M NaOH
β-Glucosidase	0.05 M p-Nitrophenyl-β-D-glucopyranoside	MUB pH 6.0[a]	0.1 M THAM pH 12.0[d]
Alkaline phosphatase	0.05 M ρ-Nitrophenyl phosphate	MUB pH 11[b]	0.5 M NaOH
Acid phosphatase	0.05 M ρ-Nitrophenyl phosphate	MUB pH 6.5[c]	0.5 M NaOH

[a]Modified universal start buffer pH 6.0 (MUB pH 6.0)
[b]Modified universal start buffer pH 11 (MUB pH 11)
[c]Modified universal start buffer pH 6.5 (MUB pH 6.5)
[d]0.1 M tris(hydroxymethyl)aminomethane (0.1 M THAM pH 12.0)

Table 2.2 Water extractable phosphorus (WEP) values for the T0 sampling time in 2024. Abbreviations include, P fertilizer treatment (TRT), control (NP), sufficiency (SUF), build and maintain (BAM), cover crop (CC), no cover (NC), number of observations (N Obs.), and standard deviation (Std Dev). The T0 sampling time was taken after CC termination and before planting.

WEP : T0						
TRT	**CC**	**N Obs.**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
BAM	**CC**	**3**	2.29	0.14154	2.2	2.45
	NC	**3**	2.14	0.45325	1.87	2.66
NP	**CC**	**3**	1.53	0.00577	1.53	1.54
	NC	**3**	1.49	0.13856	1.41	1.65
SUF	**CC**	**3**	1.73	0.06083	1.66	1.77
	NC	**3**	1.49	0.24269	1.29	1.76

Table 2.3 An ANOVA table presenting inorganic nutrient pools for 2024 and 2025. Table abbreviations include P fertilizer treatment (TRT), cover crop (CC), and sample time (TIME). Values bolded and underlined are considered significant at a significance level of ($p<0.05$).

	2024			2025		
Effect	**Inorganic N**	**NO3-N**	**NH4-N**	**Inorganic N**	**NO3-N**	**NH4-N**
TRT	**0.0307**	0.2459	**0.0025**	0.4557	0.8557	0.3132
CC	0.3533	**0.0076**	0.4984	0.0842	0.117	0.1942
TRT*CC	0.5914	0.5830	0.5974	0.2146	0.0749	0.7103
TIME	**0.0016**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
TRT*TIME	0.1667	0.2021	**0.0032**	0.3395	0.5986	**0.023**
CC*TIME	0.5087	**0.0142**	0.6924	0.232	0.2103	0.485
TRT*CC*TIME	0.8365	0.6651	0.8012	0.4159	0.4518	0.4045

Table 2.4 An ANOVA table presenting organic nutrient pools for 2024 and 2025. Table abbreviations include P fertilizer treatment (TRT), cover crop (CC), sample time (TIME), dissolved total N (DTN), dissolved organic N (DON), dissolved organic C (DOC), autoclavable citrate extractable (ACE) protein, and permanganate oxidizable C (POXC). Dissolved organic N was not measured in 2024. Values bolded and underlined are considered significant at a significance level of ($p<0.05$).

	2024					2025				
Effect	**DTN**	**DON**	**DOC**	**ACE**	**POXC**	**DTN**	**DON**	**DOC**	**ACE**	**POXC**
TRT	0.7331	.	0.5263	0.0573	**0.017**	0.5701	0.6719	0.1526	0.8636	0.125
CC	0.7417	.	0.6519	**0.023**	**<.0001**	0.104	0.5029	0.926	0.8095	0.5876
TRT*CC	0.0541	.	0.1909	0.36	0.1013	0.2401	0.5723	0.3374	0.933	0.4413
TIME	**<.0001**	.	**0.0097**	**0.0136**	**<.0001**	**<.0001**	**<.0001**	0.5239	**<.0001**	**<.0001**
TRT*TIME	0.6466	.	0.6385	0.8653	0.5705	0.3689	0.94	0.5519	0.9927	0.0587
CC*TIME	0.6295	.	0.8001	0.9767	0.7444	0.1574	0.061	0.7157	0.9933	0.7013
TRT*CC*TIME	0.1826	.	0.269	0.9268	0.1373	0.2379	0.3498	0.1851	0.9749	0.3791

Table 2.5 An ANOVA table presenting biological nutrient pools for 2024 and 2025. Table abbreviations include P fertilizer treatment (TRT), cover crop (CC), sample time (TIME), microbial biomass C (MBC), microbial biomass N (MBN), and microbial biomass P (MBP). Values bolded and underlined are considered significant at a significance level of ($p<0.05$).

	2024						2025					
Effect	**MBC**	**MBN**	**MBP**	**C:N**	**N:P**	**C:P**	**MBC**	**MBN**	**MBP**	**C:N**	**N:P**	**C:P**
TRT	**0.0004**	0.899	**0.0055**	0.8488	0.3267	0.6107	0.6721	0.9834	**<.0001**	0.5577	**0.0006**	**0.0022**
CC	**<.0001**	0.1204	0.2314	0.0816	0.6643	0.8216	0.6098	**0.0443**	0.1123	0.7462	0.9156	0.2172
TRT*CC	0.4635	0.4226	0.5315	0.3872	0.7414	0.8941	0.3685	0.3529	0.4419	0.3918	0.9049	0.6285
TIME	**<.0001**	**<.0001**	**<.0001**	**0.0001**	0.7207	0.837	0.0844	**<.0001**	**0.0004**	**0.0002**	**<.0001**	**0.0059**
TRT*TIME	**0.0074**	0.7869	**0.045**	0.8008	0.7596	0.8886	0.9051	**0.0345**	0.431	0.6994	**0.0002**	0.1334
CC*TIME	**0.004**	0.0644	0.8324	0.1509	0.9605	0.9437	0.9986	**0.0182**	0.1128	0.9905	0.3892	0.0648
TRT*CC* TIME	0.0849	0.8043	0.9711	0.593	0.8508	0.9603	0.1549	0.5332	0.3836	0.5419	0.453	0.2215

Table 2.6 An ANOVA table presenting measures of biological activity for 2024 and 2025. Table abbreviations include P fertilizer treatment (TRT), cover crop (CC), sample time (TIME), β-glucosaminidase (NAG), β-glucosidase (GLU), acid phosphatase (ACID P), alkaline phosphatase (ALK P), and soil respiration (RESP).Values boldened and underlined are considered significant at a significance level of ($p<0.05$).

	2024					2025				
Effect	**NAG**	**GLU**	**ACID P**	**ALK P**	**RESP**	**NAG**	**GLU**	**ACID P**	**ALK P**	**RESP**
TRT	0.2063	0.5624	0.5296	0.1398	0.8345	0.5325	0.1772	0.6832	0.1768	0.4675
CC	**<.0001**	**0.0005**	**0.0006**	**<.0001**	**<.0001**	0.3773	0.2468	0.2426	0.2549	0.4691
TRT*CC	**0.0373**	**0.0101**	0.2677	**0.0045**	**0.0056**	0.9931	0.1735	0.844	0.5107	0.2415
TIME	0.3021	**<.0001**	**<.0001**	**<.0001**	**0.0002**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
TRT*TIME	0.5708	0.6096	0.9342	0.4612	0.4012	0.3678	0.0532	0.717	0.6701	0.7988
CC*TIME	0.1941	0.6072	0.9818	0.4325	0.1371	0.2713	0.4764	0.7722	0.1278	0.436
TRT*CC*TIME	0.8902	0.1042	0.8122	0.5565	0.2442	0.9031	0.5138	0.9031	0.3047	0.3962

Table 2.7 Cover Crop Biomass (kg ha^{-1}) across 8 years at the KAW. Abbreviations include, P fertilizer treatment (TRT), control (NP), sufficiency (SUF), build and maintain (BAM), cover crop (CC), no cover (NC), number of observations (N Obs.), and standard deviation (Std Dev).

CC BIOMASS (kg ha^{-1})						
YEAR	**TRT**	**N Obs.**	**Mean**	**Std Dev**	**Minimum**	**Maximum**
2018	**BAM**	**3**	2237	366.873	1817.3	2496.7
	NP	**3**	2454.33	376.359	2059	2808.3
	SUF	**3**	2482.67	616.868	1823.3	3045.7
2019	**BAM**	**3**	340.9	136.093	188.3	449.7
	NP	**3**	161.867	44.4574	119.3	208
	SUF	**3**	433.467	98.0865	320.7	499
2020	**BAM**	**3**	4218.57	561.322	3726	4829.7
	NP	**3**	2680.67	226.902	2419	2823
	SUF	**3**	3918	632.641	3375	4612.7
2021	**BAM**	**3**	524.333	104.923	404	596.7
	NP	**3**	338.1	22.5062	323.3	364
	SUF	**3**	523.7	158.294	346.7	651.7
2022	**BAM**	**3**	1672	286.794	1393	1966
	NP	**3**	1302.33	272.876	1018.7	1563
	SUF	**3**	1456.33	341.011	1062.7	1662
2023	**BAM**	**3**	424.133	9.33613	413.7	431.7
	NP	**3**	119.333	45.9481	76.7	168
	SUF	**3**	292.667	101.507	181.3	380
2024	**BAM**	**3**	2104.2	274.236	1926.1	2420
	NP	**3**	2177.57	724.891	1608.2	2993.6
	SUF	**3**	2534.53	648.911	2128	3282.9
2025	**BAM**	**3**	454.667	76.8064	366	500.7
	NP	**3**	126.767	16.6512	110	143.3
	SUF	**3**	355.267	84.2514	271.3	439.8

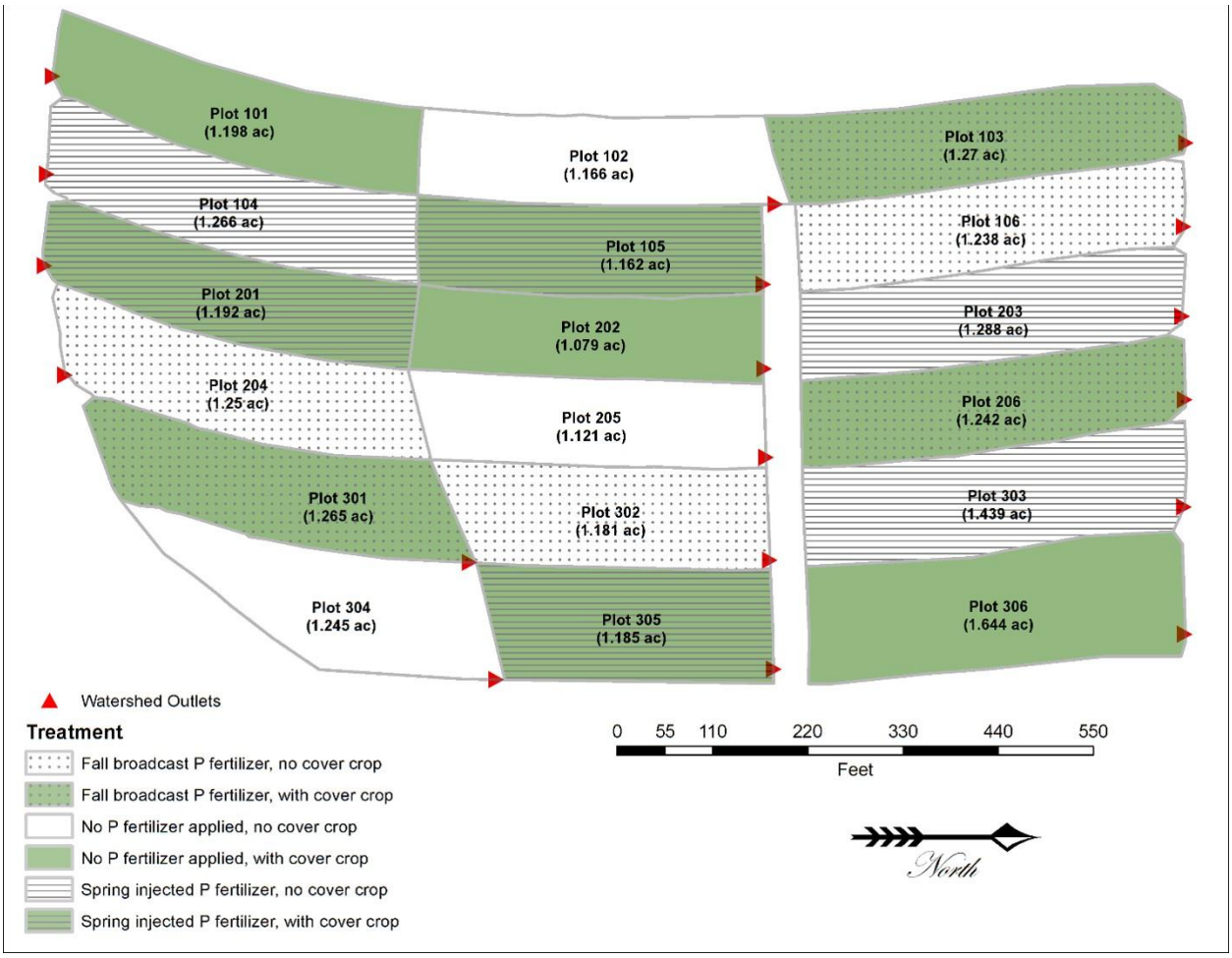


Figure 2.1 A plot map of the Kansas Agricultural Watershed (KAW) field laboratory site.

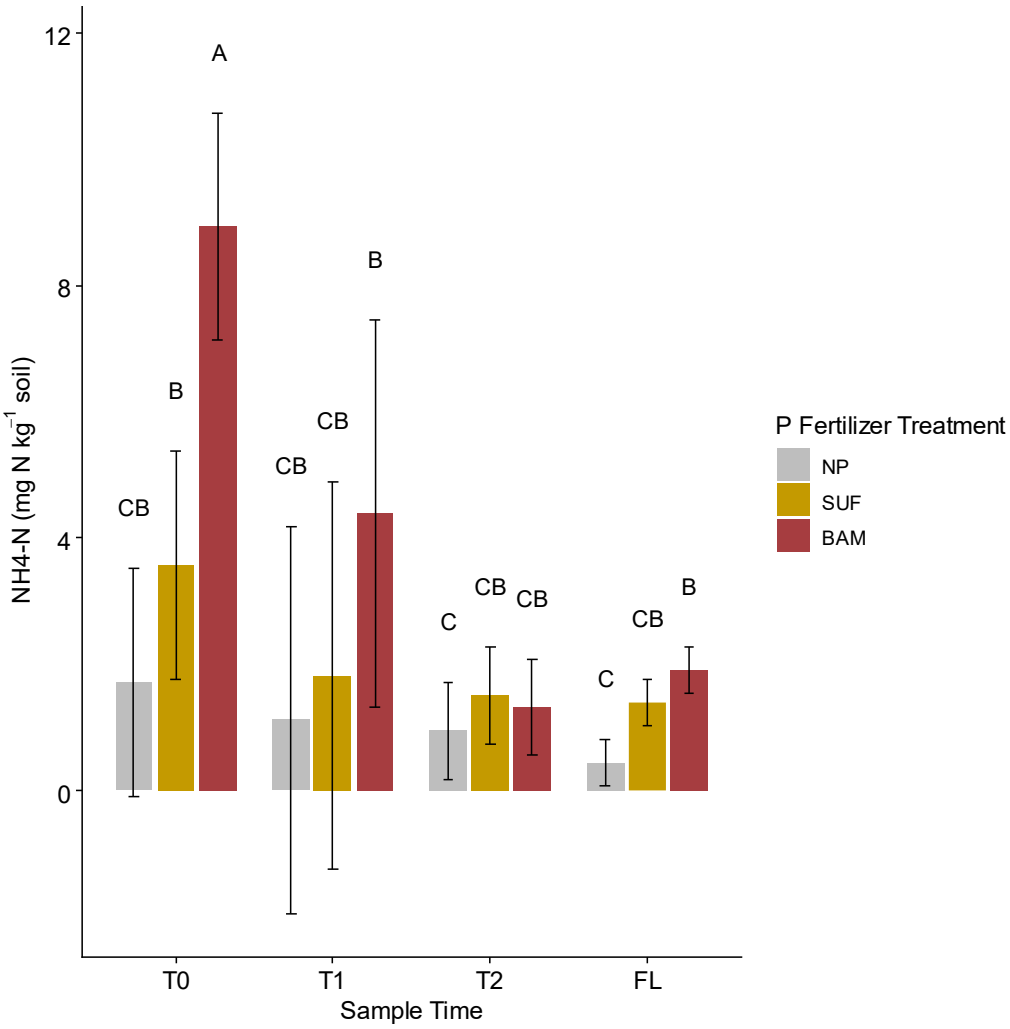


Figure 2.2 A TRT*TIME interaction in NH_4-N (mg N kg^{-1} soil) in the 2024 soybean production year that shows decreasing NH_4-N from T0 to T2 (p=0.0032). The BAM treatment at the T0 sampling time was considerably greater than all other treatments at all other sampling times. The sampling time T0 was taken after cover crop (CC) termination but before planting, T1 was taken 30 days after the initial sampling, T2 was taken 60 days after the initial sampling, and FL was taken after the grain crop harvest. Phosphorus fertilizer treatments include a control (NP), sufficiency (SUF), and build and maintain (BAM).

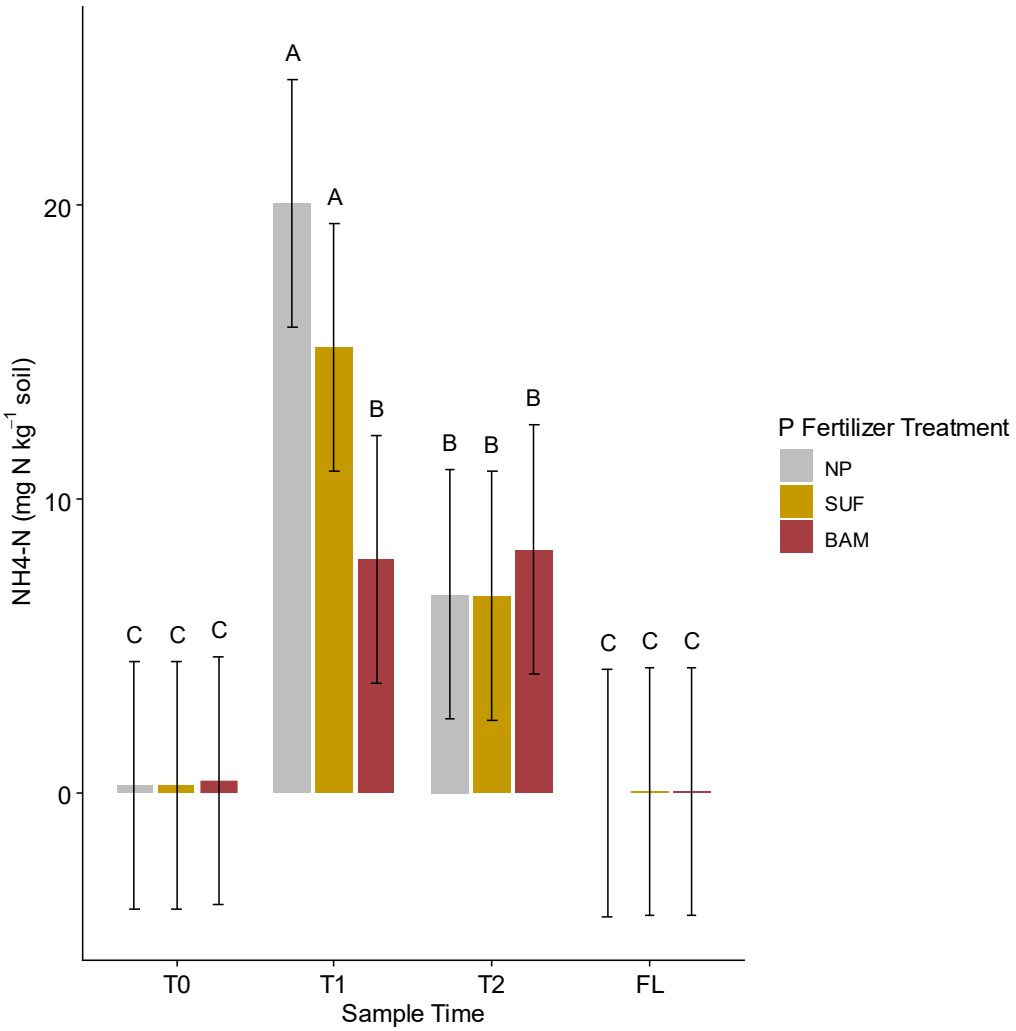


Figure 2.3 A TRT*TIME interaction in NH_4-N (mg N kg^{-1} soil) in the 2025 corn production year shows increased NH_4-N at the T1 and T2 sampling times compared to T0 and FL (p=0.023). The sampling time T0 was taken after cover crop (CC) termination but before planting, T1 was taken 30 days after the initial sampling, T2 was taken 60 days after the initial sampling, and FL was taken after the grain crop harvest. Phosphorus fertilizer treatments include a control (NP), sufficiency (SUF), and build and maintain (BAM).

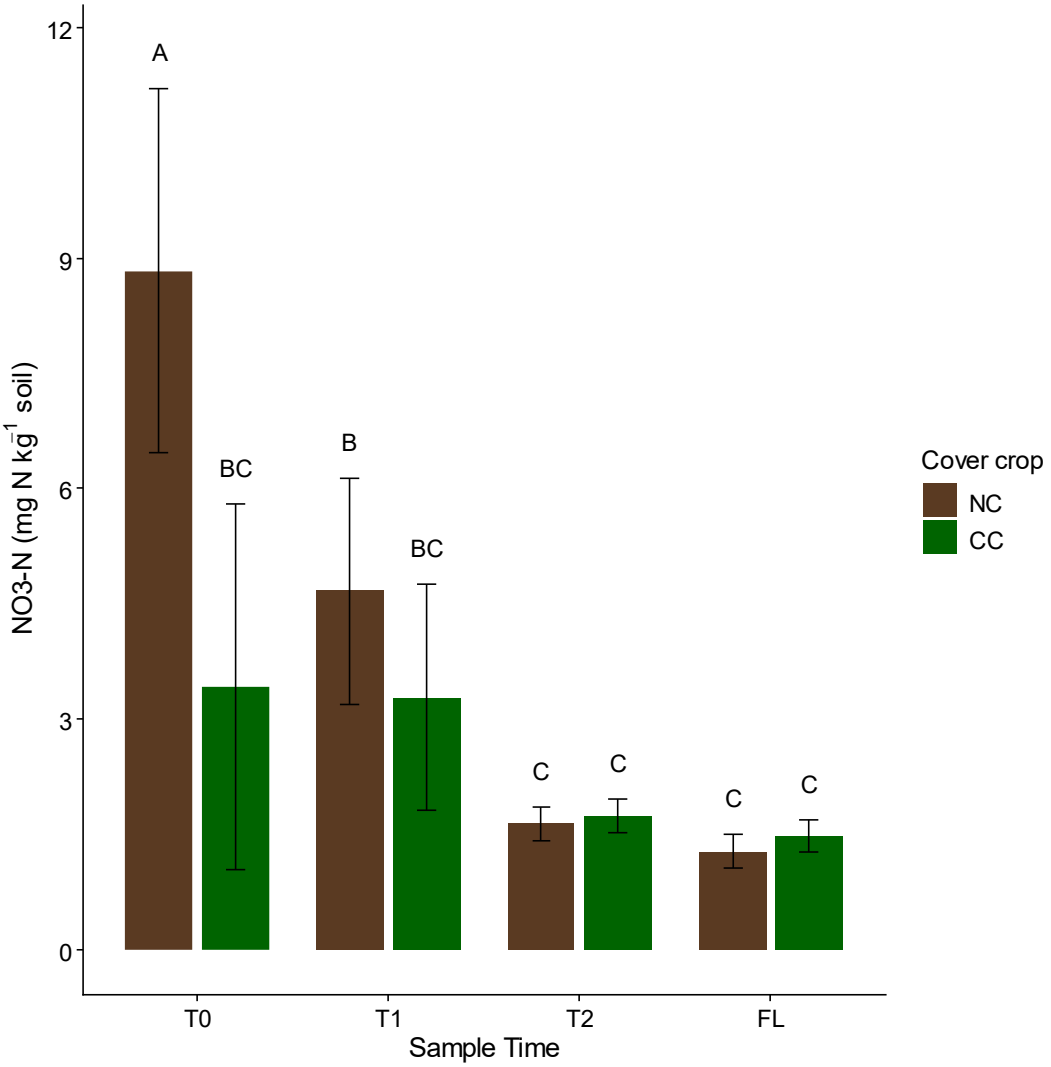


Figure 2.4 A CC*TIME interaction in NO_3-N (mg N kg^{-1} soil) in 2024 during the soybean crop year showing steady NO_3-N values in the CC treatment and decreasing NO_3-N values in the NC treatment from T0 to T2 (p=0.0142). The NC treatment had notably higher NO_3-N at the T0 sampling time compared to the CC treatment. The sampling time T0 was taken after cover crop (CC) termination but before planting, T1 was taken 30 days after the initial sampling, T2 was taken 60 days after the initial sampling, and FL was taken after the grain crop harvest.

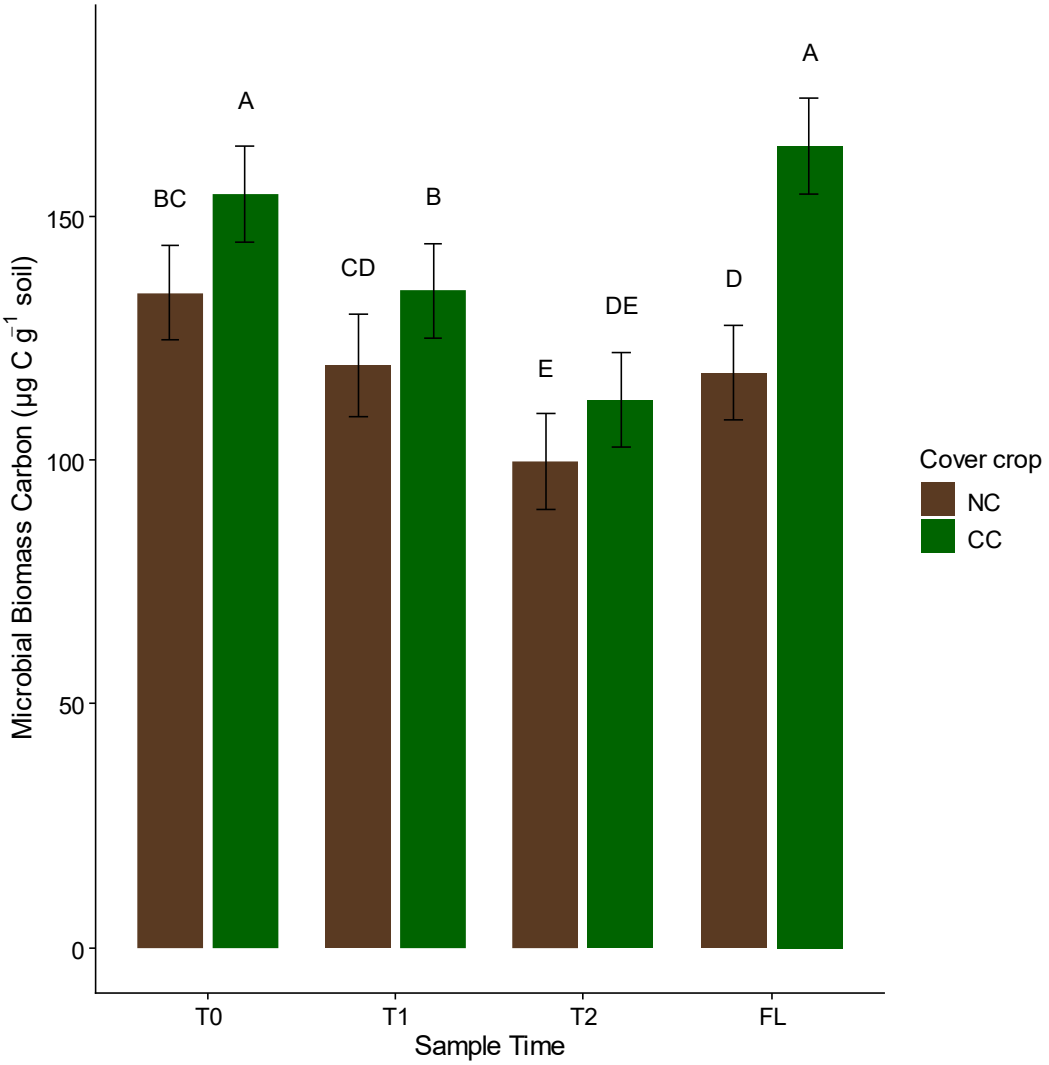


Figure 2.5 A CC*TIME interaction in MBC (µg C kg^{-1} soil) in 2024 during the soybean crop year (p=0.004). The MBC values in the CC treatment were generally greater compared to the NC treatment and both CC and NC treatments followed a similar trend of decreasing from T0 to T2 and increasing in the FL. Additionally, the increase between the CC and the NC treatments in MBC in the FL were greater than any other sampling time. The sampling time T0 was taken after cover crop (CC) termination but before planting, T1 was taken 30 days after the initial sampling, T2 was taken 60 days after the initial sampling, and FL was taken after the grain crop harvest.

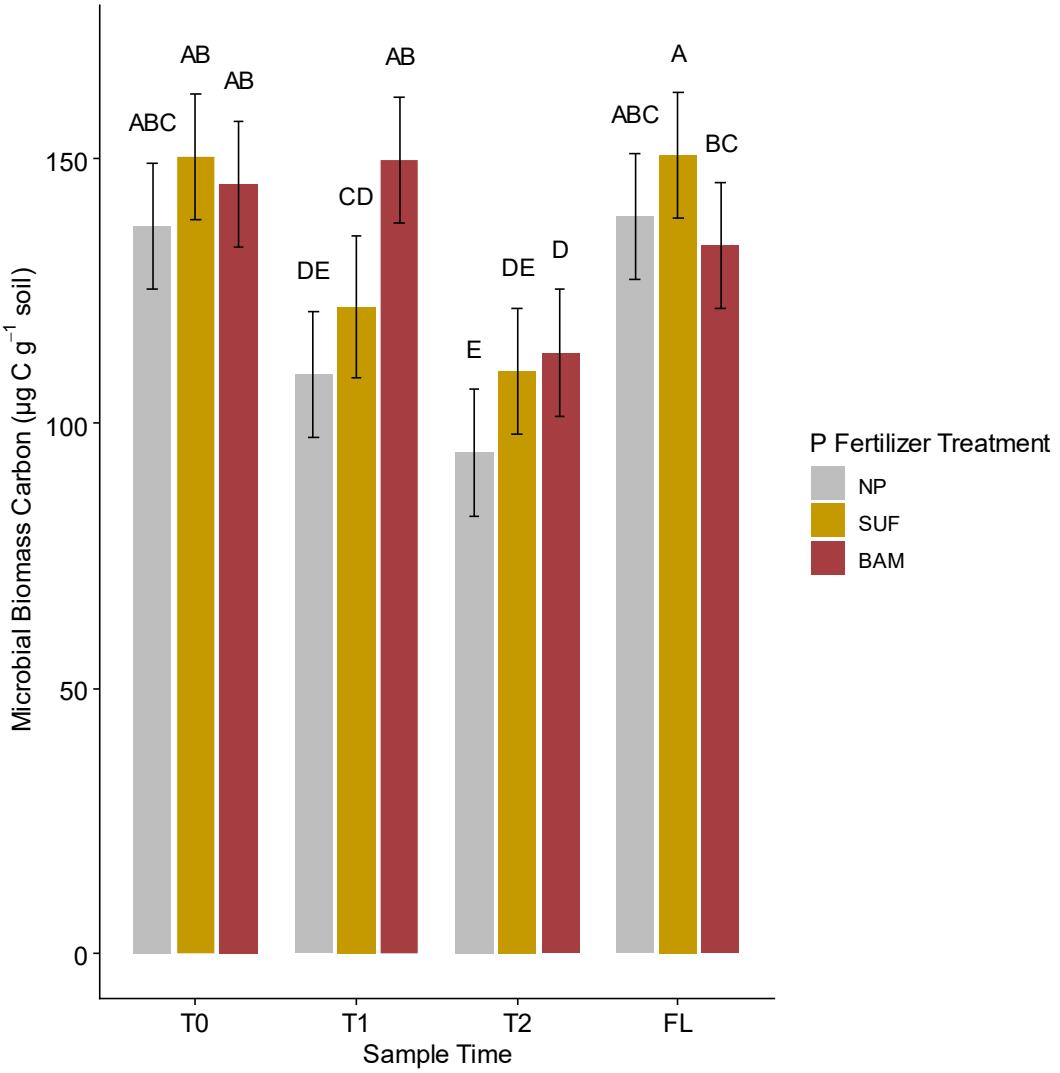


Figure 2.6 A TRT*TIME interaction in MBC (µg C g^{-1} soil) in 2024 during the soybean crop year (p=0.0074). Microbial biomass C in the NP and SUF treatments decreased from T0 to T2 and then increased in the FL. Microbial biomass C in the BAM treatment only saw a decrease in the T2 sample time. The sampling time T0 was taken after cover crop (CC) termination but before planting, T1 was taken 30 days after the initial sampling, T2 was taken 60 days after the initial sampling, and FL was taken after the grain crop harvest. Phosphorus fertilizer treatments include a control (NP), sufficiency (SUF), and build and maintain (BAM).

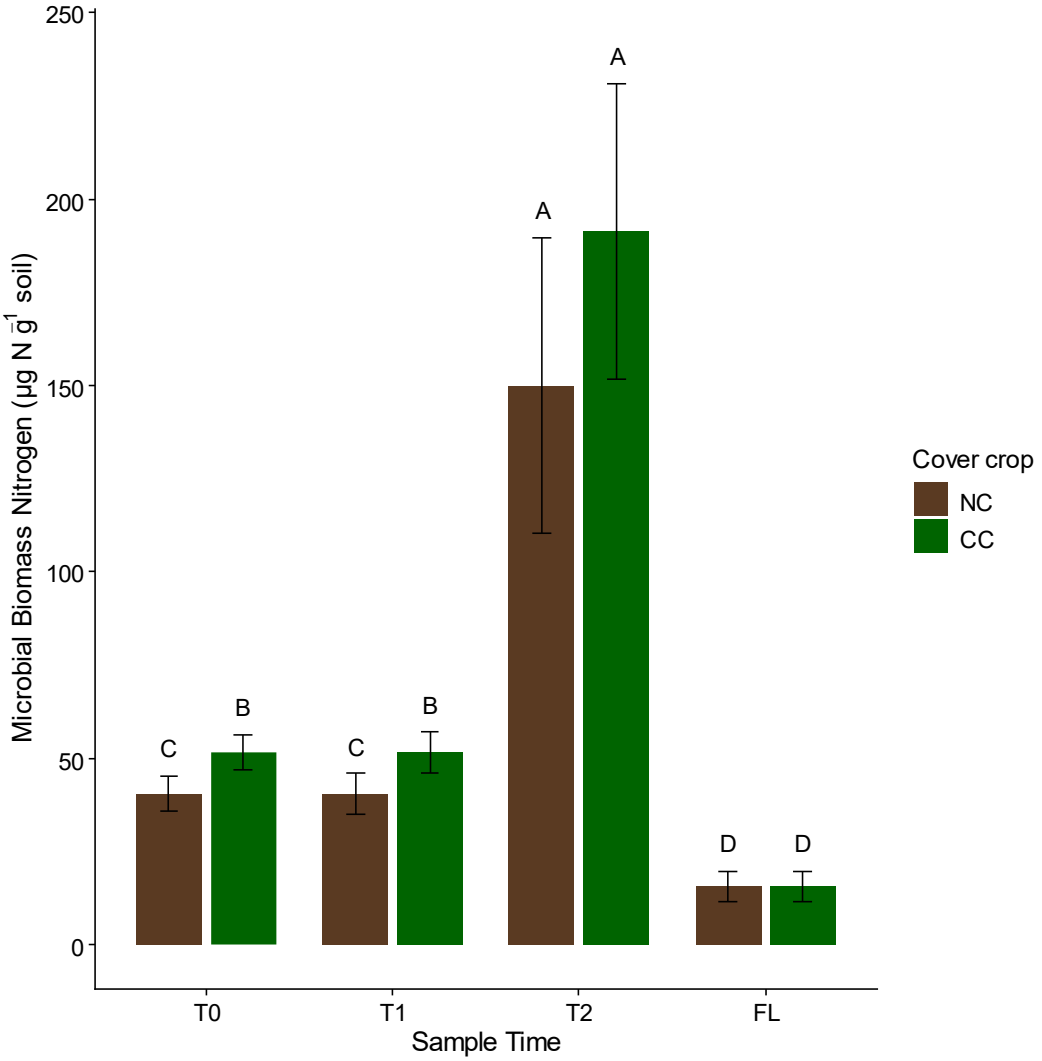


Figure 2.7 A CC*TIME interaction in MBN (µg N g^{-1} soil) in 2025 during the corn crop year (p=0.0182). Microbial biomass N is notably higher, in both the CC and NC treatments, in T2 compared to all other sampling times. Microbial biomass N is greater in the CC treatment compared to the NC treatment in the T0 and T1 sample times. There is no difference between CC and NC treatments in the T2 and FL sample times. The sampling time T0 was taken after cover crop (CC) termination but before planting, T1 was taken 30 days after the initial sampling, T2 was taken 60 days after the initial sampling, and FL was taken after the grain crop harvest.

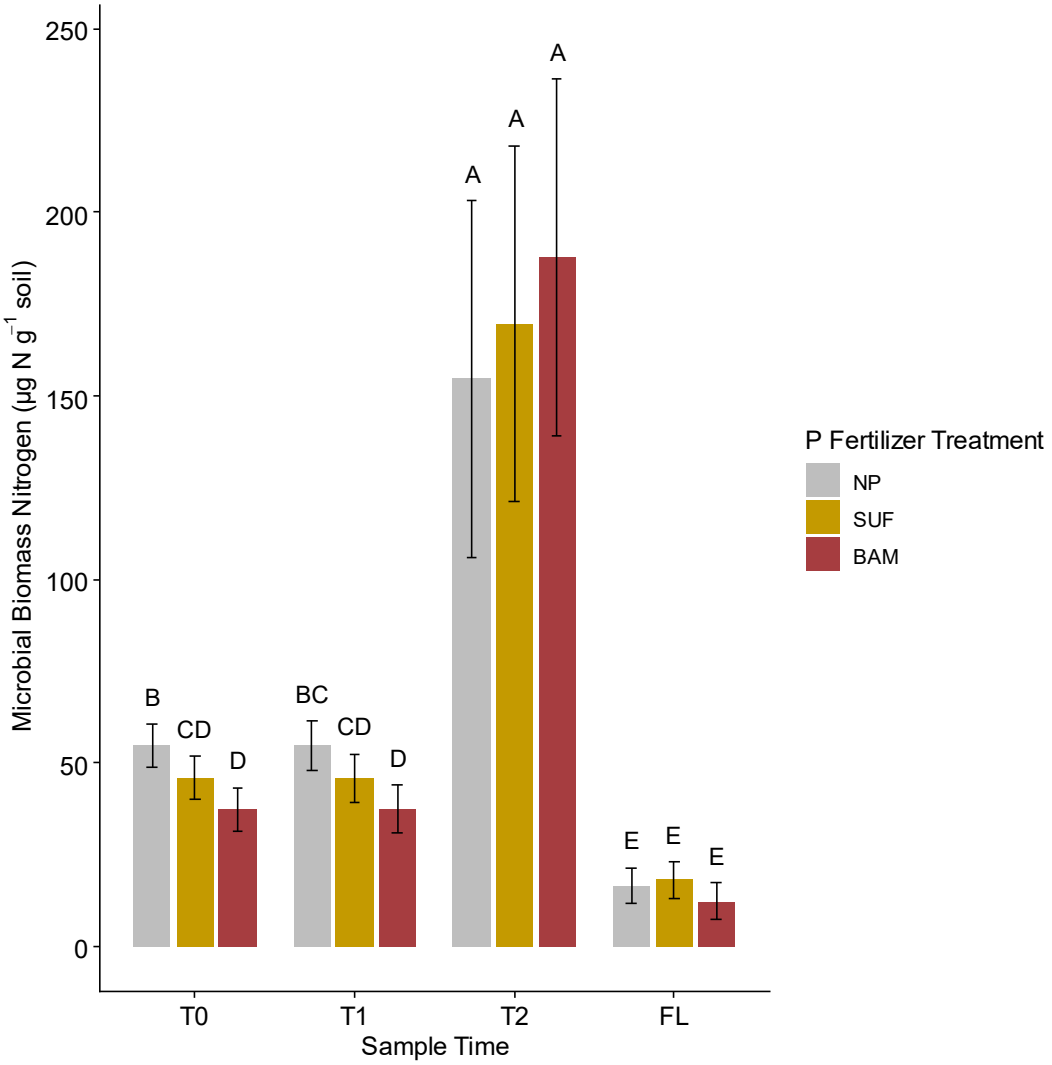


Figure 2.8 A TRT*TIME interaction in MBN (µg N g^{-1} soil) in 2025 during the corn crop year (p=0.0345). All three P fertilizer treatments had notably greater MBN values at T2 compared to all other sample times. There were minimal differences between P treatments within each sample time. The sampling time T0 was taken after cover crop (CC) termination but before planting, T1 was taken 30 days after the initial sampling, T2 was taken 60 days after the initial sampling, and FL was taken after the grain crop harvest. Phosphorus fertilizer treatments include a control (NP), sufficiency (SUF), and build and maintain (BAM).

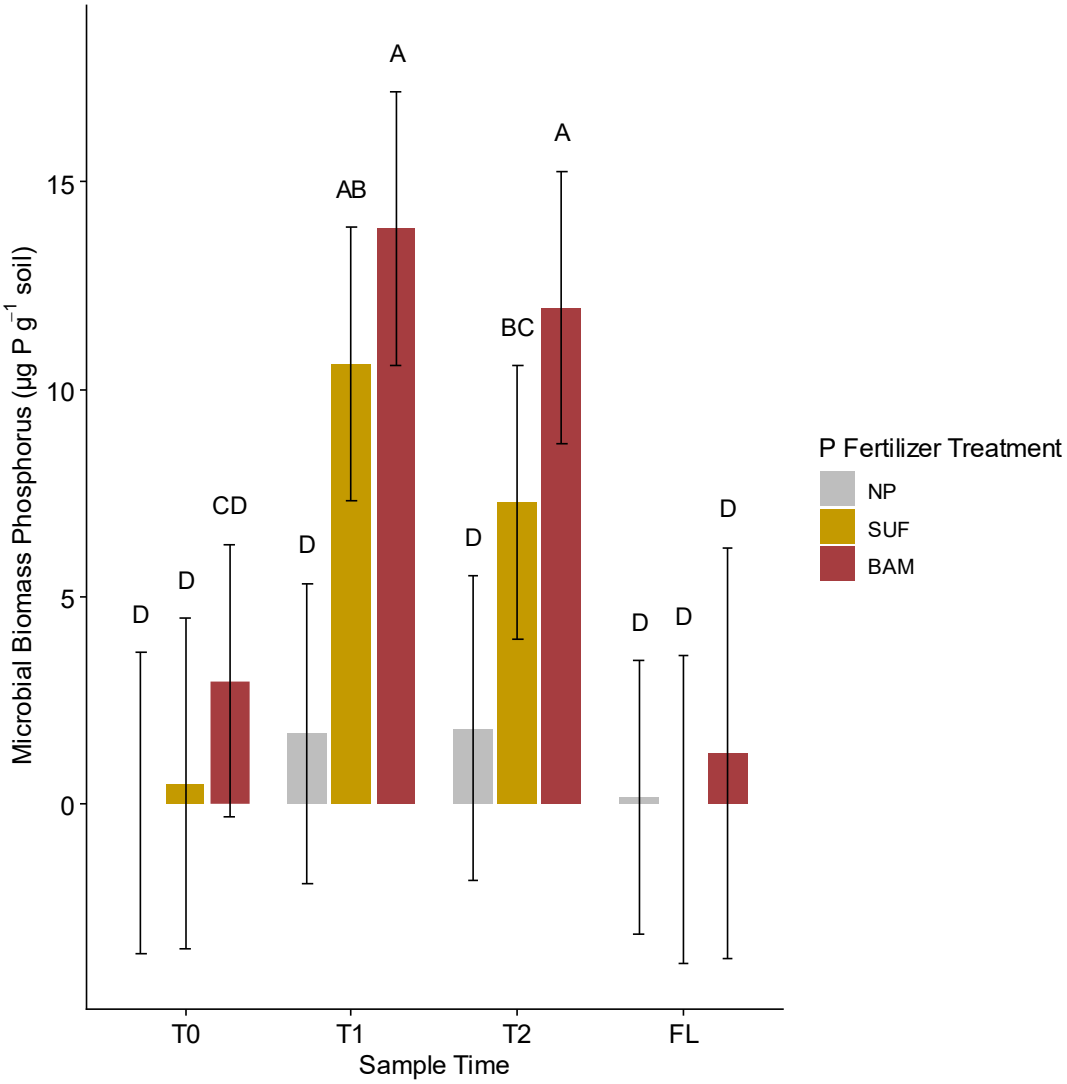


Figure 2.9 A TRT*TIME interaction in MBP (µg P g^{-1} soil) in 2024 during the soybean crop year (p=0.045). The T1 and T2 sampling times have much greater MBP values in the SUF and BAM P treatments compared to T0 and FL sampling times. There was no difference between P fertilizer treatments within the T0 and FL sampling times. The sampling time T0 was taken after cover crop (CC) termination but before planting, T1 was taken 30 days after the initial sampling, T2 was taken 60 days after the initial sampling, and FL was taken after the grain crop harvest. Phosphorus fertilizer treatments include a control (NP), sufficiency (SUF), and build and maintain (BAM).

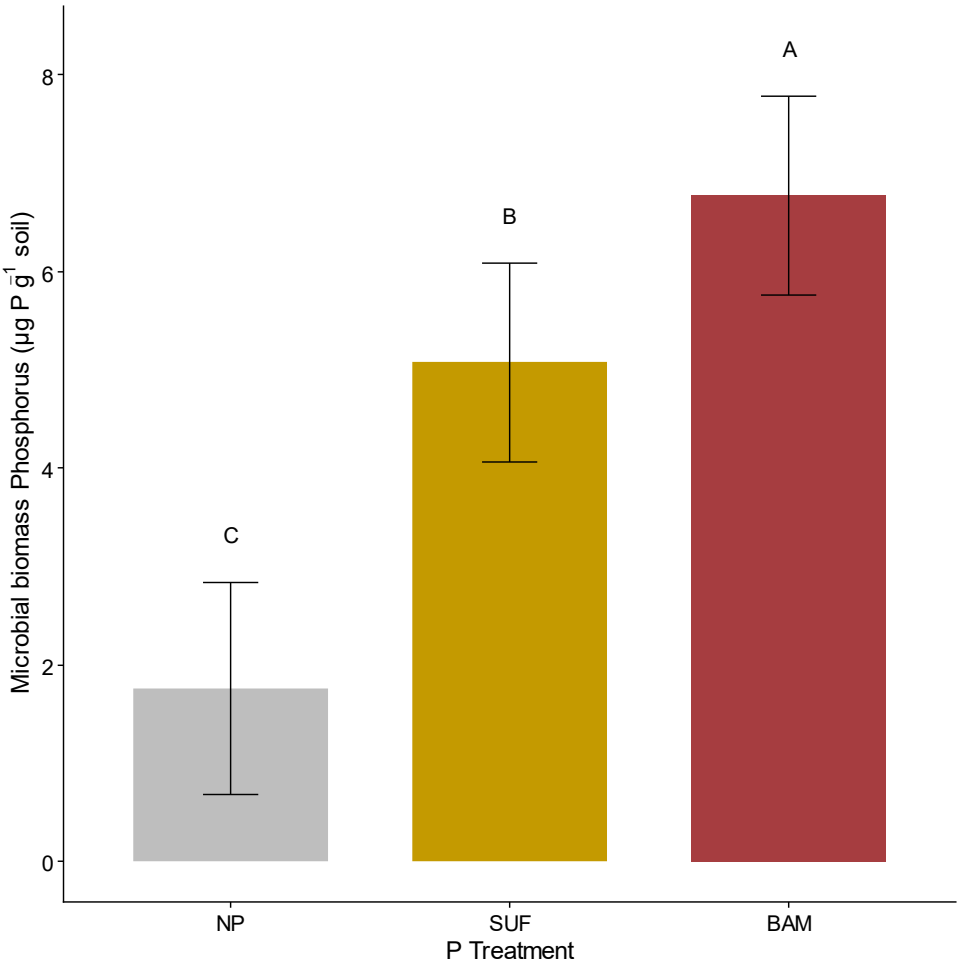


Figure 2.10 A main effect of TRT in MBP (µg P g^{-1} soil) in 2025 during the corn crop year (p=0.0001). Phosphorus fertilizer treatments include a control (NP), sufficiency (SUF), and build and maintain (BAM).

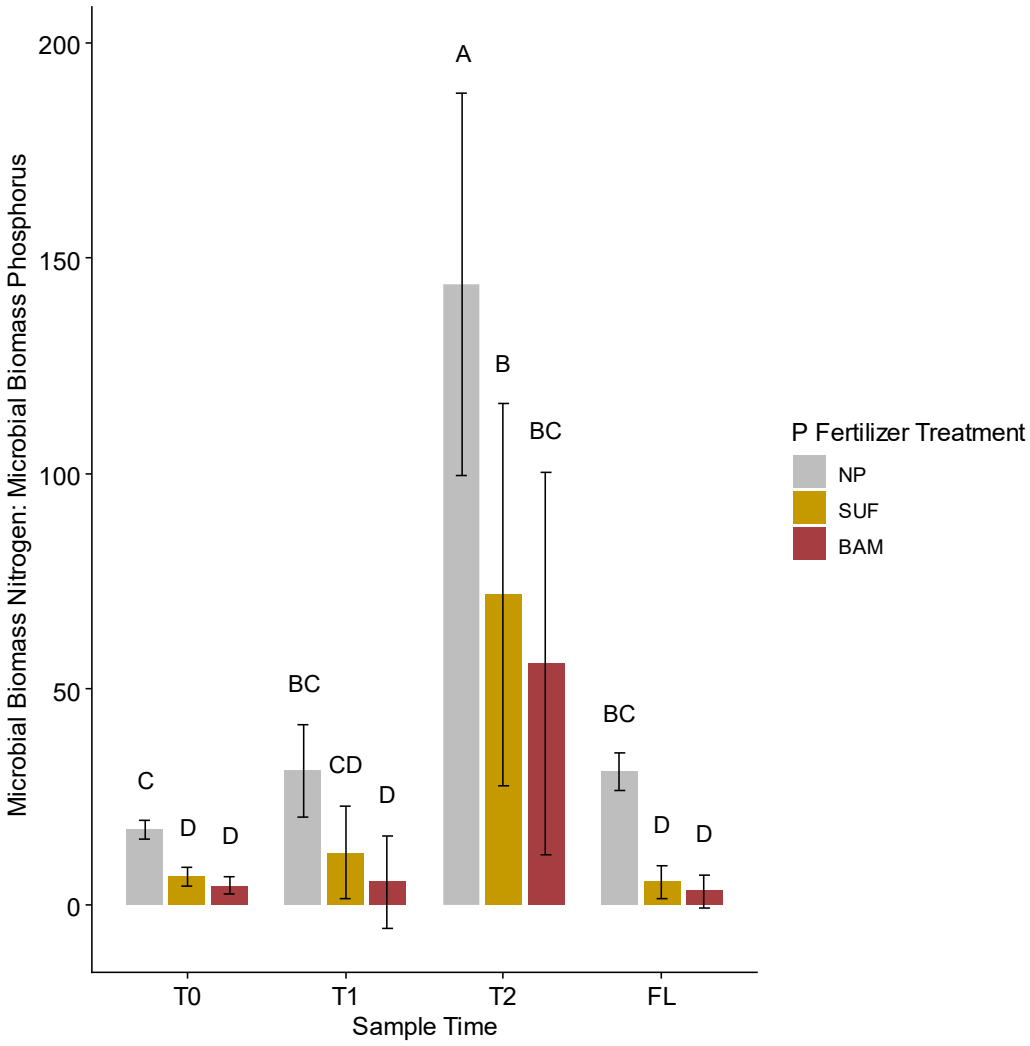


Figure 2.11 A TRT*TIME interaction in the ratio of MBN:MBP in 2025 during the corn crop year (p=0.0002). The T2 sample time had much larger MBN:MBP ratios in all P treatments compared to other sampling times. The T0, T2, and FL sampling times followed the same trend for P fertilizer treatments, with NP having a higher ratio than SUF and BAM. The sampling time T0 was taken after cover crop (CC) termination but before planting, T1 was taken 30 days after the initial sampling, T2 was taken 60 days after the initial sampling, and FL was taken after the grain crop harvest. Phosphorus fertilizer treatments include a control (NP), sufficiency (SUF), and build and maintain (BAM).

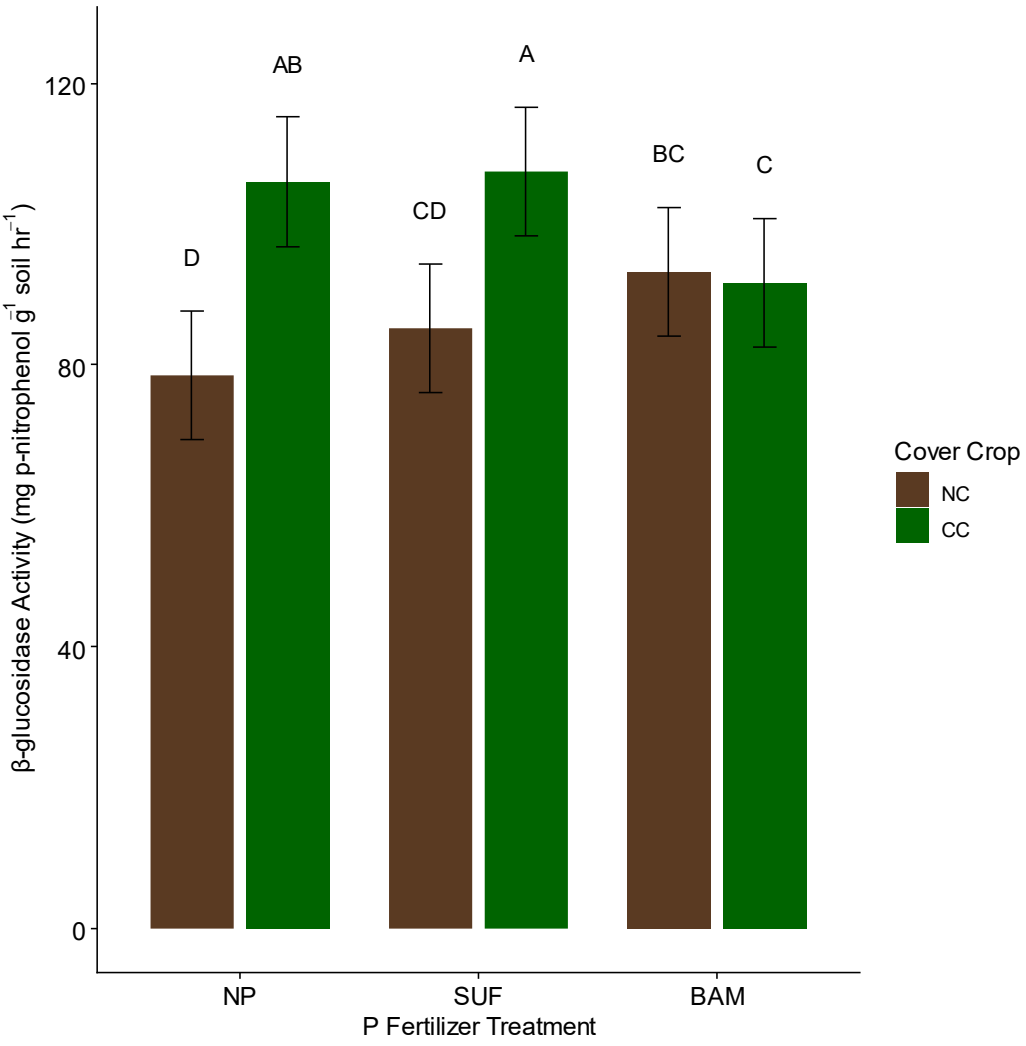


Figure 2.12 A TRT*CC interaction in GLU (mg p-nitrophenol g^{-1} soil hr^{-1}) in 2024 during the soybean crop year (p=0.0101). The NP and SUF P fertilizer treatments have greater GLU activity in the CC treatment compared to the NC treatment. In the BAM P fertilizer treatment, there is no difference in activity between the CC and NC treatments. Phosphorus fertilizer treatments include a control (NP), sufficiency (SUF), and build and maintain (BAM).

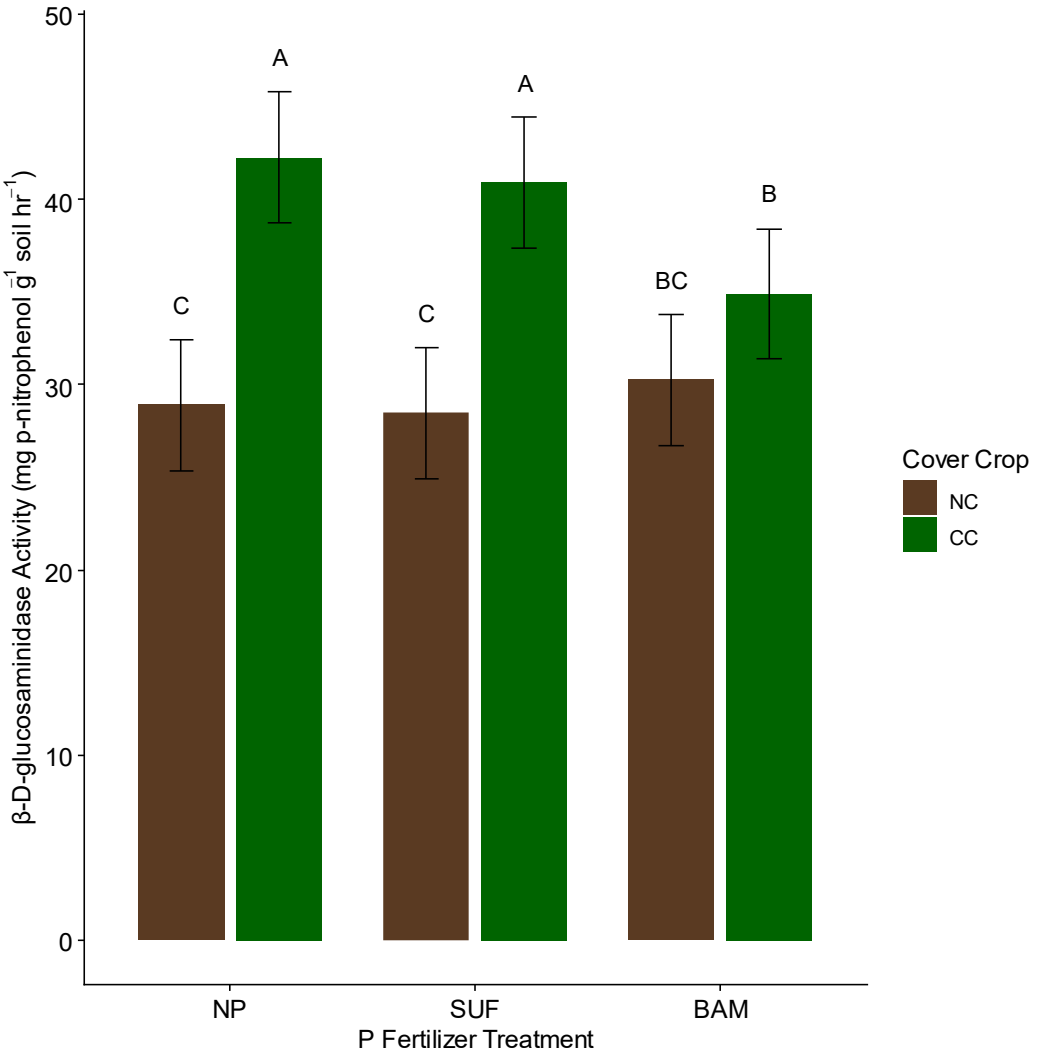


Figure 2.13 A TRT*CC interaction in NAG (mg p-nitrophenol g^{-1} soil hr^{-1}) in 2024 during the soybean crop year (p=0.0373). The NAG activity in the NP and SUF P fertilizer treatments are greater in the CC treatment compared to the NC treatment. There is no difference in NAG activity between the CC and NC treatments in the BAM P fertilizer treatment. Phosphorus fertilizer treatments include a control (NP), sufficiency (SUF), and build and maintain (BAM).

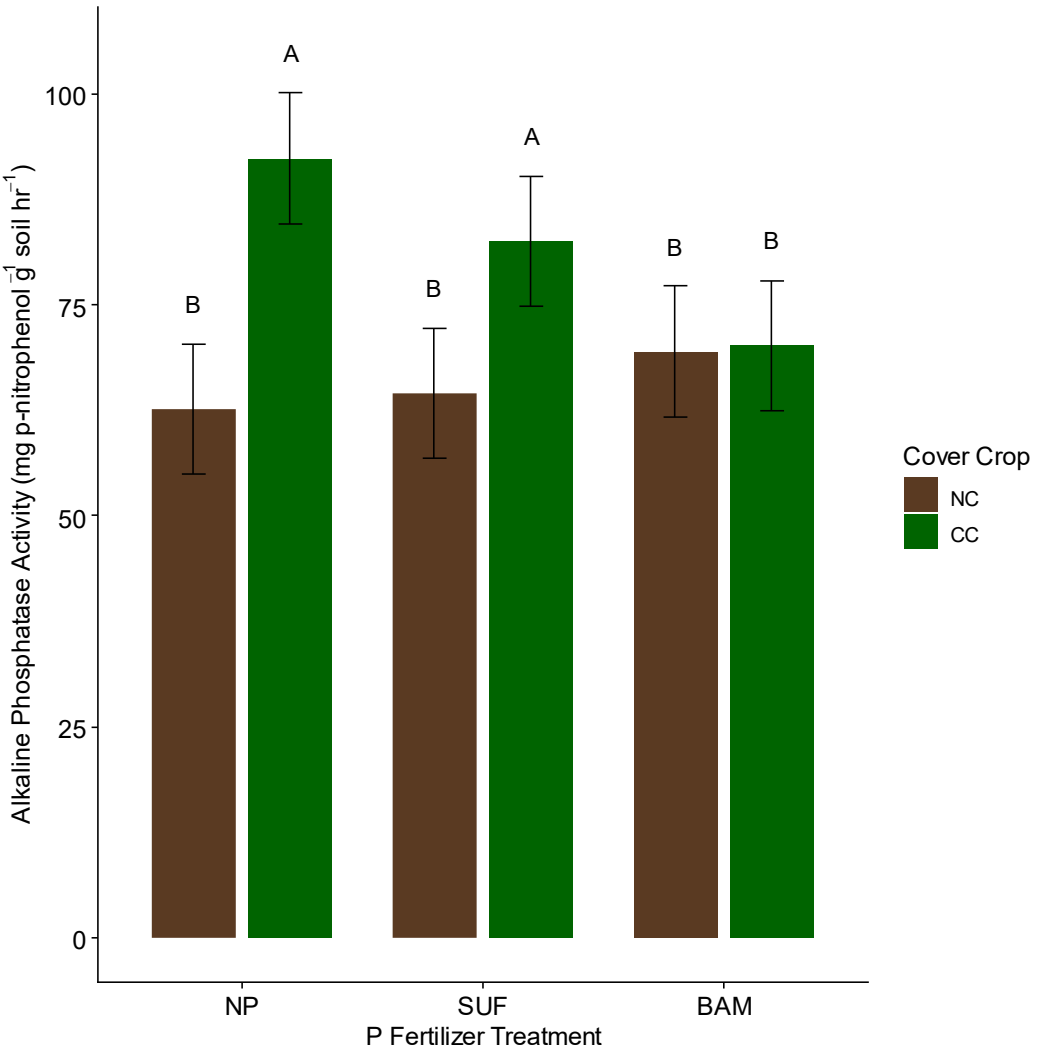


Figure 2.14 A TRT*CC interaction in alkaline phosphatase (mg p-nitrophenol g^{-1} soil hr^{-1}) in 2024 during the soybean crop year (p=0.0045). The NP and SUF P fertilizer treatments have greater alkaline phosphatase activity in the CC treatment compared to the NC treatment. In the BAM P fertilizer treatment, there is no difference in alkaline phosphatase activity between the CC and NC treatments. Phosphorus fertilizer treatments include a control (NP), sufficiency (SUF), and build and maintain (BAM).

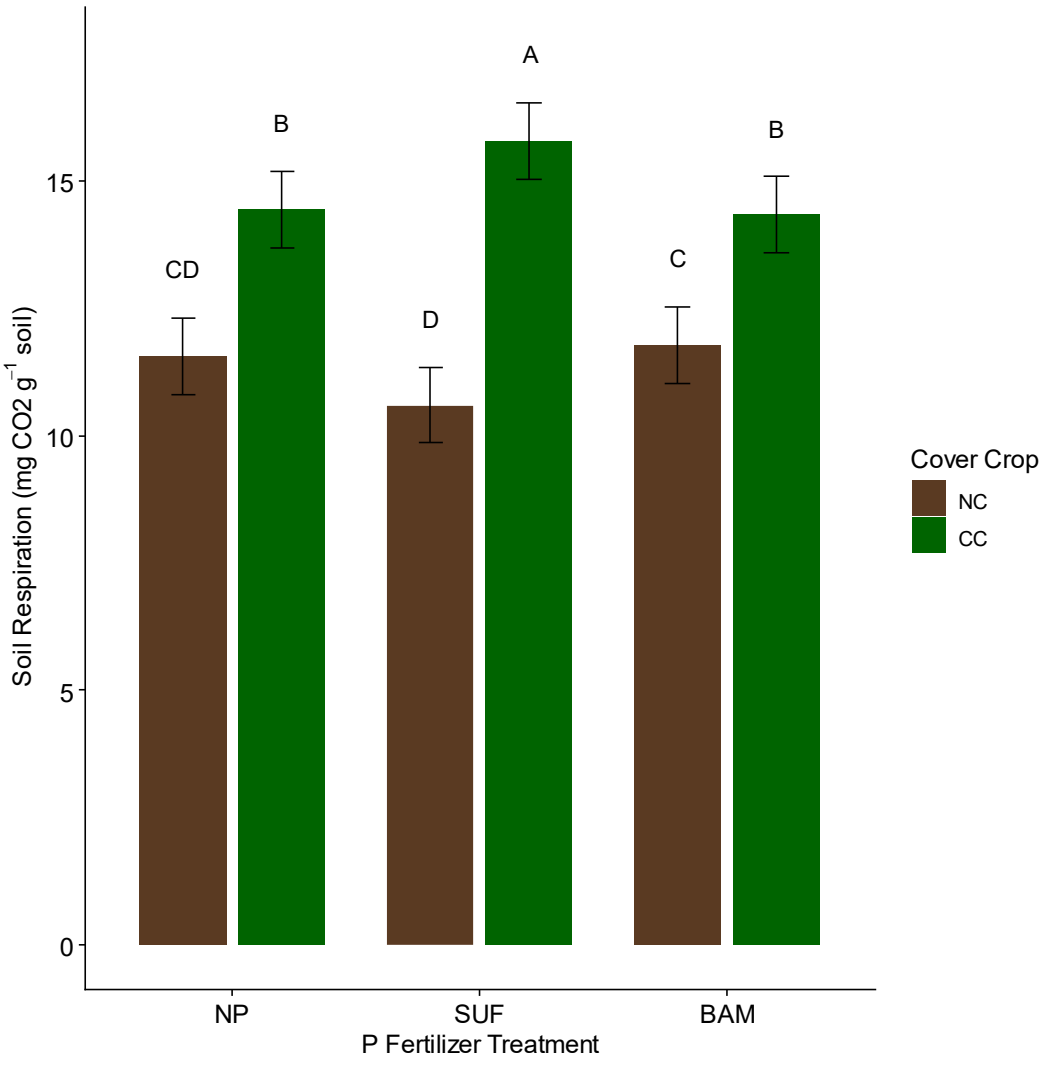


Figure 2.15 A TRT*CC interaction in soil respiration (mg CO_2 g^{-1} soil) in 2024 during the soybean crop year (p=0.0056). The SUF P fertilizer treatment has greater soil respiration in the CC treatment compared to the activity in the CC treatment at the NP and BAM treatments. All three P fertilizer treatments have greater soil respiration in the CC treatment compared to the NC treatment. Phosphorus fertilizer treatments include a control (NP), sufficiency (SUF), and build and maintain (BAM).

Chapter 3 - Evaluating the effects of cover crop and phosphorus fertilizer management on the soil microbial community using phospholipid fatty acid (PLFA) analysis

Abstract

The impacts of cover crops and phosphorus (P) fertilizer management on the soil microbial community are not fully understood. Phospholipid fatty acid (PLFA) analyses are used to estimate individual microbial groups [arbuscular mycorrhizal fungi (AMF), gram-negative and gram-positive bacteria, eukaryotes, fungi, and actinomycetes], as well as total microbial biomass, which can assess how management impacts the soil microbial community. This research aims to address how cover crops and P fertilizer management impact the size and structure of the soil microbial community. This experiment was conducted at the Kansas Agricultural Watershed Field Laboratory and was designed to explore soil health in 2018, 2020, 2022, and 2024 in soybean (*Glycine max* (L.). Samples were taken at the 0-5 cm depth in the spring (SP) and fall (FL). The cover crop treatment positively influenced microbial biomass and all microbial groups compared to the no cover treatment in all the SP and FL sampling times. An increase in abundance values were observed in the cover crop (CC) treatment compared to no cover (NC) in total microbial biomass, AMF, gram-negative, gram-positive, and actinomycetes for the FL sampling of all years analyzed. Phosphorus fertilizer management had a minimal effect on soil microbial abundances aside from FL 2024 where the highest level of P management had the greatest abundance values observed in gram-negative, gram-positive, eukaryotes, and actinomycetes. Soil microorganisms showed minimal response to P fertilizer management as seen by estimated abundance levels and community composition.

Introduction

Soil microorganisms and their influence on soil health and nutrient cycling have been a key area of research for many years. Better understanding this relationship may lead to a future of more intentional nutrient management in agriculture. Soil microorganism initiate biochemical processes that drive nutrient

cycling. However, many agricultural management practices can impact soil microorganisms, which can potentially impact soil microbial activities.

There are many ways to evaluate changes in the soil microbial community. One of the most common methods to evaluate community abundance and diversity is phospholipid fatty acid (PLFA) analysis. Phospholipid fatty acid analysis is the extraction of different lipids from a soil sample that can provide information on the soil microbial community (Zelles, 1999). Phospholipid fatty acid analysis can be used to assess many elements of microbial communities at any given point of sampling collection (Willers et al., 2015). There is a lot of utility in PLFA and analysis can provide an estimate of microbial biomass, information on biomarkers which can be used to assess community structure, and details on the functioning of the microbial community overall (Willers et al., 2015). Microbial community composition is the total of all microbial groups that constitute a community, expressed as a percentage. Community composition can be a tool used to evaluate changes in microbial groups that affect community dynamics and structure overall.

Phospholipid fatty acid profiles use biomarkers to classify soil microorganisms and determine microbial community composition. Phospholipid fatty acid biomarkers are membrane lipids and the fatty acids that they are comprised of (Zelles, 1999). Soil microbial community composition is the characterization of different types of soil microorganisms and the amount of each microbial group that make up total microbial biomass. The use of PLFA biomarkers have generally been successful in determining microbial groups (Zelles, 1999).

Researchers have used PLFA for decades to measure different metrics of soil microbial communities. Phospholipid fatty acid analysis has been one of the driving methods in which scientist have been able to make great strides in discovering more about soil microorganisms. Phospholipid fatty acid analysis can be utilized to monitor soil microbial community responses to P management and cover crop influence over time. Monitoring these shifts over time provides better insight into the effect that soil microorganisms have on nutrient cycling (Li et al., 2020).

The influence of cover crops on the soil microbial community is well documented and there is strong evidence in the literature that suggest cover crops have a positive influence on different microbial groups. A study by Chavarría et al. (2016) found that cover crops influenced PLFA in the short term, most prominently in the bacterial PLFA groups, such as gram-negative and gram-positive bacteria. Rankoth et al. (2019), found that in two out of the three years of their study, the inclusion of a cover crop had a positive effect on total microbial biomass in comparison with no cover crop. This observed increase in microbial biomass was a result of an increase in multiple microbial groups: AMF, gram-negative bacteria, gram-positive bacteria, and saprophytes (Rankoth et al., 2019).

Phospholipid fatty acid analysis allows for abundance estimates of individual microbial groups, such as AMF. Arbuscular mycorrhizal fungi (*Glomus* sp.) are a group of fungi that form symbiotic relationships with plants and increase nutrient uptake (Frąc et al., 2018; Schneider et al., 2019). It is well known that AMF can form relationships with soybean plants and enhance their uptake of P. The beneficial role that AMF play in soybean production is continuously being researched today and many scientist are trying to better understand factors that affect this relationship (Higo et al., 2018). More research needs to be conducted to understand how cover cropping and P additions to the soil specifically impact AMF and the impact on agriculture (Higo et al., 2018).

As microbial communities impact nutrient cycling, they ultimately influence crop production. Evaluating the impacts of different management practices on the soil microbial community over time will allow for more informed management decisions in agriculture. There have been great strides in research to understand soil microbial communities and there are many methods available to assess soil microorganisms. However, our knowledge is limited in this area of study and there is still much to discover about soil microbial function, specific microbial species, and the implications of combined management practices on soil microbial communities. Today many researchers are trying to understand how soil microorganisms are influenced by the management system which combines multiple management practices.

The main objective of this study was to determine cover crop and P fertilizer management impacts on microbial community abundance and structure. The three hypotheses tested were: (1) the addition of cover crops will increase the abundance of all microbial groups and total microbial biomass, (2) Reducing P fertilizer inputs will drive an increase in the abundance of all microbial groups, and (3) cover crops and P fertilizer management improve soil microbial community structure.

Materials and Methods

Experimental Site Characteristics

The study was conducted from 2018-2025 at the Kansas Agricultural Watershed (KAW) field laboratory located in Manhattan, KS. The site has a soil type of primarily Smolan silty clay loam (fine, smectitic, mesic Pachic Argiustoll) and a slope of 6-8% with soil pH ranging between 6 and 7. This site has a mean annual precipitation of 836 mm and a mean annual temperature of 13.1°C and is classified as having a hot, humid continental climate. The site consists of 18 total plots arranged in 3 blocks with each block containing 2x3 complete factorial arranged in a randomized complete block design. Each plot serves as a small, watershed that measures approximately 0.5 ha in area. The site has been managed in a no-till, corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) crop rotation since 2014. The KAW field laboratory project began in 2014 focusing research efforts on assessing different P fertilizer managements and their effects on P loss in an agricultural system. Many studies have been conducted at this site to evaluate P fertilizer management and CC effects on crop productivity, water quality, P cycling, and economic impact (Bourns et al., 2026; Nelson et al., 2023). Phosphorus fertilizer management and CC effects on soil health indicators and the soil microbial community have also been evaluated at this site (Pasket, 2023.; Starr et al., 2025; Stewart, 2021). All even years of the crop rotation were planted to corn while all odd years were planted to soybean.

The study treatments included three different levels of P management and two cover crop treatments. The cover crop treatments included: no cover crop (NC) and cover crop (CC) throughout all years. The cover crop treatment from 2018-2019 contained wheat (*Triticum aestivum* L.), triticale (*Secale*

X Triticum L.), and rapeseed (*Brassica napus*). The cover crop treatment from 2020-2025 consisted of cereal rye (*Secale cereale* L.). Phosphorus management can be viewed from two different points: P application and P management goals (Table 3.1). The three levels of P management from 2018-2019 included no phosphorus (NP), spring injected (SI), and fall surface broadcast (FB) applications. The management goal from 2018-2019 was to be a phase for building soil P in the SI and FB treatments. Both SI and FB plots received 61 kg P_2O_5 ha^{-1} while the NP treatment remained the control. The P management goal changed in 2020-2022 and aimed to maintain P in the SI plots and begin the drawdown of P in the FB plots. The maintenance P management approach included 55 kg P_2O_5 ha^{-1} applied to the SI plots, while both the drawdown P approach in the former FB plots and the NP control plots received no P fertilizer during these years. At this point, P fertilizer treatment labels shifted to no phosphorus (NP), sufficiency (SUF), and build and maintain (BAM). This shift in treatment labels better reflects the purpose and goals of each P management level from 2023 to 2025. During the years of 2023-2025, the P managements goals were to follow the Kansas State University P management build and maintain and sufficiency recommendations (Ruiz Diaz, 2024). In 2023, the SI plots (BAM) received 53 kg P_2O_5 ha^{-1}, SUF plots received 23 kg P_2O_5 ha^{-1}, and the NP plots continued to remain a no P control. Phosphorus fertilizer treatment labels in this chapter include, no phosphorus (NP), drawdown/ sufficiency (DD/SUF), and build and maintain (BAM).

Soil Sampling

Soil samples were collected during the 2018-2025 growing seasons. Sampling took place two times per year: once in the spring (SP) after cover crop termination and before planting of either corn or soybean and once in fall (FL) after the grain crop was harvested. All soil samples were taken at the 0-5 cm depth. Soil samples were collected along an established line transects in each plot. The line transects was comprised of two-line segments connecting three GPS points. The outer two points were approximately in the mid slope region of the plot, and the middle point was at the top of the terrace channel forming a V shape. Ten soil samples were randomly collected along each line segment using a

soil probe resulting in a composite sample of 20 soil cores of field-moist soil in each plot. In between each plot, soil probes were cleaned and sprayed with ethanol to sterilize and prevent contamination between plots. Immediately following sampling, approximately 10-20 g of field-moist soil was immediately separated and stored in a freezer at -80° C until further processing could take place. The soil samples were freeze-dried and ground in preparation for PLFA analysis and kept in storage at -80° C.

Phospholipid Fatty Acid Analysis

All PLFA analysis were completed at the University of Missouri, Soil Health Assessment Center (SHAC) following the high-throughput method developed by (Buyer & Sasser, 2012) which is based off of lipid extraction detailed by Blight and Dyer (1959) and modified by White et al. (1979).

Approximately 2 g of the freeze-dried and ground soil was weighed out for each soil sample and stored at -80° C until analysis. The Buyer and Sasser (2012) method contains four steps: lipid extraction, lipid separation, transesterification, and then analysis via gas chromatography. The first step, lipid extraction, began by adding 4 mL of the Bligh-Dyer extractant to each tube, which was then mixed with the soil. The Bligh-Dyer extractant contains 200 mL K_2HPO_4 in deionized water, 500 mL methanol, and 250 mL chloroform (Buyer & Sasser, 2012). The tubes were then shaken on an end-to-end shaker for 2 hours before being centrifuged for 10 minutes. The liquid extract was then transferred to new tubes and 1 mL of chloroform and 1 mL of ultrapure deionized water were added. The tubes were then vortexed for 5 seconds and centrifuged for 10 minutes. This concluded the drying and extraction, and the samples were then stored at -20° C.

Next is the lipid separation step which is completed via solid phase extraction. This step is performed with specialized Phenomenex, Cat. No. 8E-S012-DGB 96-well plates (Phenomenex, Torrence, CA, USA) that contain 50 mg of silica in each well. Each well was conditioned with 1 mL of methanol three times and 1 mL of chloroform three times. Each sample could then be mixed with 1 mL chloroform and added to the plate to pass through the silica. The tubes that contained the samples were rinsed with another 1 mL of chloroform which was added to the plate. Each well in the plate was washed with 1 mL of chloroform

and 1 mL of acetone. Phospholipids were eluded into a 1.5 mL glass vial with 0.5 mL of a 5:5:1 methanol:chloroform:H_2O mixture. The solution of phospholipids was evaporated at 70° C for 30 minutes and then remained at 37° C for 121 minutes.

The third step is transesterification, which began with 0.2 mL of the transfiguration solution being added to each vial after that they were sealed with a cap mat before being incubated at 37°C for 15 minutes. Then 0.4 mL each of 0.075 M acetic acid and chloroform were added to each vial. The phases were separated after being shaken for 10 seconds. A multichannel pipettor was used to extract the bottom 0.3 mL of the vial which was then transferred to 1 mL vials in a multi-tier microplate. The extraction process with chloroform was repeated and an additional 0.4 mL was added to the vials and mixed with the 0.3 mL of extractant. The chloroform was evaporated to dryness at room temperature. Samples were then dissolved in 0.075 mL hexane and prepped for gas chromatography by transferring to gas chromatography vials that contained conical glass inserts. Samples were stored at -20° C until analyzed. Samples were analyzed by gas chromatography in the final step using an Agilent 7890A Network Gas Chromatograph (Agilent, Santa Clara, CA, USA).

Statistical Analysis

The effects of P fertilizer treatment, CC, year, and all interactions on PLFA measurements were analyzed using SAS version 9.4 (Cary, NC, U.S.A). Phosphorus fertilizer treatment, cover crop, year, and block were all fixed effects. Data were analyzed using PROC GLIMMIX with the Kenward-Roger approximation for denominator degrees of freedom (DDFM = KR). The covariance structure was chosen based on the best fit statistics for Akaike information criterion (AIC) and Bayesian information criterion (BIC). Least-squares means estimates and standard errors were used to create all figures and tables. Spring and fall sampling times were analyzed separately and not compared statistically. Significant differences were detected using $p < 0.05$.

Results

Spring Sampling – Soybean Years

Several interactions and main effects were observed in the SP sampling time as presented in the ANOVA table (Table 3.2). There were no three-way interactions between CC, P fertilizer treatment (TRT), and year (YR) in any microbial group, total microbial biomass, or microbial group ratios in the SP sampling time of soybean years. Additionally, there were no CC*TRT interactions in the SP for any microbial group, total microbial biomass, or microbial group ratios. There was a TRT*YR effect in gram-negative bacteria (p=0.0252), gram-positive bacteria (p=0.046), and eukaryotes (p=0.0225) (Table 3.2). Aside from the TRT*YR interaction observed in gram-positive bacteria, gram-negative bacteria, and eukaryotes, there were no other significant P fertilizer effects on the microbial community. Additionally, there were no significant interactions observed in fungi, AMF, total microbial biomass, fungi:bacteria, and gram+:gram- in the SP sampling time.

In the TRT*YR interaction in gram-negative bacteria, abundance was the same across 2018, 2020, and 2022 in all three P fertilizer treatments, while in 2024, the BAM P fertilizer treatment had higher abundance levels than DD/SUF and NP in 2024 as well as all other treatments at all other sampling times (Figure 3.2). Gram-negative bacteria abundance in the BAM treatment in 2024 was 122% greater than abundance in the DD/SUF treatment and 127% greater than abundance in the NP treatment of 2024. Gram-positive bacteria and eukaryote abundance observed similar trends in the TRT*YR interaction (Figure 3.3 and 3.4). In 2024, gram-positive bacteria abundance in the BAM treatment was 117% greater than abundance in the DD/SUF treatment and 124% greater than abundance in the NP treatment. Similarly, in 2024, Eukaryote abundance in the BAM treatment was 127% greater than abundance in the DD/SUF treatment and 142% greater than abundance in the NP treatment. The SP 2020 abundance values in gram-negative and gram-positive bacteria are notably lower than all other sampling times.

A CC*YR interaction was detected in the SP influencing the abundance of actinomycete (p=0.0013), gram-negative bacteria (p=0.0482), and gram-positive bacteria (p=0.0256) (Table 3.2).

Actinomycete abundance was greater in the CC treatment compared to the NC treatment for 2020, 2022, and 2024 (Figure 3.4). Actinomycete abundance had little variability between each year within the CC and NC treatments, aside from 2020, which had lower abundance values than all other sample years. Gram-negative bacteria abundance was greater in the CC treatment compared to the NC treatment for all sampling years (Figure 3.5). The 2024 sample year had the greatest abundance values for the CC and NC treatments while 2020 was the lowest. Similar to gram-negative bacteria, gram-positive bacteria abundance was greater in the CC treatment compared to the NC treatment for all sampling years (Figure 3.6). Gram-positive bacteria abundance levels in 2020 were lower than all other sample years for both the CC and NC treatments.

Total microbial biomass ($p=0.0083$), AMF ($p=0.0001$), fungi ($p=0.0001$), and eukaryote ($p=0.0001$) abundance all had a main effect of CC in the SP of soybean years (Table 3.2). Microbial abundance of AMF, fungi, eukaryote and total microbial biomass increased with the presence of a CC. Total microbial biomass increased 119% with a CC compared to the NC treatment. Arbuscular mycorrhizal fungi abundance increased 133% with a CC compared to the NC treatment. Abundance of fungi increased 151% between the NC and CC treatments. Eukaryote abundance increased 158% with a CC compared to the NC treatment. The ratios of fungi:bacteria ($p=0.0016$) and gram-positive bacteria:gram-negative bacteria ($p=0.031$) were also impacted by the presence of a CC. The fungi:bacteria increased from 0.130 in the NC treatment to 0.164 in the CC treatment. The ratio of gram-positive bacteria to gram-negative bacteria decreased from 1.253 in the NC treatment to 1.206 in the CC treatment.

Total microbial biomass ($p=0.0001$), AMF ($p=0.0001$), and fungi ($p=0.0001$) abundance all had a main effect of YR in the SP of soybean years (Table 3.2). Total microbial biomass was lowest in 2020 and highest in 2024. There was no change in total microbial biomass in the two years of 2018 and 2022 which fell in the between 2020 and 2024. Arbuscular mycorrhizal fungi responded similarly to total microbial biomass, with the lowest abundance levels in 2020, highest abundance levels in 2024, and

abundance in 2018 and 2022 falling in between. Fungi abundance was highest in 2022 and 2024 and lowest in 2020 with 2018 in the middle between the high and low.

Phospholipid fatty acid analysis provided information on microbial community composition that was used to assess the percentage of each microbial group that sum up to be the entire community. The fraction of the microbial community was analyzed for each microbial group for the SP, including 2018, 2020, 2022, and 2024. Microbial groups that were positively influenced by the presence of a CC were eukaryotes in 2022 and 2024 as well as fungi in 2018, 2022, and 2024. The proportion of gram-positive bacteria was higher in the NC compared to the CC in 2018 and 2024, while the proportion of gram-negative bacteria was higher in the NC in 2022 compared to the CC treatment. Actinomycetes were also higher in the NC treatment compared to CC treatment in 2020 and 2024. The only P fertilizer effect in the SP was in actinomycetes in which the BAM treatment was lower than both DD/SUF and NP treatments.

Fall Sampling – Soybean Years

An ANOVA table presenting several interactions and main effects observed in the FL sampling (Table 3.3). Three-way CC*TRT*YR interactions were present for abundance of fungi ($p=0.0193$), AMF ($p=0.0345$), and total microbial biomass ($p=0.0395$). Aside from the three-way interactions, there were no other P fertilizer treatment effects on the soil microbial community for the FL, including TRT*YR, CC*TRT, and TRT effects. There were no interactions or main effects for the ratios of fungi:bacteria and gram-positive: gram-negative bacteria for the FL of 2018, 2020, 2022, and 2024. In the three-way interactions, fungi had variable results but had a positive increase in the CC treatment compared to NC in the DD/SUF P fertilizer treatment in 2020 and a positive increase in the CC treatment compared to NC in the NP P fertilizer treatment in 2024 (Table 3.4). Arbuscular mycorrhizal fungi abundance was higher in the CC treatment compared to the NC treatment for all three P fertilizer treatments, BAM, DD/SUF, and NP for the FL of 2024 (Table 3.5). Additionally, AMF abundance was greater in the CC treatment compared to NC for NP and DD/SUF P fertilizer treatments in 2018 as well as the DD/SUF treatment in 2020 and the BAM treatment in 2022. Phosphorus fertilizer treatment as influenced by CC showed the

greatest impact in total microbial biomass (Table 3.6). All three P fertilizer treatments in 2024 had greater total biomass in the CC treatment compared to the NC treatment. The NP and DD/SUF treatments in 2018 also had greater total microbial biomass in the CC treatment compared to NC, as well as the SUF and BAM treatments in 2020 and the NP and BAM treatments in 2022.

There was a CC*YR interaction for actinomycetes (p=0.009), gram-negative bacteria (p=0.0081), and gram-positive bacteria (p=0.0025) (Table 3.3). Actinomycete abundance was higher in the CC treatment compared to the NC treatment for all sample years for the FL (Figure 3.7). Actinomycete abundance was 113% higher in the CC treatment in 2018, 115% higher in the CC treatment in 2020, 120% higher in the CC treatment in 2022, and 132% higher in the CC treatment in 2024. Similarly, to actinomycetes, gram-positive bacteria abundance (Figure 3.8) and gram-negative bacteria abundance (Figure 3.9) was higher in the CC treatment compared to the NC treatment for all sample years in the FL. Gram-positive bacteria abundance in the CC treatment was 116% higher in 2018, 117% higher in 2020, 122% higher in 2022, and 136% higher in 2024 all compared to the NC treatment. Gram-negative bacteria abundance in the CC treatment was 111% higher in 2018, 114% higher in 2020, 117% higher in 2022, and 133% higher in 2024 compared to the NC treatment. Additionally, eukaryotes had a main effect of CC (p=0.0001) showing that the CC treatment was 133% greater in the CC treatment compared to the NC treatment.

Phospholipid fatty acid analysis provided information on microbial community composition that was used to assess the percentage of each microbial group that sum up to be the entire community. Similar to SP, each microbial group was analyzed individually for every sampling time for the FL including, 2018, 2020, 2022, and 2024. The FL sample times showed minimal CC and P fertilizer treatment impact on microbial community composition. There was a CC by P fertilizer interaction in 2024 for fungi and actinomycetes. In fungi, the NC*NP treatment was lower than the NP treatment with a CC while the BAM and DD/SUF treatments saw no difference with the presence of a CC. Alternatively, actinomycetes were higher in the NC*NP treatment compared to NP with a CC. The BAM and DD/SUF treatments were not impacted by the presence of a CC. There was also a CC effect for gram-positive

bacteria in 2018 and 2020 and gram-negative bacteria in 2018. Gram-positive bacteria were higher in the NC compared to the CC treatment in 2020 while in 2018 the CC treatment was higher than the NC treatment. Gram-negative bacteria were higher in the NC treatment compared to the CC treatment in 2018.

Discussion

A three-way interaction between P fertilizer treatment, CC, and year was observed for the FL sampling time in fungi (Table 3.4), AMF (Table 3.5), and total microbial biomass (Table 3.6). In fungi, the NP treatment in 2024 had greater fungi abundance in the CC treatment compared to NC. The DD/SUF treatment in 2020 also had greater fungi abundance in the CC treatment compared to the NC. Arbuscular mycorrhizal fungi had greater CC response with the NP and DD/SUF treatments in 2018, the DD/SUF treatment in 2020, and the BAM treatment in 2022 in comparison to the NC treatments within each respective P fertilizer treatment and year. All three P fertilizer treatments saw increases in abundance due to CC in 2024 in comparison to the NC treatments in AMF. A meta-analysis done by Wu et al., (2022) determined that the addition of P had a positive effect on soil microbial biomass. Rankoth et al. (2019) found increases in total microbial biomass due to CC when comparing to NC practices.

The interaction between CC*YR, observed for the FL sampling time, positively influenced total microbial biomass, AMF, gram-negative bacteria, gram-positive bacteria, and actinomycetes. This interaction proves the use of a cover crop in the crop rotation increases microbial abundance over time in total microbial biomass and select microbial groups. The BAM treatment for 2024 consistently had the highest abundance values for gram-negative and gram-positive bacteria, eukaryotes, and actinomycetes for the SP sampling time in the TRT*YR interaction. Abundance values for DD/SUF and NP treatments in all years were not different from each other.

Cover crops had a widespread impact on the soil microbial community observed by the increase in abundance of all microbial groups and total microbial biomass in the CC treatments in comparison to the NC treatments for the SP and FL sampling times. The positive CC effects observed for the SP

sampling time show the immediate effects seen shortly after CC termination. The FL sampling time shows the legacy effect that CC have on the soil microbial community in soybeans (Figure 3.12). The CC effect observed in the FL was over half a year after the termination of the CC. The difference in abundance between the CC and NC treatments was greatest in the FL sampling time of 2024 in gram-positive bacteria, gram-negative bacteria, and actinomycetes. Schmidt et al. (2018) determined the increases in microbial abundance in their study were likely due to the long term and cumulative effects of CC of increased organic C into the system. The increase in total microbial biomass and abundance of microbial groups due to the positive effects of cover crops is well documented in the literature (Blanco, 2023; Finney et al., 2017; Mendis et al., 2025; Rankoth et al., 2019; Schmidt et al., 2018). Blanco (2023) confirmed that cover crops are known to contribute to increases in labile organic matter which can stimulate microbial abundance. Schmidt et al. (2018) found that additional nutrients supplied by CC expanded microbial abundance. A study done in a corn-soybean system by Rankoth et al. (2019) found CC positively impacted gram-positive bacteria, gram-negative bacteria, and AMF leading to an increase in total microbial biomass. This research continues to highlight the impact that CC can have on the soil microbial community, especially when used in addition to no-till conservation management.

Phosphorus fertilizer management had a minimal effect on the soil microbial community in the SP, as observed by the changes in, gram-positive bacteria, gram-negative bacteria and eukaryote abundance. For all three groups, the NP treatment was significantly lower than the treatment of continual P fertilizer applications through time (BAM). The DD/SUF treatment was the same as the BAM treatment as well as the NP. This response from gram-positive bacteria, gram-negative bacteria, and eukaryotes was contradictory to the hypothesis in this study. A meta-analysis done by Wu et al. (2022) determined that bacteria, gram-positive bacteria, and gram-negative bacteria abundance increased as P fertilizer application rate was increased, and this was likely due to enhanced microbial growth and metabolism resulting in increased MBP. This finding supports the results found in this study that increased MBP was observed with increased P fertilizer treatment rate, and increased P fertilizer treatment rate increased gram-positive bacteria and gram-negative bacteria groups. No additional microbial groups, ratios, or total

microbial biomass were affected by P fertilizer for the SP. The PLFA analysis provided information on microbial community composition for the SP and FL sampling times. In the year-by-year analysis of community composition, the soil microbial community structure was not impacted by P fertilizer treatment as shown for the SP with the only group affected being actinomycetes, and for the FL with CC*TRT interactions in fungi and actinomycetes. Although changes in abundance were observed, these do not reflect in overall community composition.

Year was influential to the abundance of all microbial groups and total microbial biomass for the SP and FL sampling times. For the SP, increases in total microbial biomass, and abundance of AMF, gram-negative and gram-positive bacteria, and actinomycete groups as well as the ratios of fungi: bacteria and gram-positive: gram-negative bacteria were observed. Temperature and moisture variability in year-to-year changes or within a year highly influenced the microbial community. This annual and seasonal variability in PLFA profiles is often noted in the literature (Rankoth et al., 2019). These results agree with what we would generally expect, because seasonal and yearly environment changes can have a large impact on the microbial community. For the FL, increases in total microbial biomass and abundance of AMF, gram-negative and gram-positive bacteria, and actinomycete groups were observed. The effect of year was seen as an increase in abundance as time progressed. The abundance of microbial groups was higher in 2024 than 2018 and either higher or the same in 2022. These observations for the SP and FL were likely due to changes in in-season and year-to-year changes in temperature and moisture as well as the use of continued conservation management impacts influencing the microbial community over time.

Conclusions

Conservation management practices, such as cover crops and no-till farming, are known to have meaningful impacts on the soil microbial community. An increase in abundance of all microbial groups and total microbial biomass was observed with the presence of cover crops confirming our first hypothesis that the addition of cover crops will increase the abundance of all microbial groups and total microbial biomass. Overall, P fertilizer management had minimal effects on the soil microbial community

contradicting the second hypothesis that reducing P fertilizer inputs will drive an increase in the abundance of all microbial groups. In this study, P fertilizer management had a negligible effect on the abundance and structure of the microbial community. There were little to no changes in microbial abundance in response to differing levels of P management. Lastly, the third hypothesis that cover crops and P fertilizer management will improve soil microbial community structure cannot be confirmed. Assessing soil microbial community composition showed variability year to year for the SP and FL sampling times, and there was no clear trend of treatment effects on the different microbial groups. Although the size of the microbial community changed, as shown by changes in abundance levels of different microbial groups, the community structure remained stable. This research contributes to the observed effects of CC practices on the soil microbial community. Phosphorus fertilizer treatment had variable effects on the soil microbial community. Further research should be conducted to learn more about how P fertilizer treatment impacts microbial community abundance and shifts microbial community structure.

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Table 3.1 Phosphorus management throughout time detailing management goals and subsequent applications grouped by year.

Year	P Management Goals	P Application
2018-2019	Build Phase	Control (0 kg P_2O_5 ha^{-1}) Subsurface Injected (61 kg P_2O_5 ha^{-1}) Fall Broadcast (61 kg P_2O_5 ha^{-1})
2020-2022	Maintain/ Drawdown Phase	Control (0 kg P_2O_5 ha^{-1}) Subsurface Injected (55 kg P_2O_5 ha^{-1}) Drawdown (0 kg P_2O_5 ha^{-1})
2023-2025	Maintain/ Sufficiency Phase	Control (0 kg P_2O_5 ha^{-1}) Subsurface Injected (53 kg P_2O_5 ha^{-1}) Sufficiency (23 kg P_2O_5 ha^{-1})

Table 3.2 Analysis of variance (ANOVA) table for all microbial groups (actinomycetes, gram-positive bacteria, gram-negative bacteria, eukaryotes, fungi, and arbuscular mycorrhizal fungi), total microbial biomass, the ratio of fungi to bacteria and the ratio of gram-positive bacteria to gram-negative bacteria for all spring sample times in 2018, 2020, 2022, and 2024. Abbreviations in the table include, cover crop (CC), year (YR), P fertilizer treatment (TRT), fungi:bacteria (F:B), gram-positive bacteria:gram-negative bacteria (G+:G-), gram-positive bacteria (G+ bacteria), gram-negative bacteria (G- bacteria), arbuscular mycorrhizal fungi (AMF), and total microbial biomass (Total).[a]

Effect	F:B	G+:G-	Actinomycete	G+ bacteria	G- bacteria	Eukaryote	Fungi	AMF	Total
CC	**0.0016**	**0.031**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**0.0083**
YR	**<.0001**	**0.0006**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
TRT	0.3497	0.7796	0.0782	0.0709	0.0623	0.1667	0.1571	0.0876	0.9031
CC*TRT	0.7404	0.5161	0.1087	0.3153	0.4063	0.4418	0.8228	0.8824	0.7276
CC*YR	0.1829	0.0513	**0.0013**	**0.0256**	**0.0482**	0.4372	0.2147	0.1883	0.6642
TRT*YR	0.9769	0.8543	0.1028	**0.046**	**0.0252**	**0.0225**	0.8711	0.1088	0.4331
CC*TRT*YR	0.3154	0.9252	0.6648	0.7171	0.7948	0.3972	0.4323	0.9762	0.6947
Covariance Structure	AR (1)	UN	ARH (1)	AR (1)	AR (1)	ARH (1)	AR (1)	AR (1)	CSH

[a] Bolded and underlined p-values represent significant values evaluated at $p < 0.05$

Table 3.3 Analysis of variance (ANOVA) table for all microbial groups (actinomycetes, gram-positive bacteria, gram-negative bacteria, eukaryotes, fungi, and arbuscular mycorrhizal fungi), total microbial biomass, the ratio of fungi to bacteria and the ratio of gram-positive bacteria to gram-negative bacteria for all fall sample times in 2018, 2020, 2022, and 2024. Abbreviations in the table include, cover crop (CC), year (YR), P fertilizer treatment (TRT), fungi:bacteria (F:B), gram-positive bacteria:gram-negative bacteria (G+:G-), gram-positive bacteria (G+ bacteria), gram-negative bacteria (G- bacteria), arbuscular mycorrhizal fungi (AMF), and total microbial biomass (Total).[a]

Effect	F:B	G+:G-	Actinomycete	G+ bacteria	G- bacteria	Eukaryote	Fungi	AMF	Total
CC	0.16	0.8043	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
YR	0.4742	0.8808	0.3898	0.1669	0.0653	0.9286	0.5404	0.3469	0.348
TRT	0.11	0.0567	**<.0001**	**<.0001**	**<.0001**	0.2509	**0.0453**	**<.0001**	**<.0001**
CC*TRT	0.2634	0.3684	0.3287	0.2934	0.3064	0.1195	0.1248	0.5839	0.4803
CC*YR	0.4086	0.0979	**0.009**	**0.0025**	**0.0081**	0.1819	0.5853	**0.0053**	**0.0033**
TRT*YR	0.8695	0.5719	0.5817	0.522	0.8544	0.2171	0.9271	0.6271	0.665
CC*TRT*YR	0.2193	0.3407	0.5956	0.0659	0.0559	0.3078	**0.0193**	**0.0345**	**0.0395**
Covariance Structure	UN	UN	ARH (1)	CS	AR (1)	ARH (1)	AR (1)	AR (1)	AR (1)

[a] Bolded and underlined p-values represent significant values evaluated at $p < 0.05$.

Table 3.4 Fungi abundance (pmol C g^{-1} soil) for the CC*TRT*YR interaction in the FL sampling times (p=0.0193). The NP treatment in 2024 had greater fungi abundance in the CC treatment compared to NC. The SUF treatment in 2020 also had greater fungi abundance in the CC treatment compared to the NC. Bolded and underlined values represent least squares means that were significantly greater in the CC treatment compared to the NC treatments evaluated at $p < 0.05$.

Year	NP				SUF				BAM			
	NC		CC		NC		CC		NC		CC	
2018	2029	defg	2611	bcde	2086	cdefg	2916	abc	2426	bcdef	2399	bcdef
2020	2138	cdefg	2293	bcdefg	**1601**	**fg**	**<u>3516</u>**	**<u>a</u>**	1853	efg	2380	bcdefg
2022	2344	bcdefg	3077	ab	2726	abcd	3049	ab	2501	bcde	3093	ab
2024	**1535**	**g**	**<u>3495</u>**	**<u>a</u>**	2250	bcdefg	2625	bcde	2473	bcde	2583	bcde

Table 3.5 Arbuscular mycorrhizal fungi abundance values (pmol C g^{-1} soil) in each CC*TRT*YR interaction in the FL sampling times (p=0.0345). The NP and DD/SUF treatments saw increased AMF abundance in the CC treatment compared to the NC treatment in 2018 as well as the DD/SUF treatment in 2020. The BAM treatment saw an increase in abundance due to CC in 2022. All three P fertilizer treatments saw increases in abundance due to CC in 2024 in comparison to the NC treatments. Bolded and underlined values represent least squares means that were significantly greater in the CC treatment compared to the NC treatments evaluated at $p < 0.05$.

Year	NP				DD/SUF				BAM			
	NC		CC		NC		CC		NC		CC	
2018	**3108**	**efg**	**3840**	**cd**	**3058**	**fg**	**3688**	**cde**	3392	defg	3589	defg
2020	3019	fg	3424	defg	**2989**	**g**	**4232**	**bc**	3115	efg	3600	defg
2022	3467	defg	3857	cd	3609	def	3962	cd	**3039**	**fg**	**3903**	**cd**
2024	**3116**	**efg**	**5198**	**a**	**3813**	**cd**	**4665**	**ab**	**3480**	**defg**	**4591**	**ab**

Table 3.6 Total microbial biomass values (pmol C g^{-1} soil) in each CC*TRT*YR interaction in the FL sampling times (p=0.0395). All three P fertilizer treatments in 2024 had greater total biomass in the CC treatment compared to the NC treatment. The NP and DD/SUF treatments in 2018 also had greater total microbial biomass in the CC treatment compared to NC, as well as the SUF and BAM treatments in 2020 and the NP and BAM treatments in 2022. Bolded and underlined values represent least squares means that were significantly greater in the CC treatment compared to the NC treatments evaluated at $p < 0.05$.

Year	NP				DD/SUF				BAM			
	NC		CC		NC		CC				NC	
2018	76266	fg	**87117**	**de**	73873	fg	**91521**	**cde**	82367	def	86788	de
2020	57974	i	65063	hi	57774	i	**80638**	**ef**	60587	i	**69937**	**gh**
2022	94519	cd	**112888**	**ab**	101456	bc	116101	ab	92375	cde	**117949**	**ab**
2024	78451	efg	**124309**	**a**	90533	cde	**115548**	**ab**	92243	cde	**111942**	**ab**

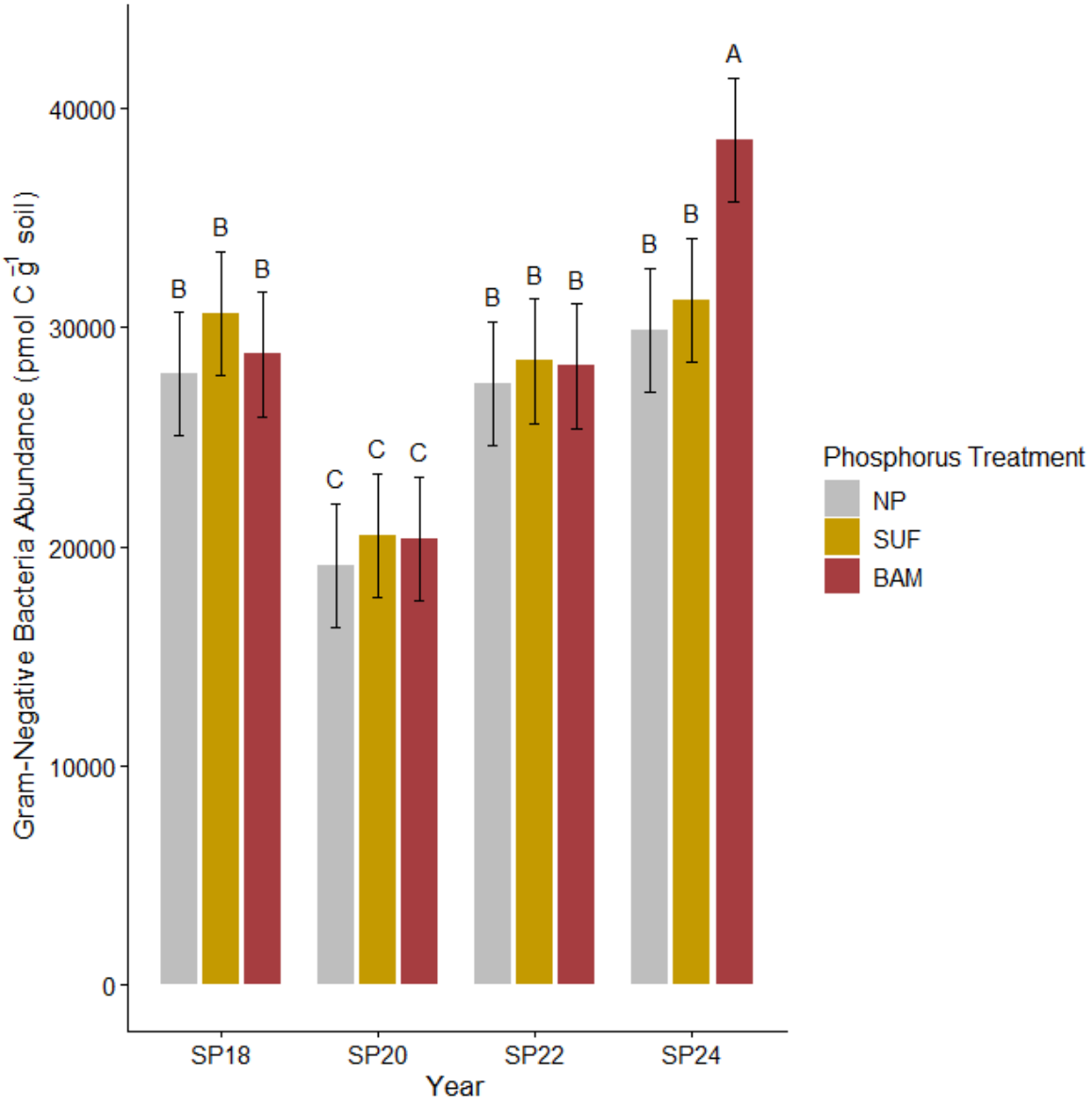


Figure 3.1 Gram-negative abundance (pmol C g^{-1} soil) measured using PLFA had influence by P fertilizer treatment by year interaction in the spring of 2018, 2020, 2022, and 2024 soybean years in Manhattan, KS managed as a no-till corn-soybean rotation. Abundance in BAM, DD/SUF, and NP treatments were the same across 2018, 2020, and 2022. While in 2024, the BAM P fertilizer treatment was higher than DD/SUF and NP in 2024 as well as all other treatments at all other sampling times ($p=0.0252$). Phosphorus fertilizer treatments include a control (NP), drawdown/ sufficiency (DD/SUF), and build and maintain (BAM).

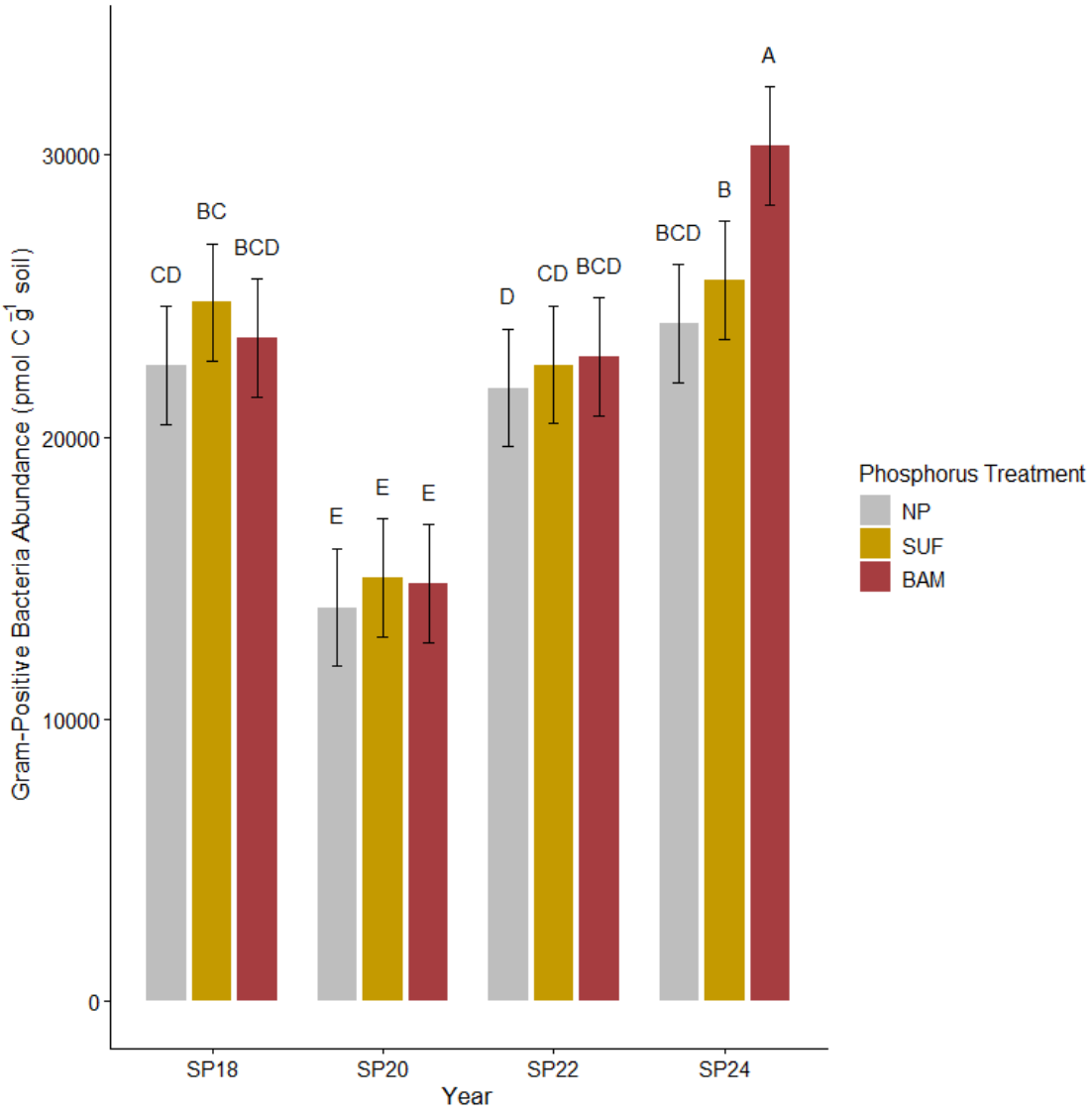


Figure 3.2 Gram-positive abundance (pmol C g^{-1} soil) measured using PLFA was affected by a P fertilizer treatment by year interaction in the spring of 2018, 2020, 2022, and 2024 soybean years in Manhattan, KS managed as a no-till corn-soybean rotation. There was no change in abundance between P treatments across 2018, 2020, and 2022. While in 2024, the BAM P fertilizer treatment was higher than DD/ SUF and NP in 2024 as well as all other treatments at all other sampling times (p=0.046). Phosphorus fertilizer treatments include a control (NP), drawdown/ sufficiency (DD/SUF), and build and maintain (BAM).

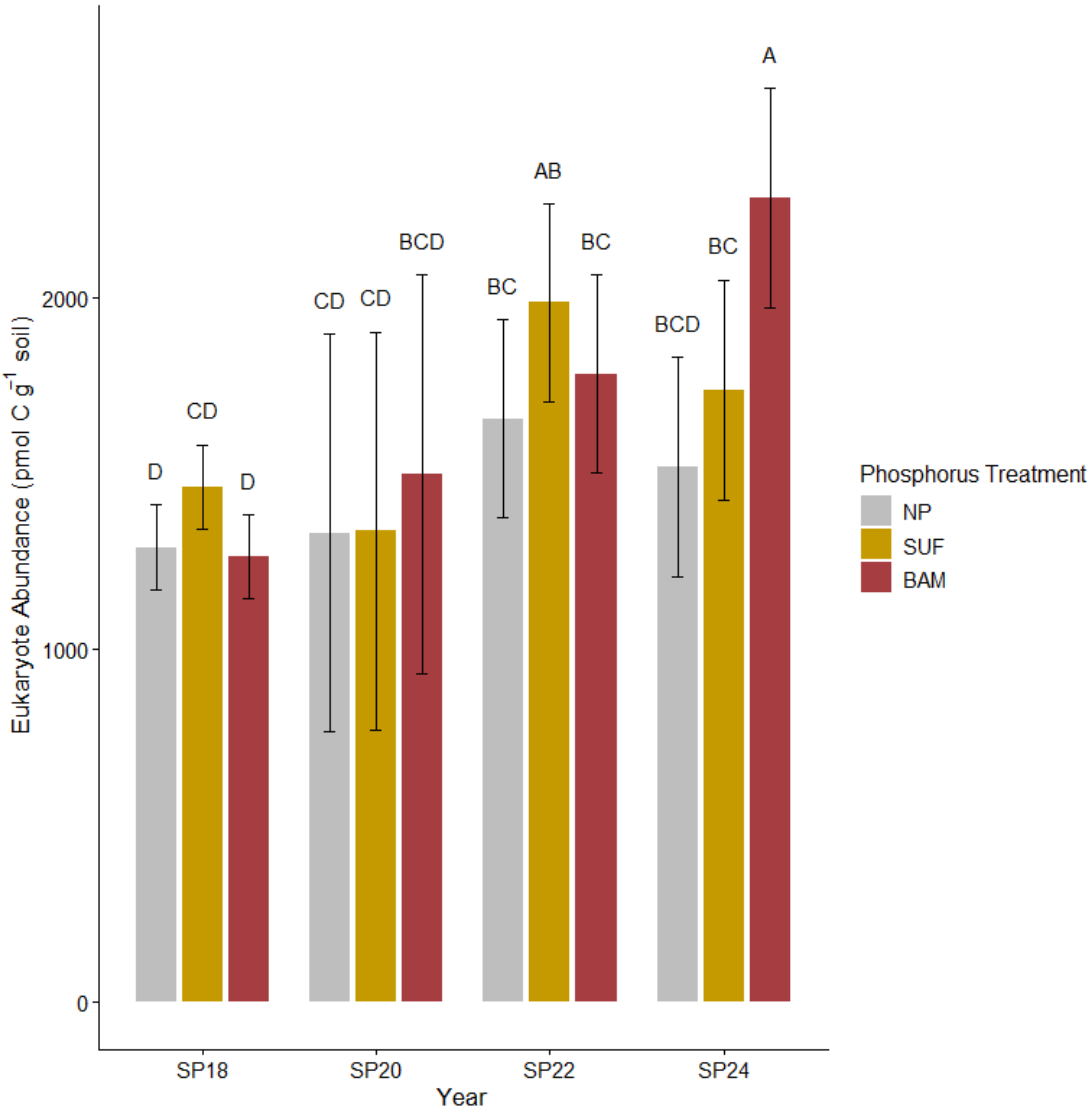


Figure 3.3 Eukaryote abundance levels (pmol C g^{-1} soil) measured using PLFA at SP sampling times in soybean years influenced by a P fertilizer treatment by year interaction in 2018, 2020, 2022, and 2024 in Manhattan, KS managed as a no-till corn-soybean rotation (p=0.0225). There was no difference in abundance level between P fertilizer treatment in 2018, 2020, and 2022. While in 2024, there was no change between the NP and DD/ SUF treatments, but an increase in abundance in the BAM treatment. Phosphorus fertilizer treatments include a control (NP), drawdown/ sufficiency (DD/SUF), and build and maintain (BAM).

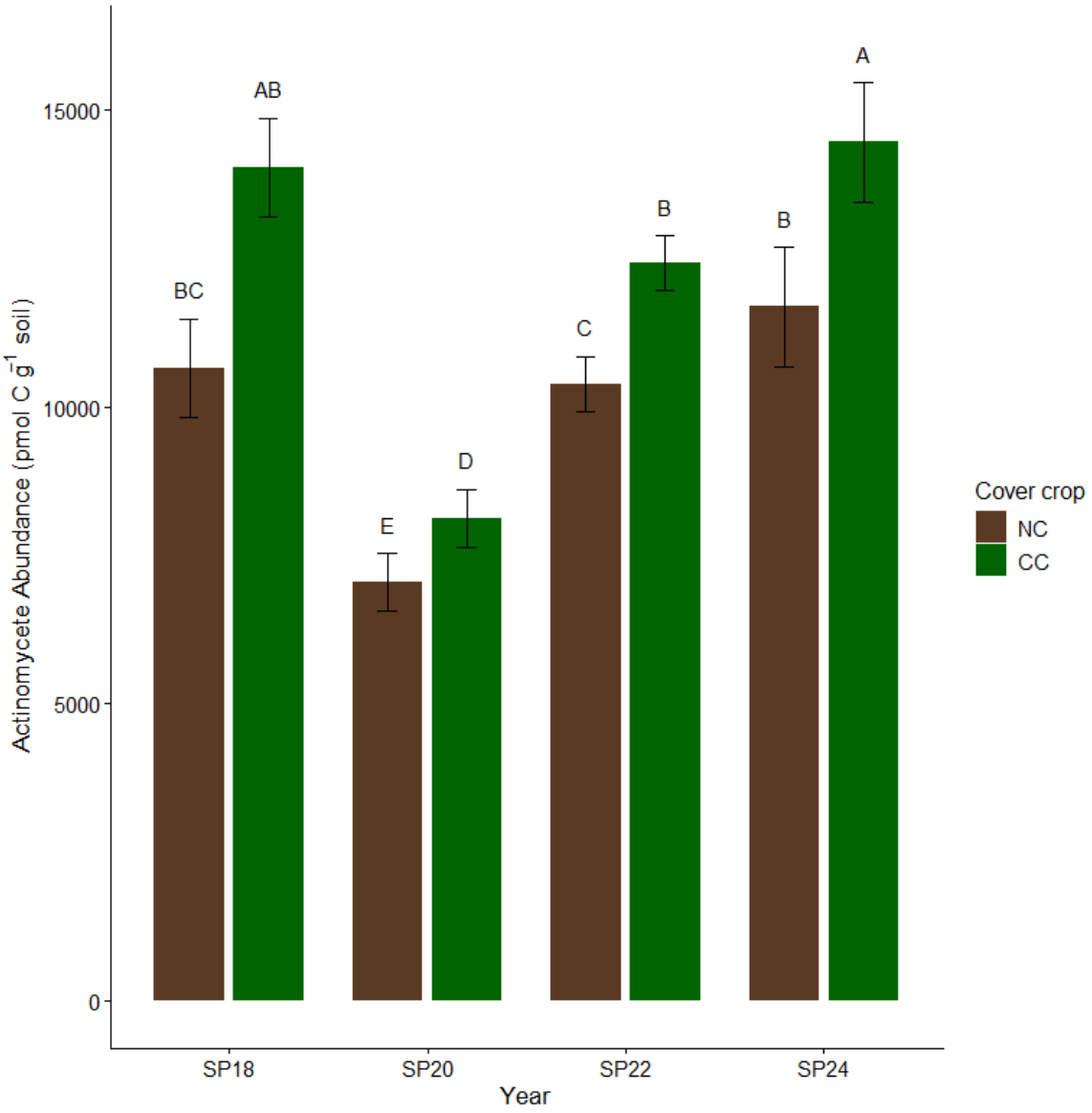


Figure 3.4 Actinomycete abundance levels (pmol C g^{-1} soil) measured using PLFA at SP sampling times in 2018, 2020, 2022, and 2024 soybean years in Manhattan, KS managed as a no-till corn-soybean rotation showing the difference between cover crop and no cover treatments (p=0.0013). Actinomycete abundance was greater in the cover crop treatment compared to the no cover treatment in 2020, 2022, and 2024.

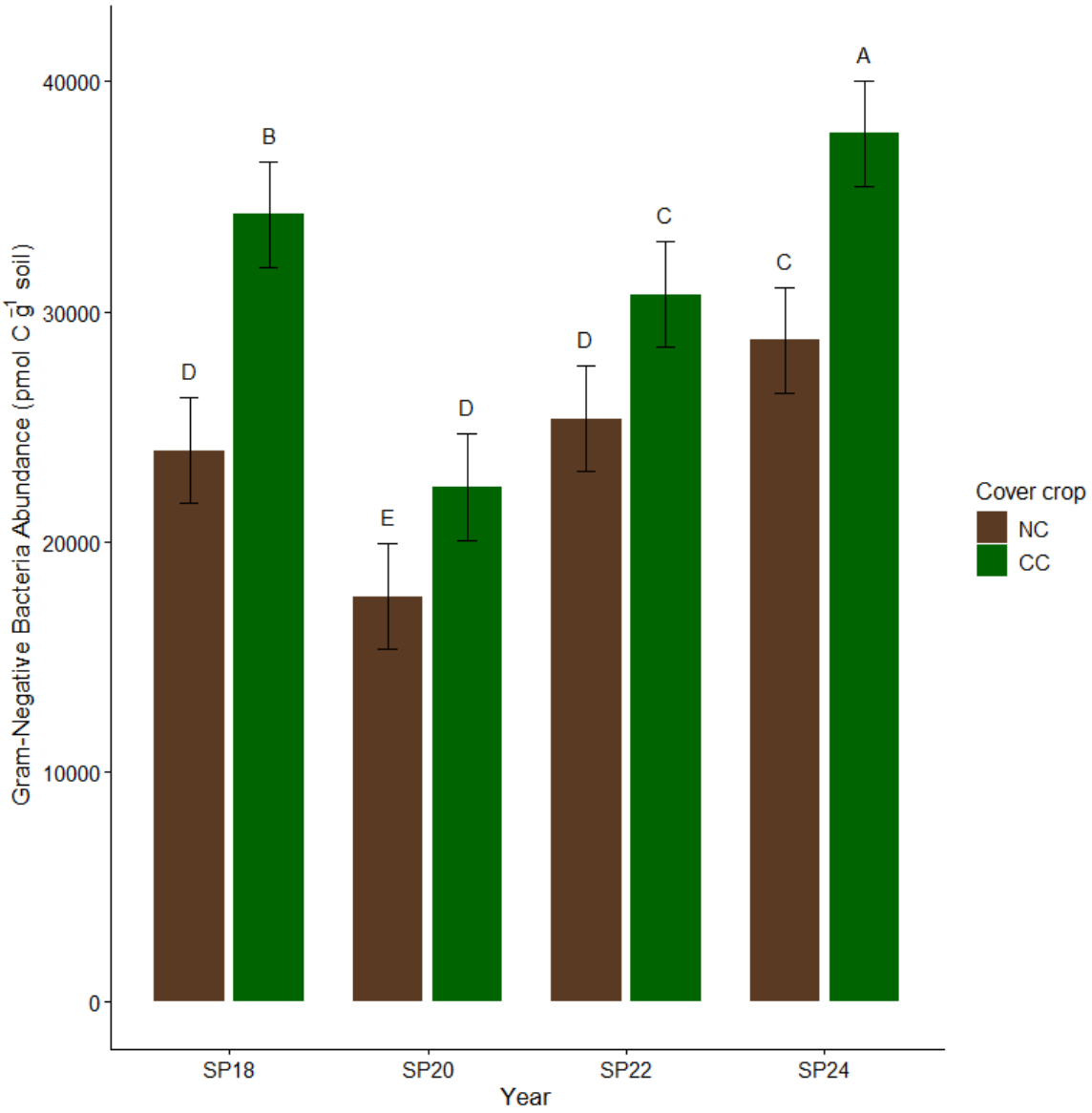


Figure 3.5 Gram-negative bacteria abundance levels (pmol C g^{-1} soil) measured using PLFA at SP sampling times in 2018, 2020, 2022, and 2024 soybean years in Manhattan, KS managed as a no-till corn-soybean rotation showing the difference between cover crop and no cover treatments (p=0.0482). For all sampling years, the abundance levels for cover crop treatment were greater than the no cover treatment of the same year.

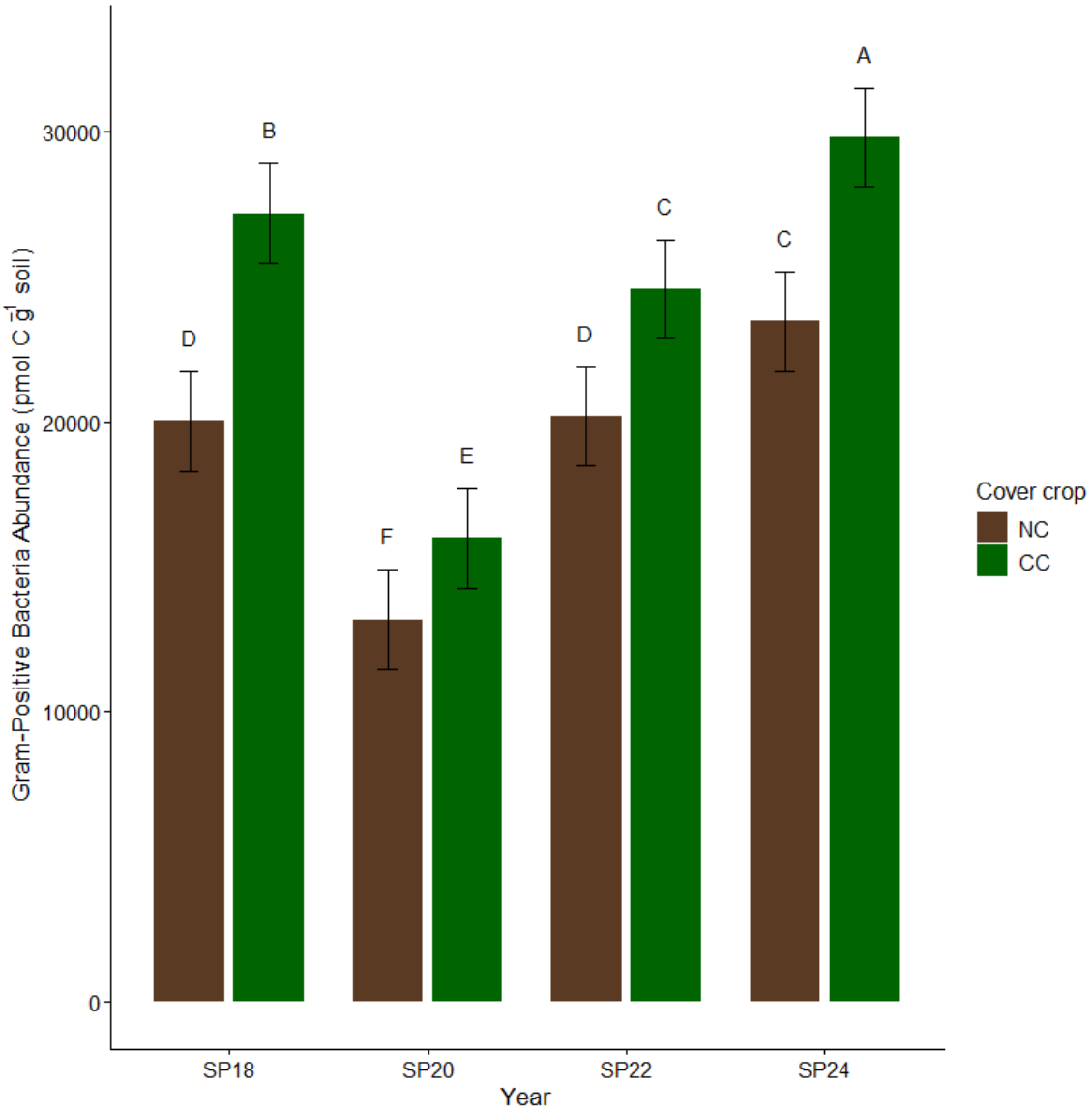


Figure 3.6 Gram-positive bacteria abundance levels (pmol C g^{-1} soil) measured using PLFA at SP sampling times in 2018, 2020, 2022, and 2024 soybean years in Manhattan, KS managed as a no-till corn-soybean rotation showing the difference between cover crop and no cover treatments (p=0.0256). For all sampling years, the abundance levels for cover crop treatment were greater than the no cover treatment of the same year.

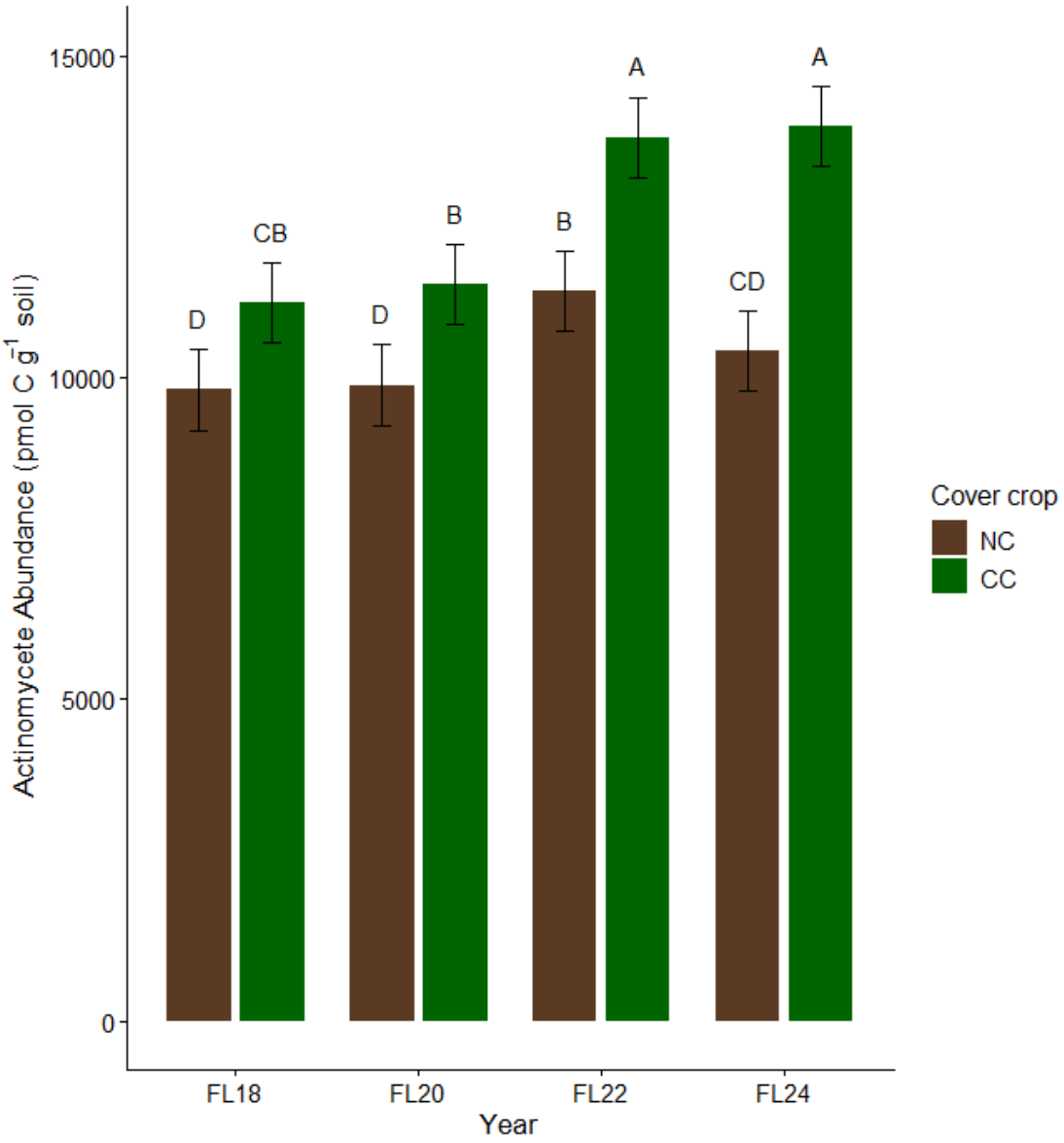


Figure 3.7 Actinomycete abundance levels (pmol C g-1 soil) measured using PLFA at FL sampling times in 2018, 2020, 2022, and 2024 soybean years in Manhattan, KS managed as a no-till corn-soybean rotation showing the cover crop by year effect (p=0.009). Abundance levels were higher in the CC treatment compared to the NC treatment in all sample years. Abundance also increases within the CC treatment as time progresses with SP22 and SP24 having the highest abundance levels.

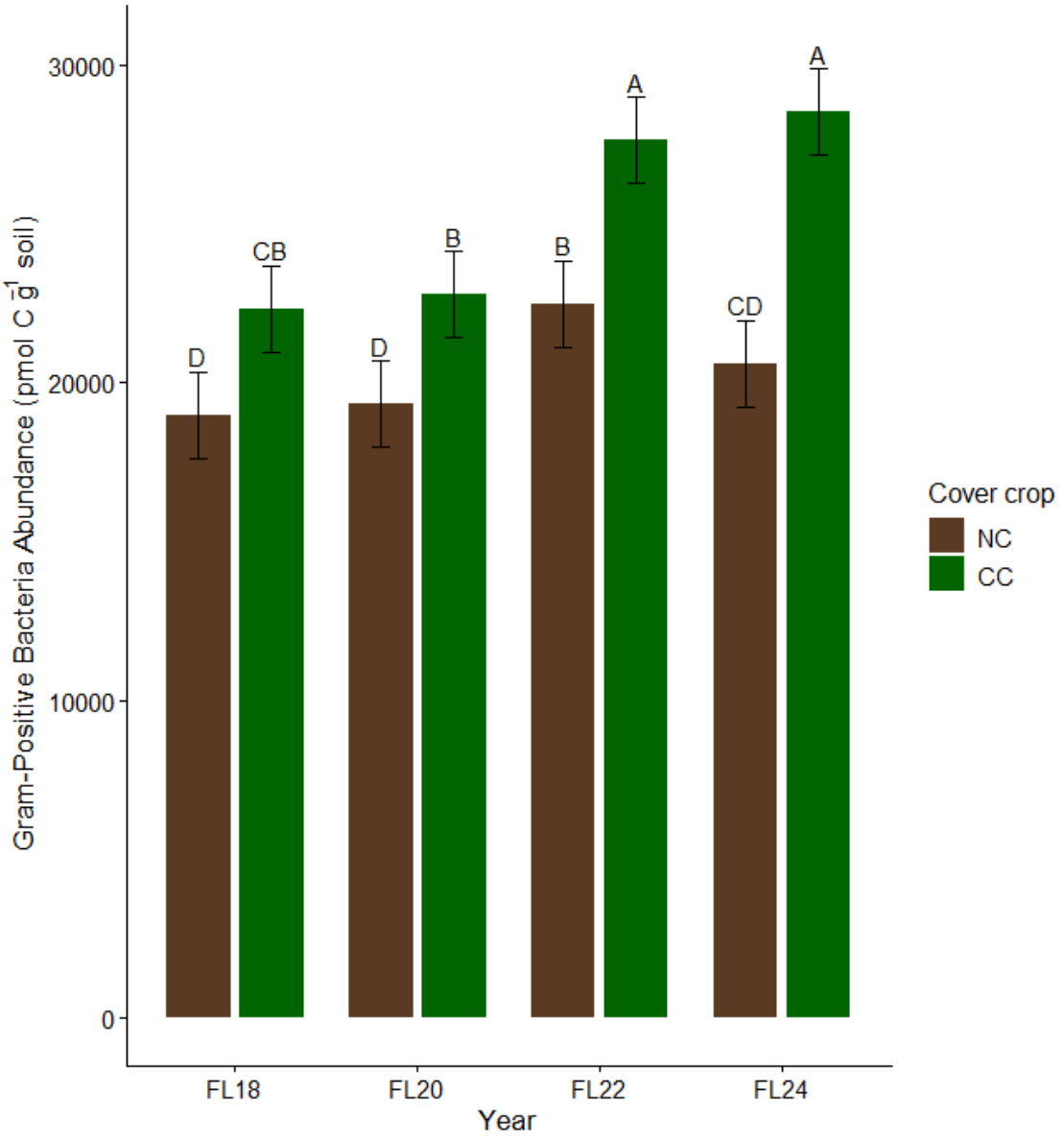


Figure 3.8 Gram-Positive abundance levels (pmol C g-1 soil) measured using PLFA at FL sampling times in 2018, 2020, 2022, and 2024 soybean years in Manhattan, KS managed as a no-till corn-soybean rotation showing the cover crop by year effect (p=0.0025). Abundance levels were higher in the CC treatment compared to the NC treatment in all sample years. Abundance also increases within the CC treatment as time progresses with SP22 and SP24 having the highest abundance levels.

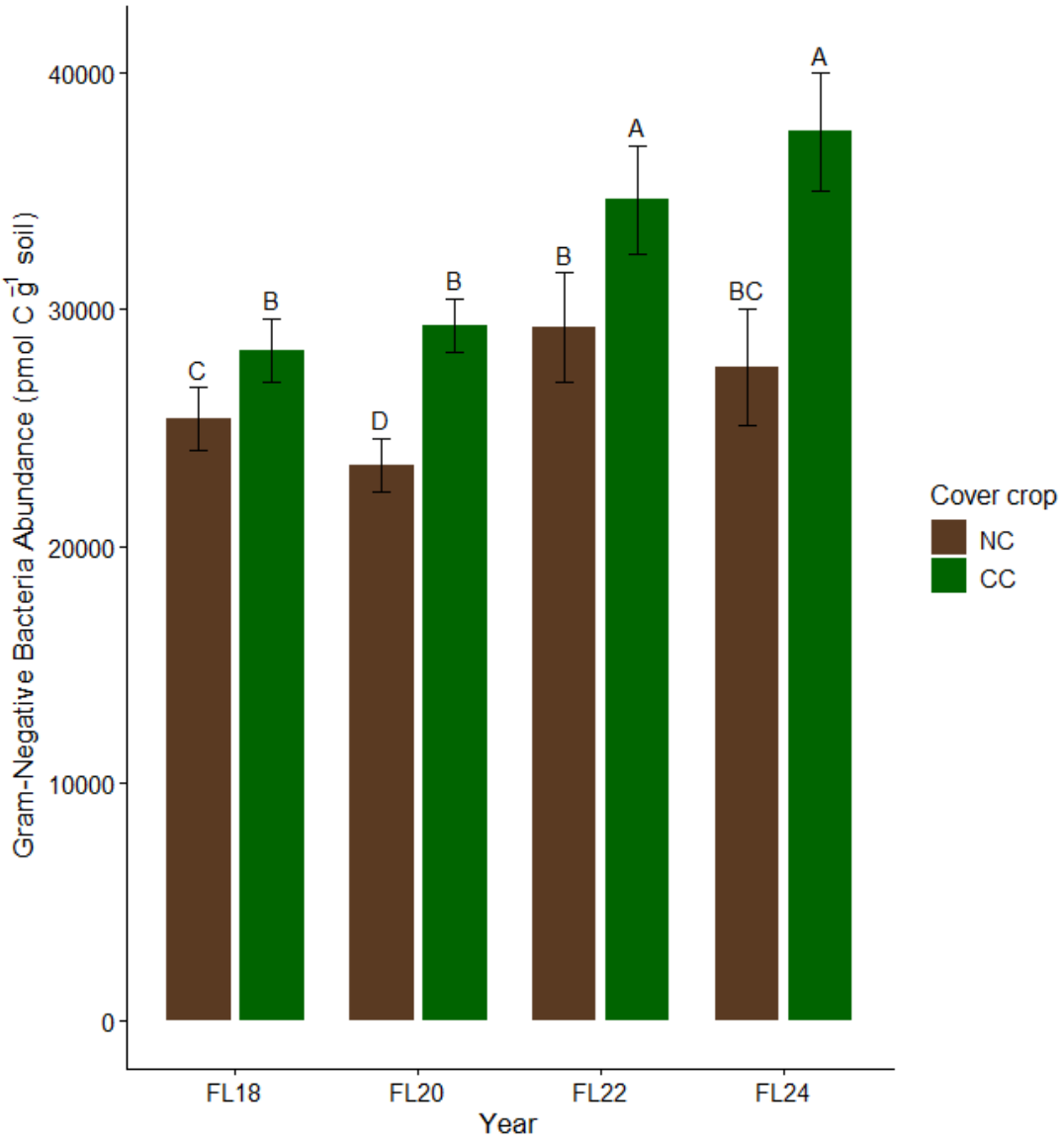


Figure 3.9 Gram-Negative abundance levels (pmol C g-1 soil) measured using PLFA at FL sampling times in 2018, 2020, 2022, and 2024 soybean years in Manhattan, KS managed as a no-till corn-soybean rotation showing the cover crop by year effect (p=0.0081). Abundance levels were higher in the CC treatment compared to the NC treatment in all sample years. Abundance also increases within the CC treatment as time progresses with SP22 and SP24 having the highest abundance levels.

Appendix A - Statistical Code

Soil Health Code

```
DATA SET1;
INPUT PLOT BLOC TRT$ CC$ time$ GLU NAG ALKP ACIDP RESP;
CARDS;

*proc print data = set1;
RUN;

**********  TRANSPOSE DATA SO ALL RESPONSE VARIABLES ARE IN A SINGLE COLUMN
**********;
proc sort data=set1; by time plot bloc trt cc;
proc transpose data=set1
     out=set2 (rename=(_Name_=rvar) rename=(col1=XXX));
   var GLU NAG    ALKP ACIDP  RESP;
   by time plot bloc trt cc;
run;
data set2; set set2; YYY=log10(XXX); *add variable YYY, which is the log-
transformation of XXX;
run;
proc print data=set2; run;


TITLE;
proc sort data=set2; by rvar time plot;
PROC MIXED DATA=SET2 PLOTS=(STUDENTPANEL);
by rvar; where rvar in ('GLU','NAG','ALKP','ACIDP','RESP');
CLASS BLOC TRT CC TIME PLOT;
MODEL XXX=BLOC TRT|CC|TIME/DDFM=KR;
REPEATED TIME/SUBJECT=PLOT TYPE=ARH(1);
LSMEANS  TRT|CC|TIME/pdiff CL;
ODS OUTPUT tests3=ANOVA LSMEANS=LSM;
STORE TEST / LABEL='Data';
RUN;
proc print data=LSM;
proc print data=ANOVA;
run;

ods graphics off;

PROC PLM RESTORE=TEST;
LSMEANS TRT|CC|TIME / PDIFF=ALL PLOT=MEANPLOT cl LINES;* adj=tukey;
ods output lsmlines=bbb lsmeans=lsm;
RUN;

***** organize output to give a table of means with letters;
proc sort data=bbb; by rvar;
data ccc; set bbb (where=(effect is not missing)); Est=(Estimate); keep rvar
effect trt cc time estimate est line: ; *Estimate in original scale;

data ccc; set ccc; array dx line:; call sortc(of line:); x = catt(of line:);
keep rvar effect trt cc time estimate est x;  *This sorts the letters
alphabetically and stores them as a single variable.;
```

```
proc print data=ccc ;
run;

proc print data=lsm; run;

proc sort data=lsm;  by rvar Effect TRT CC time;
proc sort data=ccc;  by rvar Effect TRT CC time;
data ddd; merge lsm ccc; by rvar Effect TRT CC time;
proc print data=ddd; run;

/***** use the code below to collect, store, and display the fit statistics
when selecting the covariance structures *****\
choose the best covariance structure from UN, ar(1), arh(1), cs, csh,
arma(1,1)

If there are two covariance structures that result in similar AIC/BIC/AICC
values, you can coose the one that is more
common for other variables based on the following:

1)    If the difference is less than 2, then there is substantial support for
choosing the more common structure;
2)    If the difference is between 2 and 4, there is strong support;
3)    If the difference is between 4 and 7, there is less support;
4)    If the difference is greater than 10, then there is no support for
choosing the more common structure.

This is a link that gives more details about this question:
https://stats.stackexchange.com/questions/232465/how-to-compare-models-on-
the-basis-of-aic.
\*************************************************************************
****************************************/

proc sort data=set2; by rvar time plot;
run;
ods output
    Tests3  = ANOVA
    LSMeans = LSM
    Diffs   = SoilHealth_Diffs;
%macro fit_covariances;
    %let covlist =
        UN
        AR(1)
        ARH(1)
        CS
        CSH
        %str(ARMA(1,1));
    %do i = 1 %to 6;
        %let covtype = %qscan(%superq(covlist), &i, %str( ));
        proc mixed data=SET2 plots=none;
            by rvar;
            class BLOC TRT CC TIME PLOT;
            model XXX = BLOC TRT|CC|TIME / ddfm=kr;
            repeated TIME /subject=PLOT type=%unquote(&covtype);
            ods output FitStatistics=fitout&i;
        run;
        data fitout&i; length _type_ $9;
            set fitout&i; _type_ = "%unquote(&covtype)";
```

```
        run;
    %end;
%mend fit_covariances;

%fit_covariances;

******** transpose fitstat ******;
data fitstats; set fitout1-fitout6;
    if descr='-2 Res Log Likelihood' then delete;
run;
proc sort data=fitstats; by rvar descr _type_;
proc transpose data=fitstats out=fitstat_t;
  by rvar descr;
  id _type_;
  var value;
proc print data=fitstat_t;
run;

title;
proc sort data=set2;
    by rvar time plot;
run;
/* Save model output */
ods output
    Tests3  = ANOVA
    LSMeans = LSM
    Diffs   = SoilHealth_Diffs;
PROC MIXED DATA=SET2 PLOTS=(STUDENTPANEL);
    by rvar;
    where rvar in ('GLU','NAG','ALKP','ACIDP','RESP');
    CLASS BLOC TRT CC TIME PLOT;
    MODEL XXX = BLOC TRT|CC|TIME / DDFM=KR;
    REPEATED TIME / SUBJECT=PLOT TYPE=ARH(1);
    /* All main effects and interactions */
    LSMEANS TRT|CC|TIME / PDIFF CL;

    STORE TEST / LABEL='Data';
RUN;
data SoilHealth_LSD;
    set SoilHealth_Diffs;

    /* Handle SAS standard error variable names */
    SE_Diff = input(vvaluex('StdErr'), best32.);

    if missing(SE_Diff) then
        SE_Diff = input(
            vvaluex('StandardError'), best32.
        );
    /* Fisher's unadjusted LSD */
    if DF > 0 and SE_Diff > 0 then
        LSD = tinv(0.975, DF) * SE_Diff;

    format LSD 12.3;
run;
proc means data=SoilHealth_LSD
           nway noprint;
    class rvar Effect;
```

```
    var LSD;
    output out=LSD_Summary
        n    = N_Comparisons
        min  = Minimum_LSD
        max  = Maximum_LSD
        mean = Mean_LSD;
run;

data LSD_Final;
    set LSD_Summary;

    length Status $25;

    /* Display tolerance: 0.01 units */
    if N_Comparisons > 0 and
       abs(Maximum_LSD - Minimum_LSD) < 0.01
       then do;

        Common_LSD = Mean_LSD;
        Status = "Common LSD";
    end;
    else do;

        Common_LSD = .;
        Status = "LSD varies";

    end;
    format Common_LSD Minimum_LSD
           Maximum_LSD Mean_LSD 12.3;

    keep rvar Effect N_Comparisons
         Common_LSD Minimum_LSD
         Maximum_LSD Mean_LSD Status;
run;

proc sort data=LSD_Final;
    by rvar Effect;
run;

proc print data=LSD_Final noobs label;
    by rvar;
    var Effect Common_LSD
        Minimum_LSD Maximum_LSD Status;
    label
        Effect      = "Effect"
        Common_LSD  = "LSD (0.05)"
        Minimum_LSD = "Minimum LSD"
        Maximum_LSD = "Maximum LSD"
        Status      = "LSD Status";
    title "LSD Summary for Soil Health Variables";
run;

title;

proc print data=LSD_Final noobs label;
    by rvar;
```

```
    var Effect Mean_LSD Common_LSD
        Minimum_LSD Maximum_LSD Status;
    label
        Effect      = "Effect"
        Mean_LSD    = "Mean LSD"
        Common_LSD  = "Common LSD"
        Minimum_LSD = "Minimum LSD"
        Maximum_LSD = "Maximum LSD"
        Status      = "LSD Status";
    title "LSD Summary for Soil Health Variables";
run;
```

PLFA Code for Spring

```
data aaa; input plot rep fert$  cover$ SP18 SP20 SP22 SP24;
cards;
;
proc sort data=aaa; by plot rep fert cover;
proc transpose data=aaa
out=bbb (rename=(_Name_=yr) rename=(col1=MB));
var SP18 SP20 SP22 SP24;
by plot rep fert cover;
run;
proc print data=bbb; run;

**** ANOVA including yr in model ****;
proc glimmix data = bbb noitprint order=data plots=(studentpanel);
class cover fert rep yr plot;
model MB = rep cover|fert|yr /ddfm = kr;
random yr/subject=plot type=UN residual;
lsmeans fert|cover|yr/lines cl;
*ods output LSmeans=Means Tests3=ANOVA LSMlines=lines;
/*Please check type=UN, CSH,CS, AR(1), arh(1) arma(1,1) for better fit*/
/* ARH(1) is best fit, but followed close by CS and CSH */
*ods output fitstatistics=fitout;
run;

ods output Diffs = PLFA_Diffs;

proc glimmix data=bbb noitprint order=data
             plots=(studentpanel);
    class cover fert rep yr plot;
    model MB = rep cover|fert|yr / ddfm=kr;
    random yr / subject=plot type=UN residual;
    lsmeans fert
            cover
            yr
            fert*cover
            fert*yr
            cover*yr
            fert*cover*yr
            / diff cl lines;
run;

data PLFA_LSD;
    set PLFA_Diffs;
```

```
    LSD = tinv(0.975, DF) * StdErr;
run;

proc means data=PLFA_LSD
           nway noprint;
    class Effect;
    var LSD;
    output out=LSD_Summary
        min = Minimum_LSD
        max = Maximum_LSD
        mean = Mean_LSD
        std = SD_LSD;
run;

data LSD_Final;
    set LSD_Summary;
    length Common_LSD_Status $25;
    if abs(Maximum_LSD - Minimum_LSD) < 0.01 then do;
        LSD = Mean_LSD;
        Common_LSD_Status = "Common LSD";
    end;
    else do;
        LSD = .;
        Common_LSD_Status = "LSD varies";
    end;
    format LSD Minimum_LSD Maximum_LSD
           Mean_LSD SD_LSD 12.2;
    keep Effect LSD Minimum_LSD Maximum_LSD
         Mean_LSD Common_LSD_Status;
run;

proc print data=LSD_Final noobs label;
    label
        Effect = "Effect"
        LSD = "Common LSD"
        Minimum_LSD = "Minimum LSD"
        Maximum_LSD = "Maximum LSD"
        Mean_LSD = "Mean LSD"
        Common_LSD_Status = "Status";
    title "LSD Summary for All Effects";
run;


/***** use the code below to collect, store, and display the fit statistics
when changing the covariance structures *****;

data fitout; set fitout; length _type_ $6; _type_ = "ARMA11";
run;
data fitstat; set fitstat fitout; run;

******** transpose fitstat ******;
proc sort data=fitstat; by descr _type_;
proc transpose data=fitstat out=fitstat_t;
  by descr;
  id _type_;
  var value;
```

```
proc print data=fitstat_t; run;
```

PLFA Code – Fall

```
data aaa; input plot rep fert$  cover$ FL18 FL20 FL22 FL24;
cards;
;
proc sort data=aaa; by plot rep fert cover;
proc transpose data=aaa
out=bbb (rename=(_Name_=yr) rename=(col1=MB));
var FL18 FL20 FL22 FL24;
by plot rep fert cover;
run;
proc print data=bbb; run;

**** ANOVA including yr in model ****;
proc glimmix data = bbb noitprint order=data plots=(studentpanel);
class cover fert rep yr plot;
model MB = rep cover|fert|yr /ddfm = kr;
random yr/subject=plot type=UN residual;
lsmeans fert|cover|yr/lines cl;
*ods output LSmeans=Means Tests3=ANOVA LSMlines=lines;
/*Please check type=UN, CSH,CS, AR(1), arh(1) arma(1,1) for better fit*/
/* ARH(1) is best fit, but followed close by CS and CSH */
*ods output fitstatistics=fitout;
run;

ods output Diffs = PLFA_Diffs;

proc glimmix data=bbb noitprint order=data
             plots=(studentpanel);
    class cover fert rep yr plot;
    model MB = rep cover|fert|yr / ddfm=kr;
    random yr / subject=plot type=UN residual;
    lsmeans fert
            cover
            yr
            fert*cover
            fert*yr
            cover*yr
            fert*cover*yr
            / diff cl lines;
run;

data PLFA_LSD;
    set PLFA_Diffs;
    LSD = tinv(0.975, DF) * StdErr;
run;

proc means data=PLFA_LSD
           nway noprint;
    class Effect;
    var LSD;
    output out=LSD_Summary
        min = Minimum_LSD
        max = Maximum_LSD
        mean = Mean_LSD
        std = SD_LSD;
```

```
run;

data LSD_Final;
    set LSD_Summary;
    length Common_LSD_Status $25;
    if abs(Maximum_LSD - Minimum_LSD) < 0.01 then do;
        LSD = Mean_LSD;
        Common_LSD_Status = "Common LSD";
    end;
    else do;
        LSD = .;
        Common_LSD_Status = "LSD varies";
    end;
    format LSD Minimum_LSD Maximum_LSD
           Mean_LSD SD_LSD 12.2;
    keep Effect LSD Minimum_LSD Maximum_LSD
         Mean_LSD Common_LSD_Status;
run;

proc print data=LSD_Final noobs label;
    label
        Effect = "Effect"
        LSD = "Common LSD"
        Minimum_LSD = "Minimum LSD"
        Maximum_LSD = "Maximum LSD"
        Mean_LSD = "Mean LSD"
        Common_LSD_Status = "Status";
    title "LSD Summary for All Effects";
run;
```

Appendix B - Soil Health Supplemental Material

Table B.1 Least squares (LS) means and least significant differences (LSD) for microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) for 2024, for all main effects and interactions. Table abbreviations include, phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

Effect	P TRT	Cover	Time	Microbial Biomass C	Microbial Biomass N	Microbial Biomass P
				μg C g soil^{-1}	μg C g soil^{-1}	μg P g soil^{-1}
TRT	BAM			135.5	38.8	7.5
TRT	NP			120.1	39.6	0.9
TRT	SUF			133.2	37.2	4.6
CC		CC		141.4	42.1	5.1
CC		NC		117.8	35.0	3.5
TRT*CC	BAM	CC		144.8	40.2	7.8
TRT*CC	BAM	NC		126.3	37.4	7.2
TRT*CC	NP	CC		133.1	47.4	1.1
TRT*CC	NP	NC		107.1	31.9	0.7
TRT*CC	SUF	CC		146.4	38.7	6.4
TRT*CC	SUF	NC		120.0	35.7	2.8
TIME			T0	144.3	16.9	1.2
TIME			T1	127.0	23.6	8.7
TIME			T2	105.9	20.8	7.0
TIME			FL	141.1	92.8	0.4
TRT*TIME	BAM		T0	145.3	19.3	3.0
TRT*TIME	BAM		T1	149.8	28.7	13.9
TRT*TIME	BAM		T2	113.4	15.9	12.0
TRT*TIME	BAM		FL	133.6	91.1	1.2
TRT*TIME	NP		T0	137.4	18.7	0.02
TRT*TIME	NP		T1	109.3	28.9	1.7
TRT*TIME	NP		T2	94.5	18.4	1.8
TRT*TIME	NP		FL	139.0	92.6	0.2
TRT*TIME	SUF		T0	150.4	12.7	0.5
TRT*TIME	SUF		T1	122.0	13.3	10.6
TRT*TIME	SUF		T2	109.9	28.2	7.3
TRT*TIME	SUF		FL	150.6	94.6	-0.1
CC*TIME		CC	T0	154.4	16.4	1.7
CC*TIME		CC	T1	134.7	27.6	9.5
CC*TIME		CC	T2	112.3	16.7	8.6
CC*TIME		CC	FL	164.3	107.7	0.7
CC*TIME		NC	T0	134.2	17.3	0.7
CC*TIME		NC	T1	119.4	19.7	8.0
CC*TIME		NC	T2	99.6	25.0	5.4

CC*TIME		NC	FL	117.8	77.9	0.07
TRT*CC*TIME	BAM	CC	T0	157.8	17.3	3.5
TRT*CC*TIME	BAM	CC	T1	155.2	30.3	13.6
TRT*CC*TIME	BAM	CC	T2	120.1	15.0	12.5
TRT*CC*TIME	BAM	CC	FL	146.1	98.0	1.8
TRT*CC*TIME	BAM	NC	T0	132.8	21.3	2.5
TRT*CC*TIME	BAM	NC	T1	144.5	27.2	14.1
TRT*CC*TIME	BAM	NC	T2	106.6	16.8	11.4
TRT*CC*TIME	BAM	NC	FL	121.1	84.3	0.6
TRT*CC*TIME	NP	CC	T0	148.5	19.7	0.2
TRT*CC*TIME	NP	CC	T1	117.4	36.8	2.0
TRT*CC*TIME	NP	CC	T2	91.7	11.8	2.3
TRT*CC*TIME	NP	CC	FL	174.7	121.3	0.1
TRT*CC*TIME	NP	NC	T0	126.2	17.7	-0.1
TRT*CC*TIME	NP	NC	T1	101.3	21.0	1.4
TRT*CC*TIME	NP	NC	T2	97.4	25.0	1.3
TRT*CC*TIME	NP	NC	FL	103.3	63.8	0.2
TRT*CC*TIME	SUF	CC	T0	157.1	12.3	1.3
TRT*CC*TIME	SUF	CC	T1	131.5	15.6	12.8
TRT*CC*TIME	SUF	CC	T2	125.0	23.3	11.0
TRT*CC*TIME	SUF	CC	FL	172.0	103.7	0.3
TRT*CC*TIME	SUF	NC	T0	143.7	13.0	-0.4
TRT*CC*TIME	SUF	NC	T1	112.5	10.9	8.5
TRT*CC*TIME	SUF	NC	T2	94.9	33.1	3.6
TRT*CC*TIME	SUF	NC	FL	129.1	85.6	-0.6

Table B.2 Least squares (LS) means and least significant differences (LSD) for microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) for 2025, for all main effects and interactions. Table abbreviations include, phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

Effect	P TRT	Cover	Time	Microbial Biomass C	Microbial Biomass N	Microbial Biomass P
				μg C g^{-1} soil	μg N g^{-1} soil	μg P g^{-1} soil
TRT	BAM			186.9	68.7	6.8
TRT	NP			180.5	70.2	1.8
TRT	SUF			148.5	69.9	5.1
CC		CC		181.6	77.5	5.0
CC		NC		162.3	61.7	4.1
TRT*CC	BAM	CC		170.6	83.9	7.1
TRT*CC	BAM	NC		203.2	53.5	6.5
TRT*CC	NP	CC		227.9	75.5	1.9
TRT*CC	NP	NC		133.1	64.8	1.6
TRT*CC	SUF	CC		146.4	73.0	6.1
TRT*CC	SUF	NC		150.6	66.7	4.1
TIME			T0	159.6	46.0	6.5
TIME			T1	159.9	46.0	4.8
TIME			T2	211.8	170.7	2.9
TIME			FL	156.7	15.6	3.9
TRT*TIME	BAM		T0	171.5	37.4	9.1
TRT*TIME	BAM		T1	171.2	37.4	8.0
TRT*TIME	BAM		T2	234.6	187.8	4.6
TRT*TIME	BAM		FL	170.3	12.3	5.4
TRT*TIME	NP		T0	150.9	54.7	3.2
TRT*TIME	NP		T1	151.6	54.7	2.2
TRT*TIME	NP		T2	181.0	154.7	1.1
TRT*TIME	NP		FL	238.6	16.5	0.5
TRT*TIME	SUF		T0	156.3	45.9	7.2
TRT*TIME	SUF		T1	156.9	45.9	4.3
TRT*TIME	SUF		T2	219.9	169.6	2.9
TRT*TIME	SUF		FL	61.1	18.1	5.9
CC*TIME		CC	T0	167.2	51.5	5.9
CC*TIME		CC	T1	166.9	51.5	4.9
CC*TIME		CC	T2	221.2	191.5	3.4
CC*TIME		CC	FL	171.3	15.6	5.8
CC*TIME		NC	T0	152.0	40.5	7.1
CC*TIME		NC	T1	152.8	40.5	4.8
CC*TIME		NC	T2	202.5	149.9	2.3
CC*TIME		NC	FL	142.0	15.7	2.1
TRT*CC*TIME	BAM	CC	T0	177.1	45.8	7.5

TRT*CC*TIME	BAM	CC	T1	175.2	45.8	7.6
TRT*CC*TIME	BAM	CC	T2	247.6	232.0	5.9
TRT*CC*TIME	BAM	CC	FL	82.5	12.1	7.4
TRT*CC*TIME	BAM	NC	T0	165.9	28.9	10.8
TRT*CC*TIME	BAM	NC	T1	167.2	28.9	8.3
TRT*CC*TIME	BAM	NC	T2	221.6	143.6	3.4
TRT*CC*TIME	BAM	NC	FL	258.2	12.5	3.3
TRT*CC*TIME	NP	CC	T0	197.1	63.0	3.5
TRT*CC*TIME	NP	CC	T1	197.1	63.0	2.3
TRT*CC*TIME	NP	CC	T2	162.0	158.0	1.2
TRT*CC*TIME	NP	CC	FL	355.5	18.1	0.5
TRT*CC*TIME	NP	NC	T0	104.7	46.5	2.9
TRT*CC*TIME	NP	NC	T1	106.1	46.5	2.1
TRT*CC*TIME	NP	NC	T2	200.0	151.3	1.0
TRT*CC*TIME	NP	NC	FL	121.8	14.9	0.6
TRT*CC*TIME	SUF	CC	T0	127.3	45.7	6.9
TRT*CC*TIME	SUF	CC	T1	128.4	45.7	4.8
TRT*CC*TIME	SUF	CC	T2	254.0	184.3	3.1
TRT*CC*TIME	SUF	CC	FL	76.0	16.4	9.5
TRT*CC*TIME	SUF	NC	T0	185.3	46.0	7.5
TRT*CC*TIME	SUF	NC	T1	185.3	46.0	3.9
TRT*CC*TIME	SUF	NC	T2	185.7	154.9	2.6
TRT*CC*TIME	SUF	NC	FL	46.2	19.8	2.3

Table B.3 Least squares (LS) means and least significant differences (LSD) for microbial biomass ratios of carbon: nitrogen (MBC: MBN), nitrogen: phosphorus (MBN: MBP), and carbon: phosphorus (MBC: MBP) for 2024, for all main effects and interactions. Table abbreviations include, phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

Effect	P TRT	Cover	Time	MBC:MBN	MBN:MBP	MBC:MBP
TRT	BAM			8.2	-81.9	15.4
TRT	NP			9.4	15202.0	57829.0
TRT	SUF			8.0	525.5	20.8
Mean LSD				5.7	29805.9	327940.1
CC		CC		10.6	7543.0	27583.0
CC		NC		6.5	2887.2	10994.0
Mean LSD				4.6	23491.2	234877.6
TRT*CC	BAM	CC		9.2	122.0	149.7
TRT*CC	BAM	NC		7.3	-285.9	-119.0
TRT*CC	NP	CC		13.6	22620.0	82723.0
TRT*CC	NP	NC		5.3	7783.1	32936.0
TRT*CC	SUF	CC		9.0	-113.3	-123.6
TRT*CC	SUF	NC		7.1	1164.3	165.2
Mean LSD				8.1	42646.5	400038.5
TIME			T0	17.5	14897.0	56055.0
TIME			T1	7.8	-303.4	-691.7
TIME			T2	7.3	4864.0	19737.0
TIME			FL	1.6	1402.2	2054.1
Mean LSD				7.7	42781.8	240401.5
TRT*TIME	BAM		T0	19.0	120.1	69.5
TRT*TIME	BAM		T1	5.1	-352.1	10.8
TRT*TIME	BAM		T2	7.4	1.6	10.8
TRT*TIME	BAM		FL	1.5	-97.3	-29.6
TRT*TIME	NP		T0	17.7	46018.0	174634.0
TRT*TIME	NP		T1	8.3	-347.9	-1848.1
TRT*TIME	NP		T2	9.9	10978.0	53542.0
TRT*TIME	NP		FL	1.6	4159.2	4989.9
TRT*TIME	SUF		T0	15.6	-1445.5	-6538.3
TRT*TIME	SUF		T1	10.1	-210.2	-237.7
TRT*TIME	SUF		T2	4.7	3612.9	5657.3
TRT*TIME	SUF		FL	1.8	144.7	1202.0
Mean LSD				12.4	66496.3	402798.0
CC*TIME		CC	T0	22.6	20830.0	75335.0
CC*TIME		CC	T1	8.6	10.5	-1240.9
CC*TIME		CC	T2	9.5	7174.4	32771.0

CC*TIME		CC	FL	1.6	2157.5	3467.5
CC*TIME		NC	T0	12.3	8965.3	36775.0
CC*TIME		NC	T1	7.0	-617.3	-142.4
CC*TIME		NC	T2	5.1	2553.7	6702.8
CC*TIME		NC	FL	1.7	646.9	640.6
Mean LSD				10.3	52783.8	339003.0
TRT*CC*TIME	BAM	CC	T0	21.9	9.6	57.7
TRT*CC*TIME	BAM	CC	T1	5.3	1.4	7.5
TRT*CC*TIME	BAM	CC	T2	8.2	1.5	10.9
TRT*CC*TIME	BAM	CC	FL	1.6	475.5	522.8
TRT*CC*TIME	BAM	NC	T0	16.1	230.5	81.4
TRT*CC*TIME	BAM	NC	T1	4.9	-705.6	14.2
TRT*CC*TIME	BAM	NC	T2	6.5	1.8	10.6
TRT*CC*TIME	BAM	NC	FL	1.5	-670.2	-582.1
TRT*CC*TIME	NP	CC	T0	25.8	62864.0	226730.0
TRT*CC*TIME	NP	CC	T1	12.0	28.7	-3742.8
TRT*CC*TIME	NP	CC	T2	15.0	21940.0	98607.0
TRT*CC*TIME	NP	CC	FL	1.4	5647.9	9298.5
TRT*CC*TIME	NP	NC	T0	9.7	29171.0	122538.0
TRT*CC*TIME	NP	NC	T1	4.7	-724.5	46.5
TRT*CC*TIME	NP	NC	T2	4.8	15.1	8476.6
TRT*CC*TIME	NP	NC	FL	1.8	2670.5	681.4
TRT*CC*TIME	SUF	CC	T0	20.1	-385.1	-781.7
TRT*CC*TIME	SUF	CC	T1	8.7	1.4	12.6
TRT*CC*TIME	SUF	CC	T2	5.4	-418.4	-306.6
TRT*CC*TIME	SUF	CC	FL	1.7	349.0	581.3
TRT*CC*TIME	SUF	NC	T0	11.2	-2505.8	-12295.0
TRT*CC*TIME	SUF	NC	T1	11.4	-421.9	-488.0
TRT*CC*TIME	SUF	NC	T2	4.1	7644.2	11621.0
TRT*CC*TIME	SUF	NC	FL	1.9	-59.5	1822.6
Mean LSD				17.3	90445.5	529746.1

Table B.4 Least squares (LS) means and least significant differences (LSD) for microbial biomass ratios of carbon: nitrogen (MBC: MBN), nitrogen: phosphorus (MBN: MBP), and carbon: phosphorus (MBC: MBP) for 2025, for all main effects and interactions. Table abbreviations include, phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

Effect	P TRT	Cover	Time	MBC:MBN	MBN:MBP	MBC:MBP
TRT	BAM			6.4	17.2	39.3
TRT	NP			5.2	55.8	102.3
TRT	SUF			3.0	24.0	41.4
Mean LSD				7.2	17.5	34.5
CC		CC		4.4	32.7	52.5
CC		NC		5.3	32.0	69.5
Mean LSD				5.9	14.3	28.2
TRT*CC	BAM	CC		3.7	17.7	27.1
TRT*CC	BAM	NC		9.2	16.7	51.5
TRT*CC	NP	CC		6.9	54.3	88.8
TRT*CC	NP	NC		3.5	57.4	115.8
TRT*CC	SUF	CC		2.7	26.1	41.6
TRT*CC	SUF	NC		3.3	21.8	41.1
Mean LSD				10.1	24.7	48.8
TIME			T0	3.4	9.5	30.3
TIME			T1	3.4	16.2	47.6
TIME			T2	1.5	90.6	100.9
TIME			FL	11.3	13.1	65.1
Mean LSD				6.1	15.0	37.0
TRT*TIME	BAM		T0	4.1	4.5	21.1
TRT*TIME	BAM		T1	4.1	5.3	26.1
TRT*TIME	BAM		T2	1.5	56.0	64.4
TRT*TIME	BAM		FL	16.0	3.0	45.6
TRT*TIME	NP		T0	2.7	17.5	45.2
TRT*TIME	NP		T1	2.7	31.1	74.5
TRT*TIME	NP		T2	1.3	143.9	145.5
TRT*TIME	NP		FL	14.2	30.8	144.0
TRT*TIME	SUF		T0	3.4	6.6	24.6
TRT*TIME	SUF		T1	3.4	12.1	42.3
TRT*TIME	SUF		T2	1.6	71.9	92.8
TRT*TIME	SUF		FL	3.5	5.3	5.7
Mean LSD				10.2	25.6	64.1
CC*TIME		CC	T0	3.1	10.4	34.8
CC*TIME		CC	T1	3.1	16.9	54.7
CC*TIME		CC	T2	1.3	91.4	92.4

CC*TIME		CC	FL	10.2	12.1	28.1
CC*TIME		NC	T0	3.7	8.6	25.7
CC*TIME		NC	T1	3.7	15.4	40.6
CC*TIME		NC	T2	1.6	89.8	109.4
CC*TIME		NC	FL	12.3	14.0	102.1
Mean LSD				8.4	21.0	52.4
TRT*CC*TIME	BAM	CC	T0	3.6	6.1	24.4
TRT*CC*TIME	BAM	CC	T1	3.6	6.0	25.2
TRT*CC*TIME	BAM	CC	T2	1.3	57.1	49.3
TRT*CC*TIME	BAM	CC	FL	6.3	1.6	9.7
TRT*CC*TIME	BAM	NC	T0	4.6	2.8	17.8
TRT*CC*TIME	BAM	NC	T1	4.6	4.6	26.9
TRT*CC*TIME	BAM	NC	T2	1.7	54.9	79.6
TRT*CC*TIME	BAM	NC	FL	25.7	4.4	81.6
TRT*CC*TIME	NP	CC	T0	3.1	18.2	56.9
TRT*CC*TIME	NP	CC	T1	3.1	35.1	108.7
TRT*CC*TIME	NP	CC	T2	1.1	131.3	121.5
TRT*CC*TIME	NP	CC	FL	20.5	32.5	68.0
TRT*CC*TIME	NP	NC	T0	2.2	16.8	33.4
TRT*CC*TIME	NP	NC	T1	2.2	27.1	40.2
TRT*CC*TIME	NP	NC	T2	1.6	156.5	169.6
TRT*CC*TIME	NP	NC	FL	8.0	29.1	219.9
TRT*CC*TIME	SUF	CC	T0	2.7	6.9	23.3
TRT*CC*TIME	SUF	CC	T1	2.7	9.6	30.0
TRT*CC*TIME	SUF	CC	T2	1.4	85.7	106.5
TRT*CC*TIME	SUF	CC	FL	3.9	2.1	6.6
TRT*CC*TIME	SUF	NC	T0	4.1	6.2	26.0
TRT*CC*TIME	SUF	NC	T1	4.1	14.6	54.7
TRT*CC*TIME	SUF	NC	T2	1.7	58.0	79.2
TRT*CC*TIME	SUF	NC	FL	3.1	8.5	4.8
Mean LSD				14.3	36.2	89.9

Table B.5 Least squares (LS) means and least significant differences (LSD) for inorganic nutrient measures of dissolved total nitrogen (DTN), inorganic nitrogen (N), nitrate-N (NO_3-N), and ammonium-N (NH_4-N) for 2024, for all main effects and interactions. Table abbreviations include, phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

Effect	P TRT	Cover	Time	Dissolved Total N	Inorganic N	NO_3-N	NH_4-N
				mg N kg^{-1} soil			
TRT	BAM			25.8	22.5	3.5	4.1
TRT	NP			Non-est	11.8	2.7	1.1
TRT	SUF			25.8	18.3	3.7	2.1
Mean LSD				13.7	7.6	1.4	1.5
CC		CC		Non-est	16.1	2.5	2.2
CC		NC		26.8	18.9	4.1	2.6
Mean LSD				.	6.2	1.1	1.2
TRT*CC	BAM	CC		25.3	21.1	2.9	4.2
TRT*CC	BAM	NC		26.4	23.8	4.2	4.1
TRT*CC	NP	CC		Non-est	12.2	2.1	1.0
TRT*CC	NP	NC		22.2	11.3	3.3	1.1
TRT*CC	SUF	CC		19.7	15.0	2.5	1.5
TRT*CC	SUF	NC		31.9	21.5	4.9	2.7
Mean LSD				19.0	10.8	1.9	2.1
TIME			T0	6.4	15.6	6.1	4.7
TIME			T1	43.7	30.4	4.0	2.4
TIME			T2	34.2	13.2	1.7	1.3
TIME			FL	Non-est	10.7	1.4	1.2
Mean LSD				14.4	7.9	1.2	1.3
TRT*TIME	BAM		T0	8.4	14.9	5.5	8.9
TRT*TIME	BAM		T1	49.9	45.9	5.1	4.4
TRT*TIME	BAM		T2	28.0	14.4	1.9	1.3
TRT*TIME	BAM		FL	17.0	14.7	1.5	1.9
TRT*TIME	NP		T0	4.8	15.3	5.8	1.7
TRT*TIME	NP		T1	38.1	14.1	2.0	1.1
TRT*TIME	NP		T2	38.4	11.4	1.6	1.0
TRT*TIME	NP		FL	Non-est	6.2	1.3	0.4
TRT*TIME	SUF		T0	5.9	16.6	7.1	3.6
TRT*TIME	SUF		T1	42.9	31.3	4.8	1.8
TRT*TIME	SUF		T2	36.3	13.8	1.6	1.5
TRT*TIME	SUF		FL	18.0	11.3	1.4	1.4
Mean LSD				27.6	13.3	2.1	2.3
CC*TIME		CC	T0	3.7	14.0	3.4	4.3
CC*TIME		CC	T1	42.4	26.0	3.3	2.2
CC*TIME		CC	T2	37.3	13.9	1.7	1.4

CC*TIME		CC	FL	Non-est	10.6	1.5	1.1
CC*TIME		NC	T0	9.0	17.2	8.8	5.2
CC*TIME		NC	T1	44.9	34.9	4.7	2.7
CC*TIME		NC	T2	31.2	12.5	1.6	1.2
CC*TIME		NC	FL	22.2	10.9	1.3	1.4
Mean LSD				21.3	10.9	1.7	1.9
TRT*CC*TIME	BAM	CC	T0	7.0	15.2	3.7	9.5
TRT*CC*TIME	BAM	CC	T1	38.9	40.5	4.4	4.0
TRT*CC*TIME	BAM	CC	T2	35.2	13.4	1.7	1.3
TRT*CC*TIME	BAM	CC	FL	20.0	15.4	1.6	1.9
TRT*CC*TIME	BAM	NC	T0	9.9	14.6	7.4	8.4
TRT*CC*TIME	BAM	NC	T1	60.9	51.3	5.9	4.8
TRT*CC*TIME	BAM	NC	T2	20.8	15.4	2.0	1.4
TRT*CC*TIME	BAM	NC	FL	13.9	14.0	1.4	1.9
TRT*CC*TIME	NP	CC	T0	1.1	13.4	3.1	1.3
TRT*CC*TIME	NP	CC	T1	52.3	13.9	1.8	1.1
TRT*CC*TIME	NP	CC	T2	55.5	14.9	1.9	1.4
TRT*CC*TIME	NP	CC	FL	20.8	6.7	1.4	0.4
TRT*CC*TIME	NP	NC	T0	8.5	17.3	8.4	2.2
TRT*CC*TIME	NP	NC	T1	24.0	14.4	2.1	1.1
TRT*CC*TIME	NP	NC	T2	21.3	8.0	1.4	0.6
TRT*CC*TIME	NP	NC	FL	35.0	5.7	1.1	0.5
TRT*CC*TIME	SUF	CC	T0	3.2	13.4	3.5	2.0
TRT*CC*TIME	SUF	CC	T1	36.0	23.6	3.6	1.5
TRT*CC*TIME	SUF	CC	T2	21.1	13.5	1.6	1.4
TRT*CC*TIME	SUF	CC	FL	18.4	9.6	1.4	1.0
TRT*CC*TIME	SUF	NC	T0	8.7	19.8	10.7	5.1
TRT*CC*TIME	SUF	NC	T1	49.9	39.1	6.0	2.2
TRT*CC*TIME	SUF	NC	T2	51.5	14.1	1.5	1.6
TRT*CC*TIME	SUF	NC	FL	17.6	13.0	1.3	1.8
Mean LSD				39.0	18.6	3.0	3.2

Table B.6 Least squares (LS) means and least significant differences (LSD) for inorganic nutrient measures of dissolved total nitrogen (DTN), inorganic nitrogen (N), nitrate-N (NO_3-N), and ammonium-N (NH_4-N) for 2025, for all main effects and interactions. Table abbreviations include, phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

Effect	P TRT	Cover	Time	Dissolved Total N	Inorganic N	NO3-N	NH4-N
				mg N kg^{-1} soil			
TRT	BAM			65.5	52.5	6.5	4.2
TRT	NP			78.6	66.7	6.7	6.8
TRT	SUF			68.6	57.3	6.1	5.5
Mean LSD				26.8	23.7	2.4	3.5
CC		CC		79.8	67.3	7.2	6.4
CC		NC		62.0	50.4	5.6	4.6
Mean LSD				21.9	19.3	2.0	2.8
TRT*CC	BAM	CC		86.5	71.9	8.8	5.8
TRT*CC	BAM	NC		44.4	33.1	4.2	2.6
TRT*CC	NP	CC		85.4	73.7	7.1	7.7
TRT*CC	NP	NC		71.8	59.8	6.3	5.9
TRT*CC	SUF	CC		67.5	56.2	5.6	5.8
TRT*CC	SUF	NC		69.7	58.4	6.5	5.3
Mean LSD				37.9	33.5	3.4	4.9
TIME			T0	17.2	5.9	0.7	0.3
TIME			T1	130.9	115.5	9.0	14.4
TIME			T2	120.7	102.7	13.5	7.2
TIME			FL	14.9	11.4	2.4	0.0
Mean LSD				27.2	25.8	2.7	3.3
TRT*TIME	BAM		T0	16.6	3.1	0.3	0.4
TRT*TIME	BAM		T1	90.5	74.5	7.3	8.0
TRT*TIME	BAM		T2	121.9	104.3	12.7	8.3
TRT*TIME	BAM		FL	32.8	28.2	5.7	0.0
TRT*TIME	NP		T0	20.8	10.3	1.4	0.3
TRT*TIME	NP		T1	167.5	150.6	10.4	20.1
TRT*TIME	NP		T2	121.9	105.0	14.6	6.8
TRT*TIME	NP		FL	4.2	1.1	12.7	0.0
TRT*TIME	SUF		T0	14.1	4.2	0.5	0.3
TRT*TIME	SUF		T1	134.6	121.4	9.5	15.2
TRT*TIME	SUF		T2	118.2	98.7	13.2	6.7
TRT*TIME	SUF		FL	7.6	5.0	1.1	0.1
Mean LSD				47.6	44.4	4.6	5.9
CC*TIME		CC	T0	15.2	4.8	0.6	0.3
CC*TIME		CC	T1	140.7	127.8	9.2	16.6
CC*TIME		CC	T2	140.3	117.8	15.1	8.6

CC*TIME		CC	FL	23.1	18.7	3.9	0.0
CC*TIME		NC	T0	19.1	6.9	0.9	0.3
CC*TIME		NC	T1	121.1	103.2	8.9	12.1
CC*TIME		NC	T2	101.0	87.6	11.9	5.9
CC*TIME		NC	FL	6.7	4.1	0.9	0.0
Mean LSD				38.8	36.3	3.8	4.8
TRT*CC*TIME	BAM	CC	T0	12.0	2.2	0.1	0.5
TRT*CC*TIME	BAM	CC	T1	104.9	91.9	8.5	10.2
TRT*CC*TIME	BAM	CC	T2	169.1	140.5	16.0	12.4
TRT*CC*TIME	BAM	CC	FL	59.9	53.1	10.7	0.1
TRT*CC*TIME	BAM	NC	T0	21.2	4.0	0.4	0.4
TRT*CC*TIME	BAM	NC	T1	76.1	57.0	6.0	5.7
TRT*CC*TIME	BAM	NC	T2	74.6	68.1	9.5	4.2
TRT*CC*TIME	BAM	NC	FL	5.8	3.4	0.8	0.0
TRT*CC*TIME	NP	CC	T0	23.2	11.7	1.5	0.3
TRT*CC*TIME	NP	CC	T1	193.4	178.4	11.1	24.9
TRT*CC*TIME	NP	CC	T2	120.4	102.9	15.3	5.5
TRT*CC*TIME	NP	CC	FL	4.7	1.7	0.5	0.0
TRT*CC*TIME	NP	NC	T0	18.4	8.8	1.2	0.2
TRT*CC*TIME	NP	NC	T1	141.6	122.9	9.7	15.2
TRT*CC*TIME	NP	NC	T2	123.5	107.1	13.9	8.0
TRT*CC*TIME	NP	NC	FL	3.7	0.5	0.2	0.0
TRT*CC*TIME	SUF	CC	T0	10.4	0.6	0.1	0.2
TRT*CC*TIME	SUF	CC	T1	123.6	113.0	8.1	14.8
TRT*CC*TIME	SUF	CC	T2	131.6	109.8	14.0	8.0
TRT*CC*TIME	SUF	CC	FL	4.6	1.4	0.4	0.0
TRT*CC*TIME	SUF	NC	T0	17.8	7.8	0.9	0.3
TRT*CC*TIME	SUF	NC	T1	145.5	129.8	10.9	15.5
TRT*CC*TIME	SUF	NC	T2	104.9	87.6	12.3	5.4
TRT*CC*TIME	SUF	NC	FL	10.6	8.5	1.8	0.1
Mean LSD				67.4	62.8	6.5	8.4

Table B.7 Least squares (LS) means and least significant differences (LSD) for organic nutrient measures of dissolved organic carbon (DOC), autoclavable citrate extractable protein (ACE), and permanganate oxidizable carbon (POXC) for 2024, for all main effects and interactions. Table abbreviations include, phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

Effect	P TRT	Cover	Time	Dissolved Organic C	ACE	POXC
				mg C kg^{-1} soil	g protein kg^{-1} soil	mg C kg^{-1} soil
TRT	BAM			53.3	4.1	451.1
TRT	NP			71.7	3.7	427.3
TRT	SUF			83.7	4.3	469.5
Mean LSD				57.6	0.4	27.7
CC		CC		64.6	4.3	486.2
CC		NC		74.5	3.8	412.4
Mean LSD				47.0	0.4	22.6
TRT*CC	BAM	CC		53.8	4.4	472.3
TRT*CC	BAM	NC		52.8	3.9	429.9
TRT*CC	NP	CC		89.3	3.8	465.5
TRT*CC	NP	NC		54.0	3.7	389.1
TRT*CC	SUF	CC		50.7	4.6	520.7
TRT*CC	SUF	NC		116.8	3.9	418.3
Mean LSD				81.5	0.6	39.1
TIME			T0	44.5	4.3	441.8
TIME			T1	107.8	3.8	410.3
TIME			T2	79.9	3.8	391.1
TIME			FL	46.0	4.3	554.0
Mean LSD				49.6	0.3	31.2
TRT*TIME	BAM		T0	44.8	4.4	454.2
TRT*TIME	BAM		T1	52.8	4.0	405.2
TRT*TIME	BAM		T2	67.2	3.8	407.4
TRT*TIME	BAM		FL	48.2	4.3	537.6
TRT*TIME	NP		T0	45.6	3.9	423.3
TRT*TIME	NP		T1	106.1	3.5	377.3
TRT*TIME	NP		T2	91.5	3.5	359.4
TRT*TIME	NP		FL	43.6	4.1	549.3
TRT*TIME	SUF		T0	43.1	4.5	447.8
TRT*TIME	SUF		T1	164.6	4.0	448.5
TRT*TIME	SUF		T2	81.1	4.1	406.6
TRT*TIME	SUF		FL	46.2	4.6	575.1
Mean LSD				83.7	0.6	54.3
CC*TIME		CC	T0	43.8	4.5	479.8
CC*TIME		CC	T1	83.3	4.0	452.3
CC*TIME		CC	T2	85.4	4.0	418.0

CC*TIME		CC	FL	45.8	4.6	594.7
CC*TIME		NC	T0	45.2	4.0	403.8
CC*TIME		NC	T1	132.4	3.7	368.3
CC*TIME		NC	T2	74.4	3.6	364.2
CC*TIME		NC	FL	46.2	4.1	513.3
Mean LSD				68.7	0.5	44.3
TRT*CC*TIME	BAM	CC	T0	44.9	4.7	485.1
TRT*CC*TIME	BAM	CC	T1	41.3	4.1	440.9
TRT*CC*TIME	BAM	CC	T2	78.8	4.1	422.0
TRT*CC*TIME	BAM	CC	FL	50.0	4.5	541.3
TRT*CC*TIME	BAM	NC	T0	44.6	4.1	423.3
TRT*CC*TIME	BAM	NC	T1	64.4	3.8	369.5
TRT*CC*TIME	BAM	NC	T2	55.6	3.5	392.8
TRT*CC*TIME	BAM	NC	FL	46.5	4.1	533.9
TRT*CC*TIME	NP	CC	T0	42.6	4.0	469.7
TRT*CC*TIME	NP	CC	T1	151.4	3.5	408.7
TRT*CC*TIME	NP	CC	T2	121.3	3.5	357.3
TRT*CC*TIME	NP	CC	FL	42.0	4.3	626.4
TRT*CC*TIME	NP	NC	T0	48.6	3.8	377.0
TRT*CC*TIME	NP	NC	T1	60.8	3.5	345.8
TRT*CC*TIME	NP	NC	T2	61.6	3.5	361.4
TRT*CC*TIME	NP	NC	FL	45.2	3.9	472.2
TRT*CC*TIME	SUF	CC	T0	43.9	4.8	484.5
TRT*CC*TIME	SUF	CC	T1	57.1	4.4	507.3
TRT*CC*TIME	SUF	CC	T2	56.2	4.4	474.6
TRT*CC*TIME	SUF	CC	FL	45.5	5.0	616.3
TRT*CC*TIME	SUF	NC	T0	42.3	4.1	411.1
TRT*CC*TIME	SUF	NC	T1	272.0	3.7	389.6
TRT*CC*TIME	SUF	NC	T2	106.0	3.8	338.5
TRT*CC*TIME	SUF	NC	FL	46.9	4.2	534.0
Mean LSD				117.8	0.9	76.9

Table B.8 Least squares (LS) means and least significant differences (LSD) for organic nutrient measures of dissolved organic nitrogen (DON), dissolved organic carbon (DOC), autoclavable citrate extractable protein (ACE), and permanganate oxidizable carbon (POXC) for 2025, for all main effects and interactions. Table abbreviations include, phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

Effect	P TRT	Cover	Time	Dissolved Organic N	Dissolved Organic C	ACE	POXC
				mg N kg^{-1} soil	mg C kg^{-1} soil	g protein kg^{-1} soil	mg C kg^{-1} soil
TRT	BAM			12.9	229.8	5.7	470.5
TRT	NP			11.9	365.0	5.2	457.2
TRT	SUF			11.3	184.8	5.9	489.6
Mean LSD				3.8	192.7	2.7	31.7
CC		CC		12.5	256.4	5.7	475.8
CC		NC		11.5	263.4	5.5	469.0
Mean LSD				3.1	157.4	2.2	25.8
TRT*CC	BAM	CC		14.6	306.2	6.0	482.4
TRT*CC	BAM	NC		11.3	153.5	5.3	458.5
TRT*CC	NP	CC		11.7	313.8	5.1	462.9
TRT*CC	NP	NC		12.0	416.1	5.3	451.5
TRT*CC	SUF	CC		11.3	149.1	6.0	482.2
TRT*CC	SUF	NC		11.3	220.6	5.8	497.1
Mean LSD				5.4	272.6	3.8	44.8
TIME			T0	11.3	265.9	5.8	529.6
TIME			T1	15.4	333.3	4.3	494.2
TIME			T2	18.0	269.1	6.3	494.8
TIME			FL	3.5	171.2	5.9	371.2
Mean LSD				4.4	251.5	0.9	34.6
TRT*TIME	BAM		T0	13.5	286.9	6.0	552.0
TRT*TIME	BAM		T1	16.1	244.0	4.4	506.2
TRT*TIME	BAM		T2	17.6	277.2	6.2	483.6
TRT*TIME	BAM		FL	4.6	111.3	6.0	340.0
TRT*TIME	NP		T0	10.5	291.5	5.5	512.5
TRT*TIME	NP		T1	16.9	685.6	4.1	490.1
TRT*TIME	NP		T2	16.9	175.5	6.0	483.5
TRT*TIME	NP		FL	3.1	307.2	5.5	342.6
TRT*TIME	SUF		T0	9.9	219.3	5.8	524.1
TRT*TIME	SUF		T1	13.2	70.3	4.5	486.2
TRT*TIME	SUF		T2	19.5	354.5	6.8	517.1
TRT*TIME	SUF		FL	2.7	95.2	6.3	431.1
Mean LSD				7.6	425.7	2.6	60.4

CC*TIME		CC	T0	10.4	232.4	5.9	537.3
CC*TIME		CC	T1	12.9	336.3	4.5	505.0
CC*TIME		CC	T2	22.6	225.4	6.4	496.4
CC*TIME		CC	FL	4.3	231.3	6.0	364.7
CC*TIME		NC	T0	12.3	299.4	5.6	521.9
CC*TIME		NC	T1	17.9	330.3	4.2	483.4
CC*TIME		NC	T2	13.4	312.7	6.2	493.1
CC*TIME		NC	FL	2.6	111.2	5.9	377.8
Mean LSD				6.2	349.4	1.9	49.3
TRT*CC*TIME	BAM	CC	T0	9.8	297.0	6.3	551.9
TRT*CC*TIME	BAM	CC	T1	13.0	360.8	4.7	507.8
TRT*CC*TIME	BAM	CC	T2	28.6	483.8	6.9	518.5
TRT*CC*TIME	BAM	CC	FL	6.8	83.1	6.2	351.5
TRT*CC*TIME	BAM	NC	T0	17.2	276.7	5.7	552.1
TRT*CC*TIME	BAM	NC	T1	19.1	127.2	4.2	504.7
TRT*CC*TIME	BAM	NC	T2	6.5	70.6	5.6	448.8
TRT*CC*TIME	BAM	NC	FL	2.3	139.4	5.8	328.5
TRT*CC*TIME	NP	CC	T0	11.5	81.4	5.4	542.7
TRT*CC*TIME	NP	CC	T1	15.1	581.7	4.0	499.0
TRT*CC*TIME	NP	CC	T2	17.4	127.1	5.7	468.6
TRT*CC*TIME	NP	CC	FL	2.9	464.9	5.5	341.2
TRT*CC*TIME	NP	NC	T0	9.6	501.7	5.5	482.4
TRT*CC*TIME	NP	NC	T1	18.7	789.4	4.2	481.3
TRT*CC*TIME	NP	NC	T2	16.4	223.8	6.2	498.5
TRT*CC*TIME	NP	NC	FL	3.3	149.6	5.4	344.0
TRT*CC*TIME	SUF	CC	T0	9.8	318.7	6.1	517.1
TRT*CC*TIME	SUF	CC	T1	10.6	66.4	4.8	508.3
TRT*CC*TIME	SUF	CC	T2	21.7	65.3	6.7	502.2
TRT*CC*TIME	SUF	CC	FL	3.2	145.9	6.3	401.3
TRT*CC*TIME	SUF	NC	T0	10.0	119.9	5.5	531.2
TRT*CC*TIME	SUF	NC	T1	15.8	74.2	4.2	464.1
TRT*CC*TIME	SUF	NC	T2	17.3	643.7	7.0	532.0
TRT*CC*TIME	SUF	NC	FL	2.1	44.6	6.4	460.9
Mean LSD				10.7	599.3	3.9	85.6

Table B.9 Least squares (LS) means and least significant differences (LSD) for biological activity measures of β-glucosidase (GLU), β--glucosaminidase (NAG), acid phosphatase (ACID P), alkaline phosphatase (ALK P), and soil respiration for 2024, for all main effects and interactions. Table abbreviations include, phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

Effect	P TRT	Cover	Time	GLU	NAG	ACID P	ALK P	Soil Respiration
				mg p-nitrophenol g^{-1} soil hr^{-1}				mg CO_2 g^{-1} soil
TRT	BAM			92.4	32.6	237.6	69.8	13.0
TRT	NP			92.2	35.6	240.8	77.5	13.0
TRT	SUF			96.3	34.7	253.2	73.5	13.2
Mean LSD				9.1	3.5	32.0	7.7	0.8
CC		CC		101.7	39.4	273.3	81.7	14.8
CC		NC		85.6	29.2	214.4	65.5	11.3
Mean LSD				7.5	2.9	26.1	6.3	0.6
TRT*CC	BAM	CC		91.6	34.9	253.2	70.2	14.3
TRT*CC	BAM	NC		93.2	30.2	221.9	69.5	11.8
TRT*CC	NP	CC		106.0	42.3	280.4	92.3	14.4
TRT*CC	NP	NC		78.5	28.9	201.2	62.6	11.5
TRT*CC	SUF	CC		107.5	40.9	286.4	82.6	15.8
TRT*CC	SUF	NC		85.1	28.5	220.1	64.5	10.6
Mean LSD				12.9	5.0	45.3	10.9	1.1
TIME			T0	104.3	35.4	212.5	63.1	11.0
TIME			T1	90.4	33.3	241.0	68.2	12.5
TIME			T2	74.0	33.0	287.9	72.6	14.1
TIME			FL	105.8	35.4	234.0	90.5	14.7
Mean LSD				7.3	3.0	20.8	7.7	1.6
TRT*TIME	BAM		T0	107.6	34.8	207.5	66.0	11.3
TRT*TIME	BAM		T1	87.9	31.9	230.6	65.6	12.4
TRT*TIME	BAM		T2	73.7	30.9	280.9	62.8	14.4
TRT*TIME	BAM		FL	100.2	32.7	231.2	84.8	14.1
TRT*TIME	NP		T0	99.0	35.1	209.8	63.2	11.8
TRT*TIME	NP		T1	89.7	36.1	245.8	72.3	11.4
TRT*TIME	NP		T2	73.3	33.6	276.6	78.7	13.7
TRT*TIME	NP		FL	106.9	37.5	231.0	95.8	15.1
TRT*TIME	SUF		T0	106.5	36.4	220.3	60.1	9.8
TRT*TIME	SUF		T1	93.4	31.8	246.6	66.7	13.8
TRT*TIME	SUF		T2	75.1	34.4	306.2	76.2	14.3
TRT*TIME	SUF		FL	110.3	36.1	239.8	91.0	14.8
Mean LSD				13.6	5.5	41.5	13.6	2.6
CC*TIME		CC	T0	114.3	41.7	243.7	74.3	13.0
CC*TIME		CC	T1	100.0	39.5	271.3	77.0	13.7
CC*TIME		CC	T2	80.5	36.9	313.6	77.3	16.9

CC*TIME		CC	FL	112.0	39.3	264.8	98.1	15.8
CC*TIME		NC	T0	94.4	29.2	181.4	51.9	9.0
CC*TIME		NC	T1	80.8	27.0	210.7	59.4	11.4
CC*TIME		NC	T2	67.5	29.1	262.1	67.8	11.3
CC*TIME		NC	FL	99.6	31.5	203.3	82.9	13.6
Mean LSD				10.9	4.5	32.9	11.0	2.1
TRT*CC*TIME	BAM	CC	T0	107.1	37.0	229.0	69.1	11.8
TRT*CC*TIME	BAM	CC	T1	88.5	35.7	242.9	69.7	14.0
TRT*CC*TIME	BAM	CC	T2	76.1	33.4	299.2	62.5	17.1
TRT*CC*TIME	BAM	CC	FL	94.5	33.5	241.9	79.3	14.4
TRT*CC*TIME	BAM	NC	T0	108.1	32.5	186.1	63.0	10.8
TRT*CC*TIME	BAM	NC	T1	87.4	28.1	218.3	61.5	10.9
TRT*CC*TIME	BAM	NC	T2	71.3	28.5	262.5	63.2	11.7
TRT*CC*TIME	BAM	NC	FL	105.9	31.9	220.6	90.3	13.8
TRT*CC*TIME	NP	CC	T0	122.2	42.7	247.0	83.9	13.1
TRT*CC*TIME	NP	CC	T1	103.2	43.6	286.9	83.0	11.4
TRT*CC*TIME	NP	CC	T2	82.9	39.0	311.6	90.6	16.0
TRT*CC*TIME	NP	CC	FL	115.7	43.8	276.3	111.9	17.2
TRT*CC*TIME	NP	NC	T0	75.8	27.6	172.6	42.6	10.6
TRT*CC*TIME	NP	NC	T1	76.3	28.6	204.7	61.6	11.3
TRT*CC*TIME	NP	NC	T2	63.7	28.3	241.5	66.7	11.3
TRT*CC*TIME	NP	NC	FL	98.2	31.1	185.8	79.6	13.0
TRT*CC*TIME	SUF	CC	T0	113.5	45.3	255.0	70.0	14.1
TRT*CC*TIME	SUF	CC	T1	108.1	39.3	284.2	78.2	15.7
TRT*CC*TIME	SUF	CC	T2	82.6	38.3	330.1	78.8	17.6
TRT*CC*TIME	SUF	CC	FL	125.8	40.7	276.1	103.2	15.7
TRT*CC*TIME	SUF	NC	T0	99.4	27.5	185.5	50.1	5.6
TRT*CC*TIME	SUF	NC	T1	78.7	24.3	209.1	55.1	11.9
TRT*CC*TIME	SUF	NC	T2	67.6	30.5	282.3	73.6	11.0
TRT*CC*TIME	SUF	NC	FL	94.8	31.6	203.5	78.9	13.9
Mean LSD				19.5	7.9	60.2	19.2	3.6

Table B.10 Least squares (LS) means and least significant differences (LSD) for biological activity measures of β-glucosidase (GLU), β--glucosaminidase (NAG), acid phosphatase (ACID P), alkaline phosphatase (ALK P), and soil respiration for 2025, for all main effects and interactions. Table abbreviations include, phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

Effect	P TRT	Cover	Time	GLU	NAG	ACID P	ALK P	Soil Respiration
				mg p-nitrophenol g^{-1} soil hr^{-1}				mg CO_2 g^{-1} soil
TRT	BAM			31.7	6.0	111.7	39.7	28.3
TRT	NP			30.3	6.7	102.1	43.3	30.4
TRT	SUF			33.9	6.1	105.8	37.7	31.2
Mean LSD				3.9	1.5	23.9	6.1	5.1
CC		CC		32.9	6.5	112.0	41.6	29.3
CC		NC		31.1	6.0	101.0	38.8	30.7
Mean LSD				3.2	1.2	19.5	5.0	4.2
TRT*CC	BAM	CC		34.1	6.3	113.9	40.3	26.4
TRT*CC	BAM	NC		29.3	5.7	109.5	39.1	30.2
TRT*CC	NP	CC		31.7	6.9	110.7	46.6	32.2
TRT*CC	NP	NC		29.0	6.4	93.4	40.0	28.7
TRT*CC	SUF	CC		32.8	6.3	111.3	38.0	29.2
TRT*CC	SUF	NC		35.0	5.8	100.2	37.5	33.2
Mean LSD				5.5	2.1	33.7	8.6	7.2
TIME			T0	3.5	2.0	18.3	12.3	16.8
TIME			T1	6.4	3.3	23.4	23.4	25.5
TIME			T2	8.2	2.0	31.8	29.6	31.7
TIME			FL	109.9	17.6	352.4	95.6	46.0
Mean LSD				4.0	1.3	22.3	5.5	4.3
TRT*TIME	BAM		T0	3.0	1.2	11.7	9.6	12.0
TRT*TIME	BAM		T1	7.1	4.2	23.3	23.3	25.3
TRT*TIME	BAM		T2	7.1	1.7	31.6	30.8	31.1
TRT*TIME	BAM		FL	109.7	17.0	380.1	95.1	44.9
TRT*TIME	NP		T0	5.4	2.9	23.3	16.4	18.5
TRT*TIME	NP		T1	7.6	2.6	23.6	23.6	26.3
TRT*TIME	NP		T2	9.7	2.4	32.9	31.1	32.0
TRT*TIME	NP		FL	98.6	18.8	328.4	102.0	44.9
TRT*TIME	SUF		T0	2.1	1.9	19.9	10.9	19.9
TRT*TIME	SUF		T1	4.5	3.3	23.4	23.4	24.9
TRT*TIME	SUF		T2	7.8	2.0	30.9	26.9	32.1
TRT*TIME	SUF		FL	121.3	17.1	348.8	89.8	48.1
Mean LSD				6.7	2.4	37.2	10.0	7.8
CC*TIME		CC	T0	3.1	1.8	18.7	11.7	13.6
CC*TIME		CC	T1	6.0	3.3	24.2	24.2	25.3
CC*TIME		CC	T2	8.0	2.1	32.7	28.5	31.7

CC*TIME		CC	FL	114.3	18.7	372.4	102.0	46.6
CC*TIME		NC	T0	3.8	2.1	18.0	12.9	20.1
CC*TIME		NC	T1	6.7	3.3	22.6	22.6	25.7
CC*TIME		NC	T2	8.4	1.9	31.0	30.7	31.8
CC*TIME		NC	FL	105.4	16.6	332.4	89.2	45.4
Mean LSD				5.5	1.9	30.6	8.1	6.3
TRT*CC*TIME	BAM	CC	T0	2.3	1.0	13.0	8.4	7.5
TRT*CC*TIME	BAM	CC	T1	7.4	4.1	21.5	21.5	23.7
TRT*CC*TIME	BAM	CC	T2	7.0	2.2	30.3	24.2	29.3
TRT*CC*TIME	BAM	CC	FL	119.9	18.0	390.9	107.0	45.1
TRT*CC*TIME	BAM	NC	T0	3.7	1.3	10.4	10.9	16.5
TRT*CC*TIME	BAM	NC	T1	6.7	4.2	25.2	25.2	26.8
TRT*CC*TIME	BAM	NC	T2	7.2	1.2	32.9	37.3	32.9
TRT*CC*TIME	BAM	NC	FL	99.5	16.1	369.3	83.1	44.6
TRT*CC*TIME	NP	CC	T0	5.5	3.1	20.6	16.3	21.6
TRT*CC*TIME	NP	CC	T1	6.4	2.5	27.7	27.7	29.7
TRT*CC*TIME	NP	CC	T2	10.4	2.6	35.7	32.8	32.3
TRT*CC*TIME	NP	CC	FL	104.3	19.4	358.9	109.4	45.0
TRT*CC*TIME	NP	NC	T0	5.2	2.7	26.0	16.5	15.4
TRT*CC*TIME	NP	NC	T1	8.7	2.6	19.5	19.5	22.9
TRT*CC*TIME	NP	NC	T2	9.0	2.2	30.2	29.4	31.7
TRT*CC*TIME	NP	NC	FL	93.0	18.2	297.8	94.6	44.9
TRT*CC*TIME	SUF	CC	T0	1.6	1.4	22.4	10.4	11.6
TRT*CC*TIME	SUF	CC	T1	4.1	3.5	23.5	23.5	22.4
TRT*CC*TIME	SUF	CC	T2	6.6	1.7	32.0	28.4	33.4
TRT*CC*TIME	SUF	CC	FL	118.9	18.7	367.5	89.5	49.6
TRT*CC*TIME	SUF	NC	T0	2.6	2.4	17.5	11.3	28.3
TRT*CC*TIME	SUF	NC	T1	4.8	3.1	23.2	23.2	27.4
TRT*CC*TIME	SUF	NC	T2	8.9	2.3	29.9	25.3	30.8
TRT*CC*TIME	SUF	NC	FL	123.7	15.5	330.1	90.0	46.6
Mean LSD				9.5	3.4	52.3	14.2	11.1

Appendix C - PLFA Supplemental Material

Table C.1 Least squares (LS) means and least significant differences (LSD) for total microbial biomass for 2018, 2020, 2022, and 2024, for all main effects and interactions. Table abbreviations include, phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), drawdown or sufficiency (DD/SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

					Total Microbial Biomass	
					pmol C g soil^{-1}	
Effect	**P TRT**	**P TRT**	**COVER**	**YR**	**Spring**	**Fall**
TRT	NP	NP			68982	87073
TRT	DD/SUF	DD/SUF			67088	90931
TRT	BAM	BAM			69620	89274
Mean LSD					11905.91	5379.22
CC			CC		75226	98317
CC			NC		61900	79868
Mean LSD					9721.13	4392.11
TRT*CC	NP	NP	CC		78338	97344
TRT*CC	DD/SUF	SUF	CC		72429	100952
TRT*CC	BAM	BAM	CC		74912	96654
TRT*CC	NP	NP	NC		59626	76802
TRT*CC	DD/SUF	SUF	NC		61746	80909
TRT*CC	BAM	BAM	NC		64328	81893
Mean LSD					16837.5	7607.36
YR				2018	72335	82989
YR				2020	47796	65329
YR				2022	71403	105881
YR				2024	82719	102171
Mean LSD					6987.92	4903.14
TRT*YR	NP	NP		2018	69692	81692
TRT*YR	NP	NP		2020	45644	61518
TRT*YR	NP	NP		2022	71179	103704
TRT*YR	NP	NP		2024	89412	101380
TRT*YR	DD/SUF	SUF		2018	68415	82697
TRT*YR	DD/SUF	SUF		2020	49090	69206
TRT*YR	DD/SUF	SUF		2022	73032	108779
TRT*YR	DD/SUF	SUF		2024	77813	103040
TRT*YR	BAM	BAM		2018	78898	84578
TRT*YR	BAM	BAM		2020	48653	65262
TRT*YR	BAM	BAM		2022	69996	105162
TRT*YR	BAM	BAM		2024	80933	102093
Mean LSD					14482.46	8793.41
CC*YR			CC	2018	78944	88476

CC*YR			CC	2020	53000	71879
CC*YR			CC	2022	76534	115646
CC*YR			CC	2024	92427	117266
CC*YR			NC	2018	65726	77502
CC*YR			NC	2020	42592	58778
CC*YR			NC	2022	66271	96117
CC*YR			NC	2024	73011	87076
Mean LSD					11408.63	7127.14
TRT*CC*YR	NP	NP	CC	2018	78739	87117
TRT*CC*YR	NP	NP	CC	2020	49264	65063
TRT*CC*YR	NP	NP	CC	2022	79401	112888
TRT*CC*YR	NP	NP	CC	2024	105946	124309
TRT*CC*YR	SUF	SUF	CC	2018	73035	91521
TRT*CC*YR	DD/SUF	SUF	CC	2020	56973	80638
TRT*CC*YR	DD/SUF	SUF	CC	2022	77549	116101
TRT*CC*YR	DD/SUF	SUF	CC	2024	82160	115548
TRT*CC*YR	BAM	BAM	CC	2018	85056	86788
TRT*CC*YR	BAM	BAM	CC	2020	52762	69937
TRT*CC*YR	BAM	BAM	CC	2022	72652	117949
TRT*CC*YR	BAM	BAM	CC	2024	89176	111942
TRT*CC*YR	NP	NP	NC	2018	60644	76266
TRT*CC*YR	NP	NP	NC	2020	42025	57974
TRT*CC*YR	NP	NP	NC	2022	62957	94519
TRT*CC*YR	NP	NP	NC	2024	72878	78451
TRT*CC*YR	DD/SUF	SUF	NC	2018	63795	73873
TRT*CC*YR	DD/SUF	SUF	NC	2020	41207	57774
TRT*CC*YR	DD/SUF	SUF	NC	2022	68515	101456
TRT*CC*YR	DD/SUF	SUF	NC	2024	73465	90533
TRT*CC*YR	BAM	BAM	NC	2018	72740	82367
TRT*CC*YR	BAM	BAM	NC	2020	44544	60587
TRT*CC*YR	BAM	BAM	NC	2022	67340	92375
TRT*CC*YR	BAM	BAM	NC	2024	72690	92243
Mean LSD					21139.55	12831.79

Table C.2 Least squares (LS) means and least significant differences (LSD) for arbuscular mycorrhizal fungi (AMF) abundance for 2018, 2020, 2022, and 2024, for all main effects and interactions. Table abbreviations include phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), drawdown or sufficiency (DD/SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

					AMF Abundance	
					pmol C g soil^{-1}	
Effect	**P TRT**	**P TRT**	**COVER**	**YR**	**Spring**	**Fall**
TRT	NP	NP			3219.83	3628.55
TRT	DD/SUF	SUF			3509.95	3751.97
TRT	BAM	BAM			3577.54	3588.5
Mean LSD					337.83	241.56
CC			CC		3945.43	4045.64
CC			NC		2926.12	3267.04
Mean LSD					275.84	197.23
TRT*CC	NP	NP	CC		3715.29	4079.6
TRT*CC	DD/SUF	SUF	CC		4064.06	4136.74
TRT*CC	BAM	BAM	CC		4056.95	3920.57
TRT*CC	NP	NP	NC		2724.37	3177.5
TRT*CC	DD/SUF	SUF	NC		2955.84	3367.19
TRT*CC	BAM	BAM	NC		3098.14	3256.43
Mean LSD					456	341.62
YR				2018	3317.14	3445.57
YR				2020	2572.02	3396.33
YR				2022	3621.5	3639.67
YR				2024	4232.44	4143.78
Mean LSD					340.48	247.29
TRT*YR	NP	NP		2018	3239.46	3473.7
TRT*YR	NP	NP		2020	2454.19	3221.33
TRT*YR	NP	NP		2022	3472.67	3662.17
TRT*YR	NP	NP		2024	3713	4157
TRT*YR	DD/SUF	SUF		2018	3526.38	3372.71
TRT*YR	DD/SUF	SUF		2020	2634.93	3610.33
TRT*YR	DD/SUF	SUF		2022	3747.5	3785.67
TRT*YR	DD/SUF	SUF		2024	4131	4239.17
TRT*YR	BAM	BAM		2018	3185.57	3490.32
TRT*YR	BAM	BAM		2020	2626.93	3357.33
TRT*YR	BAM	BAM		2022	3644.33	3471.17
TRT*YR	BAM	BAM		2024	4853.33	4035.17
Mean LSD					595.08	431.57
CC*YR			CC	2018	3963.53	3705.43
CC*YR			CC	2020	2887.76	3751.78
CC*YR			CC	2022	4075.67	3907.56

CC*YR			CC	2024	4854.78	4817.78
CC*YR			NC	2018	2670.74	3185.72
CC*YR			NC	2020	2256.28	3040.89
CC*YR			NC	2022	3167.33	3371.78
CC*YR			NC	2024	3610.11	3469.78
Mean LSD					484.94	351.8
TRT*CC*YR	NP	NP	CC	2018	3910.15	3839.73
TRT*CC*YR	NP	NP	CC	2020	2652.65	3423.67
TRT*CC*YR	NP	NP	CC	2022	3940.67	3857.33
TRT*CC*YR	NP	NP	CC	2024	4357.67	5197.67
TRT*CC*YR	SUF	SUF	CC	2018	4278.36	3687.64
TRT*CC*YR	DD/SUF	SUF	CC	2020	3046.56	4232
TRT*CC*YR	DD/SUF	SUF	CC	2022	4142.67	3962.33
TRT*CC*YR	DD/SUF	SUF	CC	2024	4788.67	4665
TRT*CC*YR	BAM	BAM	CC	2018	3702.07	3588.93
TRT*CC*YR	BAM	BAM	CC	2020	2964.06	3599.67
TRT*CC*YR	BAM	BAM	CC	2022	4143.67	3903
TRT*CC*YR	BAM	BAM	CC	2024	5418	4590.67
TRT*CC*YR	NP	NP	NC	2018	2568.76	3107.66
TRT*CC*YR	NP	NP	NC	2020	2255.74	3019
TRT*CC*YR	NP	NP	NC	2022	3004.67	3467
TRT*CC*YR	NP	NP	NC	2024	3068.33	3116.33
TRT*CC*YR	DD/SUF	SUF	NC	2018	2774.39	3057.78
TRT*CC*YR	DD/SUF	SUF	NC	2020	2223.29	2988.67
TRT*CC*YR	DD/SUF	SUF	NC	2022	3352.33	3609
TRT*CC*YR	DD/SUF	SUF	NC	2024	3473.33	3813.33
TRT*CC*YR	BAM	BAM	NC	2018	2669.08	3391.71
TRT*CC*YR	BAM	BAM	NC	2020	2289.8	3115
TRT*CC*YR	BAM	BAM	NC	2022	3145	3039.33
TRT*CC*YR	BAM	BAM	NC	2024	4288.67	3479.67
Mean LSD					843.05	611.23

Table C.3 Least squares (LS) means and least significant differences (LSD) for fungi abundance for 2018, 2020, 2022, and 2024, for all main effects and interactions. Table abbreviations include phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), drawdown or sufficiency (DD/SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

					Fungi Abundance	
					pmol C g soil^{-1}	
Effect	**P TRT**	**P TRT**	**COVER**	**YR**	**Spring**	**Fall**
TRT	NP	NP			2812.98	2440.34
TRT	DD/SUF	SUF			2875.68	2596.13
TRT	BAM	BAM			3400.02	2463.5
Mean LSD					670.2	313.32
CC			CC		3722.15	2836.43
CC			NC		2336.96	2163.55
Mean LSD					547.22	255.82
TRT*CC	NP	NP	CC		3393.84	2869.11
TRT*CC	DD/SUF	SUF	CC		3645.41	3026.51
TRT*CC	BAM	BAM	CC		4127.21	2613.66
TRT*CC	NP	NP	NC		2232.12	2011.56
TRT*CC	DD/SUF	SUF	NC		2105.95	2165.75
TRT*CC	BAM	BAM	NC		2672.82	2313.33
Mean LSD					947.81	443.1
YR				2018	2632.81	2411.23
YR				2020	1655.7	2296.78
YR				2022	4141.94	2798.5
YR				2024	3687.78	2493.44
Mean LSD					743.63	354.67
TRT*YR	NP	NP		2018	2437.68	2320.02
TRT*YR	NP	NP		2020	1442.9	2215.5
TRT*YR	NP	NP		2022	3848.33	2710.67
TRT*YR	NP	NP		2024	3523	2515.17
TRT*YR	DD/SUF	SUF		2018	2468.87	2501.18
TRT*YR	DD/SUF	SUF		2020	1789.69	2558.5
TRT*YR	DD/SUF	SUF		2022	4128	2887.67
TRT*YR	DD/SUF	SUF		2024	3116.17	2437.17
TRT*YR	BAM	BAM		2018	2991.89	2412.49
TRT*YR	BAM	BAM		2020	1734.5	2116.33
TRT*YR	BAM	BAM		2022	4449.5	2797.17
TRT*YR	BAM	BAM		2024	4424.17	2528
Mean LSD					1278.44	609.09
CC*YR			CC	2018	3280.08	2641.93
CC*YR			CC	2020	1924.99	2729.67
CC*YR			CC	2022	5191.56	3073.22

CC*YR			CC	2024	4492	2900.89
CC*YR			NC	2018	1985.55	2180.53
CC*YR			NC	2020	1386.41	1863.89
CC*YR			NC	2022	3092.33	2523.78
CC*YR			NC	2024	2883.56	2086
Mean LSD					1045.52	498.23
TRT*CC*YR	NP	NP	CC	2018	3286.36	2610.78
TRT*CC*YR	NP	NP	CC	2020	1567.33	2293.33
TRT*CC*YR	NP	NP	CC	2022	4203.67	3077.33
TRT*CC*YR	NP	NP	CC	2024	4518	3495
TRT*CC*YR	SUF	SUF	CC	2018	3129.84	2916.37
TRT*CC*YR	DD/SUF	SUF	CC	2020	2475.8	3516
TRT*CC*YR	DD/SUF	SUF	CC	2022	5476.67	3049
TRT*CC*YR	DD/SUF	SUF	CC	2024	3499.33	2624.67
TRT*CC*YR	BAM	BAM	CC	2018	3424.02	2398.65
TRT*CC*YR	BAM	BAM	CC	2020	1731.84	2379.67
TRT*CC*YR	BAM	BAM	CC	2022	5894.33	3093.33
TRT*CC*YR	BAM	BAM	CC	2024	5458.67	2583
TRT*CC*YR	NP	NP	NC	2018	1588.99	2029.26
TRT*CC*YR	NP	NP	NC	2020	1318.48	2137.67
TRT*CC*YR	NP	NP	NC	2022	3493	2344
TRT*CC*YR	NP	NP	NC	2024	2528	1535.33
TRT*CC*YR	DD/SUF	SUF	NC	2018	1807.9	2086
TRT*CC*YR	DD/SUF	SUF	NC	2020	1103.57	1601
TRT*CC*YR	DD/SUF	SUF	NC	2022	2779.33	2726.33
TRT*CC*YR	DD/SUF	SUF	NC	2024	2733	2249.67
TRT*CC*YR	BAM	BAM	NC	2018	2559.77	2426.33
TRT*CC*YR	BAM	BAM	NC	2020	1737.17	1853
TRT*CC*YR	BAM	BAM	NC	2022	3004.67	2501
TRT*CC*YR	BAM	BAM	NC	2024	3389.67	2473
Mean LSD					1805.34	859.94

Table C.4 Least squares (LS) means and least significant differences (LSD) for gram-negative bacteria abundance for 2018, 2020, 2022, and 2024, for all main effects and interactions. Table abbreviations include phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), drawdown or sufficiency (DD/SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

					Gram-Negative Bacteria Abundance	
					pmol C g soil^{-1}	
Effect	**P TRT**	**P TRT**	**COVER**	**YR**	**Spring**	**Fall**
TRT	NP	NP			26076	28571
TRT	DD/SUF	SUF			27717	30305
TRT	BAM	BAM			29000	29428
Mean LSD					2401.16	1444.04
CC			CC		31274	32448
CC			NC		23921	26422
Mean LSD					1960.54	1179.06
TRT*CC	NP	NP	CC		29874	31573
TRT*CC	DD/SUF	SUF	CC		32090	33872
TRT*CC	BAM	BAM	CC		31857	31898
TRT*CC	NP	NP	NC		22279	25569
TRT*CC	DD/SUF	SUF	NC		23344	26738
TRT*CC	BAM	BAM	NC		26142	26959
Mean LSD					3395.75	2042.18
YR				2018	29093	26842
YR				2020	20004	26399
YR				2022	28047	31949
YR				2024	33246	32549
Mean LSD					2231.66	2012.74
TRT*YR	NP	NP		2018	27869	26709
TRT*YR	NP	NP		2020	19115	24868
TRT*YR	NP	NP		2022	27420	30875
TRT*YR	NP	NP		2024	29902	31833
TRT*YR	DD/SUF	SUF		2018	30630	26463
TRT*YR	DD/SUF	SUF		2020	20528	28017
TRT*YR	DD/SUF	SUF		2022	28476	33312
TRT*YR	DD/SUF	SUF		2024	31232	33428
TRT*YR	BAM	BAM		2018	28779	27354
TRT*YR	BAM	BAM		2020	20368	26312
TRT*YR	BAM	BAM		2022	28247	31659
TRT*YR	BAM	BAM		2024	38605	32388
Mean LSD					3960.65	3349.59
CC*YR			CC	2018	34221	28304
CC*YR			CC	2020	22389	29344
CC*YR			CC	2022	30747	34634

CC*YR			CC	2024	37738	37508
CC*YR			NC	2018	23964	25380
CC*YR			NC	2020	17619	23455
CC*YR			NC	2022	25348	29263
CC*YR			NC	2024	28755	27590
Mean LSD					3217.18	2758.82
TRT*CC*YR	NP	NP	CC	2018	33084	28356
TRT*CC*YR	NP	NP	CC	2020	20572	26313
TRT*CC*YR	NP	NP	CC	2022	30447	32308
TRT*CC*YR	NP	NP	CC	2024	35392	39315
TRT*CC*YR	SUF	SUF	CC	2018	36976	28829
TRT*CC*YR	DD/SUF	SUF	CC	2020	23981	33185
TRT*CC*YR	DD/SUF	SUF	CC	2022	30849	36237
TRT*CC*YR	DD/SUF	SUF	CC	2024	36552	37238
TRT*CC*YR	BAM	BAM	CC	2018	32603	27728
TRT*CC*YR	BAM	BAM	CC	2020	22613	28533
TRT*CC*YR	BAM	BAM	CC	2022	30944	35358
TRT*CC*YR	BAM	BAM	CC	2024	41270	35972
TRT*CC*YR	NP	NP	NC	2018	22654	25062
TRT*CC*YR	NP	NP	NC	2020	17657	23424
TRT*CC*YR	NP	NP	NC	2022	24393	29442
TRT*CC*YR	NP	NP	NC	2024	24412	24350
TRT*CC*YR	DD/SUF	SUF	NC	2018	24284	24097
TRT*CC*YR	DD/SUF	SUF	NC	2020	17076	22849
TRT*CC*YR	DD/SUF	SUF	NC	2022	26102	30388
TRT*CC*YR	DD/SUF	SUF	NC	2024	25913	29618
TRT*CC*YR	BAM	BAM	NC	2018	24954	26981
TRT*CC*YR	BAM	BAM	NC	2020	18123	24091
TRT*CC*YR	BAM	BAM	NC	2022	25549	27960
TRT*CC*YR	BAM	BAM	NC	2024	35939	28804
Mean LSD					5627.57	4699.25

Table C.5 Least squares (LS) means and least significant differences (LSD) for gram-positive bacteria abundance for 2018, 2020, 2022, and 2024, for all main effects and interactions. Table abbreviations include phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), drawdown or sufficiency (DD/SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

					Gram-Positive Bacteria Abundance	
					pmol C g soil^{-1}	
Effect	**P TRT**	**P TRT**	**COVER**	**YR**	**Spring**	**Fall**
TRT	NP	NP			20559	22095
TRT	DD/SUF	SUF			21971	23517
TRT	BAM	BAM			22868	22835
Mean LSD					1964.36	1527.69
CC			CC		24389	25296
CC			NC		19210	20336
Mean LSD					1603.9	1247.35
TRT*CC	NP	NP	CC		23373	24694
TRT*CC	DD/SUF	SUF	CC		25134	26500
TRT*CC	BAM	BAM	CC		24661	24693
TRT*CC	NP	NP	NC		17745	19496
TRT*CC	DD/SUF	SUF	NC		18809	20535
TRT*CC	BAM	BAM	NC		21075	20976
Mean LSD					2778.03	2160.48
YR				2018	23601	20642
YR				2020	14584	21034
YR				2022	22379	25046
YR				2024	26633	24541
Mean LSD					1558.91	1277
TRT*YR	NP	NP		2018	22529	20108
TRT*YR	NP	NP		2020	13949	19577
TRT*YR	NP	NP		2022	21741	23971
TRT*YR	NP	NP		2024	24017	24724
TRT*YR	DD/SUF	SUF		2018	24770	20731
TRT*YR	DD/SUF	SUF		2020	15006	22297
TRT*YR	DD/SUF	SUF		2022	22551	26265
TRT*YR	DD/SUF	SUF		2024	25558	24776
TRT*YR	BAM	BAM		2018	23504	21087
TRT*YR	BAM	BAM		2020	14798	21228
TRT*YR	BAM	BAM		2022	22845	24901
TRT*YR	BAM	BAM		2024	30325	24122
Mean LSD					2884.78	2316.22
CC*YR			CC	2018	27185	22310
CC*YR			CC	2020	15987	22753
CC*YR			CC	2022	24574	27621

CC*YR			CC	2024	29811	28498
CC*YR			NC	2018	20017	18974
CC*YR			NC	2020	13182	19316
CC*YR			NC	2022	20185	22471
CC*YR			NC	2024	23456	20583
Mean LSD					2323.1	1872.92
TRT*CC*YR	NP	NP	CC	2018	26227	21741
TRT*CC*YR	NP	NP	CC	2020	14913	20329
TRT*CC*YR	NP	NP	CC	2022	24400	26157
TRT*CC*YR	NP	NP	CC	2024	27950	30550
TRT*CC*YR	SUF	SUF	CC	2018	29285	23253
TRT*CC*YR	DD/SUF	SUF	CC	2020	17095	25355
TRT*CC*YR	DD/SUF	SUF	CC	2022	24497	28990
TRT*CC*YR	DD/SUF	SUF	CC	2024	29659	28400
TRT*CC*YR	BAM	BAM	CC	2018	26042	21937
TRT*CC*YR	BAM	BAM	CC	2020	15953	22574
TRT*CC*YR	BAM	BAM	CC	2022	24824	27716
TRT*CC*YR	BAM	BAM	CC	2024	31824	26545
TRT*CC*YR	NP	NP	NC	2018	18832	18474
TRT*CC*YR	NP	NP	NC	2020	12984	18825
TRT*CC*YR	NP	NP	NC	2022	19081	21786
TRT*CC*YR	NP	NP	NC	2024	20085	18899
TRT*CC*YR	DD/SUF	SUF	NC	2018	20255	18210
TRT*CC*YR	DD/SUF	SUF	NC	2020	12918	19239
TRT*CC*YR	DD/SUF	SUF	NC	2022	20606	23539
TRT*CC*YR	DD/SUF	SUF	NC	2024	21457	21151
TRT*CC*YR	BAM	BAM	NC	2018	20965	20238
TRT*CC*YR	BAM	BAM	NC	2020	13643	19883
TRT*CC*YR	BAM	BAM	NC	2022	20866	22087
TRT*CC*YR	BAM	BAM	NC	2024	28825	21699
Mean LSD					4130.79	3304.51

Table C.6 Least squares (LS) means and least significant differences (LSD) for eukaryote abundance for 2018, 2020, 2022, and 2024, for all main effects and interactions. Table abbreviations include phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), drawdown or sufficiency (DD/SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

					Eukaryote Abundance	
					pmol C g soil^{-1}	
Effect	**P TRT**	**P TRT**	**COVER**	**YR**	**Spring**	**Fall**
TRT	NP	NP			1448.4	1572.53
TRT	DD/SUF	SUF			1629.36	1576.4
TRT	BAM	BAM			1705.79	1540
Mean LSD					276.58	211.19
CC			CC		1989.97	1799.31
CC			NC		1199.07	1326.64
Mean LSD					225.83	172.43
TRT*CC	NP	NP	CC		1804.61	1905.86
TRT*CC	DD/SUF	SUF	CC		2125.94	1836.04
TRT*CC	BAM	BAM	CC		2039.35	1656.04
TRT*CC	NP	NP	NC		1092.19	1239.2
TRT*CC	DD/SUF	SUF	NC		1132.78	1316.76
TRT*CC	BAM	BAM	NC		1372.22	1423.97
Mean LSD					391.14	298.66
YR				2018	1337.87	1542.63
YR				2020	1388.42	1539.33
YR				2022	1806.78	1504.83
YR				2024	1845	1665.11
Mean LSD					270.71	218.85
TRT*YR	NP	NP		2018	1290.02	1543.95
TRT*YR	NP	NP		2020	1331.59	1611.17
TRT*YR	NP	NP		2022	1654.17	1436.17
TRT*YR	NP	NP		2024	1517.83	1698.83
TRT*YR	DD/SUF	SUF		2018	1460.68	1429.1
TRT*YR	DD/SUF	SUF		2020	1336.08	1423.83
TRT*YR	DD/SUF	SUF		2022	1984.33	1633
TRT*YR	DD/SUF	SUF		2024	1736.33	1819.67
TRT*YR	BAM	BAM		2018	1262.91	1654.84
TRT*YR	BAM	BAM		2020	1497.58	1583
TRT*YR	BAM	BAM		2022	1781.83	1445.33
TRT*YR	BAM	BAM		2024	2280.83	1476.83
Mean LSD					474.58	397.07
CC*YR			CC	2018	1693.97	1661.36
CC*YR			CC	2020	1677.68	1857.56
CC*YR			CC	2022	2271.56	1709.67

CC*YR			CC	2024	2316.67	1968.67
CC*YR			NC	2018	981.77	1423.91
CC*YR			NC	2020	1099.16	1221.11
CC*YR			NC	2022	1342	1300
CC*YR			NC	2024	1373.33	1361.56
Mean LSD					386.5	310.65
TRT*CC*YR	NP	NP	CC	2018	1642.95	1671.44
TRT*CC*YR	NP	NP	CC	2020	1681.5	2116.67
TRT*CC*YR	NP	NP	CC	2022	1970.67	1636
TRT*CC*YR	NP	NP	CC	2024	1923.33	2199.33
TRT*CC*YR	SUF	SUF	CC	2018	1945.14	1620.49
TRT*CC*YR	DD/SUF	SUF	CC	2020	1874.94	1847.67
TRT*CC*YR	DD/SUF	SUF	CC	2022	2531.67	1818.67
TRT*CC*YR	DD/SUF	SUF	CC	2024	2152	2057.33
TRT*CC*YR	BAM	BAM	CC	2018	1493.82	1692.15
TRT*CC*YR	BAM	BAM	CC	2020	1476.6	1608.33
TRT*CC*YR	BAM	BAM	CC	2022	2312.33	1674.33
TRT*CC*YR	BAM	BAM	CC	2024	2874.67	1649.33
TRT*CC*YR	NP	NP	NC	2018	937.08	1416.47
TRT*CC*YR	NP	NP	NC	2020	981.69	1105.67
TRT*CC*YR	NP	NP	NC	2022	1337.67	1236.33
TRT*CC*YR	NP	NP	NC	2024	1112.33	1198.33
TRT*CC*YR	DD/SUF	SUF	NC	2018	976.22	1237.71
TRT*CC*YR	DD/SUF	SUF	NC	2020	797.23	1000
TRT*CC*YR	DD/SUF	SUF	NC	2022	1437	1447.33
TRT*CC*YR	DD/SUF	SUF	NC	2024	1320.67	1582
TRT*CC*YR	BAM	BAM	NC	2018	1032	1617.54
TRT*CC*YR	BAM	BAM	NC	2020	1518.56	1557.67
TRT*CC*YR	BAM	BAM	NC	2022	1251.33	1216.33
TRT*CC*YR	BAM	BAM	NC	2024	1687	1304.33
Mean LSD					672.74	564.5

Table C.7 Least squares (LS) means and least significant differences (LSD) for actinomycete abundance for 2018, 2020, 2022, and 2024, for all main effects and interactions. Table abbreviations include phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), drawdown or sufficiency (DD/SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

					Actinomycete Abundance	
					pmol C g soil^{-1}	
Effect	**P TRT**	**P TRT**	**COVER**	**YR**	**Spring**	**Fall**
TRT	NP	NP			10661	11204
TRT	DD/SUF	SUF			11196	11660
TRT	BAM	BAM			11461	11514
Mean LSD					708.51	704.39
CC			CC		12264	12558
CC			NC		9948.34	10360
Mean LSD					578.5	575.13
TRT*CC	NP	NP	CC		12043	12418
TRT*CC	DD/SUF	SUF	CC		12568	12935
TRT*CC	BAM	BAM	CC		12182	12321
TRT*CC	NP	NP	NC		9279.75	9990.7
TRT*CC	DD/SUF	SUF	NC		9824.48	10384
TRT*CC	BAM	BAM	NC		10741	10706
Mean LSD					1001.99	996.15
YR				2018	12353	10486
YR				2020	7591.59	10663
YR				2022	11406	12533
YR				2024	13074	12155
Mean LSD					681.03	567.03
TRT*YR	NP	NP		2018	12095	10321
TRT*YR	NP	NP		2020	7352.64	10025
TRT*YR	NP	NP		2022	11315	12296
TRT*YR	NP	NP		2024	11883	12175
TRT*YR	DD/SUF	SUF		2018	12713	10486
TRT*YR	DD/SUF	SUF		2020	7794.52	11299
TRT*YR	DD/SUF	SUF		2022	11558	12721
TRT*YR	DD/SUF	SUF		2024	12719	12133
TRT*YR	BAM	BAM		2018	12252	10650
TRT*YR	BAM	BAM		2020	7627.6	10665
TRT*YR	BAM	BAM		2022	11344	12582
TRT*YR	BAM	BAM		2024	14621	12157
Mean LSD					1217.59	1053.04
CC*YR			CC	2018	14043	11163
CC*YR			CC	2020	8133.43	11444
CC*YR			CC	2022	12420	13730

CC*YR			CC	2024	14460	13895
CC*YR			NC	2018	10664	9808.2
CC*YR			NC	2020	7049.75	9882
CC*YR			NC	2022	10391	11336
CC*YR			NC	2024	11688	10415
Mean LSD					1209.24	847.4
TRT*CC*YR	NP	NP	CC	2018	13869	11036
TRT*CC*YR	NP	NP	CC	2020	7876.73	10587
TRT*CC*YR	NP	NP	CC	2022	12655	13540
TRT*CC*YR	NP	NP	CC	2024	13772	14508
TRT*CC*YR	SUF	SUF	CC	2018	14898	11512
TRT*CC*YR	DD/SUF	SUF	CC	2020	8499.85	12502
TRT*CC*YR	DD/SUF	SUF	CC	2022	12477	13967
TRT*CC*YR	DD/SUF	SUF	CC	2024	14396	13760
TRT*CC*YR	BAM	BAM	CC	2018	13361	10942
TRT*CC*YR	BAM	BAM	CC	2020	8023.71	11243
TRT*CC*YR	BAM	BAM	CC	2022	12130	13681
TRT*CC*YR	BAM	BAM	CC	2024	15213	13417
TRT*CC*YR	NP	NP	NC	2018	10321	9606.13
TRT*CC*YR	NP	NP	NC	2020	6828.56	9462.67
TRT*CC*YR	NP	NP	NC	2022	9974.67	11052
TRT*CC*YR	NP	NP	NC	2024	9994.67	9841.67
TRT*CC*YR	DD/SUF	SUF	NC	2018	10527	9459.52
TRT*CC*YR	DD/SUF	SUF	NC	2020	7089.2	10096
TRT*CC*YR	DD/SUF	SUF	NC	2022	10639	11475
TRT*CC*YR	DD/SUF	SUF	NC	2024	11042	10506
TRT*CC*YR	BAM	BAM	NC	2018	11144	10359
TRT*CC*YR	BAM	BAM	NC	2020	7231.5	10087
TRT*CC*YR	BAM	BAM	NC	2022	10559	11482
TRT*CC*YR	BAM	BAM	NC	2024	14029	10897
Mean LSD					1732.45	1508.84

Table C.8 Least squares (LS) means and least significant differences (LSD) for the ratio of gram-positive bacteria: gram-negative bacteria for 2018, 2020, 2022, and 2024, for all main effects and interactions. Table abbreviations include phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), drawdown or sufficiency (DD/SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

					Gram-Positive Bacteria: Gram-Negative Bacteria	
Effect	**P TRT**	**P TRT**	**COVER**	**YR**	**Spring**	**Fall**
TRT	NP	NP			1.2042	1.1752
TRT	DD/SUF	SUF			1.2046	1.1739
TRT	BAM	BAM			1.1917	1.1803
Mean LSD					0.05	0.03
CC			CC		1.1764	1.1779
CC			NC		1.2239	1.1751
Mean LSD					0.04	0.02
TRT*CC	NP	NP	CC		1.1942	1.1862
TRT*CC	DD/SUF	SUF	CC		1.1775	1.176
TRT*CC	BAM	BAM	CC		1.1575	1.1713
TRT*CC	NP	NP	NC		1.2142	1.1641
TRT*CC	DD/SUF	SUF	NC		1.2317	1.1717
TRT*CC	BAM	BAM	NC		1.2258	1.1893
Mean LSD					0.07	0.04
YR				2018	1.2561	1.1692
YR				2020	1.1167	1.21
YR				2022	1.2156	1.1867
YR				2024	1.2122	1.14
Mean LSD					0.04	0.04
TRT*YR	NP	NP		2018	1.2617	1.1507
TRT*YR	NP	NP		2020	1.12	1.1933
TRT*YR	NP	NP		2022	1.2183	1.1833
TRT*YR	NP	NP		2024	1.2167	1.1733
TRT*YR	DD/SUF	SUF		2018	1.2467	1.1871
TRT*YR	DD/SUF	SUF		2020	1.125	1.2167
TRT*YR	DD/SUF	SUF		2022	1.2067	1.1783
TRT*YR	DD/SUF	SUF		2024	1.24	1.1133
TRT*YR	BAM	BAM		2018	1.26	1.1697
TRT*YR	BAM	BAM		2020	1.105	1.22
TRT*YR	BAM	BAM		2022	1.2217	1.1983
TRT*YR	BAM	BAM		2024	1.18	1.1333
Mean LSD					0.08	0.07
CC*YR			CC	2018	1.2178	1.1937
CC*YR			CC	2020	1.0844	1.1711
CC*YR			CC	2022	1.2178	1.2056

CC*YR			CC	2024	1.1856	1.1411
CC*YR			NC	2018	1.2944	1.1447
CC*YR			NC	2020	1.1489	1.2489
CC*YR			NC	2022	1.2133	1.1678
CC*YR			NC	2024	1.2389	1.1389
Mean LSD					0.06	0.06
TRT*CC*YR	NP	NP	CC	2018	1.23	1.1681
TRT*CC*YR	NP	NP	CC	2020	1.1167	1.18
TRT*CC*YR	NP	NP	CC	2022	1.2367	1.24
TRT*CC*YR	NP	NP	CC	2024	1.1933	1.1567
TRT*CC*YR	SUF	SUF	CC	2018	1.2067	1.2175
TRT*CC*YR	DD/SUF	SUF	CC	2020	1.0767	1.1467
TRT*CC*YR	DD/SUF	SUF	CC	2022	1.2133	1.1967
TRT*CC*YR	DD/SUF	SUF	CC	2024	1.2133	1.1433
TRT*CC*YR	BAM	BAM	CC	2018	1.2167	1.1954
TRT*CC*YR	BAM	BAM	CC	2020	1.06	1.1867
TRT*CC*YR	BAM	BAM	CC	2022	1.2033	1.18
TRT*CC*YR	BAM	BAM	CC	2024	1.15	1.1233
TRT*CC*YR	NP	NP	NC	2018	1.2933	1.1333
TRT*CC*YR	NP	NP	NC	2020	1.1233	1.2067
TRT*CC*YR	NP	NP	NC	2022	1.2	1.1267
TRT*CC*YR	NP	NP	NC	2024	1.24	1.19
TRT*CC*YR	DD/SUF	SUF	NC	2018	1.2867	1.1567
TRT*CC*YR	DD/SUF	SUF	NC	2020	1.1733	1.2867
TRT*CC*YR	DD/SUF	SUF	NC	2022	1.2	1.16
TRT*CC*YR	DD/SUF	SUF	NC	2024	1.2667	1.0833
TRT*CC*YR	BAM	BAM	NC	2018	1.3033	1.144
TRT*CC*YR	BAM	BAM	NC	2020	1.15	1.2533
TRT*CC*YR	BAM	BAM	NC	2022	1.24	1.2167
TRT*CC*YR	BAM	BAM	NC	2024	1.21	1.1433
Mean LSD					0.11	0.1

Table C.9 Least squares (LS) means and least significant differences (LSD) for the ratio of fungi: bacteria for 2018, 2020, 2022, and 2024, for all main effects and interactions. Table abbreviations include phosphorus (P), P fertilizer treatment (TRT), year (YR), no phosphorus (NP), build and maintain (BAM), drawdown or sufficiency (DD/SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

				Fungi: Bacteria	
Effect	**P TRT**	**COVER**	**YR**	**Spring**	**Fall**
TRT	NP			0.1286	0.1231
TRT	DD/SUF			0.1305	0.1205
TRT	BAM			0.1363	0.1198
Mean LSD				0.01	0.01
CC		CC		0.1399	0.1228
CC		NC		0.1237	0.1195
Mean LSD				0.01	0
TRT*CC	NP	CC		0.1343	0.1274
TRT*CC	DD/SUF	CC		0.1395	0.1216
TRT*CC	BAM	CC		0.1458	0.1195
TRT*CC	NP	NC		0.1229	0.1188
TRT*CC	DD/SUF	NC		0.1214	0.1195
TRT*CC	BAM	NC		0.1268	0.1201
Mean LSD				0.02	0.01
YR			2018	0.1139	0.1268
YR			2020	0.1232	0.1233
YR			2022	0.1567	0.1167
YR			2024	0.1333	0.1178
Mean LSD				0.01	0.01
TRT*YR	NP		2018	0.1117	0.1273
TRT*YR	NP		2020	0.1193	0.1267
TRT*YR	NP		2022	0.15	0.12
TRT*YR	NP		2024	0.1333	0.1183
TRT*YR	DD/SUF		2018	0.1083	0.1272
TRT*YR	DD/SUF		2020	0.1235	0.1233
TRT*YR	DD/SUF		2022	0.1583	0.1167
TRT*YR	DD/SUF		2024	0.1317	0.115
TRT*YR	BAM		2018	0.1217	0.1258
TRT*YR	BAM		2020	0.1268	0.12
TRT*YR	BAM		2022	0.1617	0.1133
TRT*YR	BAM		2024	0.135	0.12
Mean LSD				0.02	0.01
CC*YR		CC	2018	0.1211	0.129
CC*YR		CC	2020	0.1261	0.1289
CC*YR		CC	2022	0.1733	0.1156

CC*YR		CC	2024	0.1389	0.1178
CC*YR		NC	2018	0.1067	0.1245
CC*YR		NC	2020	0.1203	0.1178
CC*YR		NC	2022	0.14	0.1178
CC*YR		NC	2024	0.1278	0.1178
Mean LSD				0.02	0.01
TRT*CC*YR	NP	CC	2018	0.1233	0.1329
TRT*CC*YR	NP	CC	2020	0.1203	0.1267
TRT*CC*YR	NP	CC	2022	0.1533	0.1233
TRT*CC*YR	NP	CC	2024	0.14	0.1267
TRT*CC*YR	SUF	CC	2018	0.1133	0.1295
TRT*CC*YR	DD/SUF	CC	2020	0.1347	0.1367
TRT*CC*YR	DD/SUF	CC	2022	0.18	0.11
TRT*CC*YR	DD/SUF	CC	2024	0.13	0.11
TRT*CC*YR	BAM	CC	2018	0.1267	0.1247
TRT*CC*YR	BAM	CC	2020	0.1233	0.1233
TRT*CC*YR	BAM	CC	2022	0.1867	0.1133
TRT*CC*YR	BAM	CC	2024	0.1467	0.1167
TRT*CC*YR	NP	NC	2018	0.1	0.1217
TRT*CC*YR	NP	NC	2020	0.1183	0.1267
TRT*CC*YR	NP	NC	2022	0.1467	0.1167
TRT*CC*YR	NP	NC	2024	0.1267	0.11
TRT*CC*YR	DD/SUF	NC	2018	0.1033	0.1248
TRT*CC*YR	DD/SUF	NC	2020	0.1123	0.11
TRT*CC*YR	DD/SUF	NC	2022	0.1367	0.1233
TRT*CC*YR	DD/SUF	NC	2024	0.1333	0.12
TRT*CC*YR	BAM	NC	2018	0.1167	0.127
TRT*CC*YR	BAM	NC	2020	0.1303	0.1167
TRT*CC*YR	BAM	NC	2022	0.1367	0.1133
TRT*CC*YR	BAM	NC	2024	0.1233	0.1233
Mean LSD				0.03	0.02