Seasonal dynamics and impact of P fertilizer treatments and cover crops on soil health indicators in a no-till corn-soybean system

by

Amber Marie Pasket

B.S., Texas A&M University, 2017
M.S., Oklahoma State University, 2020

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2023

Abstract

While phosphorus (P) is an essential nutrient for crop growth, it can be one of the most limiting nutrients in crop production as it is relatively immobile in soil. Therefore, P amendments (such as synthetic fertilizer or animal manure) are needed to meet crop P demand. The overapplication of P can lead to adverse environmental conditions, namely eutrophication. Soil testing prior to nutrient applications is important as it defines the agronomic need for nutrient applications to support crop yields while reducing the risks of overapplication. Soil testing laboratories use two different approaches to make P fertilizer recommendations. The build and maintain (BAM) approach builds soil test P to a critical level then maintains soil test P in a target range with subsequent fertilizer applications. In the sufficiency (SUF) approach, a critical level of soil test P is determined to achieve 90-95% maximum yield, and P is only applied when a crop response is expected. In efforts to improve soil health, conservation practices such as no-till management or the implementation of cover crops can be introduced into a cropping system. Soil health indicators are measurable properties of soil that can provide insights on how well the soil can function. In addition, seasonal dynamics of soil health indicators are widely inconsistent in literature due to differences in climate, cover crops, crop rotations, and tillage practices. As a consequence of the seasonal dynamics of soil health indicators, especially for biological indicators, recommendations of the correct time to soil sample are difficult.

A study was initiated in 2020 to determine the effects of cover crop and P fertilizer treatment impacts on biological soil heath indicators and to assess the seasonal dynamics of these indicators during a growing season. This study occurred at the Kansas Agricultural Watershed (KAW) site near Manhattan, KS. The site is a 2x3 randomized complete block design in a no-till corn (*Zea mays*)-soybean (*Glycine max*) rotation that is replicated three times. There were three

levels of P fertilizer managements; treatments under BAM and SUF managements and a no P/control (NP) treatment. There were two levels of cover crop treatments; the presence (CC)/absence (NC) of a cereal rye (*Secale cereale*) cover crop. During the three-year duration of the experiment, ammonium polyphosphate was added to the BAM treatment at maintenance rates of 17, 31, and 54 kg P_2O_5 ha^{-1} in 2020, 2021, and 2022, respectively. No P has been applied to the SUF treatment since December 2018, therefore, the SUF treatment has been in a P draw down phase during the duration of this experiment. No P had been applied to the NP treatment since 2014. Nitrogen (N) applications were not balanced in 2020 and 2022. As a result, only the BAM treatment received N fertilizer through the ammonium polyphosphate application in 2020 and 2022.

Composite soil samples were collected (0-5 cm) six times during the 2021 and 2022 growing season. Sampling times included a sampling prior to cover crop termination, four sampling during crop growth, and one sampling post-harvest. Soil health indicators measured were microbial biomass carbon (C), N, and P, inorganic N, dissolved total N, dissolved organic N, autoclaved citrate extractable (ACE) protein, citrate-extractable P, four soil enzyme activities (β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase), soil respiration, active C, and dissolved organic C.

Significant P fertilizer treatment by sampling time interactions were observed for N pools in both years, likely due to differences in soil moisture and temperature, crop N uptake, biological transformations of N, and fertilizer applications. There was a significant cover crop by sampling time interaction for dissolved total N and NO_3-N in 2022. Cover crops reduced dissolved total N and NO_3-N concentrations during the first two sampling times when a cover crop was still living and when the cover crop had been terminated three days prior. It was likely

that cereal rye was able to scavenge N from the soil and assimilate N into the biomass, reducing the risk of N loss through leaching. Cover crops influenced C pools and measures of microbial function and activity in both years. Cover crops likely provided additional above- and belowground inputs that increased substrate availability to the soil microbial community. In addition, a significant P fertilizer by cover crop interaction was observed for alkaline phosphatase in 2021 and 2022 and glucosidase in 2022. The presence of a cover crop significantly increased enzyme activity in the NP and SUF treatment, likely reflecting increased nutrient cycling in system at the margins of deficiency. Sampling time influenced most soil health indicators in both years, however, the lack of cover crop by sampling time interactions for most soil health indicators observed in either year likely reflect that other environmental factors such as precipitation and temperature were the primary driver of seasonal trends of soil health indicators.

Although no-till management can improve soil health, nutrient stratification can lead to decreased microbial activity in the subsurface. In order to assess the effect of sample depth (as well as cover crop and P fertilizer treatment) on selected soil health indicators, soil samples were also collected at 0-5, 5-10, and 10-15 cm post-harvest in 2020, 2021, and 2022. Soil health indicators assessed included active C and ACE protein, soil respiration, and four soil enzyme activities (β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase). Sampling depth significantly influenced all soil health indicators in all three years. Nutrient pools and measures of microbial activity were significantly higher at 0-5 cm compared to 5-10 and 10-15 cm, reflecting nutrient stratification at the study site. Phosphorus availability significantly influenced soil protein concentrations in all three years. Cover crop biomass production and well as precipitation and temperature conditions during the three harvest years likely resulted in

inconsistent cover crop effects on soil health indicators. Only soil respiration was significantly increased by cover crop presence in all three years of this study.

The overall results of this study, highlight the potential to improve nutrient cycling by cover crop implementation. Cover crops can promote the soil microbial community to meeting the challenges of meeting crop nutrient demand while minimizing nutrient loss by managing a nutrient such as P closer to the margins of deficiency.

Seasonal dynamics and impact of P fertilizer treatments and cover crops on soil health indicators in a no-till corn-soybean system

by

Amber Marie Pasket

B.S., Texas A&M University, 2017
M.S., Oklahoma State University, 2020

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2023

Approved by:

Major Professor
Peter Tomlinson

Copyright

Abstract

While phosphorus (P) is an essential nutrient for crop growth, it can be one of the most limiting nutrients in crop production as it is relatively immobile in soil. Therefore, P amendments (such as synthetic fertilizer or animal manure) are needed to meet crop P demand. The overapplication of P can lead to adverse environmental conditions, namely eutrophication. Soil testing prior to nutrient applications is important as it defines the agronomic need for nutrient applications to support crop yields while reducing the risks of overapplication. Soil testing laboratories use two different approaches to make P fertilizer recommendations. The build and maintain (BAM) approach builds soil test P to a critical level then maintains soil test P in a target range with subsequent fertilizer applications. In the sufficiency (SUF) approach, a critical level of soil test P is determined to achieve 90-95% maximum yield, and P is only applied when a crop response is expected. In efforts to improve soil health, conservation practices such as no-till management or the implementation of cover crops can be introduced into a cropping system. Soil health indicators are measurable properties of soil that can provide insights on how well the soil can function. In addition, seasonal dynamics of soil health indicators are widely inconsistent in literature due to differences in climate, cover crops, crop rotations, and tillage practices. As a consequence of the seasonal dynamics of soil health indicators, especially for biological indicators, recommendations of the correct time to soil sample are difficult.

A study was initiated in 2020 to determine the effects of cover crop and P fertilizer treatment impacts on biological soil heath indicators and to assess the seasonal dynamics of these indicators during a growing season. This study occurred at the Kansas Agricultural Watershed (KAW) site near Manhattan, KS. The site is a 2x3 randomized complete block design in a no-till corn (*Zea mays*)-soybean (*Glycine max*) rotation that is replicated three times. There were three

levels of P fertilizer managements; treatments under BAM and SUF managements and a no P/control (NP) treatment. There were two levels of cover crop treatments; the presence (CC)/absence (NC) of a cereal rye (*Secale cereale*) cover crop. During the three-year duration of the experiment, ammonium polyphosphate was added to the BAM treatment at maintenance rates of 17, 31, and 54 kg P_2O_5 ha^{-1} in 2020, 2021, and 2022, respectively. No P has been applied to the SUF treatment since December 2018, therefore, the SUF treatment has been in a P draw down phase during the duration of this experiment. No P had been applied to the NP treatment since 2014. Nitrogen (N) applications were not balanced in 2020 and 2022. As a result, only the BAM treatment received N fertilizer through the ammonium polyphosphate application in 2020 and 2022.

Composite soil samples were collected (0-5 cm) six times during the 2021 and 2022 growing season. Sampling times included a sampling prior to cover crop termination, four sampling during crop growth, and one sampling post-harvest. Soil health indicators measured were microbial biomass carbon (C), N, and P, inorganic N, dissolved total N, dissolved organic N, autoclaved citrate extractable (ACE) protein, citrate-extractable P, four soil enzyme activities (β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase), soil respiration, active C, and dissolved organic C.

Significant P fertilizer treatment by sampling time interactions were observed for N pools in both years, likely due to differences in soil moisture and temperature, crop N uptake, biological transformations of N, and fertilizer applications. There was a significant cover crop by sampling time interaction for dissolved total N and NO_3-N in 2022. Cover crops reduced dissolved total N and NO_3-N concentrations during the first two sampling times when a cover crop was still living and when the cover crop had been terminated three days prior. It was likely

that cereal rye was able to scavenge N from the soil and assimilate N into the biomass, reducing the risk of N loss through leaching. Cover crops influenced C pools and measures of microbial function and activity in both years. Cover crops likely provided additional above- and belowground inputs that increased substrate availability to the soil microbial community. In addition, a significant P fertilizer by cover crop interaction was observed for alkaline phosphatase in 2021 and 2022 and glucosidase in 2022. The presence of a cover crop significantly increased enzyme activity in the NP and SUF treatment, likely reflecting increased nutrient cycling in system at the margins of deficiency. Sampling time influenced most soil health indicators in both years, however, the lack of cover crop by sampling time interactions for most soil health indicators observed in either year likely reflect that other environmental factors such as precipitation and temperature were the primary driver of seasonal trends of soil health indicators.

Although no-till management can improve soil health, nutrient stratification can lead to decreased microbial activity in the subsurface. In order to assess the effect of sample depth (as well as cover crop and P fertilizer treatment) on selected soil health indicators, soil samples were also collected at 0-5, 5-10, and 10-15 cm post-harvest in 2020, 2021, and 2022. Soil health indicators assessed included active C and ACE protein, soil respiration, and four soil enzyme activities (β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase). Sampling depth significantly influenced all soil health indicators in all three years. Nutrient pools and measures of microbial activity were significantly higher at 0-5 cm compared to 5-10 and 10-15 cm, reflecting nutrient stratification at the study site. Phosphorus availability significantly influenced soil protein concentrations in all three years. Cover crop biomass production and well as precipitation and temperature conditions during the three harvest years likely resulted in

inconsistent cover crop effects on soil health indicators. Only soil respiration was significantly increased by cover crop presence in all three years of this study.

The overall results of this study, highlight the potential to improve nutrient cycling by cover crop implementation. Cover crops can promote the soil microbial community to meeting the challenges of meeting crop nutrient demand while minimizing nutrient loss by managing a nutrient such as P closer to the margins of deficiency.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to extend my gratitude to my advisor Dr. Peter Tomlinson for his support, understanding, and patience. I would also like to thank my committee members Drs. Nathan Nelson, Ganga Hettiarachchi, and John Blair for their insight and guidance on this project. I would like to acknowledge Dr. Qing Kang and the Statistical Consulting Laboratory for their help with statistical analysis as well as Kathy Lowe and the K-State Soil Testing Lab for soil sample analysis. I would also like to acknowledge the funding sources of this project: Kansas Soybean Commission, Kansas Corn Commission, Foundation for Food and Agriculture Research, the USDA, USDA-NRSC, and the 4-R Research Fund.

To Kathy Gehl, my sounding board during my PhD. Thank you for the advice and all the support that you have given me. I am eternally grateful that I was able to be in grad school the same time that you were. You have helped me more that you will ever know! From the gut-busting laugher to trying to explain all the weirdness that seems to happen when we are together, there has never been a dull moment in our hallway. I will always treasure our friendship! To Alexis Correira, Sarah Frye, Abigail Kortokrax, and Kendra Stahl, thank you for your comradery. I will treasure the bonds we have created during our time as grad students! Finally, thank you to Laura Starr and Catherine Stewart for showing me the ropes.

Lastly, to the KAW team, Cropping Systems, Soil Pedology, and Soil Fertility lab groups for their assistance in field work and soil processing. I extend my upmost gratitude to the Environmental Quality lab group. To Katie, Cam, Ryan, Kye, and Molly, without your help, this project would have never been completed.

Dedication

For my parents, thank you for your unwavering support and love

Chapter 1 – General Introduction

Nutrient cycling by the soil microbial community is an important ecosystem service that can affect the availability of nutrients. Increased agricultural production to meet the needs of an increasing world population requires additional inputs to meet crop nutrient demand. However, overapplication of synthetic fertilizer and/or animal manure can lead to adverse effects on the environment. Utilizing soil testing prior to nutrient additions allows for crop nutrient demand to be met while reducing the risks of nutrient loss through runoff or leaching. Additionally, management practices such as conventional tillage, residue removal, and monocultural crop production have led to decreased soil quality/health (Doran 2002). Conservation practices such as the inclusion of cover crops in a cropping system and no-till management can increase soil health. Cover crops can promote nutrient cycling in systems with low nutrient availability. Therefore, enhanced nutrient cycling by the soil microbial community in systems receiving less fertilizer inputs can potentially increase nutrient availability without the environmental risks of overapplication. However, the response of the soil microbial community to increased additions can vary due to differences in precipitation and temperature and crop residue amounts.

Phosphorus Management

Phosphorus (P) is an essential nutrient for plant growth, but is often one of the most limiting nutrients as less than 1% of total soil P is plant available (Hansen et al., 2002). Plants take up P from the soil solution in orthophosphate forms ($H_2PO_4^-$ and HPO_4^{2-}), however most P is immobile in soil due to absorption of P onto mineral surfaces and the precipitation of P as secondary P minerals (Daniel et al., 1994; Warren et al., 2017). As a result, only 0.01% of total P required to sustain plant growth is available in soil solution at any point in time (Brady and Weil, 2008). Soil pH plays an important role in P availability, as maximum P availability occurs in a

pH range of 5.5 to 7.2. In soils with pH lower than 5.5, iron (Fe) and aluminum (Al) reacts with P to form insoluble phosphates, while soil with a pH greater than 7.2, relatively insoluble calcium (Ca) phosphates are formed. Additionally, microbial biomass P represents up to 40% total organic P (Bunemann, 2015). Mineralization of organic P by biological and biochemical mechanisms can increase plant available P concentrations in soil. Microbial biomass can act as a source or sink for P availability due to mineralization and immobilization processes (Schneider et al., 2019).

Eutrophication is the process of increased enrichment of biological productivity due to excess amounts of nutrients that promote dense plant and algal growth. Eutrophication can lead to harmful algae blooms, and as the algae die, microbial decomposition depletes dissolved oxygen concentrations and creates a hypoxic/anoxic environment for animal life, leading to fish kills (Schindler, 2006). Thus, eutrophication of freshwater systems resulting from P loss is a major environmental concern (Sharpley et al., 1994). Critical values of surface water concentrations of inorganic P and total P in which eutrophication is accelerated are 0.01 and 0.02 mg L^{-1}, respectively (Daniel et al., 1998). The amount of P lost to the environment is dependent on soil P concentration, rate, method and timing of P applications (Kleinman et al., 2002). Lehmann et al. (2005) estimated that soil exhausted the ability to adsorb additional amounts of P when soil total P reached 1000-2000 mg kg^{-1}. Loss of P from fields occurs primarily through erosion and surface runoff, however, P leaching can occur if soils are amended with excess animal manure, have coarser textures, or have low P adsorption capacities (Kleinman et al., 2008). Loss of P through runoff or erosion occurs in both the dissolved and sediment-bound forms of P. Sediment-bound P includes P adsorbed to soil particles and constitutes the major portion of P transported from land under conventional tillage (Daniel et al., 1994), while

dissolved P is generally found in runoff from agricultural systems and is immediately available to biological uptake (Daniel et al., 1998).

Farmers and producers often rely on nutrient recommendations from soil testing laboratories to determine fertilizer application rates. There are two approaches that soil testing laboratories use for P recommendations; sufficiency or build and maintain. Common to both approaches is the importance of critical levels of soil test P in determining application rate. The critical soil test value is defined as the soil test level at which there is a relatively low probability of obtaining a yield response to added crop nutrients and about 90-95% of maximum yield will be obtained if the nutrient is not applied (Leikam et al., 2003). The sufficiency approach aims to add just enough P fertilizer to maximize profitability and is typically designed to provide 90 to 95% of maximum yield; only when a crop response is expected will fertilizer be applied in the sufficiency approach (Leikam et al., 2003). The build and maintain approach aims to add enough P fertilizer so that crop needs are met while also building up soil test P values to a critical value. After the critical value is reached, nutrient recommendations are made to maintain soil test levels in a target range (Macnack et al., 2011), typically equal to the amount of P removed in grain. The build and maintain approach is designed to feed the soil, while the sufficiency approach is designed to feed the crop (Zhang et al., 2021).

Several studies have shown that P fertilization can influence soil microbial community composition by shifting microbial community composition and increasing abundance and activity (Beauregard et al., 2010; Wakelin et al., 2012; Cheng et al., 2020). In addition, influences of P additions on biological soil health indicators have also been reported. Microbial biomass P concentrations have been reported to increase with increasing P fertilizer application rates (Tang et al., 2016; Shi et al., 2020). In a meta-analysis of soil respiration response to P

additions, it was observed that soil respiration increased by 31.7% in response to P additions in cropland (Feng and Zhu, 2019). Positive correlations between soil protein and P availability have also been reported in previous studies (Wu et al., 2011; Guo et al., 2012; Qiu et al., 2021; Wang et al., 2022), suggesting greater amounts of soil protein with increased P availability.

Conservation and Soil Health

Soil degradation due to intense agricultural practices to maximize crop productivity has led to efforts designed to preserve soil quality and health through principals of regenerative agriculture (Laishram et al., 2012). These include no-till management, introduction of cover crops, utilizing crop rotations, and the retention of crop residues on soil surface (Lehman et al., 2015). Soil health benefits can be greatly improved when several conservation approaches are used simultaneously. For example, Alhameid et al. (2019) found that microbial biomass carbon (C) was significantly higher in a no-till, four-year crop rotation compared with the same four-year rotation under conventional tillage. The addition of a cover crop to a cropping rotation was shown to increase total soil C by 8.6%, total nitrogen (N) by 12.8%, microbial biomass C by 20.7%, and microbial biomass N by 26.1% (McDaniel et al., 2014). The benefits of cover crops, including increased soil C and N inputs and functional diversity, have been more quickly identified under no-till management due to reduced decomposition rates since there is less contact between soil microorganisms and residues compared to conventional tillage (Adetunji et al., 2020). Wegner et al. (2018) found a significant interaction between cover crop addition and residue retention as residue removal significantly decreased soil organic matter (SOM) when no cover crop is present, while cover crops in high residue removal rates increased SOM when a cover crop was present.

Cover crops

The implementation of cover crops in replacement of fallow periods allows for a crop to be continuously grown during the year. The addition of cover crops can influence soil health in various ways, including increased organic inputs (Brennan and Acosta-Martinez, 2017), improvement of soil aggregation (Nivelle et al., 2016), increased fungal to bacterial ratios (Wegner et al., 2018), and improved soil moisture and temperature control (Adetunji et al., 2020). Utilizing diverse cropping rotations allows for greater crop residue inputs, improved nutrient cycling through increased SOM, and can be used as a cultural control for weeds, insects, and diseases through integrated pest management (Barzman et al., 2015; Agomoh et al., 2021).

The impacts of cover crops on the soil biological community has also been widely reported in the literature. Strickland et al. (2019) found that cover crops increased bioavailable C by 37%, increase NH_4-N by 64%, and decreased NO_3-N by 30%. Brennan and Acosta-Martinez (2019) found that cover crops increased β-glucosidase and β-glucosaminidase activity at least 66% and 79%, respectively, while Chavarria et al. (2016) found that acid phosphatase activities were between 12.1 and 21.9% higher in cover cropped plots. Nunes et al. (2018) found that cover crops increased soil respiration by 30% and autoclaved citrate extractable (ACE) protein content by 8%.

The presence of a cover crop in cropping systems can also influence soil P cycling. Differences in cover crop species affect P acquisition strategies, as legumes are generally known for P mobilization while grasses are considered a catch crop and are able to scavenge P (Hallama et al., 2019). Hallama et al. (2022) detailed that cover crops improve P availability through the cycling of P through their biomass (biomass pathway), through the enhancement of the soil microbial community (microbial pathway), and through the mining of sparingly-available P

forms (biochemical modification). Strategies used by cover crops to access poorly-available P include exploration of a greater soil volume, mobilization of sparingly-soluble inorganic and organic P forms, and mineralization of organic P (Hallama et al., 2019). Exploration of a greater volume of soil is facilitated by cover crop root architecture and morphology. In the case of root architecture, topsoil exploration and root hair density are the most important traits for improved P uptake (Hunter et al., 2014). P availability also can influence the architectural complexity of root systems in terms of the number, density, and growth rate of lateral roots (Hunter et al., 2014). Cover crops with more extensive root systems scavenge P from a larger and deeper soil volume. Cover crops can also exude low-molecular-weight organic anions to dissolve precipitates and chelate metal cations in order to facilitate P mobilization in soils. These carboxylates can also block binding sites for P, increasing the P concentration in the soil solution (Guppy et al., 2005). Factors that facilitate competition between binding sites include the composition, concentration, sorption, and persistence of root exudates in soils. Monocarboxylic low molecular weight acids such as acetate, formate, and lactate are present in greater concentration compared to di- and tri-carboxylic acids. Sorption of low molecular weight acids is governed by ligand exchange with sorption following the series of phosphate >>> oxalate > citrate > malate >> acetate (Guppy et al., 2005). Soil pH also plays an important role in sorption of organic molecules to soil, as competitive inhibition of P sorption increases with decreasing pH. Microorganisms also use plant exudates to produce P-solubilizing compounds in the rhizosphere to complement P mobilization by roots. Finally, mineralization of organic P through enzymatic processes help increase plant available P, but soil particles can bind enzymes, causing the inactivation of these enzymes. Due to the complex interactions with soil, microbial

degradation, and interception of the enzyme producers, increased phosphatase activity does not always translate into more rapid P uptake.

The conservation practices of no-till management and cover crop utilization can also have adverse effects on crop productivity and environmental quality. For example, no-till can cause nutrient stratification since most of the changes in soil properties occur near the surface (Blanco-Canqui and Ruis, 2018). Increased application rates of herbicides can also occur in no-till to combat weed emergence, leading to concerns of environmental safety. Cover crops can immobilize nutrients and water needed for cash crops if not managed effectively (Bakker et al., 2016; Tyler and Locke, 2019; Tyler, 2020). Cover crop termination date is an important management consideration, too, as late termination can result in immobilization of soil N which then is not available to the subsequent crop (Blanco-Canqui et al., 2015). Cover crops can also affect yields, as lower yields of subsequent crops, especially corn, have been reported (Nevins et al., 2018).

Corn and Soybean Rotations

More than 85% of corn and soybeans grown in the Unites States is produced in the Corn Belt. Of the 85%, more than 65% of the corn and soybeans are grown in a 2-year crop rotation (Grassini et al., 2015). Benefits of corn and soybean production in rotations compared to monocultural production are prevalent for both crops. Agomoh et al. (2021) found significantly higher soybean yields in a corn-soybean rotation compared to monoculture soybeans. Corn often produces more grain when grown in rotation with soybean than in continuous monoculture, due partially to greater N availability from soybean residue (Omay et al., 1998; Gentry et al. 2001). Soybean also may scavenge residual N after corn, thus reducing N leaching. Gentry et al. (2001) found that soil inorganic N in top 30 cm was higher when the previous crop was nodulated

soybeans compared to corn (24 vs 14 kg N ha^{-1}), and concluded that degradation rate of the crop residues was responsible for the difference.

Recommendations for N fertilizer application on corn crops following soybean in a rotation are generally less than reported for monoculture corn (Vanotti and Bundy, 1995; Gentry et al., 2001). Hesterman et al. (1987) found that corn derived 12% of its N from soybean residue after one year of decomposition. Though variation can occur between years due to environmental and soil conditions, soybean contributes approximately 20 lb ac^{-1} to the following cash crop as an N credit (Warren et al., 2017). About 55% of total N accumulated in aboveground biomass is attributed to N fixation (Ciampitti and Salvagiotti, 2018). Cordova et al. (2019) found that biological N fixation was maximized early during the seed filling phase, while the maximum N accumulation was reached after early seed filling and was contributed to soil inorganic N. The authors concluded that N uptake is favorable when N availability in soil increases since biological N fixation is energy intensive (Schipanski et al., 2010). Gentry et al. (2001) found that the period when mineralization rates were most affected by previous crop coincided with the time of linear N uptake (V10– R2) by the corn crop. Cumulative mineralization values showed that the soil released a total of 112 kg N ha^{-1} when nodulated soybean was the previous crop, compared to 92 kg N ha^{-1} for corn following non-nodulated soybean and 72 kg N ha^{-1} for corn following corn (Gentry et al., 2001). While there is a general consensus that application of N fertilizer on legume species is not needed, studies have been conducted to see what impacts N fertilization might have on soybeans. Gelfand and Robertson (2015) reported that N fertilization reduced the amount of N fixation and had no impact on yields. These authors accessed the effects of N application on biological N fixation and found that the contribution of biologically

fixed N to grain N. vegetative aboveground biomass N, and root N decreased linearly with increased applications of N.

Importance of Cover Crops in Low Residue Yielding Cropping Systems

Decomposition rates of crop residues is dependent on the C:N ratio of the residue. It is well established that the optimal C:N ratio in residue material is 30:1 (Vanhie et al., 2015). A C:N ratio above this level will result in immobilization of nutrients while a C:N ratio below the optimal will result in mineralization of N (Turmel et al., 2015). Residues with low C:N ratios contain more readily available compounds that decompose quicker than those with high C:N ratios (Thapa et al., 2021), while lignin and other complex organic compounds in plant residues are rate limiting in the later stages of litter decomposition (Mangalassery et al. 2015; Turmel et al., 2015).

Soybean produces only one-third the amount of residue as corn and, decomposes rapidly due to low lignin content and have lower C:N ratios than corn (Hsiao et al., 2019). The C:N ratio of corn residue is 47-57:1 (Vanhie et al., 2015), while the C:N ratio of soybean residues is generally 20:1 (Gentry et al., 2001). In a litterbag study in South America, Vachon and Oelbermann (2011) found that only 11% of crop residue C remained in soybean residues, while 19% remained in the corn residues following nine months of decomposition. Comparing crop residue N, these authors found that soybean residues had a significantly lower amount of N remaining compared with the corn residues during the same time period (9% vs. 27%, respectively). Burgess et al. (2001) found that surface residues of corn lost 40% of the initial mass after one year in a litterbag study in Southwestern Quebec.

Due to the increased decomposition rate of soybean residues compared to corn, the introduction of a cover crop in soybean-corn rotations can potentially be beneficial to promote a

more sustainable agroecosystem. Presence of cover crops can increase the rate of decomposition of soybean residue, but this effect can be nullified due to the amount of residue that is generated by the cover crop, which can lead to greater residue inputs overall (Varela et al., 2014). Cereal rye is the most winter-hardy of all small grains and is often chosen as an overwintering cover crop in corn-soybean rotations in the Midwest United States (Moore et al., 2014). However, there are concerns about planting non-legume cover crops ahead of corn, as yields are generally negatively impacted. In a study that analyzed impacts of cover crops on corn yield in Midwest crop agroecosystems, Qin et al. (2021) reported the impact of cereal rye termination date on corn yield and found yield decreases of 6.4% and 3% when termination was one day before corn planting or one month, respectively. Immobilization of N can occur after a non-legume cover crop is terminated and can limit N uptake by corn. Cereal rye residues have higher C:N ratios compared to corn and the slower decomposition, compared to soybean, can facilitate N immobilization (Sievers and Cook, 2018). However, N immobilization due to cereal rye decomposition might not result in less N available for soybeans since soybeans can generally overcome the limitation due to biological N fixation.

No-till and Nutrient Stratification

No-till practices result in increased residue retention on soil surface that will translate to greater accumulation of organic inputs in the soil (Malobane et al., 2020), thus increasing the amount of substrate that is available to the microbial community (Balota et al., 2004a). The decomposition of residue on soil surface will promote accumulation of SOM that can stimulate the microbial community (Green et al., 2007; Nivelle et al., 2016). Benefits of residue retention on the soil surface includes improved soil structure, increased SOM, increased soil microbial community activity, and increased nutrient availability (Turmel et al., 2015; Wegner et al.,

2018). Tillage and residue retention practices go hand-in-hand, as conventional tillage buries surface residues deeper in the soil profile (incorporating the residues), potentially stimulating the microbial community at the subsurface level, while no-till systems benefits are generally reported for the soil surface (Turmel et al., 2015). The accumulation of crop residues at the soil surface in no-till systems can result in isolation of crop residues in the surface and leads to nutrient stratification (Franzluebbers 2002). Stratification of dynamic soil health indicators also can occur in no-till systems (Green et al., 2007).

Soil Health Indicators

Soil health indicators are measurements that are sensitive to changes in physical, chemical, and biological functioning under various management strategies. Criteria that are needed to determine effective soil health indicators includes sensitivity to variations in soil management, correlations to other indicators that can be used to predict soil functioning, repeatability in analyses, and are cost and user-ease (Laishram et al., 2012; Hurisso et al., 2018a). Total SOM and total soil organic C (SOC) content are the most widely measured indicators of soil health (Bunemann et al., 2018). However, changes in overall SOM are hard to detect due to the size and quantification of SOM, instead measurements of specific fractions of SOM have been suggested, including hot water extractable C, permanganate oxidizable C (active C), and particulate organic matter (Bunemann et al., 2018). The active or biological-active pool of SOM is small, but extremely important because it reflects recent C additions as well as the size and composition of the microbial community (Culman et al., 2021). Permanganate oxidation mimics microbial decomposition of organic matter (Christy et al., 2022). Active C is an operationally-defined pool of easily-oxidizable C; with Weil et al. (2003) defining active C as a labile pool of C. Culman et al. (2012) found that active C was closely related with smaller and

heavier particulate organic C fractions, indicating that active C pool reflects a relatively processed or stabilized pool of active soil C. Microbial biomass is mainly composed of bacteria and fungi and makes up <5% of SOM (Sparling, 1992). Since microbial biomass responds quickly to management changes compared to SOM, it is considered an important soil health indicator. Soil respiration measures carbon dioxide (CO_2) released from soil due to the decomposition of SOM and plant litter or through plant roots and soil fauna, and can be used to estimate biomass activity (Laishram et al., 2012).

The largest pool of organic N in soil is proteins, as measures of soil protein can reflect the size of the organic N pool being depolymerized and can serve as a reservoir of N that is subsequently released through mineralization (Roberts and Jones, 2008). The neutral citrate extraction method was first described by Wright and Upadhyaya (1996) and thought to measure glomalin produced by arbuscular mycorrhizal fungi (AMF) (Hurisso et al., 2018b). It is now widely recognized that the extraction procedure extracts large quantities of non-mycorrhizal proteins (Redmile-Gordon et al., 2013), resulting in proteins extracted from this method to be operationally defined as glomalin related soil protein. Hurisso et al. (2018b) proposed that the pool of proteins extracted in the Wright and Upadhyaya (1996) method be referred as autoclaved citrate extractable (ACE) protein and viewed more broadly as a soil health indicator that reflects the primary pools of organically-bound N, and thus as a measure of potential available organic N.

Other important soil health indicators reflect soil chemical properties (Turmel et al., 2015; Crookston et al., 2021). Measures of nutrient availability are important chemical indicators of soil health. Included in these measures are dissolved total N and inorganic N pools as well as measures of organic and inorganic P. In comparison to other chemical extractants, citrate appears

to extract a more enzyme-labile pool of organic P, and is therefore more likely to be representative of organic P likely to be utilized by plants (Darch et al., 2016). Dissolved organic nutrients are often the dominant elemental pool in many soils, especially dissolved organic N and P (Jones and Willett, 2006).

Soil enzyme activities are an important measure of soil health because they provide a snapshot of catalysts for nutrient cycling. Enzyme production can be regulated by substrate availability, end-product repression, de-repression due to insufficient nutrient supply, and constitutive production (Geisseler et al., 2010). Soil pH can affect enzyme activities because the sensitivity of amino acid functional groups can alter binding and catalysis of these amino acids (Dick et al., 2000). β-glucosidase hydrolyzes oligosaccharides and releases monosaccharides and catalyzes the final step in the breakdown of cellulose (Luo et al., 2017). β-N-acetyl-β-glucosaminidase catalyzes the terminal reaction in cellulose degradation and can also facilitate the release of carbon. β-glucosaminidase also plays a pivotal role in the mineralization and cycling of N in soil. Phosphatases hydrolyze phosphomonoesters and phosphodiesters that release inorganic P. There are two types of phosphatase enzymes found in soil (acid and alkaline) which are expressed at different pHs. Alkaline phosphatase is derived solely from microorganisms since plants are not known to produce these enzymes (Tabatabai, 1994).

Correlations between different soil health indicators and SOM, nutrient availability, other indicators, and climatic variables are commonly reported in literature. SOM has been reported to be positively correlated with active C (Nunes et al., 2018; Hsiao et al., 2019; Crookston et al., 2021), soil enzyme activities (Luo et al., 2017; Tyler, 2020), microbial biomass (Nair and Ngouajio, 2012), soil respiration (Fine et al., 2017), and ACE protein (Nunes et al., 2018). Significant correlations between SOC and active C (Culman et al., 2012), soil enzyme activities

(Balota et al., 2004b; Gong et al., 2015), and soil respiration (Bastida et al., 2006) have been reported. Reported positive correlations with N availability in soil and various soil health indicators includes active C (Nunes et al., 2018), soil respiration (Nunes et al., 2018), β-glucosidase activity (Green et al., 2006; Gong et al., 2015) and acid phosphatase (de la Paz Jimenez et al., 2002; Green et al., 2006). Relationships between available N and β-glucosaminidase activity are inconsistently reported, as both positive and negative effects on activity can occur with increased N availably (Luo et al., 2017). While it has been widely reported that phosphatase activity is negatively correlated with soil P availability (Sinsabaugh and Follstad Shah, 2012; Fernandez et al., 2016), other studies have shown positive relationships (Shi et al., 2013; Gong et al., 2015; Brennen and Acosta-Martinez, 2019) or no significant relationship between available P and phosphatase activity (Wick et al., 2002; Balota et al., 2004b; Margelef et al., 2017).

Different indicators of various C pools in soil are often interrelated, as significant positive correlations between soil respiration, active C, microbial biomass C, and dissolved organic C are often found (Lou et al., 2004; Wang et al., 2013; Nunes et al., 2018). Relationships between individual measures of C in soil and other soil health indicators include positive correlations between microbial biomass C and soil enzyme activity (Balota et al., 2004b; Laishram et al., 2012; Tyler, 2020), active C and ACE protein (Nunes et al., 2018), and dissolved organic N and respiration (Iqbal et al., 2010). Soil enzyme activities are often positively correlated with each other (Smith et al., 2015; Fernandez et al., 2016; Hsiao et al., 2019). However, there are reported cases in which this is not true, as Carpenter-Boggs et al. (2003) found that alkaline phosphatase activity was not significantly correlated with acid phosphatase activity in a study under various tillage managements in central South Dakota.

The impact of climatic factors have on soil health indicators cannot be understated as these relationships often dictate nutrient cycling in soil. McDaniel et al. (2014) conducted a meta-analysis on soil microbial biomass and organic matter dynamics and determined that mean annual temperature positively correlated with total C and microbial biomass N while mean annual precipitation positively correlated with total C and N as well as with microbial biomass N. Correlations between enzyme activities and climatic factors have yielded contradictory results. Baldrian et al. (2013) and Gong et al. (2015) determined that phosphatase activity was positively correlated to soil temperature. However, others have reported that β-glucosidase and phosphatase activities were not correlated with soil temperature (Zibilske and Makus, 2009; Jiao et al., 2011; Kotroczo et al., 2014). Kramer and Green (2000) found no correlation between phosphatase activities and soil temperature and soil moisture. Luo et al. (2017) reported that β-glucosidase, β-glucosaminidase, and phosphatase activity all had negative correlations with mean annual temperature, while Sinsabaugh et al. (2008) reported that β-glucosaminidase activity was positively correlated with mean annual temperature. The authors also reported that β-glucosidase and phosphatase activity both were positively correlated with mean annual precipitation.

Though soil health indicators can be an effective tool to assess soil quality, there are drawbacks that can limit the quantification and relevance of these indicators. The determination of indicator values often requires large amounts of labor, time, and costs. Many of the indicators proposed are not readily measured compared to SOM or pH. Many of the biological indicators give results that are contradictory and are not easily explained (Bunemann et al., 2018). Enzyme activities, in particular, are especially difficult to compare among studies due to lack of standard procedures and variable conditions such as optimal pH and substrate utilization (Nannipieri et

al., 2012). There is a need to determine adequate reference levels for soil health indicators in order to compare values.

Seasonal Dynamics of Soil Health Indicators

The importance of soil testing prior to nutrient application is crucial to ensure that enough fertilizer is applied to meet yield goals while reducing the risk of nutrient loss due to overapplication. Due to variations in climate and organic matter dynamics, no single sampling time can be recommended as the most appropriate for assessing the status of all soil biological properties (Liebig et al., 2006). Recommendations of soil sampling include both preplant and post-harvest sampling. Liebig et al. (2006) found that both preplant and post-harvest sampling yielded similar results as weather and soil conditions are most stable during these times of the year. However, Benintende et al. (2015) found that post-harvest samplings in the fall are preferred relative to spring preplant samplings as biological activity was most stable during the fall. Therefore, soil samples should be taken at the same time annually and weather conditions should be documented at the time of sampling.

In addition, seasonal fluctuations in biological analyses makes it difficult to determine an index of soil health (Bandick and Dick, 1999), as several factors can impact various soil properties. Differences in climatic factors such as temperature and moisture, geographic location, implementation of cover crops, tillage practices, residue management, and crop rotation all can have significant impacts on microbial activity. Therefore, direct comparison of trends and activities between studies can be difficult. For example, phosphatase enzymes are regulated by primarily microclimate and soil chemical factors, whereas lignocellulose degrading enzymes such as β-glucosidases are regulated by substrate availability (Sinsabaugh et al., 1992, Sinsabaugh et al., 1993; Boerner et al., 2005).

Reported trends over the growing season have been inconsistent for all soil health indicators. Hargreaves and Hofmockel (2014) found that β-glucosidase activity significantly increased from spring into mid-summer, followed by a decrease in activity into the late summer. Nevins et al. (2020) found that β-glucosidase activity linearly increased following cover crop termination (April to June), followed by a decrease until harvest in Indiana, in a cover crop residue study. These examples are consistent with other reports that β-glucosidase activity decreased throughout the summer growing season (Zibilske and Mankus, 2009; Kotroczo et al., 2014; Singh and Kumar, 2020; Tyler, 2020). Other studies have shown that β-glucosidase activity was either relatively constant during the growing season (Debosz et al., 1999; McDaniel and Grandy, 2016) or the β-glucosidase activity was inconsistent. Geisseler and Horwath (2009b) found that β-glucosidase activity tended to decrease in the summer under standard tillage practices, while activity tended to increase through the summer in conservative tillage. Hsiao et al. (2019) found that β-glucosidase activity tended to increase from October to February during winter wheat and fallow portions of two corn-winter wheat-soybean-fallow cropping systems, and showed inconsistent results during the summer growing season. For β-glucosaminidase activity trends, McDaniel and Grandy (2016) reported that β-glucosaminidase activity increased over the growing season in five different cropping rotations in Michigan, while Hsiao et al. (2019) reported that β-glucosaminidase activity tended into increase from October to April in winter wheat, then decline during soybean growth from August to October. Hargreaves and Hofmockel (2014) found that β-glucosaminidase activity significantly increased from spring until mid-summer, followed by a decrease in activity into the late summer.

Phosphatase activities showed similar inconsistencies as other soil health indicators. McDaniel and Grandy (2016) found that acid phosphatase activity increased from spring to

summer, then decreased from summer to fall in various cropping systems in Michigan, with activity being 25% and 99% higher in the summer compared to fall and spring, respectively. Tyler (2020) and Hsiao et al. (2019) reported inconsistencies within the growing season of acid phosphatase activity, in studies from Mississippi and Kansas, respectively. Kotroczo et al. (2014) and Singh and Kumar (2020) found that acid phosphatase activity decreased throughout the growing season. However, while Kramer and Green (2000) also observed a decrease in acid phosphatase activity during the growing season, the authors noted an increase from fall to winter in one-seed juniper-galleta woodland in northern Arizona. In both organic and conventionally managed olive oil farms in southern Spain, Garcia-Ruiz et al. (2009) found that alkaline phosphatase activity increased from spring into fall, regardless of tillage practices, fertilizer applications, or weed residue management. Kramer and Green (2000) observed that alkaline phosphatase activity increased from fall to winter, decrease from spring into early summer, before decreasing in the summer in one-seed juniper-galleta woodland in northern Arizona. Zibilske and Mankus (2009) observed that alkaline phosphatase activity decreased over time from June to August.

Microbial biomass variations in C, N, and P during the growing season have been reported in several studies. For microbial biomass C, Debosz et al. (1999) found various temporal trends in microbial biomass C, with concentrations tending to decrease during late summer/early fall. Tyler (2020) observed that microbial biomass C concentrations were inconsistent over a three-year period. However, microbial biomass C tended to increase during April and May. Singh and Kumar (2020) found that microbial biomass C increased from early spring into early fall in all rotations, tillage practices, and in both presence and absence of cover crops. Zibilske and Makus (2009) found that microbial biomass C tended to increase from June

into August in various mowing treatments of black oat cover crop. Spedding et al. (2004) found that microbial biomass C tended to slightly decrease through the summer. McDaniel and Grandy (2016) found that microbial biomass C tended to be twice as high in fall, compared to spring and summer in five different cropping rotations in Michigan. Jiang et al. (2013) found that microbial biomass C concentrations decreased from October to January, increased from January to Apr, then decreased from April to July.

For microbial biomass N, Spedding et al., 2004 and Tyler (2020) found that microbial biomass N was inconsistent during the growing season, while Geisseler and Horwath (2009a) found microbial biomass N decreased in the summer under standard tillage practices, and increased in the summer until the later part of the growing season under conservational tillage practices in a corn-tomato rotation in Davis, California. Singh and Kumar (2020) found that microbial biomass N initially increased from early spring into June, then decreased until the fall in all rotations, tillage management, and both the presence and absence of cover crops, while McDaniels and Grandy (2016) found that microbial biomass N tended to decreased from spring into summer, before increasing in fall. For microbial biomass P, Halecki and Gasiorek (2015) found that microbial biomass P increased from spring into summer, then decreased from summer to fall human-altered urban soils in southern Poland, while Ullah et al. (2013) observed highest concentrations of microbial biomass P in summer compared to spring and fall in both winter wheat-corn and winter wheat-mungbean cropping systems in northern Pakistan.

Novara et al. (2020) found that NO_3-N concentrations increased from March into July in soils, regardless of cover crop presence or tillage management. McDaniel and Grandy (2016) found that NO_3-N concentrations increased from spring into summer, then decreased from summer into fall. Omer et al. (2018) found NO_3-N concentrations decreased from fall to spring

from 20.65 to 7.52 mg kg^{-1}, then increased to 15.68 mg kg^{-1} in the summer. McDaniel and Grandy (2016) found that NH_4-N concentrations increased from spring into summer, then decreased from summer into fall. Burke et al. (2019) found that NH_4-N concentrations increased during the fall, decreased through the winter, then increased again in the spring. Zibilske and Mankus (2009) observed that total inorganic N concentrations were drastically reduced from early June into late June. Burke et al. (2019) found that total inorganic N concentrations showed various trends, but tended to decrease in the fall and winter and then increase in the spring. Spedding et al. (2004) found inconsistent concentrations of dissolved organic N during the growing season in a crop residue experiment in Quebec. McDaniel and Grandy (2016) found that dissolve organic N concentrations were six times higher in summer than in spring and fall.

For active C trends over the growing season, concentrations increased during the fall and winter months (Burke et al., 2019; Hsiao et al., 2019) but inconsistencies were observed for active C trends in the summer with Burke et al. (2019) noting that concentrations tended to increase in the summer growing season, while Hsiao et al. (2019) found that different crop sequences had different trends. Omer et al. (2018) found that active carbon significantly decreased from fall (508.96 mg kg^{-1}) to summer (361.7 mg kg^{-1}). Rochette et al. (1991) found that soil respiration was highest in periods of higher soil temperature and plant growth. Respiration tended to decrease later in the growing season (late summer). Hargreaves and Hofmockel (2014) found that soil respiration was consistent from spring into late summer. Zibilske and Mankus (2009) found that dissolved organic C concentrations were inconsistent over time, with concentrations decreasing from early June into late June, then increasing into August. McDaniel and Grandy (2016) found that dissolved organic C concentrations increased from spring until fall, with highest concentrations observed in the fall.

Research Rational

While research exists supporting the benefits and drawbacks of several phosphorus (P) fertilization approaches, the differences in these approaches and their impacts on the soil microbial community has not been well established. This research has the unique opportunity to provide data on the effects of each fertilization approach in comparison to each other. This work seeks to understand how the microbial community responds to a range of soil P management approaches including build up and maintenance, draw down phase of sufficiency, and no P additions. Does a production system with P managed at the margins of deficiency or under a no P addition scenario enhance nutrient cycling? Does excess application of P suppress microbial activity and function or promote luxury consumption of P? In addition, this research will also provide insight to the response of the soil microbial community to the presence of a cover crop. How do residues left from the previous cover crop influence microbial activity and function? Is there an increase in microbial community dynamics as the cover crop is decomposing? How does weather influence decomposition rates and microbial response? Do cover crops support greater soil enzyme activities and provide additional above- and belowground carbon inputs that can stimulate the microbial community? The simultaneous response of the microbial community to the presence of cover crops under different P fertilization regimes can provide insights on enhanced nutrient cycling in response to P availability. Will the presence of cover crops enhance nutrient cycling when the systems are at the margins of deficiency?

In relation to fertilization approach and cover crop implementation, seasonal trends in various soil health indicators are important. While there are many studies that analyze trends during the growing season or even the whole year, the results are often contradictory due to variations in climate, amount of plant residue, tillage practices, and cropping rotation. This study

will provide one of the first reports on how soil health indicators vary seasonally, while also looking at the response of P fertilization approaches and cover crop implementation during the growing season. This work aims to understand how the microbial community responds to seasonal variations in climate, while also examining the influence of cover crop decomposition and nutrient release and P fertilizer approaches on microbial activity and function. How does cover crop decomposition affect microbial activity and function and does the response vary annually? When during ta crop growing season is there a decline in microbial response? Are the seasonal dynamics of soil health indicators similar between years? Are there certain indicators that are more robust during the growing season?

This research was conducted at the Kansas Agricultural Watershed (KAW) field research facility at the Ashland Bottoms Research Farms, Kansas State University, Manhattan, KS. This research aimed to analyze the impact of P fertilization approach and cover crop usage on microbial activity and function while determining seasonal trends in microbial activity and function in response the P fertilization approach and cover crop usage during both corn and soybean phases of the crop rotation. The finding of this research will contribute to the following objectives:

Microbial response to P fertilization approaches and cover crops

Hypothesis 1

1. Cover crops will result in increased microbial activity and labile nutrient concentrations.
2. The sufficiency approach will enhance nutrient cycling (as measured by soil enzyme activities) when a system is at the margins of deficiency.
3. In addition, the presence of cover crops will promote enhanced nutrient cycling when a system is that the margins of deficiency.

Objective 1

To assess the impacts of P fertilizer management approaches and cover crops on soil microbial activity and function.

Approach 1

This hypothesis was tested using several different soil health indicators as a measurement for nutrient cycling in all plots at the KAW. These include different indicators of N pools in soil (autoclavable citrate extractable protein (ACE), dissolved organic N, dissolved total N, and inorganic N (NO_3-N, and NH_4-N), carbon pools (active C and dissolved organic C), citrate-extractable P, microbial biomass (microbial biomass C, N, and P), soil enzyme assays (acid and alkaline phosphatase, β-glucosaminidase, and β-glucosidase), and soil respiration.

Seasonal trends of various soil health indicators during cover crop decomposition

Hypothesis 2

1. Sampling time will greatly influence all measures of soil health.
2. Cover crop decomposition and variations in soil temperature and moisture throughout the growing season will influence seasonal trends.

Objective 2

To assess seasonal dynamics in soil health indicators

Approach 2

Various soil health indicators were measured 20-, 40-, 60-, and 80-days post cover crop termination. Soil samples taken before cover crop termination helped establish a baseline in values. Extractable nutrients, soil enzyme activities, active C, microbial biomass C, N, and P, soil respiration and ACE protein before cover crop termination and at the five in season sampling times.

Differences in dynamic soil health indicators due to sampling depth

Hypothesis 3

Microbial activity will decrease in subsurface soils due to nutrient stratification are a result of no-till management.

Objective 3

To assess the effect of sampling depth on dynamics soil health indicators

Approach 3

This hypothesis was tested by analysis of active C, ACE protein, soil enzyme activities, and soil respiration at sampling depths of 0-5 cm, 5-10 cm, and 10-15 cm.

References

Adetunji, A.T., Ncube, B., Mulidzi, R., Lewu, F.B., 2020. Management impact and benefit of cover crops on soil quality: a review. Soil Till. Res. 204, 104717.

Agomoh, I.V., Drury, C.F., Yang, X., Phillips, L.A., Reynolds, W.D., 2021. Crop rotation enhances soybean yields and soil health indicators. Soil Sci. Soc. Am. J. 85. 1185-1195.

Alhameid, A., Singh, J., Sekaran, U., Kumar, S., Singh, S., 2019. Soil biological health: influence on crop rotational diversity and tillage on soil microbial properties. Soil Sci. Soc. Am. J. 83, 1431-1442.

Bakker, M.G., Acharya, J., Moorman, T.B., Robertson, A.E., Kaspar, T.C., 2016. The potential for cereal rye cover crops to host corn seedling pathogens. Phytopathology 106, 591–601.

Baldrian, P., Snajdr, J., Merhautova, V., Dobiasova, P., Cajthaml, T., Valaskova, V., 2013. Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biol. Biochem. 56, 60-68.

Balota, E.L., Filho, A.C., Andrade, D.S., Dick, R.P., 2004a. Long-term tillage and crop rotation effects on microbial biomass and C and N mineralization in a Brazilian Oxisol. Soil Till. Res. 77, 137-145.

Balota, E.L., Kanashiro, M., Filho, A.C., Andrade, D.S., Dick, R.P., 2004b. Soil enzyme activities under long-term tillage and crop rotation systems in subtropical agro-ecosystems. Brazilian J. Microbiol. 35, 300-306.

Bandick, A.K., and Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biol. Biochem. 31, 1471-1479.

Barzman, M., Bàrberi, P., Birch, A.N.E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J.E., Kiss, J., Kudsk, P., Lamichhane, J.R., Messéan, A., Moonen, A.-C., Ratnadass, A., Ricci, P., Sarah, J.-L., Sattin, M., 2015. Eight principles of integrated pest management. Agron. Sustain. Dev. 35, 1199–1215.

Bastida, F., Moreno, J.L., Hernández, T., García, C., 2006. Microbiological activity in a soil 15 years alter its devegetation. Soil Biol. Biochem. 38, 2503–2507.

Beauregard, M.S., Hamel, C. and St-Arnaud, M., 2010. Long-term phosphorus fertilization impacts soil fungal and bacterial diversity but not AM fungal community in alfalfa. Microb. Ecol. 59. 379-389.

Benintende, S., Benintende, M., Sterren, M., Saluzzio, M. and Barbagelata, P., 2015. Biological variables as soil quality indicators: effect of sampling time and ability to classify soils by their suitability. Ecol. Indic. 52, 147-152.

Blanco-Canqui, H., and Ruis, S.J., 2018. No-tillage and soil physical environment. Geoderma 326, 164-200.

Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A., Hergert, G.W., 2015. Cover crops and ecosystem services: insights from studies in temperate soils. Agron. J. 107, 2449-2474.

Boerner, R.E.J., Brinkman, J.A., Smith, A., 2005. Seasonal variations in enzyme activity and organic carbon in soil of a burned and unburned hardwood forest. Soil Biol. Biochem. 37, 1419-1426.

Brady, N.C., and Weil, R.R., 2008. The nature and properties of soils (Vol. 14). Upper Saddle River, NJ: Prentice Hall.

Brennan, E.B., and Acosta-Martinez, V., 2017. Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production. Soil Biol. Biochem. 109, 188-204.

Brennan, E.B., and Acosta-Martinez, V., 2019. Cover crops and compost influence soil enzymes during six years of tillage-intensive organic vegetable production. Soil Sci. Soc. Am. J. 83, 624-637.

Bunemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mader, P., Pulleman, M., Sukkel, W., van Groenigen, J.W., Brussaardm, L., 2018. Soil quality-a critical review. Soil Biol. Biochem. 120, 105-125.

Burgess, M.S., Mehuys, G.R., Madramootoo, C.A. 2001. Decomposition of grain-corn residues (Zea mays L.): A litterbag study under three tillage systems. Can. J. Soil Sci. 82, 127-138.

Burke, J.A., Lewis, K.L., Richie, G.L., Moore-Kucera, J., DeLaune, P.B., Keeling, J.W., 2019. Temporal variability of soil carbon and nitrogen in cotton production on the Texas high plains. Agron. J. 111, 2218-2225.

Caldwell, B.A., 2005. Enzyme activities as a component of soil biodiversity: a review. Pedobiologia 49, 637-644.

Carpenter-Boggs, L., Stahl, P.D., Lindstrom, M.J., Schumacher, T.E., 2003. Soil microbial properties under permanent grass, conventional tillage, and no-till management in South Dakota. Soil Till. Research 71, 15-23.

Chavarría, D.N., Verdenelli, R.A., Serri, D.L., Restovich, S.B., Andriulo, A.E., Meriles, J.M., Vargas-Gil, S., 2016. Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability. Eur. J. Soil Biol. 76, 74-82.

Cheng, H., Yuan, M., Duan, Q., Sun, R., Shen, Y., Yu, Q. and Li, S., 2020. Influence of phosphorus fertilization patterns on the bacterial community in upland farmland. Ind. Crops Prod., 155, p.112761.

Christy, I., Moore, A., Myrold, D., Kleber, M., A mechanistic inquiry into the applicability of permanganate oxidizable carbon as a soil health indicator. Soil Sci. Soc. Am. J. doi:10.1002/saj2.20569

Ciampitti, I.A., and Salvagiotti, F., 2018. New insights into soybean biological nitrogen fixation. Agron. J. 100, 704-710.

Cleveland, C.C., and Liptzin, D., 2006. C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochem. 85, 235-252.

Cordova, S.C., Castellano, M.J., Dietzel, R., Licht, M.A., Togliatti, K., Martinez-Feria, R., Archontoulis, S.V., 2019. Soybean nitrogen fixation dynamics in Iowa, USA. Field Crops Res. 236, 165-176.

Crookston, B.S., Yost, M.A., Bowman, M., Veum, K., Cardon, G., Norton, J., 2021. Soil health spatial-temporal variation influence soil security on Midwestern, U.S. farms. Soil Security 3, 100005

Culman, S.W., Hurisso, T.T., Wade, J., 2021. Permanganate oxidizable carbon: An indicator of biologically active soil carbon. Soil Health Series: Volume 2 Laboratory Methods for Soil Health Analysis, pp.152-175.

Culman, S.W., Snapp, S.S., Freeman, M.A., Schipanski, M.E., Beniston, J., Lal, R., Drinkwater, L.E., Franzluebbers, A.J., Glover, J.D., Grandy, A.S., Lee, J., Six, J., Maul, J.E., Mirsky, S.B., Spargo, S.B., Wander, M.M., 2012. Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management. Soil Sci. Soc. Am. J. 76, 494-504.

Daniel, T.C., Sharpley, A.N., Edwards, D.R., Wedepohl, R., Lemunyon, J.L., 1994. Minimizing surface water eutrophication from agriculture by phosphorus management. J. Soil Water Conserv. 49, 30-38.

Daniel, T.C., Sharpley, A.N., Lemunyon, J.L., 1998. Agricultural phosphorus and eutrophication: a symposium overview. J. Environ. Qual. 27, 251–257.

Darch, T., Blackwell, M.S.A., Chadwick, D., Haygarth, P.M., Hawkins, J.M.B., Turner, B.L., 2016. Assessment of bioavailable organic phosphorus in tropical forest soils by organic acid extraction and phosphatase hydrolysis. Geoderma 284, 93-102.

De la Paz Jimenez, M., De la Horra, A.M., Pruzzo, L., Palma, R.M., 2002. Soil quality: a new index based on microbiological and biochemical parameters. Biol. Fert. Soils 35, 302–306.

Debosz, K., Rasmussen, P.H., Pedersen, A.R., 1999. Temporal variations in microbial biomass C and cellulolytic enzyme activity in arable soils: effects of organic matter input. Appl. Soil Ecol. 13, 209-218.

Doran, J.W., 2002. Soil heatlh and global sustainability: translating science into practice. Agric. Ecosys. Environ. 88, 119-127.

Dick, W.A., Cheng, L., Wang, P., 2000. Soil acid and alkaline phosphatase activity as pH adjustment indicators. Soil Biol. Biochem. 32, 1915-1919.

Feng, J., and Zhu, B., 2019. A global meta-analysis of soil respiration and its components in response to phosphorus addition. Soil Biol. Biochem. 135, 38-47.

Fernandez, A.L., Sheaffer, C.C., Wyse, D.L., Staley, C., Gould, T.J., Sadowsky, M.J., 2016. Associations between soil bacterial community structure and nutrient cycling functions in long-term organic farm soils following cover crop and organic fertilizer amendment. Sci. Total Environ. 566-567, 949-959.

Fine, A.K., van Es, H.M., Schindelbeck, R.R., 2017. Statistics, Scoring Functions, and Regional Analysis of a Comprehensive Soil Health Database. Soil Sci. Soc. Am. J. 81, 589-601.

Franzluebbers, A.J., 2002. Soil organic matter stratification ratio as an indicator of soil quality. Soil Till. Res. 66, 95-106.

Garcia-Ruiz, R., Ochoa, V., Vinegla, B., Hinojosa, M.B., Pena-Santiago, R., Liebanas, G., Linares, J.C., Carreira, J.A., 2009. Soil enzymes, nematode community and selected physio-chemical properties as soil quality indicators in organic and conventional olive oil farming: influence of seasonality and site features. Appl. Soil Ecol. 41, 305-314.

Geisseler, D., and Horwath, W.R., 2009a. Relationship between carbon and nitrogen availability and extracellular enzyme activities in soil. Pedobiologia 53, 87-98.

Geisseler, D., and Horwath, W.R., 2009b. Short-term dynamics of soil carbon, microbial biomass, and soil enzyme activities as compared to longer-term effects of tillage in irrigated row crops. Biol. Fertil. Soils 46, 65-72.

Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B., 2010. Pathways of nitrogen utilization by soil microorganism-a review. Soil Biol. Biochem. 42, 2058-2067.

Gelfand, I., and Robertson, G.P., 2015. A reassessment of the contribution of soybean biological nitrogen fixation to reactive N in the environment. Biogeochem. 123, 175-184.

Gentry, L.E., Below, F.E., David, M.B., Bergerou, J.A., 2001. Source of the soybean N credit in maize production. Plant Soil 236, 175-184.

Gong, S., Zhang, T., Guo, R., Cao, H., Shi, L., Guo, J., Sun, W., 2015. Response of soil enzyme activity to warming and nitrogen addition in a meadow steppe. Soil Res. 53, 242-252.

Grassini, P., Specht, J.E., Tollenaar, M., Ciampitti, I., Cassman, K.G., 2015. High-yield maize-soybean cropping systems in the US Corn Belt. In: Sadra, V.O., Calderini, D.F. (Eds) Crop Physiology-Applications for Genetic Improvements and Agronomy., second ed. Elsevier, Netherlands

Green, V.S., Stott, D.E., Cruz, J.C., Curi, N., 2007. Tillage impacts on soil biological activity and aggregation in a Brazilian Cerrado Oxisol. Soil Till. Res. 92, 114-121.

Guo, H. He, X., Li, Y., 2012. Spatial distribution of arbuscular mycorrhiza and glomalin in the rhizosphere of Caragana korshinskii Kom. in the Otindag sandy land, China. Afr. J. Microbiol. Res. 6, 5745-5753.

Guppy, C.N., Menzies, N.W., Moody, P.W., Blamey, F.P.C., 2005. Competitive sorption reactions between phosphorus and organic matter in soil: a review. Soil Res. 43, 189-202.

Halecki, W., Gasiorek, M., 2015. Seasonal variability of microbial biomass phosphorus in urban soils. Sci Total Environ. 502, 42-47.

Hallama, M., Pekrun, C., Lambers, H., Kandeler, E., 2019. Hidden miners-the roles of cover crops and soil microorganisms in phosphorus cycling. Plant Soil 434, 7-45.

Hallama, M., Pekrun, C., Mayer-Gruner, P., Uksa, M., Abdullaeva, Y., Pilz, S., Schloter, M., Lambers, H., Kandeler, E., 2022. The role of microbes in the increase of organic phosphorus availability in the rhizosheath of cover crops. Plant Soil 476, 353-373.

Hansen, N.C., Daniel, T.C., Sharpley, A.N., Lemunyon, J.L., 2002. The fate and transport of phosphorus in agricultural systems. J. Soil Water Conserv. 57, 408-417.

Hargreaves, S.K., and Hofmockel, K.S., 2014. Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. Biogeochem. 117, 67-79.

Hesterman, O.B., Russelle, M.P., Sheaffer, C.C., Heichel, G.H., 1987. Nitrogen utilization from fertilizer and legume residues in a legume-corn rotation. Agron. J. 79, 726-731.

Hsiao, C-J., Sassenrath, G.F., Zeglin, L.H., Hettiarachchi, G.M., Rice, C.W., 2019. Temporal variation of soil microbial properties in a corn-wheat-soybean system. Soil Biol. Biochem. 83, 1696-1711.

Hunter, P.J., Teakle, G.R., Bending, G.D., 2014. Root traits and microbial community interactions in relation to phosphorus availability and acquisition, with particular reference to Brassica. Front. Plant Sci. 5, 27.

Hurisso, T.T., Culman, S.W., Zhao, K., 2018a. Repeatability and Spatiotemporal Variability of Emerging Soil Health Indicators Relative to Routine Soil Nutrient Tests. Soil Sci. Soc. Am. J. 82, 939-948.

Hurisso, T.T., Moebius-Clune, D.J., Culman, S.W., Moebius-Clune, B.N., Thies, J.E.,van Es, H.M., 2018b. Soil protein as a rapid soil health indicator of potentially available organic nitrogen. Agric. Environ. Lett. 3, 180006.

Iqbal, J., Hu, R., Feng, M., Lin, S., Malghani, S., Ali, I.M., 2010. Microbial biomass, and dissolved organic carbon and nitrogen strongly affect soil respiration in different land uses: a case study at Three Gorges Reservoir Area, South China. Agricult. Ecosy. Environ. 137, 294e307

Jiang, X., Shi, X., Wright, A.L., 2013. Seasonal variability of microbial biomass associated with aggregates in a rice-based ecosystem. Eur. J. Soil Biol. 56, 84-88.

Jiao, X-G., Gao, C-S., Lu, G-H., Sui, Y-Y., 2011. Effect of long-term fertilization on soil enzyme activities under different hydrothermal conditions in Northeast China. J. Integr. Agric. 10, 412-422.

Jones, D.L. and Willett, V.B. 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem. 38, 991-999.

Kleinman, P.J.A., Sharpley, A.N., Moyer, B.G., Elwinger, G.F., 2002. Effect of mineral and manure phosphorus sources on runoff phosphorus. J. Environ. Qual. 31, 2026-2033.

Kleinman, P.J.A., Sharpley, A.N., Saporite, L.S., Buda, A.R., Bryant, R.B., 2008. Application of manure to no-till soils: phosphorus losses by sub-surface and surface pathways. Nutr. Cycl. Agroecosyst. 84, 215-227.

Kotroczo, Z., Veres, Z., Fekete, I., Krakomperger, Z., Attila Toth, J., Lajtha, K., Tothmeresz, B., 2014. Soil enzyme activity in response to long-term organic matter manipulation. Soil Biol. Biochem. 70, 237-243.

Kramer, S., and Green, D.M., 2000. Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in a semiarid woodland. Soil Biol. Biochem. 32, 179-188.

Laishram, J., Saxena, K.G., Maikhuri, R.K., Rao, K.S., 2012. Soil quality and soil health: a review. Int. J. Ecol. Environ. Sci. 38, 19-37.

Lehman, R.M., Cambardella, C.A., Stott, D.E., Acosta-Martinez, V., Manter, D.K., Buyer, J.S., Maul, J.E., Smith, J.L., Collins, H.P., Halvorson, J.J., Kremer, R.J., Lundgren, J.G., Ducey, T.F., Jin, V.L., Karlen. D.L., 2015.Understanding and enhancing soil biological health: The solution for reversing soil degradation. Sustainability 7, 988-1027.

Lehmann, J., Lan, Z., Hyland, C., Sato, S., Solomon, D., Ketterings, Q., 2005. Long-term dynamics of phosphorus forms and retention in manure-amended soils. Environ. Sci. Technol. 39,6672-6680.

Leikam, D.F., Lamond, R.E., Mengel, D.B., 2003. Providing flexibility in phosphorus and potassium fertilizer recommendations. Better Crops 87, 6-10

Liebig, M., Carpenter-Boggs, L., Johnson, J.M.F., Wright, S., Barbour, N., 2006. Cropping system effects on soil biological characteristics in the Great Plains. Renew. Agric. Food Syst. 21, 36-48.

Lou, Y., Li, Z., Zhang, T., Liang, Y., 2004. CO2 emissions from subtropical arable soils of China. Soil Biol. Biochem. 36, 1835-1842.

Luo, L., Meng, H., Gu, J-D., 2017. Microbial extracellular enzymes in biogeochemical cycling of ecosystems. J. Environ. Manage. 197, 539-549.

Macnack, N., Khin Chim, B., Amedy, B., Arnall, B., 2011. Fertilization based on sufficiency, build-up and maintenance concept. Oklahoma Cooperative Extension Service Publication, PSS-2266.

Malobane, M.E., Nciizah, A.D., Nyambo, P., Mudau, F.N., Wakindiki, I.I.C., 2020. Microbial biomass carbon and enzyme activities as influenced by tillage, crop rotation and residue management in a sweet sorghum cropping system in marginal soils of South Africa. Heliyon 6, e05513.

Mangalassery, S., Mooney, S.J., Sparkes, Fraser, W.T., Sjogersten, S., 2015. Impacts of zero tillage on soil enzyme activities, microbial characteristics and organic matter functional chemistry in temperate soils. Eur. J. Soil Biol. 68, 9-17.

Margelef, O., Sardans, J., Fernandez-Martinez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., Goll, D., Aichter, A., Obersteiner, M., Asensio, D., Penuelas, J., 2017. Global patterns of phosphatase activity in natural soils. Scientific Rep.7, 1-13.

McDaniel, M.D., and Grandy, A.S., 2016. Soil microbial biomass and function are altered by 12 years of crop rotation. SOIL 2, 583-599.

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecol. Appl. 24, 560-570.

Moore, E.B., Wiedenhoeft, M.H., Kaspar, T.C., Cambardella, C.A., 2014. Rye Cover Crop Effects on Soil Quality in No-Till Corn Silage–Soybean Cropping Systems. Soil Sci. Soc. Am. J. 78, 968-976.

Nair, A., and Ngouajio, M., 2012. Soil microbial biomass, functional microbial diversity, and nematode community structure as affected by cover crops and compost in an organic vegetable production system. Appl. Soil Ecol. 58, 45-55.

Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccaanti, B., Masciandaro, G., Fornasier, F., Moscatelli, M.C., Marinari, S., 2012. Soil enzymology: classical and molecular approaches. Biol. Fertil. Soils 48, 743-762.

Nevins, C.J., Lacey, C., Armstrong, S., 2020. The synchrony of cover crop decomposition, enzyme activity, and nitrogen availability in a corn agroecosystem in the Midwest United States. Soil Till. Res. 197, 104518.

Nevins, C.J., Nakatsu, C., Armstrong, S., 2018. Characterization of microbial community response to cover crop residue decomposition. Soil Biol. Biochem. 127, 39-49.

Nivelle, E., Verzeaux, J., Habbib, H., Kuzyakov, Y., Decocq, G., Roger, D., Lacoux, J., Duclercq, J., Spicher, F., Nava-Saucedo, J.E., Catterou, M., 2016. Functional response of soil microbial communities to tillage, cover crops and nitrogen fertilization. Appl. Soil Ecol. 108, 147-155.

Novara, A., Catania, V., Tolone, M., Gristina, L., Laudicina, V.A., Quatrini, P., 2020. Cover crop impact on soil organic carbon, nitrogen dynamics and microbial diversity in a Mediterranean semiarid vineyard. Sustainability 12, 3256.

Nunes, M.R., van Es, H.M., Schindelbeck, R., Ristow, A.J.., Ryan, M., 2018. No-till and cropping system diversification improve soil health and crop yield. Geoderma 328, 30-43.

Omay, A.B., Rice, C.W., Maddux, L.D., Gordon, W.B., 1998. Corn yield and nitrogen uptake in monoculture and in rotation with soybean. Soil Sci. Soc. Am. J. 62, 1596-1603.

Omer, M., Idowu, O.J., Ulery, A.L., VanLeeuwen, D., Guldan, S.J., 2018. Seasonal changes of soil quality indictors in selected arid cropping systems. Agriculture 8, 124.

Qin, X., Huang, T., Lu, C., Dang, P., Zhang, M., Guan, X.K., Wen, P.F., Wang, T.C., Chen, Y., Siddique, K.H., 2021. Benefits and limitations of straw mulching and incorporation on maize yield, water use efficiency, and nitrogen use efficiency. Agric. Water Manag. 256, 107128.

Qiu, L., Lin, H., Song, B., Kong, T., Sun, W., Sun, X., Zhang, Y., Li, B., 2021. Glomalin-related soil protein (GRSP) in metal sequestration at Pb/Zn-contaminated sites. J. Soils Sed. 22, 577-593.

Redmile-Gordon, M.A., Armenise, E., White, R.P., Hirsch, P.R. and Goulding, K.W.T., 2013. A comparison of two colorimetric assays, based upon Lowry and Bradford techniques, to estimate total protein in soil extracts. Soil Biol. Biochem. 67, 166-173.

Reich, P.B., and Oleksyn, J., 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proc. Natl. Acad. Sci. USA 101, 11011-11006.

Roberts, P., and Jones, D.L., 2008. Critical evaluation of methods for determining total protein in soil solution. Soil Biol. Biochem. 40, 1485e1495.

Rochette, P., Desjardins, R.L., Pattey, E., 1991. Spatial and temporal variability of soil respiration in agricultural fields. Can. J. Soil Sci. 71, 189-196.

Schindler, D.W., 2006. Recent advances in the understanding and management of eutrophication. Limnol. Oceanogr. 51, 356–363.

Schipanski, M.E., Drinkwater, L.E., Russelle, M.P., 2010. Understanding the variability in soybean nitrogen fixation across agroecosystems. Plant Soil 329, 379-397.

Schneider, K.D., Thiessen Martens, J.R., Zvomuya, F., Reid, D.K., Fraser, T.D., Lynch, D.H., O'Halloran, I.P., Wilson, H.F., 2019. Options for improved phosphorus cycling and use in agriculture at the field and regional scales. J. Environ. Qual. 48, 1247-1264.

Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., Reddy, K.R., 1994. Managing agricultural phosphorus for protection of surface waters: issues and options. J. Environ. Qual. 23, 437-451.

Shi, Y., Lalande, R., Hamel, C., Ziadi, N., Gagnon, B., Hu, Z., 2013. Seasonal variation of microbial biomass, activity, and community structure in soil under different tillage and phosphorus management practices. Biol. Fertil. Soils 49, 803-818.

Shi, Y., Ziadi, N., Hamel, C., Bélanger, G., Abdi, D., Lajeunesse, J., Lafond, J., Lalande, R., Shang, J., 2020. Soil microbial biomass, activity and community structure as affected by mineral phosphorus fertilization in grasslands. Appl. Soil Ecol. 146, 103391.

Sievers, T., and Cook, R.L., 2018. Aboveground and root decomposition of cereal rye and hairy vetch cover crops. Soil Sci. Soc. Am. J. 82, 147–155.

Singh, J., and Kumar, S., 2020. Seasonal changes of soil carbon fractions and enzyme activities in response to winter cover crops under long-term rotation and tillage systems. Eur. J. Soil Sci. 1-14.

Sinsabaugh, R.L., and Follstad Shah, J.J., 2012. Ecoenzyme stoichiometry and ecological theory. Annu. Rev. Ecol. Evol. Syst. 43, 313-343.

Sinsabaugh, R.L., Antibus, R.K., Linkins, A.E., McClaugherty, C.A., Rayburn, L., Weiland, T., 1993. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecol. 74, 1586–1593

Sinsabaugh, R.L., Hill, B.H., Follstad Shah, J.J., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462, 795-798.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252-1264.

Smith, A.P., Marin-Spiotta, E., Balser, T., 2015. Successional and seasonal variations in soil and litter microbial community structure and function during tropical postagricultural forest regeneration: a multiyear study. Global Change Biology 21, 3532-3547.

Sparling, G.P., 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Aust. J. Soil Res. 30, 195-207.

Spedding, T.A., Hamel, C., Mehuys, G.R., Madramootoo, C.A., 2004. Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. Soil Biol. Biochem. 36, 499-512.

Strickland, M.S., Thomason, W.E., Avera, B., Franklin, J., Minick, K., Yamada, S., Badgley, B.D., 2019. Short-term effects of cover crops on soil microbial characteristics and biogeochemical processes across actively managed farms. Agrosyst. Geosci. Environ. 2, 180064.

Tabatabai, M.A., 1994. Soil enzymes. In: Weaver, R.W., Angle, J.S., Bottomly, P.S. (eds.). Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, USA. PP. 775-833.

Tang, X., Placella, S.A., Daydé, F., Bernard, L., Robin, A., Journet, E.P., Justes, E., Hinsinger, P., 2016. Phosphorus availability and microbial community in the rhizosphere of intercropped cereal and legume along a P-fertilizer gradient. Plant Soil, 407, 119-134.

Thapa, V.R., Ghimire, R., Acosta-Martinez, V., Marsalis, M.A., Schipanski, M.E., 2021. Cover crop biomass and species composition affect soil microbial community structure and enzyme activities in semiarid cropping systems. Appl. Soil Ecol. 157, 103735.

Turmel, M-S., Speratti, A., Baudron, F., Verhulst, N., Govaerts, 2015. Crop residue management and soil health: a systems analysis. Agricul. Sys. 134, 6-16.

Tyler, H.L., 2020. Winter cover crops and no till management enhance enzyme activities in soybean field soils. Pedobiologia 81-82, 150666.

Tyler, H.L., and Locke, M.A., 2019. Effects of weed management on soil ecosystems. In: Korres, N.E., Burgos, N.R., Duke, S.O. (Eds.), Weed Control: Sustainability Hazards, and Risks in Cropping Systems Worldwide. Taylor & Francis Group, Boca Raton, FL, pp. 32–61.

Ullah, R., Lone, M.I., Ullah, K.S., Mehdi, S.M., Qazi, M.A., 2013. Effect of cropping system and seasonal variations on soil microbial biomass and enzymatic activities in arid soils. Journal of Animal and Plant Sciences 23, 493-499.

Vachon, K., and Oelbermann, M., 2011. Crop residue input and decomposition in a temperate maize-soybean intercrop system. Soil Sci. 176, 157-163.

Vanhie, M., Deen, W., Lauzon, J.D., Hooker, D.C., 2015. Effects of increasing levels of maize (Zea mays L.) residue on no-till soybean (Glycine max Merr.) in Northern production regions: a review. Soil Till. Res. 150, 201-210.

Vanotti, M.B., and Bundy, L.G., 1995. Soybean effects on soil nitrogen availability in crop rotations. Agron. J. 87, 676-680.

Varela, M.F., Scianca, C.M., Taboada, M.A., Rubio, G., 2014. Cover crop effects on soybean residue decomposition and P release in no-tillage systems of Argentina. Soil Till. Res. 143, 59-66.

Wakelin, S., Mander, C., Gerard, E., Jansa, J., Erb, A., Young, S., Condron, L., O'Callaghan, M., 2012. Response of soil microbial communities to contrasted histories of phosphorus fertilisation in pastures. Appl. Soil Ecol. 61, 40-48.

Wang, Q., Xiao, F., He, T., Wang, S., 2013. Responses of labile soil organic carbon and enzyme activity in mineral soils to forest conversion in the subtropics. Annals of Forest Sci. 70, 579-587.

Wang, X., Cao, Q., Yang, W., Zhu, X., 2022. Spatial changes in glomalin-related soil protein and their correlation with soil properties in the black soil region of northeast China. Agronomy 12, 2165.

Warren, J., Raun, B., Zhang, H., Arnall, B., Penn, C., Bushlong, J., Abit, J., 2017. Oklahoma soil fertility handbook. Oklahoma Cooperative Extension Service. E-1039.

Wegner, B.R., Osborne, S.L., Lehman, R.M., Kumar, S., 2018. Sever-year impact of cover crops on soil health when corn residue is removed. Bioenergy Res. 11, 239-248.

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. Am. J. Alternative Agric. 18, 3–17.

Wick, B., Kuhne, R.F., Vielhauer, K., Vleck, P.L.G., 2002. Temporal variability of selected soil microbiological and biochemical indicators under different soil quality conditions in south-western Nigeria. Biol. Fertil. Soils 35, 155-167.

Wright, S.F., and Upadhyaya, A., 1996. Quantification of arbuscular mycorrhizal fungi activity by the glomalin concentration on hyphal traps. Mycorrhiza 8, 283–285.

Wu, F., Dong, M., Liu, Y., Ma, X., An, L., Young, J.P.W., Feng, H., 2011. Effects of long-term fertilization on AM fungal community structure and Glomalin-related soil protein in the Loess Plateau of China. Plant Soil, 342, 233-247.

Zhang, H., Antonangelo, J., Grove, J., Osmond, D., Slaton, N.A., Alford, S., Florence, R., Huluka, G., Hardy, D.H., Lessl, J., Maguire, R., 2021. Variation in soil-test-based phosphorus and potassium rate recommendations across the southern USA. Soil Sci. Soc. Am. J. 85, 975-988.

Zibilske, L.M., and Makus, D.J., 2009. Black oat cover crop management effects on soil temperature and biological properties on a Mollisol in Texas, USA. Geoderma 149, 379-385.

Chapter 2 - Seasonal dynamics and effect of phosphorus fertilizer and cover crops on biological soil health indicators during a corn year in a no-till corn soybean rotation

Abstract

Seasonal dynamics of soil health indicators are widely inconsistent in literature due to differences in climate, cover crops, crop rotations, and management practices. A study was initiated in 2020 to determine the effects of three phosphorus (P) fertilizer management approaches: (no P (NP), build and maintain (BAM), and sufficiency (SUF)), as well as the presence (CC)/absence (NC) of a cereal rye (*Secale cereale*) cover crop, on different soil health indicators. All treatments were balanced for nitrogen (N) inputs. Composite soil samples were collected (0-5 cm) six times during the 2021 corn growing season at the Kansas Agricultural Watershed Research Facility near Manhattan, KS. Sampling times included a sampling prior to cover crop termination (T0), four samplings during the corn growing season (T1-T4), and one sampling post-harvest (T5). Soil health indicators included measures of active nutrient pools (active carbon (C) and autoclaved citrate extractable (ACE) protein), as well as N (dissolved total N, inorganic N (NH_4-N and NO_3-N), and dissolved organic N) and P pools (citrate-extractable P). Indicators that assess microbial activity and function including microbial biomass C, N, and P, soil respiration, and four soil enzyme activities (β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase). A significant P treatment by sampling time interaction was observed for all soil N pools and followed similar trends in all P fertilizer treatments; showing an increase in concentrations from prior to termination until June (T3), followed by a decrease throughout the rest of the growing season. A significant P fertilizer treatment by cover crop

interaction was observed for alkaline phosphatase activity, as activity increased with the inclusion of a cover crop in both NP and SUF, while no increase was observed for the BAM treatment with the inclusion of a cover crop, suggesting that the inclusion of cover crops in P deficient systems can promote increase nutrient cycling. Measures of labile C and N, as well as measures of microbial function and activity responded positively to the inclusion of cover crops. No significant effect of cover crops was detected for soil N pools, suggesting that N fertilizer application possibly masked any measurable effects. Phosphorus availability dictated significant changes in ACE protein, dissolved organic N, and soil respiration, implying that plant productivity might have been responsible for these trends. Sampling time significantly impacted all measures of soil health in this study, however very few cover crop by sampling time interactions were observed, suggesting that seasonal dynamics were primarily driven by changes in precipitation and temperature. Increased nutrient cycling when the system is trending towards to margin of deficiency implies that systems can overcome limited nutrient additions and access residual nutrients that might not be readily available, thereby potentially reducing annual P fertilizer requirements.

Introduction

Seasonal fluctuations in biological analyses makes it difficult to assess soil health as several factors, including differences in climate (such as temperature and moisture), tillage practices, residue management strategies, and utilization of crop rotations and cover crops, can all have significant impacts on microbial activity and function. (Bandick and Dick, 1999). An assessment of seasonal trends for soil health indicators (including active carbon (C), soil protein, and soil respiration) and routine soil nutrient tests (Mehlich-3 extractable nutrients) during a corn growing season demonstrated the tendency for both biological and nutrient tests to vary over a

growing season (Hurisso et al., 2018a). Therefore, no single sampling time can be recommended as being the most appropriate for assessing the status of all soil biological properties (Liebig et al., 2006), underscoring the importance of soil sample collection and testing occurring at the same time annually (Hurisso et al., 2018a).

While phosphorus (P) is an essential nutrient for plant growth, it is often the most limiting nutrient as less than 1% of total soil P is in plant available forms (Hansen et al., 2002). Although 50-90% of P found in soil is in inorganic form, P fixation renders a majority of inorganic P immobile in soil (Daniel et al., 1994), therefore, supplemental amount of P must be added to meet crop demand. When P fertilizer is applied at excess rates, the risks of P loss through surface runoff and leaching are increased. Eutrophication is the process of enrichment of biological productivity due to excess amounts of nutrients that promotes a hypoxic environment for aquatic life causing great environmental concern (Sharpley et al., 1994). The amount of P lost to the environment is dependent on soil P concentrations, rate, timing and method of P fertilizer applications (Kleinman et al., 2002).

Soil testing is a crucial tool in reducing excess fertilizer application by quantifying the amount of nutrients in soil and matching fertilizer rates with crop demand (Ulrich-Schad et al., 2017). Phosphorus fertilizer recommendations from soil testing laboratories are based on two different management approaches; build and maintain (BAM) and sufficiency (SUF). In the BAM approach, soil test P is built up to a pre-determined critical level then maintained at the critical level with subsequent fertilizer applications equal the crop removal rates (Macnack et al., 2011). The SUF approach allows for just enough P fertilizer to be added to maximize profitability while maintaining 90-95% of maximum yield (Leikam et al., 2003). Only when a

crop response is expected (i.e., soil test is below the critical level) will P fertilizer be applied under the SUF approach.

Influences of P additions on biological soil health indicators have been reported. Microbial biomass P concentrations have been reported to increase with increasing P fertilizer application rates (Tang et al., 2016; Shi et al., 2020). In a meta-analysis of soil respiration response to P additions, it was observed that soil respiration increased by 31.7% in response to P additions in cropland (Feng and Zhu, 2019). Positive correlations between soil protein and P availability have also been reported in previous studies (Wu et al., 2010; Guo et al., 2012; Qiu et al., 2021; Wang et al., 2022), suggesting greater amounts of soil protein with increased P availability.

While intensified agricultural practices have resulted in increases in food production, the adverse effects of these practices on environmental health are becoming well known (Kopittke et al., 2019). Conservation practices such as cover crop implementation, crop rotations, and no-till management are known to increase soil health and build soil organic matter (Blanco-Canqui et al., 2011; Blanco-Canqui et al., 2015). Cover crops within a no-till system can enhance microbial activity and benefits are detectable more rapidly under no-till management (Blanco-Canqui et al., 2015; Alhameid et al., 2019). Above- and belowground inputs from cover crops can increase substrate availability for soil microorganisms and stimulate growth and microbial biomass (Blanco-Canqui et al., 2015; Chavarria et al., 2016; Muhammad et al., 2021).

The impacts of cover crops on the soil biological community has been widely reported in the literature. Strickland et al. (2019) found that cover crops increased bioavailable C by 37%, increase NH_4-N by 64%, and decreased NO_3-N by 30%. Brennan and Acosta-Martinez (2019) found that cover crops increased β-glucosidase and β-glucosaminidase activity at least 66% and

79%, respectively, while Chavarria et al. (2016) found that acid phosphatase activities were between 12.1 and 21.9% higher in cover cropped treatments. Nunes et al. (2018) found that cover crops increased soil respiration by 30% and autoclaved citrate extractable (ACE) protein content by 8%.

More than 85% of corn and soybeans grown in the Unites States is produced in the Corn Belt. Of the 85%, more than 65% of the corn and soybeans are grown in a 2-year crop rotation (Grassini et al., 2015). Though variation can occur between years due to environmental and soil conditions, it is estimated that soybean contributes approximately 22.4 kg ha^{-1} to the following cash crop as an N credit (Warren et al., 2017). In aboveground soybean biomass, about 55% of total N accumulated is attributed to N fixation (Ciampitti and Salvagiotti, 2018).

Understanding the implications of cover crop presence and P fertilizer management on various biological soil health indicators as well as the seasonal dynamics of biological soil health indicators can gain insight into P cycling and availability and dynamics of the soil microbial community. Therefore, the objectives for this study were to (1) assess the impacts of P fertilizer management approaches and the presence and absence of cover crops on various indicators of nutrient pools and microbial activity and function, and to (2) assess seasonal dynamics of biological soil health indictors in a no-till corn and soybean cropping rotation. The hypotheses of the study were (1) that the presence of cover crops would increase measure of labile nutrient as well as measures of microbial activity and function (microbial biomass, soil respiration, and soil enzyme activities), (2) increased nutrient cycling in systems undergoing P nutrient drawdown, (3) climatic factors will drive the seasonal dynamics of biological soil health indicators.

Materials and Methods

Experimental Site, Design, and Agricultural Management:

The experimental site was located at the Kansas Agricultural Watershed (KAW) Field Laboratory near Manhattan, Kansas. The site consists of primarily erode Smolan silty clay loam (fine, smectitic, mesic Pachic Argiustoll) with a 6-8% slope, with soil pH ranging from 6 to 7. The site has a hot, humid continental climate, with a mean annual temperature of 12.9°C and mean annual precipitation of 889 mm. There are 18 terraced watersheds that are approximately 0.5 ha each. The site has been in a continuous no-till, corn-soybean rotation since 2014. From 2014 to 2019 a five-year study evaluating P fertilizer source, timing and placement and the presence or absence of a cover crop on crop response and surface water quality was conducted (Carver et al., 2022).

The experiment is a 2x3 complete factorial, arranged in a randomized complete block design. The blocks were assigned based on landscape position and all treatments are replicated three times (n = 18). There are three levels of P fertilizer management; no P control (NP), build BAM, and SUF, and two levels of cover crop management; presence (CC) or absence (NC) of a cereal rye (*Secale cereale*) cover crop. Nitrogen (N) fertilizer applications were balanced between all treatments for a total N application rate of 180 kg N ha^{-1}. In the SUF and NP treatments, 28% urea-ammonium nitrate was applied on 22 April 2021. In the BAM treatment 20.1 kg N ha^{-1} was applied as ammonium polyphosphate and the balance was applied as 28% urea-ammonium nitrate (160 kg N ha^{-1}). Ammonium polyphosphate (10-34-0) was applied to BAM plots at an application rate of 71 kg P_2O_5 ha^{-1}. No P fertilizer has been applied to SUF plots since December 2018, while NP plots have not received P fertilizer since 2014. Cereal rye

was planted immediately following harvest of the main soybean crop on 13 October 2020 and was chemically terminated with Glyphosate, 2,4-D, and Dicamba on 14 April 2021. Corn was planted on 22 April 2021 and harvested on 16-17 September 2021.

Soil Sampling:

Composite soil samples were collected at 0-5 cm depth six times during the corn growing season in 2021. Soil samples were collected prior to cover crop termination, four times during the corn growing season, and at harvest. Sampling dates were 12 April (pre-termination-T0), 4 May (T1), 26 May (T2), 14 June (T3), 17 July (T4), which correspond to 21, 43, 65, 85 days after cover crop termination, respectively, and at corn harvest on 17 September (T5). 40 cores measuring 18 mm in diameter were taken from each plot, then separated into field-moist and air-dried subsamples.

All field-moist soil was processed using a 2 mm sieve and stored at 4°C until analysis were performed. Moist soil samples were analyzed for microbial biomass C, N, and P, extractable inorganic N, dissolved total N, dissolved organic C and N. Soil moisture was determined by oven-drying a subsample of field-moist soil for 48 hr at 90°C. Air-dried soil samples were dried at room temperature then ground and sieved to pass a 2 mm sieve. Air-dried soils were analyzed for active C, autoclaved citrate extractable (ACE) protein, soil respiration, β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase.

Methodology:

Microbial biomass C and N, dissolved total N, inorganic N, and dissolved organic N was determined from a single extraction (Jones and Willett, 2006). Microbial biomass C and N were determined by the chloroform fumigation extraction method (Brookes et al., 1982; Vance et al., 1987). Briefly, two 8 g (dry weight equivalent) samples of moist soil were weighed into 250-mL

glass jars. One of the two jars was fumigated with chloroform in a desiccator for 24 hr. Both samples were then extracted with 40 mL of 0.5 M K_2SO_4 and shaken for 30 min. The samples were then filtered through Ahlstrom 74 filter paper into 40-mL glass vials. Samples were analyzed for non-purgeable organic C on a Total Organic Carbon (TOC) analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). Microbial biomass C was determined be calculating the difference between the fumigated and unfumigated samples. Dissolved organic C was determined from the extractant of the unfumigated microbial biomass C/N samples and measured with the TOC for non-purgeable organic carbon (Jones and Willett, 2006).

Dissolved total N was determined by oxidizing an aliquot of the fumigated and unfumigated filtrate using the potassium persulfate oxidation method (Cabrera and Beare, 1993). $K_2S_2O_8$ 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). Inorganic N (NO_3-N and NH_4-N) was measured by the Kansas State Soil Testing Lab. Dissolved organic N was determined by calculating the difference between dissolved total N and inorganic N. Microbial biomass N was determined by the difference in dissolved total N in the fumigated and unfumigated samples.

Microbial biomass P was determined using a 24-hr chloroform fumigation followed by citrate extraction (Darch et al., 2016). Briefly, two 8 g (dry weight equivalent) moist soil samples were weighed into two 250-mL glass jars. One jar was fumigated with chloroform for 24 hours, while the other jar was not fumigated. Unfumigated and fumigated samples were extracted with 40 mL 2 mM citric acid pH 5. Samples were shaken for 30 mins, centrifuged at 10,000 rpm for 10 mins, and filtered with Ahlstrom 74 filter paper into 40-mL glass vials. The amount of

molybdate reactive P was determined colormetrically at 880 nm using the Murphy Riley Method (Murphy and Riley, 1962; O'Halloran and Cade-Menun, 2008). Microbial biomass P was determined by the difference in molybdate reactive P between fumigated and unfumigated samples.

Active C was determined using the permanganate oxidizable C method described by Weil et al. (2003). Briefly, 2.5 g of air-dried soil were weighed into 50-mL Falcon tubes; 18 mL of deionized water was added and 2 mL of 0.2 M $KMnO_4$ was added to the Falcon tube. Samples were then shaken for 2 min, and left to settle for 8 min before 0.2 mL of each sample was added to 20 mL deionized water to stop the reaction. Samples are analyzed colormetrically on a spectrophotometer at 550 nm. Bioavailable N was determined using the autoclaved citrate extractable (ACE) protein method (Wright and Upadhyaya, 1996, 1998; Hurisso et al., 2018b). Briefly, two sets of 3 g of soil was weighed out into 50-mL centrifuge tubes. 24 mL of 20 mM sodium citrate pH 7 is added to samples then shaken at 180 rpm for 5 min. Samples are then autoclaved at 121°C and 15 psi for 30 min. The samples were then allowed to cool, shaken for one min and a 1.75-mL aliquot from each sample was transferred added to a clean 2-mL microcentrifuge tube. The aliquot was centrifuged at 10000 x g for 3 mins and then 1 mL of the cleared extract was transferred to a 1.5-ml microcentrifuge tube. For quantification, a dry heat block (VWR heat block, 97043-610, USA) was heated to 61.5˚C and Pierce bicinchoninic acid (BCA) working solution was prepared. Using a multichannel pipettor, 10 µL of standard were added to reaction plate, followed by two replicate columns of each strip of eight sample tubes into plate wells. 200 µL of working reaction was added into each well of the reaction plate, which is then sealed with tape seal and placed on heat block for one hour. Tape was then

removed, and the plate was allowed to cool for 10 min. The plate was then read with a plate reader (BioTek Synergy H1, USA) at 562 nm.

Soil respiration was determined using the alkali trap method described by (Haney and Haney 2010). Briefly, 20 g of air-dried soil was weighed into an aluminum weigh boat (diameter 51 mm) that was perforated nine times (three by three array) and place onto two filter papers (qualitative 413-VWR North America). The alkali trap was then supported above the soil surface on a plastic pizza stool and filled with 9 mL of 0.5 M KOH and 7.5 mL of deionized water was added to the inside edge of the jar to wet the filter papers and soil. Jars were then incubated for 4 days at room temperature. Electrical conductivity of the KOH solution changes in proportion to the amount of CO_2 trapped; therefore, the amount of CO_2 trapped is calculated from the change in conductivity and used to estimate the amount of CO_2 evolved from the samples.

Activities of β-glucosidase (Eivazi and Tabatabai, 1988), N-acetyl-β-glucosaminidase (Parham and Deng, 2000), and acid and alkaline phosphatases (Eivazi and Tabatabai, 1977) were determined colormetrically at 400 nm. Briefly, three subsamples of 0.5 g of air-dried soil were weighed out into 20-mL vials. Each sample had two replicates (A and B) and one control (C). 2 mL of start buffer was added to all A, B, and C samples. Start and stop buffers as well as p-nitrophenol substrates used for each enzyme assay are listed in Table 2.1. Vials were capped and shaken gently by hand before being incubated at 37˚C for 1 hr. To stop the reaction, 0.5 mL of 0.5 M CaCl2 was added to each vial, followed by 2 mL of stop buffer. Substrate was then added to the control only.

Statistical Analysis:

The effects of P fertilizer treatment, cover crop, sampling time, and their interactions on various biological soil health indicators were analyzed using a repeated measured analysis of

variance in linear mixed effects model via Statistical Analysis Software (SAS version 9.4; Cary, NC) with option DDFM = KR (Denominator Degrees of Freedom = Kenward Rogers). Fixed effects of the model included P fertilizer treatment, cover crop treatment, sampling time, and their interactions. Natural logarithm, log10, or square root transformations were performed on certain soil health indicators to satisfy assumptions of normality and homogeneity of variance. Microbial biomass P and dissolved organic C and N were transformed using square root function, ACE protein was transformed using natural logarithm, while C:N ratios and citrate-extractable phosphorus was transformed using log10. Back transformed least squares (LS) means and standard errors are reported in tables and figures. Time was treated as a repeated measure with covariance structures were determined by fit statistics, in which the structure that resulted in the lowest Akaike information criterion and Bayesian information criterion was used. Covariance structures selected for this study included compound symmetry, heterogenous compound symmetry, first-order autoregressive, heterogenous first-order autoregressive, or unstructured. Three-way analysis of variance was first conducted for all soil health indicators. The three-way interaction term of P fertilizer, cover crop, and sampling time was then dropped from the model statement, and two-way analysis of variance was conducted. If no significant two-way interactions were observed, the interaction term was dropped from the model statement and the model was rerun only analyzing main effects. Two-way analysis of variance was used for soil moisture, acid and alkaline phosphatase, dissolved total N, inorganic N, NH_4-N, NO_3-N, dissolved organic N, and citrate-extractable P. One-way analysis of variance was used for glucosidase, glucosaminidase, ACE protein, soil respiration, C:N ratio, microbial biomass C and N, active C, soil respiration, and dissolved organic C.

Results

Weather Data

Cumulative precipitation between sampling times as well as soil moisture and minimum and maximum daily air temperatures at each sampling time are reported in Table 2.2. Monthly minimum and maximum daily air temperatures for the 2021 harvest year as well as 30-year normals are presented in Fig. 2.1, while cumulative precipitation for the 2021 harvest year and 30-year normals are presented in Fig. 2.2. The average maximum and minimum daily air temperatures recorded during the growing season were within normal range of the 30-year normal for Riley County (Weather Data Library, Kansas Mesonet). Cumulative precipitation decreased between T0 and T1, then increased between T1 and T2. Precipitation greatly decreased between T2 and T3 and from T3 to T4. Cumulative precipitation greatly increased 20 days before the T5 sampling. Overall cumulative precipitation for the 2021 harvest year was 13% less than 30-year normals, while cumulative precipitation during the growing season was 21% less than 30-year normal (Fig. 2.2).

There was a significant P fertilizer treatment by cover crop interaction for soil moisture (Table 2.3). The presence of a cover crop in the BAM and SUF treatments significantly increased soil moisture by 9 and 13%, respectively, while there was no significant effect of cover crop in the NP treatment (Fig. 2.3). There was a significant main effect sampling time for soil moisture (Table 2.3). Soil moisture was significantly greater at T0 compared to all other sampling times. Soil moisture significantly decreased between T0 and T1, followed by an increase in soil moisture at T2. Soil moisture significantly decreased at T3, followed by a significant increase from T3 to T4 and from T4 to T5. Soil moisture was significantly lower at T3 compared to all other sampling times (Table 2.4).

Extractable Nutrients:

Carbon:

Both sampling time and cover crop had significant effects on active C concentrations (Table 2.3). The presence of a cover crop increased active C concentrations by 14% (Table 2.4). Active C concentrations significantly decreased from T0 to T1 and from T1 to T2. Active C concentrations increased towards the latter portion of the growing season. There was no statistical difference in active C concentrations from T3 to T5 (Table 2.4). Active C concentrations at T0, T3, and T4 were not significantly different (Table 2.4).

Due to methodology difficulties as a result of dry conditions, no data for dissolved organic C is reported at T3. Both sampling time and cover crop significantly impacted dissolved organic C concentrations (Table 2.3). The presence of a cover crop increased dissolved organic C concentrations by 12% (Table 2.4). There was a significant increase in dissolved organic C from T0 to T1, followed by a significant decrease in concentrations at T2. Concentrations peaked at T4, while concentrations had significantly decreased by T5 (Table 2.4).

Phosphorus:

There was a significant two-way interaction between P fertilizer treatment and sampling time on citrate-extractable P concentrations (Table 2.3, Fig. 2.4). Regardless of sampling time, citrate-extractable P concentrations were always significantly higher in the BAM treatment compared to SUF and NP treatments (Fig. 2.4). In the BAM treatment, citrate-extractable P was similar at T0 and T1, followed by a peak in concentrations at T2. Concentrations at T3 and T4 were not significantly different from each other, however citrate-extractable P at both of these sampling times was significantly lower than at T2. There was a significant decrease in concentrations at T5. For the SUF and NP treatments, citrate-extractable P remained relatively

consistent through the growing season. Only at T0 and T4 was there a significant difference between the SUF and NP treatments; concentrations at T0 and T4 were significantly higher in the SUF treatment compared to the NP treatment (Fig. 2.4).

Nitrogen:

Main effects of P fertilizer treatment, cover crop, and sampling time significantly influenced ACE protein concentrations (Table 2.5). Protein concentrations were significantly lower in the NP treatment compared to BAM and SUF treatments (Table 2.6). The presence of a cover crop increased ACE protein by 12% (Table 2.6). ACE protein concentrations significantly decreased from T0 to T1, then significantly increased from T1 to T2. ACE protein significantly increased from T2 to T3, followed by a significant decrease in concentrations at T4. There was no significant difference in ACE protein concentrations at T4 and T5. ACE protein concentrations peaked significantly at T3 (Table 2.6).

There was a significant two-way interaction between P fertilizer treatment and sampling time for dissolved total N, inorganic N (NO_3-N + NH_4-N), and dissolved organic N (Table 2.5). For all P fertilizer treatments, there was a significant increase in dissolved total N concentrations between T0 and T1 (Fig. 2.5). In the BAM treatment, concentrations increased from T1 to T3, where a peak in concentrations occurred at T3. A peak in concentrations was also occurred at T3 in the NP treatment, while concentrations were not significantly different between T1 and T4 in the SUF treatment. For all P fertilizer treatments, concentrations decreased from T3 to T4 and from T4 to T5. With regard to sampling time, only T2 and T3 significant differed between P fertilizer treatments. At T2 and T3, dissolved total N concentrations were significantly higher in the BAM treatment compared to NP and SUF treatments. Concentrations were not significantly

different at T0 and T5 for all P fertilizer treatments; concentrations at T0 and T5 were significantly lower compared to all other sampling times (Fig. 2.5).

Ammonium-N concentrations increased significantly from T0 to T1 in all P fertilizer treatments (Fig. 2.6). In the BAM treatment, NH_4-N concentrations were not statistically different from T1 through T4. Within the NP and SUF treatments, there was not a significant difference in NH_4-N concentrations from T1 through the remainder on the growing season. In comparing sampling times, NH_4-N concentrations only differed significantly between P fertilizer treatments at T2; NH_4-N concentrations at T2 in the BAM treatment were significantly higher than NP and SUF treatments, while no significant difference occurred between the NP and SUF treatments. NH_4-N concentrations were similar at T0 and T5 for all P fertilizer treatments (Fig. 2.6).

Nitrate-N concentrations increased significantly from T0 to T1 in all P fertilizer treatments (Table 2.6, Fig. 2.7). In the NP and BAM treatments, concentrations increased from T1 to T3, while no significant difference occurred between T1 and T3 in the SUF treatment. There was a decrease in concentrations from T3 to T4 and from T4 to T5 in all P fertilizer treatments. Concentrations at T2 were significantly greater in the BAM treatment compared to NP and SUF treatments, while concentrations were significant greater at T3 in the BAM treatment compared to the SUF treatment. There was no significant difference in concentrations at T0 and T5 in all P fertilizer treatments; concentrations at T0 and T5 were significantly lower compared to all other sampling times.

For total inorganic N, in the BAM treatment, there was a significant increase in concentrations from T0 to T1 (Table 2.6, Fig. 2.8). Concentrations increased from T1 to T3 in the BAM treatment, where a peak in concentrations occurred at T3. Concentrations in the SUF

treatment were not significantly different between T1 and T4. Concentrations in the NP treatment increased from T2 to T3. Concentrations decreased from T3 to T4 and from T4 to T5 in all P fertilizer treatments. In comparisons among sampling times, the only significant difference among fertilizer treatments was at T2 and T3. Concentrations in the BAM treatment were significantly higher at T2 and T3 compared to NP and SUF treatments, with no significant difference between the NP and SUF treatments. Inorganic N concentrations were not significantly different at T0 and T5 (Fig. 2.8).

Dissolved organic N concentrations remained stable from T2 through T4 in the NP and SUF treatments (Table 2.6, Fig. 2.9). There was a significant increase in dissolved organic N from T2 to T3 followed by a significant decrease in concentrations from T3 to T4 in the BAM treatment. There was no significant difference between the NP and SUF treatments at any sampling time. The BAM treatment had significantly less dissolved organic N at T0 and T2 compared to NP and SUF treatments, while concentrations at T3 were significantly higher in the BAM treatment compared to NP and SUF treatments (Fig. 2.9).

There was a significant P fertilizer by cover crop interaction for dissolved organic N (Table 2.5, Fig 2.10). The presence of a cover crop significantly increased dissolved organic N concentrations by 12% and 19% in the NP and SUF treatments, respectively. There was no significant difference in dissolved organic N concentrations when a cover crop was present in the BAM treatment (Fig. 2.10).

Microbial Activity and Function:

Microbial Biomass C, N, and P:

Microbial biomass C concentrations were significantly influenced by cover crop and sampling time (Table 2.7 & 2.8). The presence of a cover crop increased microbial biomass C

concentrations by 20% (Table 2.8). Microbial biomass C concentrations significantly decreased from T0 to T1, followed by a significant increase in concentrations from T1 to T2. There was no significant difference in concentrations from T2 and T4. Concentrations were significantly higher at T5 compared to all other sampling times (Table 2.8).

Sampling time was the only significant effect for microbial biomass N concentrations (Table 2.7 & 2.8). Overall, microbial biomass N concentrations tended to be relatively stable throughout the growing season. Microbial biomass N concentrations significantly decreased from T0 to T1, followed by a significant increase in concentrations from T1 to T2. Concentrations at T2, T4, and T5 were not significantly different. There was no difference in biomass N concentrations at T0 and T5 (Table 2.8).

Microbial biomass P concentrations were influenced by both P fertilizer treatment and sampling time (Table 2.7 & 2.8). Microbial biomass P concentrations were significantly lower in the NP treatment compared to BAM and SUF treatments, while the BAM treatment had significantly higher concentrations compared to the SUF treatment. Overall, microbial biomass P concentrations in the BAM and SUF treatments were 146% and 111% higher than NP, respectively (Table 2.8). There was no significant difference in biomass P concentrations from T0 through T3. Concentrations at T4 and T5 were significantly higher compared to T0 (Table 2.8)

There were no main effects of P fertilizer treatment, cover crop, or sampling time on microbial biomass C:N ratios (Table 2.7). Both microbial biomass N:P and C:P ratios had a significant main effect of P fertilizer treatment (Table 2.7 & 2.8). Microbial biomass N:P and C:P ratios were significantly lower in the BAM treatment compared to SUF and NP treatments,

while ratios in the SUF treatment were significantly lower than in the NP treatment (Table 2.8). There was no main effect of cover crop or sampling time for N:P and C:P ratios (Table 2.7).

Soil Respiration:

Phosphorus fertilizer treatment, cover crops, and sampling time all significantly influenced soil respiration (Table 2.7 & 2.8). The presence of a cover crop increased soil respiration by 21% (Table 2.8). Respiration was significantly lower in the NP treatment compared to BAM and SUF treatments; there was no statistical difference in soil respiration between the BAM and SUF treatments (Table 2.8). Soil respiration was similar at T0 and T1, followed by a decrease in soil respiration at T2. Respiration was not significantly different at T2 and T3. Respiration increased significantly from T3 to T4 and from T4 to T5. Soil respiration was significantly greater at T5 compared to all other sampling times (Table 2.8).

Soil Enzyme Activities:

A significant cover crop by sampling time interaction occurred for acid phosphatase activity (Table 2.9, Fig. 2.11). Regardless of sampling time, acid phosphatase activity was always significantly higher in the cover crop treatment compared to the no cover crop treatment. In the cover crop treatments, there was a significant decrease in acid phosphatase activity between T0 and T1. There was no significant difference in acid phosphatase activity from T1 through T3. Activity significant increased from T3 to T4, followed by a significant decrease in activity from T4 to T5. There was no significant difference in acid phosphatase activity in the cover crop treatment at T0 and T5. For the no cover crop treatment, acid phosphatase activity was relatively stable throughout the growing season. Activity at T3 was significantly lower compared to T0, T1, and T5. There was no significant difference in activity in the no cover crop treatment at T0 and T5 (Fig. 2.11).

There was a significant P treatment by cover crop interaction for alkaline phosphatase activity (Table 2.9, Fig. 2.12). The presence of a cover crop in the NP treatment significantly increased alkaline phosphatase activity by 53%, while the presence of a cover crop in the SUF treatment significantly increased activity by 33%. There was no significant increase in alkaline phosphatase activity in the BAM treatment when a cover crop was present (Fig. 2.12).

Both cover crop and sampling time had significant effects on glucosidase activity (Table 2.9 & 2.10). The presence of a cover crop increased activity by 32% (Table 2.10). Glucosidase activity significantly decreased from T0 to T1, while there was no significant difference at T1 and T2. Activity then significantly increased at T3, where activity was not significantly different from T3 through T5. Activity was significantly higher at T5 compared to T0 (Table 2.10).

There was a significant main effect of cover crop and sampling time for glucosaminidase activity (Table 2.9 & 2.10). The presence of a cover crop resulted in an 39% increase in glucosaminidase activity (Table 2.10). Glucosaminidase activity significantly decreased from T0 to T1 and from T1 to T2. There was a significant increase in activity from T2 to T3, where activity was not significantly different at T3 and T4. Activity significantly increased from T4 to T5; activity at T5 was significantly higher compared to all other sampling times (Table 2.10).

Discussion

Cover crop impacts on soil health indicators

Dissolved organic C & N, Active C, and ACE protein:

In this study, the presence of cover crops significantly increased both dissolved organic C and N concentrations (Tables 2.4 & 2.6). The positive effect of cover crops on dissolved organic C and N is consistent with several studies (Steenwerth and Belina, 2008; Dinesh et al., 2009; Zhou et al., 2012). Extractable organic C and N pools in systems with cover crops are derived

from the decomposition of soil organic matter, belowground crop residues, and labile soluble organic matter that is produced during decomposition of aboveground residues or through root exudation (Zhou et al., 2012). Dissolved organic C and N influences the mobility and bioavailability of nutrients that interact with organic matter and undergo biological transformations processes. (Bolan et al., 2011). Plant residues are the major source of dissolved organic C and N, which consists of low molecular weight carboxylic acids, amino acids, carbohydrates, and fulvic acids (Bolan et al., 2011). Labile C is rapidly incorporated into the microbial biomass via the dissolved organic matter pathway; dissolved organic C and N leaching from litter followed by high microbial incorporation of this input is the dominant pathway of SOM formation from litter decomposition in the initial phase (Cotrufo et al., 2015).

Active C is proposed as a measure to assess the most bioavailable dissolved organic C. The response of the active C pool to cover crops has been mixed with some reporting increases in active C concentrations due to the inclusion of cover crops in an agroecosystem (Jokela et al., 2009; White et al., 2020; Islam et al., 2021; Marshall et al., 2021), while other studies that conclude that cover crops do not influence concentrations due to differences in climatic and soil factors. For example, Pokhrel et al. (2021) found that neither single nor mixed species cover crops significantly increased active carbon concentrations compared to a non-cover control treatment in a no-till dryland soybean system in Mississippi. Likewise, Steele et al. (2012) found that winter annual cereal cover crops significantly increased active C concentrations in one of two years in coastal plains soils while no significant increases were observed in Piedmont soils in Maryland. The coarser soil texture in Pokhrel et al. (2021), and drought conditions that limited cover crop biomass in coastal soils and better soil structure and overall greater amount of soil organic matter in the Piedmont loam in Steele et al. (2012) were suggested as reasons that

increases in active C were not observed in those studies. Differences in active C in response to soil texture was reported by Fine et al. (2017), who found that coarse-soils had significantly lower active C concentrations compared to fine-textured soil. It is also possible that soils with high levels of organic C could reach an equilibrium in C saturation where additional C inputs would not equate to increased C sequestration, causing negligible effects of cover crops on labile C (Six et al., 2002; Chung et al., 2010; Lucas and Weil, 2012, 2021). The active C pool of soil organic matter is comprised on relatively recent C additions that provide nutrient-rich substrates for the soil microbial community that promote organic matter accumulation and stabilization (Hurisso et al., 2016). Increased active C concentrations due to the presence of a cover crop can also reflect the contribution of root exudates to labile forms of C (Wang et al., 2017). Increases in active C concentrations under cover cropping in this study suggests that cover crops contributed more readily available forms of C to the soil system (Table 2.4). Therefore, it is possible that the finer soil texture, soil organic C (Stahl 2023), and favorable climatic conditions at the site in the current study helped facilitate increases in active C concentrations under cover cropping.

Proteins are the largest pool of organic N in soil organic matter (Hurisso et al., 2018b). Protein measurements can provide estimates of the organic N pool that can be depolymerized, and can serve as a reservoir of N that is subsequently released through mineralization (Roberts and Jones, 2008; Hurisso et al., 2018b). Inconsistent effects of cover crops on ACE protein concentrations are presented in the literature. Studies have found positive effects (Balota et al., 2014; Nunes et al., 2018; Chahal and Van Eerd, 2019; Marshall et al. 2021; Feng et al., 2021) as well as no effect (Pokhrel et al., 2021; Wood and Bowman, 2021) of cover crops on ACE protein concentrations. Significantly positive correlations between ACE protein and measures of labile C

pools and activity (soil respiration and soil enzyme activity) have been reported (Fine et al., 2017; Caudle et al., 2020). Marshall et al. (2021) reported that ACE protein aligned better with measures of C compared to measures of N pools. Therefore, the higher ACE protein due to the presence of a cover crop in the present study (Table 2.6) might be caused by stimulation of microbial activity by increased substrate availability (Feng et al., 2021).

Lack of cover crop impacts on N pools and biomass N:

Nonlegume cover crop species are known for their ability of scavenging residual nutrients in soil (Adetunji et al., 2020). Grass cover crops can assimilate and recycle N and P back to the soil, which could reduce the risk of nitrate leaching or P loss from surface runoff (Blanco-Canqui et al., 2015; Koudahe et al., 2022). A potential drawback of grass cover crops is the risks of N immobilization, due to wide C:N ratios, during decomposition, which would require subsequent fertilizer applications to alleviate low N availability during peak crop demand for the succeeding crop (Adetunji et al., 2020). In the present study, the cover crop did not have a significant effect on dissolved total N or inorganic N (NH_4-N and NO_3-N). This finding was contradictory to Strickland et al. (2019) who found a 64% increase in NH_4-N concentrations and 30% decrease in NO_3-N concentrations due to cover cropping practices. The authors suggested that the increase in NH_4-N and decrease in NO_3-N indicated a shift towards less mobile forms of N, therefore, greater N retention due to cover cropping. It is possible that the application of N fertilizer at corn planting masked any impacts that cover crop had on these N pools in the present study. Additionally, the C:N ratio of the cereal rye biomass (averaged between P treatments) was 20:1 (Nelson-Personal Communication) in the present study, suggesting that N released from the cereal rye biomass should undergo mineralization, increasing the amount of inorganic N in cover treatments. This further suggests that the N fertilizer application masked any cover crop effect.

The present study did not find a significant increase in microbial biomass N concentrations due to cover cropping (Table 2.8). As with microbial biomass C, residue quantity and quality, soil parameters, tillage practices, and climatic conditions all can affect microbial biomass N dynamics. While some studies report increases in microbial biomass N due to cover cropping, those authors often found that only legume cover crops resulted in increased microbial biomass N; in comparing nonlegumes and fallow treatments, many of these same studies do not report a significant increase microbial biomass N (Wang et al., 2007; Balota et al., 2014; Mbuthia et al., 2015). Using ^{15}N labeled cover crop, Jackson (2000) reported that very little of the observed increases in microbial biomass N were derived from cover crop decomposition, suggesting that microbial demand for N was alleviated with soil N instead of residue N from cover crops. Roth et al. (2023) found that ^{15}N-labeled cereal rye residue only contributed to 1.5% of the microbial biomass N, showing a peak at 20 days post incubation.

Microbial biomass C, soil respiration, and soil enzyme activities:

Increases in C inputs from cover crops suggests that soil microorganisms will have access to additional organic resources in cover cropped soils that will promote increases in the microbial biomass (Hao et al., 2023; St. Aime et al., 2023). Similarly, increased C substrate availability due to cover cropping is thought to increase soil respiration, an overall measure of microbial community activity (St. Aime et al., 2023) and increases in soil respiration due to presence of cover crops has been reported previously (Nunes et al., 2018; Chahal and Van Eerd, 2019; Wood and Bowman, 2021; Crookston et al., 2023). Although microbial biomass only accounts for up to 5% of soil organic C, it is an important indicator of the size of the microbial community (Balota et al., 2014). Turnover of microbial biomass contributes to nutrient cycling within soil and can be used as an indicator of soil health as it is sensitive to management changes (Bunemann 2018).

Increased root biomass due to cover crops can also increase microbial biomass (Blanco-Canqui et al., 2015). Root exudates that contain labile C compounds can be incorporated into the microbial biomass to stimulate growth (McDaniel et al., 2014). In fact, Brennan and Acosta-Martinez (2017) speculated that belowground inputs of labile C (roots and root exudates) was the primary driver for increased microbial biomass in their study on intensive vegetable production in California. Increased microbial biomass C in the present study (Table 2.8), is in agreement with several studies (Wang et al., 2007; Acosta-Martinez et al., 2011; Balota et al., 2014; Mukumbareza et al., 2015; Frasier et al., 2016b; Muhammad et al., 2021). Residue quantity and quality, soil parameters (i.e. soil texture and soil organic matter), tillage practices, and climatic conditions all can affect microbial biomass dynamics in soil (Blanco-Canqui et al., 2015). Low C:N cover crop ratios promote faster decomposition of residues, while high C:N ratios promote slower decomposition in grasses (at full maturity) which can result in greater soil C levels (Roth and Waite, 2021). Differences in the response of microbial biomass to cover cropping due to species variation have been reported. Balota et al. (2014) found that microbial biomass C was 28% higher in legume cover crop treatments compared to grasses, while Muhammad et al. (2021) in a meta-analysis of 81 studies found a marked increase in microbial biomass C under nonlegume cover crops. Focusing on the introduction of cereal rye as cover crop in a dryland cotton-sorghum rotation in the Texas High Plains, Acosta-Martinez et al. (2011) found 50% higher microbial biomass C under cereal rye cover crops compared to fallow treatments. For the present study, it is possible that increased C substrate availability from the cereal rye that contributed to increases in the microbial community as proposed by Muhammad et al. (2021).

In general, the inclusion of cover crops increased all measures of soil enzyme activity in the present study (Table 2.10). Increased activities due to cover cropping is in agreement with

numerous studies (Chavarria et al., 2016; Brennan and Acosta-Martinez, 2019; Tyler, 2020; Thapa et al., 2021; Shu et al., 2021). Studies on resource allocation of soil enzymes suggests that enzyme production can also be induced in the presence of a substrate when adequate substrate is available (Geisseler et al., 2010; Allison et al., 2011). Conservation practices such as implementation of cover crops and no-till management result in increased substrate availability, especially in surface soils (Balota et al., 2004; Tyler 2020). Glucosidase hydrolyzes oligosaccharides and releases monosaccharides and catalyzes the final step in the breakdown of cellulose (Luo et al., 2017). The release of simple sugars by glucosidase provides an energy source for the soil microbial community (de Almeida et al., 2015). Glucosaminidase catalyzes the hydrolysis of N-acetyl-β-G-glucosamine residues from the terminal non-reducing ends of chitooligosaccharides (Ekenler and Tabatabai, 2002). This hydrolysis of chitin into amino sugars is important in C and N cycling because amino sugars are one of the major sources of mineralizable N in soil. Phosphatases hydrolyze phosphomonoesters and phosphodiesters to release inorganic P (Luo et al., 2017). Enhanced phosphatase activity due to cover cropping can reflect residual release of phosphatases by cover crop roots, changes in the abundance or structure of the soil microbial community, or to an overall substrate-driven increase in activity due to P-rich cover crop residues in the soil (Hallama et al., 2019). Increased soil enzyme activity due to cover cropping in this study (Table 2.10) likely reflects the increased presence of substrates from cover crops that induced enzyme synthesis (Jiao et al., 2011; Chavarria et al., 2016).

The general consensus in the literature is that phosphatase activity increases when P availability is low, and is depressed with applications of P fertilizer (Olander and Vitousek, 2000; Marklein and Houlton, 2011; Sinsabaugh and Follstad Shah, 2012). Alkaline phosphatase

activity was significantly influenced by P fertilizer treatment in this study (Table 2.10). In the absence of P fertilizer application, alkaline phosphatase activity was significantly higher compared to both BAM and SUF-based management practices that do receive some form of P application. Resource allocation models suggest that induction and/or depression of enzyme synthesis are regulatory mechanisms that affect enzyme production only when it is beneficial to the producer (Allison et al., 2011). Since P availability is low in treatments not receiving P fertilizer, it would be beneficial for microorganisms to induce enzyme production to increase the amount of P available in soil, resulting in increases in phosphatase activity as observed in this study. Considering resource allocation, for alkaline phosphatase activity, it is not surprising to see significant increases in activity for both the NP and SUF treatments due to the presence of a cover crop, while activity in the BAM treatment was similar in both cover and non-cover treatments. Increased substrate availability from decomposing cover crop residue would promote the production of phosphatases if P availability in soil is low (NP and SUF-based systems) (Allison et al., 2011).

In the present study, acid phosphatase activity was relatively stable in the NC treatments, while activity in CC treatments was more variable, with a significant increase in activity observed at T4. Acid phosphatase is thought to be regulated primarily by microclimate and soil chemical factors (Boerner et al., 2005). It is possible that the low soil moisture conditions at T3 constrained acid phosphatase production activity and probably hindered decomposition of cover crop residues. As soil moisture significantly increased at T4 in comparison to T3, it is possible that enzyme production increased once moisture constraints were reduced (Table 2.2). Acid phosphatase has been reported to increase with increasing soil moisture (Baldrian et al., 2010) and be positively correlated with mean annual precipitation (Sinsabaugh et al., 2008).

P fertilizer treatment effects on ACE protein, citrate-extractable P, microbial biomass P, and soil respiration

Autoclaved citrate extractable protein concentrations were significantly lower in the NP treatment compared to BAM and SUF in the present study. Several studies have found that soil protein (measured as glomalin related soil protein) was positively correlated with soil P concentrations (Wu et al., 2010; Guo et al., 2012; Qiu et al., 2021; Wang et al., 2022). A positive response to P availability suggests that soil protein measured were not of arbuscular mycorrhizal fungi (AMF) origin, as AMF populations decrease with increasing P availability (Staunton et al., 2020; Law and Maherali, 2023), further verifying that the ACE protein procedure extracts a wide range of proteins, not just glomalin (Hurisso et al., 2018b).

Citrate-extractable P and microbial biomass P concentrations were significantly higher in the BAM treatment that receive annual applications of P fertilizer compared to the SUF and NP treatments, while the SUF treatment tended to have significantly higher concentrations compared to NP in the present study (Table 2.4). Citrate extractable P is implicated in root and microbial P mobilization in soil through the cycling of organically bound soil P through the chelation of bridging cations (DeLuca et al., 2015; Menezes-Blackburn et al., 2016). The type of P fertilizer used could influence citrate-extractable P as Yuan et al. (2022) found increased citrate-extractable P in soils following ammonium polyphosphate applications in comparisons to monoammonium phosphate applications. Citrate-extractable P has been positively correlated with Olsen P (DeLuca et al., 2015), and known to increase in response to P fertilizer additions (Yu et al., 2022). Microbial biomass P is known to increase due to P fertilization and be positively correlated with Mehlich-3 P (Shi et al., 2013).

Soil respiration was significantly lower in the NP treatment compared to the BAM and SUF treatments in the present study (Table 2.8). This finding is in agreement with Feng and Zhu (2019) who found, in a global meta-analysis on soil respiration response to P additions, that P additions increased soil respiration by 31.7% in cropland. It is likely that higher soil respiration in response to P additions can be attributed to increased litterfall caused by improved net primary productivity (Sun et al., 2022), as P limitation has been reported to reduce net primary productivity (Wang et al., 2010). Corn yield was significantly affected by P fertilizer treatments in the present study; the BAM and SUF treatments increased corn yield by 16% (1420 kg ha^{-1}) in comparison to the NP treatment (Nelson – Personal Communication). Therefore, it is likely that crop response to P availability was the primary driver of soil respiration differences between P fertilizer treatments

Dissolved organic N concentrations were significantly increased when a cover crop was present in the SUF and NP treatments (Table 2.6). Higher dissolved organic N in P deficient conditions is agreement with Wang et al. (2022), who found that in a global meta-analysis, a medium P application rate (50-100 kg ha^{-1} yr^{-1}) decreased dissolved organic N concentrations. Likewise, significant negative correlations between dissolved organic N and soil total P have been reported (Jiang et al., 2021; Sun et al., 2022). It is possible that P availability influenced dissolved organic N trends in the present study as it controlled N mineralization/immobilization processes. It is also possible that greater primary production resulted in a larger microbial community that resulted in faster turnover of organic N in the BAM treatment, resulting in less dissolved organic N present (Chen et al., 2016; Sun et al., 2022).

Seasonal Dynamics of Soil Health Indicators

Effects of soil moisture and temperature on various soil health indicators:

Sampling time significantly influenced all soil health indicators in the present study. Seasonal dynamics of soil health indicators are probably attributed to differences in soil moisture and temperature. Soil temperature controls decomposition of crop residues since it can influence soil microbial activity (Turmel et al., 2015). Short-term increases in temperature can increase rates of enzyme-mediated reactions and affect the size of the enzyme pool by controlling both the microbial production and the rate of enzyme turnover (Conant et al., 2011). Adequate soil moisture increases microbial biomass and activity; however, microbial activity will decline when soil becomes saturated due to limited oxygen availability (Zhang et al., 2005). Decreased soil moisture content can negatively affect microbial activity due to the reduced diffusion of enzymes and nutrients, which can reduce substrate availability and nutrient cycling (Papendick and Campbell, 1981).

Seasonal variation in soil moisture can influence microbial biomass C (Debosz et al., 1999; Baldrian et al., 2010; Baldrian et al., 2013; Shi et al., 2013), microbial biomass N (Steenwerth and Belina, 2008; Geisseler and Horwath, 2009; Shi et al., 2013), enzyme activity (Baldrian et al., 2010; Kotroczo et al., 2014; Singh and Kumar, 2020), and soil respiration (Baldrian et al., 2010; Baldrian et al., 2013). However, increases in certain indicators during drought periods have been reported as well. Increased respiration during periods of drought can indicate a stressed microbial community (Bastida et al., 2006), and reduced moisture induces production of extracellular enzymes due to inhibited microbial access to soil resources (Smith et al., 2015). The influence of soil temperature on seasonal variability of soil health indicators has been reported for active C (Omer et al., 2018; Burke et al., 2019), microbial biomass C (Jiang et

al., 2013; McDaniel and Grandy, 2016), soil enzyme activity (Baldrian et al., 2013; Nevins et al., 2020), and soil respiration (Rochette et al., 1991).

During the present study, sampling time T3 recorded the lowest soil moisture of the study, and although the maximum daily air temperature continued to increase following T3, the low moisture coupled with higher temperatures probably contributed to the seasonal fluctuations. Soil enzyme activity at T3 significantly increased, suggesting that drought conditions induced production of extracellular enzymes (Smith et al., 2015), followed by higher activity during the latter part of the growing season. It is possible that higher activity was sustained towards the end of the growing season due to increases in belowground inputs. A marked decrease in microbial biomass P was also observed at T3. It is possible that drought conditions contributed to the decrease in size of the microbial community, and following rewetting, increased substrate availability due to necromass was utilized, resulting in significantly higher biomass P concentrations towards to latter part of the growing season. Interestingly, both active C and ACE protein showed significant increases from T2 to T3, with ACE protein concentrations peaking at T3. It is possible that substrate availability was the primary driving factor of this change instead of climatic factors. As no significant increases occurred before T3, it is possible that as the decomposition of cover crop residues was ongoing between T2 and T3 there was release of labile forms of C and N from cover crop residues. Using ^{15}N labeled cereal rye during an incubation study, Roth et al. (2023) found that 53% of labeled cereal rye biomass N remained in organic form, suggesting that N was released from decomposing residues, but mineralization processing had not occurred after 120 days after incubation. However, Strickland et al. (2019) found no significant difference in dissolved organic C or N concentrations between cover and non-cover treatments, while Frasier et al. (2016a) observed inconsistent impacts of cover crops on dissolved

organic carbon while simultaneously observing that dissolved organic N concentrations were not influenced by cover crop presence. Strong correlations between soil organic carbon pools and climate suggests that temperature plays a key role in the release of dissolved organic C (Kalbitz et al., 2000). Drying and rewetting cycles that are often observed throughout the crop growing season also contribute to fluxes in dissolved organic C. The rewetting of soil following drier periods leads to increased levels of dissolved organic carbon present in soil (Kalbitz et al., 2000). Therefore, it is possible that the positive effects of cover crops on dissolved organic C and N found in this study were driven by more favorable climatic conditions towards to early part of the growing season that promoted the decomposition of cover crop residues that released dissolved forms of organic C and N.

Effect of sampling time on dissolved total N, inorganic N, and dissolved organic N:

Differences in soil moisture and temperature, plant uptake of N, and N additions can also influence seasonal N dynamics (Nieder et al., 2011). Additionally, seasonal dynamics of N in soil can be influenced by biochemical transformations of N (such as ammonification and nitrification) and physical-chemical processes involved in the adsorption and fixation of NH_4-N (Kowalenko and Cameron, 1976).

Increased soil moisture can reduce NH_4^+ fixation as clay minerals are expanded under wet conditions, however, in drier conditions, the interlayer space is reduced and NH_4^+ fixation increases (Allison et al., 1953; Nieder et al., 2011). Ammonium fixation can build up the available N pool in soils as the NH_4^+ ions after penetration into the clay mineral interlayers are excluded from nitrification (Guo et al., 1983). Decreased soil moisture conditions at T3 and T4 relative to other sampling times could have promoted increased NH_4^+ fixation in the present study.

The majority of crop N uptake in corn occurs during the rapid growth stages of corn (V6-VT) (English et al., 2017). In the present study, the growth stage of corn was V8/V9 at the T3 sampling time. Culman et al. (2013) found that in a corn-soybean-wheat rotation, soil NO_3-N peaked at V5, and was attributed to a greater portion of inorganic N coming from organic matter mineralization. Salinas-Garcia et al. (1997) found that potential N mineralization at flowering and harvest was increased by greater N fertilization, possible because fertilizer N that was immobilized into microbial biomass early in the season was later mineralized. Likewise, Nevins et al. (2020) reported a peak in inorganic N at VT.

The significant decrease in N concentrations following the T3 (Table 2.6, Fig 2.7) sampling time could be contributed to rapid crop N uptake. It is also possible that the decreases in N after peak uptake could be a result of increased immobilization of N (Franzluebbers et al., 1996). The increases in microbial biomass C and N concentrations towards the latter part of the growing season could indicate increased immobilization of N by the microbial biomass in the present study. Likewise, Nevins et al. (2020) attributed N immobilization as the reason for the lack of response of inorganic N to significantly greater soil enzyme activity immediately following peak residue decomposition and C release. These results are contradictory to the present study as a peak in soil enzyme activity was observed towards the latter part of the growing season. The observed increases of enzyme activities correspond to increases in the microbial biomass, further suggesting that increase immobilization of N is occurring towards to latter part of the growing season.

Significant increases in dissolved total N and inorganic N from T0 to T1 can be explained by the addition of N fertilizer between the sampling times. Ammonium added by fertilizer applications can be quickly fixed by clay minerals and later released slowly during the crop

growth season due to increased crop demand with concomitant decrease in NH_4-N concentrations in the soil solution (Nieder et al., 2011). In addition, N additions are known to increase rates of soil N mineralization (Lu et al., 2011; Chen et al., 2018). In a meta-analysis of 64 studies on soil N-acquisition enzyme activities, Chen et al. (2018) found that increased soil organic matter quality can stimulate soil organic N mineralization, even if soil enzyme activities do not increase due to lower C:N ratios.

Phosphorus availability could have contributed to trends within each P fertilizer treatment. Although N pools peaked at T3 for all P fertilizer treatments, the differences within each P treatments were not always significant, especially in SUF and NP treatments. In the BAM treatment, there is a steady increase in N concentrations until T3 followed by a sharp decrease in concentrations throughout the latter part of the growing season. Furthermore, a significant P fertilizer by sampling time interaction was observed for citrate-extractable P in the present study. While citrate-extractable P was relatively stable in SUF and NP treatments throughout the growing season, there was a significant trend in the BAM treatment that was similar that of the N pools. White and Reddy (2000) found that the potential mineralizable N was significantly correlated with total P. Therefore, it is possible that P availability could explain the differences between all three P fertilizer treatments during the growing season.

Differences in soil health indicators at T0 and T5 – When is the best time to soil sample?

Common recommendations for soil testing are to soil sample pre-plant in the spring or post-harvest in the fall (Liebig et al., 2006). In the present study, comparisons between soil health indicators at T0 (which would coincide with pre-plant soil sampling) and T5 (post-harvest) yielded differing results. While several indicators did not significantly differ between T0 and T5 including dissolved organic C, citrate-extractable P, microbial biomass N, and acid and

alkaline phosphatases (Tables 2.4, 2.6, & 2.10). On the other hand, labile nutrients (active C and ACE protein) were significantly higher at T5 compared to T0 (Tables 2.4 & 2.6). Most measures of soil microbial activity and function were significantly higher at T5 compared to T0 (microbial biomass C and P, soil respiration, and glucosidase and glucosaminidase). Nitrogen pools (dissolved total N, dissolved organic N, NH_4-N, NO_3-N, and inorganic N) were inconsistent in trends between T0 and T5 samplings (Table 2.6). There was no significant difference in dissolved total N or NO_3-N at either of these sampling times, while NH_4-N and inorganic N concentrations were significantly higher at T5. As mentioned previously, it is likely that moisture and temperature trends are dictating the response of soil health indicators as very few cover crop by sampling time interactions were observed in this study. The results of the present study further demonstrate the seasonal dynamics of soil health indicators, suggesting that the period of soil sampling (spring or fall) is less important than soil sampling during a consistent season annually (Liebig et al., 2006; Hurisso et al., 2018a).

Conclusion

The presence of cover crops increased active C, ACE, dissolved organic C and N, as well as all measures of microbial activity and function, thereby supporting the first hypothesis. An increase in alkaline phosphatase activity due to the inclusion of a cover crop on P-limited treatments, resulted in a significant increase in activity. However, no significant increase was observed for the BAM treatment with the inclusion of a cover crop. This suggests that enhanced nutrient cycling is possible when P availability is low, adding support for the second hypothesis that increased nutrient cycling occurs in systems undergoing P nutrient draw-down. Finally, it was observed that seasonal variability occurred in all measured soil health indicators. As very few cover crop by sampling time interactions were observed for various soil health indicators,

this suggests that climatic factors were the primary driver of seasonal dynamics in this study. Therefore, the third hypothesis that climatic factors will drive the seasonal dynamics of biological soil health indicators was generally supported.

References

Acosta-Martínez, V., Lascano, R., Calderón, F., Booker, J.D., Zobeck, T.M., Upchurch, D.R., 2011. Dryland cropping systems influence the microbial biomass and enzyme activities in a semiarid sandy soil. Biol. Fertil. Soils, 47, 655-667.

Adetunji, A.T., Ncube, B., Mulidzi, R., Lewu, F.B., 2020. Management impact and benefit of cover crops on soil quality: a review. Soil Tillage Res. 204, 104717.

Alhameid, A., Singh, J., Sekaran, U., Kumar, S., Singh, S., 2019. Soil biological health: influence on crop rotational diversity and tillage on soil microbial properties. Soil Sci. Soc. Am. J. 83, 1431-1442.

Allison, F.E., Doetsch, J.H., Roller, E.M., 1953. Availability of fixed ammonium in soil containing different clay minerals. Soil Sci. 75, 373-381.

Allison, S.D., Weintraub, P.M., Gartner, T.B., Waldrop, M.P., 2011. Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. Soil Enzymology, Springer, pp. 229-243.

Baldrian, P., Merhautova, V., Petrankova, M., Cajthaml, T., Snajdr, J., 2010. Distribution of microbial biomass and activity on extracellular enzymes in a hardwood forest reflect soil moisture content. Appl. Soil Ecol. 46, 177-182.

Baldrian, P., Snajdr, J., Merhautova, V., Dobiasova, P., Cajthaml, T., Valaskova, V., 2013. Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biol. Biochem. 56, 60-68.

Balota, E.L., Calegari, A., Nakatani, A.S., Coyne, M.S., 2014. Benefits of winter cover crops and no-tillage for microbial parameters in a Brazilian Oxisol: A long-term study. Agric. Ecosyst. Environ. 197, 31-40.

Balota, E.L., Filho, A.C., Andrade, D.S., Dick, R.P., 2004. Long-term tillage and crop rotation effects on microbial biomass and C and N mineralization in a Brazilian Oxisol. Soil Tillage Res. 77, 137-145.

Bandick, A.K., and Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biol. Biochem. 31, 1471-1479.

Bastida, F., Moreno, J.L., Hernández, T., García, C., 2006. Microbiological degradation index of soils in a semiarid climate. Soil Biol. Biochem. 38, 3463-3473.

Blanco-Canqui, H., Mikha, M.M., Presley, D.R., Claassen, M.M., 2011. Addition of cover crops enhances no-till potential for improving soil physical properties. Soil Sci. Soc. Am. J. 75, 1471-1482.

Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A., Hergert, G.W., 2015. Cover crops and ecosystem services: insights from studies in temperate soils. Agron. J. 107, 2449-2474.

Boerner, R.E.J., Brinkman, J.A., Smith, A., 2005. Seasonal variations in enzyme activity and organic carbon in soil of a burned and unburned hardwood forest. Soil Biol. Biochem. 37, 1419-1426.

Bolan, N.S., Adriano, D.C., Kunhikrishnan, A., James, T., McDowell, R., Senesi, N., 2011. Dissolved organic matter: biogeochemistry, dynamics, and environmental significance in soils. Adv. Agron. 110, 1-75.

Brennan, E.B., and Acosta-Martinez, V., 2017. Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production. Soil Biol. Biochem. 109, 188-204.

Brennan, E.B., and Acosta-Martinez, V., 2019. Cover crops and compost influence soil enzymes during six years of tillage-intensive organic vegetable production. Soil Sci. Soc. Am. J. 83, 624-637.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S. 1982. Measurement of microbial biomass phosphorus in soil. Soil Biol. Biochem. 14, 319-329.

Burke, J.A., Lewis, K.L., Richie, G.L., Moore-Kucera, J., DeLaune, P.B., Keeling, J.W., 2019. Temporal variability of soil carbon and nitrogen in cotton production on the Texas high plains. Agron. J. 111, 2218-2225.

Cabrera, M.L., and Beare, M.H., 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. Am. J. 57, 1007–1012.

Carver, R.E., Nelson, N.O., Roozeboom, K.L., Kluitenberg, G.J., Tomlinson, P.J., Kang, Q., Abel, D.S., 2022. Cover crop and phosphorus fertilizer management impacts on surface water quality from a no-till corn-soybean rotation. J. Environ. Manage. 301, 113818.

Caudle, C., Osmond, D., Heitman, J., Ricker, M., Miller, G., Wills, S., 2020. Comparison of soil heath metrics for a Cecil soil in the North Caroline Piedmont. Soil Sci. Soc. Am. J. 84, 978-993.

Chahal, I., and Van Eerd, L.L., 2019. Quantifying soil quality in a horticultural-cover cropping system. Geoderma 352, 38-48.

Chavarría, D.N., Verdenelli, R.A., Serri, D.L., Restovich, S.B., Andriulo, A.E., Meriles, J.M., Vargas-Gil, S., 2016. Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability. Eur. J. Soil Biol. 76, 74-82.

Chen, G., Yuan, J., Wang, S., Liang, Y., Wang, D., Zhu, Y., Wang, Y., 2023. Soil and microbial C:N:P stoichiometry play a vital role on regulating P transformation in agricultural ecosystems. Pedosphere doi:10.1016/j.pedsph.2023.06.002

Chen, H., Li, D., Zhao, J., Xiao, K., Wang, K., 2018. Effects of nitrogen addition on activities of soil nitrogen acquisition enzymes: A meta-analysis. Agric. Ecosyst. Environ. 252, 126-131.

Chen, Y., Sun, T-T., Qian, H-Y., Fan, J-B., He, Y-Q., Sun, B., 2016. Nitrogen mineralization as a result of phosphorus supplementation in long-term phosphate deficient soil. Appl. Soil Ecol. 106, 24-32.

Chung, H., Ngo, K.J., Plante, A., Six., 2010. Evidence for carbon saturation in a highly structured and organic-matter-rich soil. Soil. Sci. Soc. Am. J. 74, 130-138.

Ciampitti, I.A., and Salvagiotti, F., 2018. New insights into soybean biological nitrogen fixation. Agron. J. 100, 704-710.

Conant, R.T., Ryan, M.G., Ågren, G.I., Birge, H.E., Davidson, E.A., Eliasson, P.E., Evans, S.E., Frey, S.D., Giardina, C.P., Hopkins, F.M., Hyvönen, R., 2011. Temperature and soil organic matter decomposition rates–synthesis of current knowledge and a way forward. Glob. Change Biol. 17, 3392-3404.

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nat. Geosci. 8, 776-779.

Crookston, B.S., Yost, M.A., Bowman, M., Veum, K., Stevens, J.R., 2023. Microbial respiration gives early indication of soil health improvement following cover crops. J. Soil Water Conserv. 78, 272-281.

Culman, S.W., Snapp, S.S., Green, J.M. and Gentry, L.E., 2013. Short-and long-term labile soil carbon and nitrogen dynamics reflect management and predict corn agronomic performance. Agron. J. 105, 493-502.

Daniel, T.C., Sharpley, A.N., Edwards, D.R., Wedepohl, R., Lemunyon, J.L., 1994. Minimizing surface water eutrophication from agriculture by phosphorus management. J. Soil Water Conserv. 49, 30-38.

Darch, T., Blackwell, M.S.A., Chadwick, D., Haygarth, P.M., Hawkins, J.M.B., Turner, B.L. 2016. Assessment of bioavailable organic phosphorus in tropical forest soils by organic acid extraction and phosphatase hydrolysis. Geoderma 284, 93-102.

de Almeida, R.F., Naves, E.R., da Mota, R.P., 2015. Soil quality: enzymatic activity of soil β-glucosidase. Glob. J. Agric. Res. Rev. 3, 146-150.

Debosz, K., Rasmussen, P.H., Pedersen, A.R., 1999. Temporal variations in microbial biomass C and cellulolytic enzyme activity in arable soils: effects of organic matter input. Appl. Soil Ecol. 13, 209-218.

DeLuca, T.H., Glanville, H.C., Harris, M., Emmett, B.A., Pingree, M.R., de Sosa, L.L., Cerdá-Moreno, C., Jones, D.L., 2015. A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. Soil Biol. Biochem. 88, 110-119.

Dinesh, R., Chaudhuri, S.G., Shiva, K.N., 2009. Soil microbial activity and biomass is stimulated by leguminous cover crops. J. Plant. Nutr. Soil Sci. 172, 288-296.

Eivazi, F., and Tabatabai, M.A., 1977. Phosphatases in soils. Soil Biol. Biochem. 9, 167–172.

Eivazi, F., and Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606.

Ekenler, M., and Tabatabai, M.A., 2002. β-glucosaminidase activity of soils: effect of cropping systems and its relationship to nitrogen mineralization. Biol. Fertil. Soils 36, 367-376.

English, E., Ketterings, Q., Czymmek, K., Gabriel, A., Flis, F., Lawrence, J., 2017. Nitrogen Uptake by Corn. Cornell University Cooperative Extension. Fact Sheet # 98.

Feng, H., Sekaran, U., Wang, T., Kumar, S., 2021. On-farm assessment of cover cropping effects on soil C and N pools, enzyme activities, and microbial community structure. J. Agric. Sci. 159, 216–226.

Feng, J., and Zhu, B., 2019. A global meta-analysis of soil respiration and its components in response to phosphorus addition. Soil Biol. Biochem. 135, 38-47.

Fine, A.K., van Es, H.M., Schindelbeck, R.R., 2017. Statistics, Scoring Functions, and Regional Analysis of a Comprehensive Soil Health Database. Soil Sci. Soc. Am J. 81, 589-601.

Franzluebbers, A.J., Hons, F.M., Zuberer, D.A., 1996. Seasonal dynamics of active soil carbon and nitrogen pools under intensive cropping in conventional and no tillage. J. Plant Nutr. Soil Sci. 159, 343-349.

Frasier, I., Noellemeyer, E., Figuerola, E., Erijman, L., Permingeat, H., Quiroga, A., 2016a. High quality residues from cover crops favor changes in microbial community and enhances C and N sequestration. Glob. Ecol. Conserv. 6, 242-256.

Frasier, I., Quiroga, A., Noellemeyer, E., 2016b. Effect of difference cover crops on C and N cycling in sorghum NT systems. Sci. Total Environ. 562, 628-639.

Geisseler, D., and Horwath, W.R., 2009. Short-term dynamics of soil carbon, microbial biomass, and soil enzyme activities as compared to longer-term effects of tillage in irrigated row crops. Biol. Fertil. Soils 46, 65-72.

Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B., 2010. Pathways of nitrogen utilization by soil microorganisms – A review. Soil Biol. Biochem. 42, 2058-2067.

Grassini, P., Specht, J.E., Tollenaar, M., Ciampitti, I., Cassman, K.G., 2015. High-yield maize-soybean cropping systems in the US Corn Belt. In: Sadra, V.O., Calderini, D.F. (Eds)

Crop Physiology-Applications for Genetic Improvements and Agronomy., second ed. Elsevier, Netherlands

Guo, H. He, X., Li, Y., 2012. Spatial distribution of arbuscular mycorrhiza and glomalin in the rhizosphere of Caragana korshinskii Kom. in the Otindag sandy land, China. Afr. J. Microbiol. Res. 6, 5745-5753.

Guo, P-C., Bohring, J., Scherer, H.W., 1983. Behavior of fertilizer NH_4 in soils with different clay mineral compositions in an incubation experiment. J. Plant Nutr. Soil Sci. 146, 752-759.

Hallama, M., Pekrun, C., Lambers, H., Kandeler, E., 2019. Hidden miners-the roles of cover crops and soil microorganisms in phosphorus cycling. Plant Soil 434, 7-45.

Haney, R.L., and Haney, E.B., 2010. Simple and rapid laboratory method for rewetting dry soil for incubations. Commun. Soil Sci, Plant Anal. 41, 1493–1501.

Hansen, N.C., Daniel, T.C., Sharpley, A.N., Lemunyon, J.L., 2002. The fate and transport of phosphorus in agricultural systems. J. Soil Water Conserv. 57, 408-417.

Hao, X., Najm, M.A., Steenwerth, K.L., Nocco, M.A., Basset, C., Daccache, A., 2023. Are there universal soil responses to cover cropping? A systematic review. Sci. Total Environ. 861, 160600

Hurisso, T.T., Culman, S.W., Horwath, W.R., Wade, J., Cass, D., Beniston, J.W., Bowles, T.M., Grandy, A.S., Franzluebbers, A.J., Schipanski, M.E., Lucas, S.T., 2016. Comparison of permanganate-oxidizable carbon and mineralizable carbon for assessment of organic matter stabilization and mineralization. Soil Sci. Soc. Am. J. 80, 1352-1364.

Hurisso, T.T., Culman, S.W., Zhao, K., 2018a. Repeatability and Spatiotemporal Variability of Emerging Soil Health Indicators Relative to Routine Soil Nutrient Tests. Soil Sci. Soc. Am. J. 82, 939-948.

Hurisso, T.T., Moebius-Clune, D.J., Culman, S.W., Moebius-Clune, B.N., Thies, J.E.,van Es, H.M., 2018b. Soil protein as a rapid soil health indicator of potentially available organic nitrogen. Agric. Environ. Lett. 3, 180006.

Islam, K.R., Roth, G., Rahman, M.A., Didenko, N.O., Reeder, R.C., 2021. Cover crop complements flue gas desulfurized gypsum to improve no-till soil quality. Comm. Soil Sci. Plant Analy. 52, 926-947.

Jackson, L.E., 2000. Fates and losses of nitrogen from a nitrogen-15-labeled cover crop in an intensively managed vegetable system. Soil Sci. Soc. Am. J. 64, 1404-1412.

Jiang, L., Wang, S., Pang, Z., Wang, C., Meng, F., Lan, Z., Zhou, X., Li, Y., Zhang, Z., Luo, C., Jones, D.L., 2021. Abiotic and biotic controls of soil dissolved organic nitrogen along a precipitation gradient on the Tibetan plateau. Plant Soil 459, 65-78.

Jiang, X., Shi, X., Wright, A.L., 2013. Seasonal variability of microbial biomass associated with aggregates in a rice-based ecosystem. Eur. J. Soil Biol. 56, 84-88.

Jiao, X-G., Gao, C-S., Lu, G-H., Sui, Y-Y., 2011. Effect of long-term fertilization on soil enzyme activities under different hydrothermal conditions in Northeast China. J. Integr. Agric. 10, 412-422.

Jokela, W.E., Grabber, J.H., Karlen, D.L., Balser, T.C., Palmquist, D.E., 2009. Cover crop and liquid manure effects on soil quality indicators in a corn silage system. Agron. J. 101, 727-737.

Jones, D. L., Willett, V. B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem. 38, 991–999.

Kalbitz, K., Solinger, S., Park, J.H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: a review. Soil Sci. 165, 277-304.

Kleinman, P.J.A., Sharpley, A.N., Moyer, B.G., Elwinger, G.F., 2002. Effect of mineral and manure phosphorus sources on runoff phosphorus. J. Environ. Qual. 31, 2026-2033.

Kopittke, P.M., Menzies, N.W., Wang, P., McKenna, B.A., Lombi, E., 2019. Soil and the intensification of agriculture for global food security. Environ. Int. 132, 105078.

Kotroczo, Z., Veres, Z., Fekete, I., Krakomperger, Z., Attila Toth, J., Lajtha, K., Tothmeresz, B., 2014. Soil enzyme activity in response to long-term organic matter manipulation. Soil Biol. Biochem. 70, 237-243.

Koudahe, K., Allen, S.C., Djaman, K., 2022. Critical review of the impact of cover crops on soil properties. Int. Soil Water Conserv. Res. 10, 343-354.

Kowalenko, C.G., and Cameron, D.R., 1976. Nitrogen transformations in an incubated soil as affected by combinations of moisture content and temperature and adsorption-fixation of ammonium. Can. J. Soil Sci. 56, 63-70.

Law, S.M., and Maherali, H., 2023. Variation in glomalin-related soil protein and plant growth response to arbuscular mycorrhizal fungi along a nutrient gradient in temperate grasslands. Plant Soil 487, 623-637.

Leikam, D.F., Lamond, R.E., Mengel, D.B., 2003. Providing flexibility in phosphorus and potassium fertilizer recommendations. Better Crops 87, 6-10.

Liebig, M., Carpenter-Boggs, L., Johnson, J.M.F., Wright, S., Barbour, N., 2006. Cropping system effects on soil biological characteristics in the Great Plains. Renew. Agric. Food Syst. 21, 36-48.

Lu, M., Yang, Y., Luo, Y., Fang, C., Zhou, X., Chen, J., Yang, X. and Li, B., 2011. Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. New Phytol. 189, 1040-1050.

Lucas, S., and Weil, R., 2021. Can permanganate oxidizable carbon predict soil function responses to soil organic matter management? Soil Sci. Soc. Am. J. 85, 1768-1784.

Lucas, S., and Weil, R.R., 2012. Can a labile carbon text be used to predict crop responses to improved soil organic matter management? Agron. J. 104, 1160-1170.

Luo, L., Meng, H., Gu, J-D., 2017. Microbial extracellular enzymes in biogeochemical cycling of ecosystems. J. Environ. Manag. 197, 539-549.

Macnack, N., Khin Chim, B., Amedy, B., Arnall, B., 2011. Fertilization based on sufficiency, build-up and maintenance concept. Oklahoma Cooperative Extension Service Publication, PSS-2266.

Marklein, A.R., and Houlton, B.Z., 2011. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystem. New Phytol. 193, 696-704.

Marshall, C.B., Burton, D.L., Lynch, D.H., 2021. Cover crops improve some, but not all, soil health indicators in horticultural rotations. Can. J. Plant Sci. 102, 1-10.

Mbuthia, L.W., Acosta-Martinez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N., 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. Soil Biol. Biochem. 89, 24-34.

McDaniel, M.D., and Grandy, A.S., 2016. Soil microbial biomass and function are altered by 12 years of crop rotation. SOIL 2, 583-599.

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecol. Appl. 24, 560-570.

Menezes-Blackburn, D., Paredes, C., Zhang, H., Giles, C.D., Darch, T., Stutter, M., George, T.S., Shand, C., Lumsdon, D., Cooper, P., Wendler, R., Brown, L., Blackwell, M., Wearing, C., Haygarth, P.M., 2016. Organic acids regulation of chemical-microbial phosphorus transformations in soils. Environ. Sci. Technol. 50, 11521-11531.

Muhammad, I., Wang, J., Sainju, U.M., Zhang, S., Zhao, F., Khan, A., 2021. Cover cropping enhances soil microbial biomass and affects microbial community structure: A meta-analysis. Geoderma 381, 114696.

Mukumbareza, C., Muchaonyerwa, P., Chiduza, C., 2015. Effects of oats and grazing vetch cover crops and fertilisation on microbial biomass and activity after five years of rotation with maize. S. Afr. J. Plant Soil 32, 189-197.

Murphy, J., and Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica. Chimica. Acta. 27, 31-36.

Nevins, C.J., Lacey, C., Armstrong, S., 2020. The synchrony of cover crop decomposition, enzyme activity, and nitrogen availability in a corn agroecosystem in the Midwest United States. Soil Tillage Res. 197, 104518.

Nieder, R., Benbi, D.K., Scherer, H.W., 2011. Fixation and defixation of ammonium in soils: a review. Biol. Fertil. Soils 47, 1-14.

Nunes, M.R., van Es, H.M., Schindelbeck, R., Ristow, A.J.., Ryan, M., 2018. No-till and cropping system diversification improve soil health and crop yield. Geoderma 328, 30-43.

O'Halloran, I. P., and Cade-Menun, B.J., 2008. Chapter 24 - Total and organic phosphorus. In Carter, M.R., and Gregorich, E.G. (Eds), Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, Taylor & Francis Group, pp. 279-280

Olander, L.P., and Vitousek, P.M., 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49, 175-190.

Omer, M., Idowu, O.J., Ulery, A.L., VanLeeuwen, D., Guldan, S.J., 2018. Seasonal changes of soil quality indictors in selected arid cropping systems. Agric. 8, 124.

Papendick, R.I., and Campbell, G.S., 1981. Theory and measurement of water potential. In Parr, J.F., Gardner, W.R., Elliott, L.F., (eds) Water potential relations in soil microbiology, Special Publication No. 9, Soil Science Society of America, Madison, WI, pp.1-22.

Parham, J.A., and Deng, S.P., 2000. Detection, quantification and characterization of β-glucosaminidase activity in soil. Soil Biol. Biochem., 32, 1183–1190.

Pokhrel, S., Kingery, W.L., Cox, M.S., Shankle, M.W., Shanmugam, S.G., 2021. Impact of cover crops and poultry litter on selected soil properties and yield in dryland soybean production. Agronomy 11, 119.

Qiu, L., Lin, H., Song, B., Kong, T., Sun, W., Sun, X., Zhang, Y., Li, B., 2021. Glomalin-related soil protein (GRSP) in metal sequestration at Pb/Zn-contaminated sites. J. Soils Sed. 22, 577-593.

Roberts, P., and Jones, D.L., 2008. Critical evaluation of methods for determining total protein in soil solution. Soil Biol. Biochem. 40, 1485-1495.

Rochette, P., Desjardins, R.L., Pattey, E., 1991. Spatial and temporal variability of soil respiration in agricultural fields. Can. J. Soil Sci. 71, 189-196.

Roth, R.T., Lacey, C.G., Camberato, J.J., Armstrong, S.D., 2023. Quantifying the fate of nitrogen from cereal rye root and shoot biomass using 15N. Nutr. Cycling Agroecosyst. 125, 219-234.

Roth, T. and Waite, J., 2021. Early Spring Carbon to Nitrogen Ratios of Cereal Rye Varieties. United States Department of Agriculture-National Resource Conservation Service. Assessed on: 10-10-2023: Assessed at: https://www.nrcs.usda.gov/plantmaterials/kspmcsr13883.pdf

Salinas-Garcia, J.R., Hons, F.M., Matocha, J.E. and Zuberer, D.A., 1997. Soil carbon and nitrogen dynamics as affected by long-term tillage and nitrogen fertilization. Biol. Fertil. Soils 25, 182-188.

Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., Reddy, K.R., 1994. Managing agricultural phosphorus for protection of surface waters: issues and options. J. Environ. Qual. 23, 437-451.

Shi, Y., Lalande, R., Hamel, C., Ziadi, N., Gagnon, B., Hu, Z., 2013. Seasonal variation of microbial biomass, activity, and community structure in soil under different tillage and phosphorus management practices. Biol. Fertil. Soils 49, 803-818.

Shi, Y., Ziadi, N., Hamel, C., Bélanger, G., Abdi, D., Lajeunesse, J., Lafond, J., Lalande, R., Shang, J., 2020. Soil microbial biomass, activity and community structure as affected by mineral phosphorus fertilization in grasslands. Appl. Soil Ecol. 146, 103391.

Shu, X., Zou, Y., Shaw, L.J., Todman, L., Tibbett, M., Sizmur, T., 2021. Cover crop residue diversity enhances microbial activity and biomass with additive effects on microbial structure. Soil Res. 60, 349-359.

Singh, J., and Kumar, S., 2020. Seasonal changes of soil carbon fractions and enzyme activities in response to winter cover crops under long-term rotation and tillage systems. Eur. J. Soil Sci. 1-14.

Sinsabaugh, R.L., and Follstad Shah, J.J., 2012. Ecoenzymatic stoichiometry and ecological theory. Annu. Rev. Ecol. Evol. Syst. 43, 313-343.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252-1264.

Six, J., Conan, R.T., Paul, E.A., Paustain, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soil. Plant Soil 241, 155-176.

Smith, A.P., Marin-Spiotta, E., Balser, T., 2015. Successional and seasonal variations in soil and litter microbial community structure and function during tropical postagricultural forest regeneration: a multiyear study. Glob. Change Biol. 21, 3532-3547.

St. Aime, R., Bridges Jr, W.C., Narayanan, S., 2023. Fall–winter cover crops promote soil health and weed control in the southeastern clayey soils. Agron. J. 115, 242-260.

Stahl, K., 2023. The effect of cover crops and P management strategies on soil physical properties and soil organic carbon (Master Thesis).

Staunton, S., Saby, N.P.A., Arrouays, D., Quiquampoxi, H., 2020. Can soil properties and land use explain glomalin-related soil protein (GRSP) accumulation? A nationwide survey in France. Catena 193, 104620.

Steele, M.K., Coale, F.J., Hill, R.H., 2012. Winter annual cover crop impacts on no-till soil physical properties and organic matter. Soil Sci. Soc. Am. J. 76, 2164-2173.

Steenwerth, K., and Belina, K.M., 2008. Cover crops and cultivation: impacts on soil N dynamics and microbiological function in a Mediterranean vineyard agroecosystem. Appl. Soil Ecol. 40, 370-380.

Strickland, M.S., Thomason, W.E., Avera, B., Franklin, J., Minick, K., Yamada, S., Badgley, B.D., 2019. Short-term effects of cover crops on soil microbial characteristics and biogeochemical processes across actively managed farms. Agrosyst. Geosci. Environ. 2, 180064.

Sun, Y., Wang, C., Chen, X., Liu, S., Lu, X., Chen, H.Y.H., Ruan, H., 2022. Phosphorus additions imbalance terrestrial ecosystem C:N:P stoichiometry. Glob. Change Biol. 28, 7353-7365.

Tang, X., Placella, S.A., Dayde, F., Bernard, L., Robin, A., Journet, E-P., Justes, E., Hinsinger, P., 2016. Phosphorus availability and microbial community in the rhizosphere of intercropped cereal and legume along a P-fertilizer gradient. Plant Soil 407, 119-134.

Thapa, V.R., Ghimire, R., Acosta-Martinez, V., Marsalis, M.A., Schipanski, M.E., 2021. Cover crop biomass and species composition affect soil microbial community structure and enzyme activities in semiarid cropping systems. Appl. Soil Ecol. 157, 103735.

Turmel, M-S., Speratti, A., Baudron, F., Verhulst, N., Govaerts, B., 2015. Crop residue management and soil health: A systems analysis. Agric. Sys. 134, 6-16.

Tyler, H.L., 2020. Winter cover crops and no till management enhance enzyme activities in soybean field soils. Pedobiologia 81-82, 150666.

Ulrich-Schad, J.D., De Jalón, S.G., Babin, N., Pape, A., Prokopy, L.S., 2017. Measuring and understanding agricultural producers' adoption of nutrient best management practices. J. Soil Water Conserv. 72, 506-518.

Vance, E.D., Brookes, P.C., Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703-707.

Wang, F., Weil, R.R., Nan, X., 2017. Total and permanganate-oxidizable organic carbon in the corn rooting zone of US Coastal Plain soils as affected by forage radish cover crops and N fertilizer. Soil Tillage Res. 165, 247-257.

Wang, Q.R., Li, Y.C. and Klassen, W., 2007. Changes of soil microbial biomass carbon and nitrogen with cover crops and irrigation in a tomato field. J. Plant Nutr. 30, 623-639.

Wang, R., Bicharanloo, B., Hou, E., Jiang, Y. and Dijkstra, F.A., 2022a. Phosphorus supply increases nitrogen transformation rates and retention in soil: a global meta-analysis. Earth's Future, 10, e2021EF002479.

Wang, X., Cao, Q., Yang, W., Zhu, X., 2022b. Spatial changes in glomalin-related soil protein and their correlation with soil properties in the black soil region of northeast China. Agronomy 12, 2165.

Wang, Y.P., Law, R.M., Pak, B., 2010. A global model of carbon, nitrogen and phosphorus cycles for the terrestrial biosphere. Biogeosciences 7, 2261-2282.

Warren, J., Raun, B., Zhang, H., Arnall, B., Penn, C., Bushlong, J., Abit, J., 2017. Oklahoma soil fertility handbook. Oklahoma Cooperative Extension Service. E-1039.

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. Am. J. Alter. Agric. 18, 3-17.

White, J.R., and Reddy, K.R., 2000. Influence of phosphorus loading on organic nitrogen mineralization of everglade soils. Soil. Sci. Soc. Am. J. 64, 1525-1534.

White, K.E., Brennen, E.B., Cavigelli, M.A., Smith. R.F., 2020. Winter cover crops increase readily decomposable soil carbon, but compost drives total soil carbon during eight years of intensive, organic vegetable production in California. PLOS One 15, e0228677.

Wood, S.A., and Bowman, M., 2021. Large-scale farmer-led experiment demonstrates positive impact of cover crops on multiple soil health indicators. Nat. Food, 2, 97-103.

Wright, S.F., and Upadhyaya, A., 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci., 161, 575–586.

Wright, S.F., and Upadhyaya, A., 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil 198, 97–107.

Wu, F., Dong, M., Liu, Y., Ma, X., An, L., Young, J.P.W., Feng, H., 2011. Effects of long-term fertilization on AM fungal community structure and Glomalin-related soil protein in the Loess Plateau of China. Plant Soil, 342, 233-247.

Yu, Q., Ma, S., Ni, X., Ni, X., Guo, Z., Tan, X., Zhong, M., Hanif, M.A., Zhu, J., Ji, C., Zhu, B., 2022. Long-term phosphorus addition inhibits phosphorus transformations involved in soil arbuscular mycorrhizal fungi and acid phosphatase in two tropical rainforests. Geoderma, 425, 116076.

Yuan, T., Chen, S., Zhang, Y., Ji, L., Dari, B., Sihi, D., Xu, D., Zhang, Z., Yan, Z., Wang, X., 2022. Mechanism of increased soil phosphorus availability in a calcareous soil by ammonium polyphosphate. Biol. Fertil. Soils 58, 649-665.

Zhang, J-S., Guo, J-F., Chen. G-S., Qian, W., 2005. Soil microbial biomass and its controls. J. For. Res. 16, 327-330.

Zhou, X., Chem, C., Wu, H., Xu, Z., 2012. Dynamics of soil extractable carbon and nitrogen under difference cover crop residues. J. Soils Sediments 12, 844-853.

Table 2.1 p-nitrophenol substrates and start and stop buffer for β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase enzyme assays

Enzyme	Substrate	Start Buffer	Stop Buffer
β-glucosidase	0.05 M p-nitrophenyl-β-D glucopyranoside	MUB pH 6	0.1 M THAM pH 12
β-glucosaminidase	0.01 M p-nitrophenyl-N-acetyl-β-D-glucosaminide	0.1 M Acetate Buffer	0.5 M NaOH
Acid Phosphatase	0.05 M p-nitrophenyl phosphate	MUB pH 6.5	0.5 M NaOH
Alkaline Phosphatase	0.05 M p-nitrophenyl phosphate	MUB pH 11	0.5 M NaOH

Table 2.2 Sampling dates, soil moisture, and weather conditions at time of sample collection in 2021

Time	Sampling Date	Days after cover crop termination	Cumulative Precip. (20-d) (mm)	Soil Moisture* ($g\ g^{-1}$)	Minimum Daily Air Temperature (°C)	Maximum Daily Air Temperature (°C)
T0	12 April	-	71.63	0.216a	4.57	18.27
T1	4 May	21	20.56	0.179c	6.78	18.46
T2	26 May	43	118.87	0.185b	12.42	22.57
T3	17 June	65	33.53	0.062f	15.72	28.90
T4	7 July	85	24.38	0.141e	19.62	31.78
T5	17 September	157	95.76	0.163d	17.75	30.82

*Letters indicate significant differences in soil moisture at $p < 0.05$; Cumulative precipitation, minimum and maximum daily air temperature were revived from the Kansas Mesonet station near the field site in Ashland Bottoms. Cumulative precipitation reported at T0 and T5 represents precipitation received 20-days before sample collection. Cumulative precipitation reported at T1 through T4 represents precipitation received between the sampling times.

Table 2.3 Analysis of variance (ANOVA) table for soil moisture, active carbon (C), dissolved organic C, and citrate-extractable phosphorus (P) in 2021. Table abbreviations include block (BLOC), P fertilizer treatments (TRT), cover crop (CC), and sampling time (TIME). Bolded values represent statistical significance at $p < 0.05$

	Moisture	**Active C**	**Dissolved Organic C**	**Citrate-P**
BLOC[a]	0.0013	0.0514	0.0331	0.5527
TRT	0.3339	0.1775	0.8833	**<.0001**
CC	**0.0038**	**<.0001**	**0.0327**	0.6244
TIME	**<.0001**	**<.0001**	**<.0001**	**<.0001**
TRT*CC	**0.0166**	-	-	0.0746
TRT*TIME	0.6601	-	-	**<.0001**
CC*TIME	0.1779	-	-	0.7711
Covariance Structure[b]	CSH	CSH	AR(1)	ARH(1)

[a]p value presented in table represent two-way ANOVA (soil moisture and citrate-extractable P) and one-way ANOVA results (active C and dissolved organic C). If no significant two-way interactions were observed, the interaction terms were removed from the model and only main effects were analyzed; [b]Covariance structure abbreviations: heterogeneous compound symmetry (CSH), autoregressive (AR(1)), and first-order autoregressive (ARH(1))

Table 2.4 Least squares (LS) means table for soil moisture, active carbon (C), dissolved organic C, and citrate-extractable phosphorus (P) in 2021. Table abbreviations include, P fertilizer treatments (TRT), no P (NP), build and maintain (BAM), sufficiency (SUF) presence of cover crop (CC), absence of cover crop (NC). Letters indicate significant difference at $p < 0.05$.

Effect	TRT	Cover	Time	Moisture	Active C	Dissolved Organic C	Citrate-P
				g g soil^{-1}	mg C kg soil^{-1}	mg C kg soil^{-1}	mg P kg soil^{-1}
TRT	**NP**			0.154	320.9	49.22	0.73C
TRT	**BAM**			0.160	329.54	50.28	6.46A
TRT	**SUF**			0.158	336.64	48.56	1.99B
CC		**CC**		0.162A	350.58A	52.34A	2.03
CC		**NC**		0.153B	307.48B	46.45B	2.19
TIME			**T0**	0.216A	344.36B	41.28C	1.26B
TIME			**T1**	0.179C	282.37C	54.67B	1.02B
TIME			**T2**	0.185B	252.32D	42.24C	4.41A
TIME			**T3**	0.062F	365.15AB	-	3.09A
TIME			**T4**	0.141E	359.11AB	62.74A	3.72A
TIME			**T5**	0.163D	370.86A	47.42C	1.36B

[a]Sampling times: 12-Apr (T0), 4-May (T1), 26-May (T2), 17-Jun (T3), 7-Jul (T4), and 17-Sept (T5)

Table 2.5 Analysis of variance (ANOVA) table for autoclaved citrate extractable (ACE) protein, dissolved total nitrogen (N), NO_3-N, NH_4-N, inorganic N, and dissolved total N in 2021. Table abbreviations include block (BLOC), phosphorus (P) fertilizer treatments (TRT), cover crop (CC), and sampling time (TIME). Bolded values represent statistical significance at $p < 0.05$.

	ACE Protein	**Dissolved Total N**	**NH_4-N**	**NO_3-N**	**Inorganic N**	**Dissolved Organic N**
BLOC[a]	0.4160	0.6428	0.3871	0.1866	0.0656	0.0003
TRT	**0.0206**	**<.0001**	**0.0005**	**0.0005**	**<.0001**	**<.0001**
CC	**0.0013**	0.5368	0.9989	0.7755	0.6537	**0.0430**
TIME	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
TRT*CC	-	0.7465	0.9466	0.1316	0.0664	**0.0393**
TRT*TIME	-	**<.0001**	**0.0314**	**<.0001**	**<.0001**	**0.0003**
CC*TIME	-	0.9414	0.6779	0.9291	0.8929	0.4612
Covariance Structure[b]	CS	ARH(1)	ARH(1)	ARH(1)	ARH(1)	CSH

[a]p value presented in table represent two-way ANOVA (dissolved total N, NO3-N, NH4-N, and dissolved organic N) and one-way ANOVA results (ACE protein). If no significant two-way interactions were observed, the interaction terms were removed from the model and only main effects were analyzed.

[b]Covariance structure abbreviations: compound symmetry (CS), first-order autoregressive (ARH(1)), and heterogenous compound symmetry

Table 2.6 Least squares (LS) means table for autoclaved citrate extractable (ACE) protein, dissolved total nitrogen (N), NO_3-N, NH_4-N, inorganic N, and dissolved total N in 2021. Table abbreviations include, phosphorus (P) fertilizer treatments (TRT), no P (NP), build and maintain (BAM), sufficiency (SUF) presence of cover crop (CC), absence of cover crop (NC). Letters indicate significant difference at $p < 0.05$.

Effect	TRT	Cover	Time	ACE Protein	Dissolved Total N	NH_4-N	NO_3-N	Inorganic N	Dissolved Organic N
				mg protein kg soil^{-1}	mg N kg soil^{-1}	mg NO3-N kg soil^{-1}	mg NH4-N kg soil^{-1}	mg N kg soil^{-1}	mg N kg soil^{-1}
TRT	**NP**			3914B	56.45B	9.82B	34.34B	43.55B	15.81A
TRT	**BAM**			4282A	82.40A	28.04A	49.91A	76.80A	12.15C
TRT	**SUF**			4275A	56.08B	14.74B	33.28B	47.60B	12.90B
CC		**CC**		4380A	66.44	17.41	39.66	57.02	14.46A
CC		**NC**		3938B	63.51	17.66	38.69	54.94	12.78B
TIME			**T0**	3977C	12.11C	2.78C	3.56D	6.35D	6.34C
TIME			**T1**	3512D	73.63B	30.37A	42.88BC	72.04B	-
TIME			**T2**	3785C	82.67B	18.49AB	55.61B	70.93B	14.6B
TIME			**T3**	4877A	137.32A	24.33A	91.95A	116.28A	21.03A
TIME			**T4**	4544B	70.31B	20.9A	37.47C	58.37B	12.49B
TIME			**T5**	4381B	13.84C	8.34B	3.60D	11.94C	-

[a]Sampling times: 12-Apr (T0), 4-May (T1), 26-May (T2), 17-Jun (T3), 7-Jul (T4), and 17-Sept (T5)

Table 2.7 One-way analysis of variance (ANOVA) table for microbial biomass carbon (C), nitrogen (N), phosphorus (P), biomass C:N, N:P, and C:P ratios and soil respiration in 2021. Table abbreviations include block (BLOC), P fertilizer treatments (TRT), cover crop (CC), and sampling time (TIME). Bolded values represent statistical significance at $p < 0.05$.

	Microbial Biomass C	**Microbial Biomass N**	**Microbial Biomass P**	**C:N Ratio**	**N:P Ratio**	**C:P Ratio**	**Soil Respiration**
BLOC	0.0441	0.2905	0.8284	0.0637	0.5217	0.4592	0.0053
TRT	0.2943	0.9083	**<.0001**	0.5931	**<.0001**	**<.0001**	**<.0001**
CC	**0.0224**	0.3610	0.7478	0.7719	0.6459	0.8930	**<.0001**
TIME	**<.0001**	**<.0001**	**<.0001**	0.1223	0.7951	0.6178	**<.0001**
Covariance Structure[a]	CS	CSH	CSH	ARH(1)	ARH(1)	CSH	AR(1)

[a]Covariance structure abbreviations: compound symmetry (CS), heterogeneous compound symmetry (CSH), and first-order autoregressive (ARH(1))

Table 2.8 Least squares (LS) means table for microbial biomass carbon (C), nitrogen (N), phosphorus (P), biomass C:N, N:P, and C:P ratios and soil respiration in 2021. Table abbreviations include, P fertilizer treatments (TRT), no P (NP), build and maintain (BAM), sufficiency (SUF) presence of cover crop (CC), absence of cover crop (NC). Letters indicate significant difference at $p < 0.05$.

Effect	TRT	Cover	Time	Microbial Biomass C	Microbial Biomass N	Microbial Biomass P	C:N Ratio	N:P Ratio	C:P Ratio	Soil Respiration
				mg C kg soil^{-1}	mg N kg soil^{-1}	mg P kg soil^{-1}				mg CO2 g soil^{-1}
TRT	**NP**			94.05	14.86	0.39C	8.42	15.70A	169.71A	12.88B
TRT	**BAM**			104.67	12.06	2.49A	9.23	3.66C	36.67C	14.48A
TRT	**SUF**			108.09	13.03	1.35B	10.28	6.06B	77.95B	14.76A
CC		**CC**		111.65A	14.58	1.19	9.44	7.24	78.16	15.58A
CC		**NC**		92.89B	12.05	1.32	9.11	6.83	78.94	12.61B
TIME			**T0**	116.33B	17.57A	0.75C	7.10	-	-	14.36B
TIME			**T1**	55.16D	6.63C	1.07BC	8.88	5.55	69.92	14.90B
TIME			**T2**	95.82C	11.80B	1.24BC	9.01	7.63	78.67	10.72C
TIME			**T3**	-	-	1.05BC		-	-	11.26C
TIME			**T4**	106.98BC	14.19AB	1.50AB	12.84	7.41	98.11	13.71B
TIME			**T5**	137.06A	16.39AB	2.13A	9.40	7.80	70.57	21.41A

[a]Sampling times: 12-Apr (T0), 4-May (T1), 26-May (T2), 17-Jun (T3), 7-Jul (T4), and 17-Sept (T5)

Table 2.9 Analysis of variance (ANOVA) table for acid phosphatase, alkaline phosphatase, β-glucosidase, and β-glucosaminidase in 2021. Table abbreviations include block (BLOC), phosphorus (P) fertilizer treatments (TRT), cover crop (CC), and sampling time (TIME). Bolded values represent statistical significance at $p < 0.05$.

	Acid Phosphatase	**Alkaline Phosphatase**	**Glucosidase**	**Glucosaminidase**
BLOC[a]	0.0035	0.1929	0.0735	0.0183
TRT	0.7848	**0.0193**	0.5823	0.5302
CC	**0.0003**	**0.0005**	**0.0020**	**<.0001**
TIME	**<.0001**	**<.0001**	**<.0001**	**<.0001**
TRT*CC	0.3057	**0.0001**	-	-
TRT*TIME	0.5209	0.5865	-	-
CC*TIME	**0.0034**	0.2938	-	-
Covariance Structure[b]	CS	CSH	CSH	CS

[a]p value presented in table represent two-way ANOVA (acid and alkaline phosphatase) and one-way ANOVA results (glucosidase and glucosaminidase). If no significant two-way interactions were observed, the interaction terms were removed from the model and only main effects were analyzed.

[b]Covariance structure abbreviations: compound symmetry (CS) and heterogeneous compound symmetry (CSH)

Table 2.10 Least squares (LS) means table for acid phosphatase, alkaline phosphatase, β-glucosidase, and β-glucosaminidase in 2021. Table abbreviations include, phosphorus (P) fertilizer treatments (TRT), no P (NP), build and maintain (BAM), sufficiency (SUF) presence of cover crop (CC), absence of cover crop (NC). Letters indicate significant difference at $p < 0.05$.

Effect	TRT	Cover	Time	Acid Phosphatase	Alkaline Phosphatase	Glucosidase	Glucosaminidase
TRT	**NP**			291.27	105.79A	112.27	35.31
TRT	**BAM**			289.21	89.71B	116.47	33.86
TRT	**SUF**			299.22	91.09B	122.52	35.56
CC		**CC**		326.14A	106.21A	128.15A	40.58A
CC		**NC**		260.31B	84.84B	106.02B	29.25B
TIME			**T0**	308.41A	104.28A	113.44B	36.99B
TIME			**T1**	290.02BC	92.22B	97.02C	33.32C
TIME			**T2**	276.78C	78.37C	98.35C	27.94D
TIME			**T3**	276.98C	95.53B	124.79AB	34.66BC
TIME			**T4**	305.68AB	92.92B	131.06A	34.74BC
TIME			**T5**	301.50AB	109.83A	137.86A	41.83A

[a]Sampling times: 12-Apr (T0), 4-May (T1), 26-May (T2), 17-Jun (T3), 7-Jul (T4), and 17-Sept (T5)

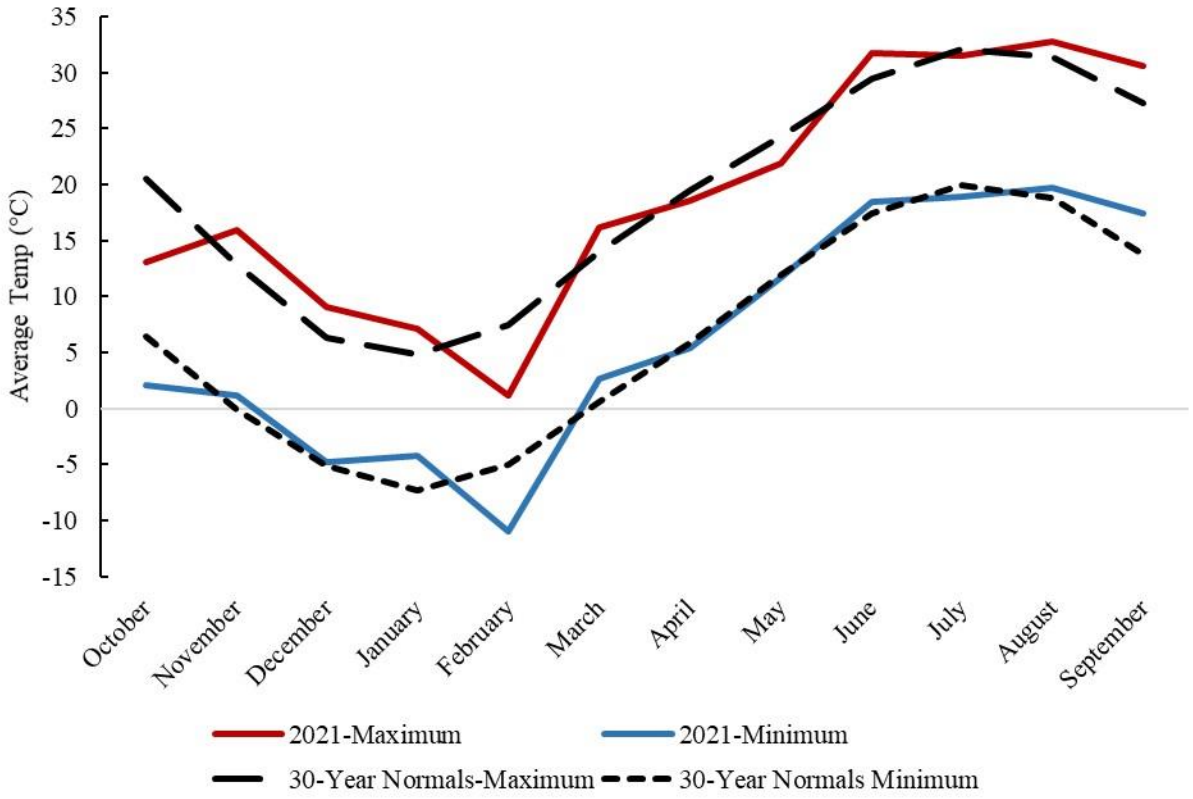

Figure 2.1 Monthly maximum and minimum daily air temperatures for 2021 harvest year as recorded by Kansas Mesonet station nearby field site in Ashland Bottoms (operated by Kansas State University). 30-years minimum and maximum daily air temperatures represent normals for Riley County, KS.

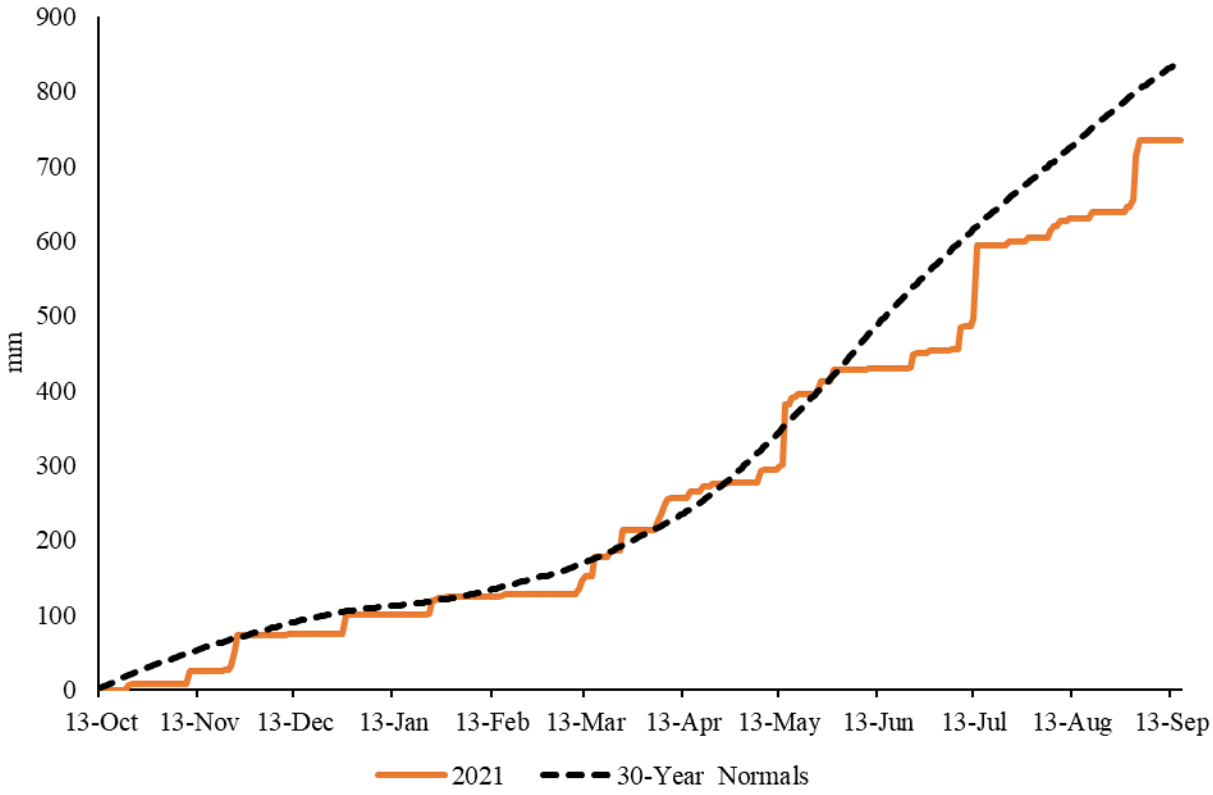

Figure 2.2 Cumulative precipitation during 2021 harvest year as reported by the Kansas Mesonet station nearby field site in Ashland Bottoms (operated by Kansas State University). 30-years minimum and maximum daily air temperatures represent normals for Riley County, KS.

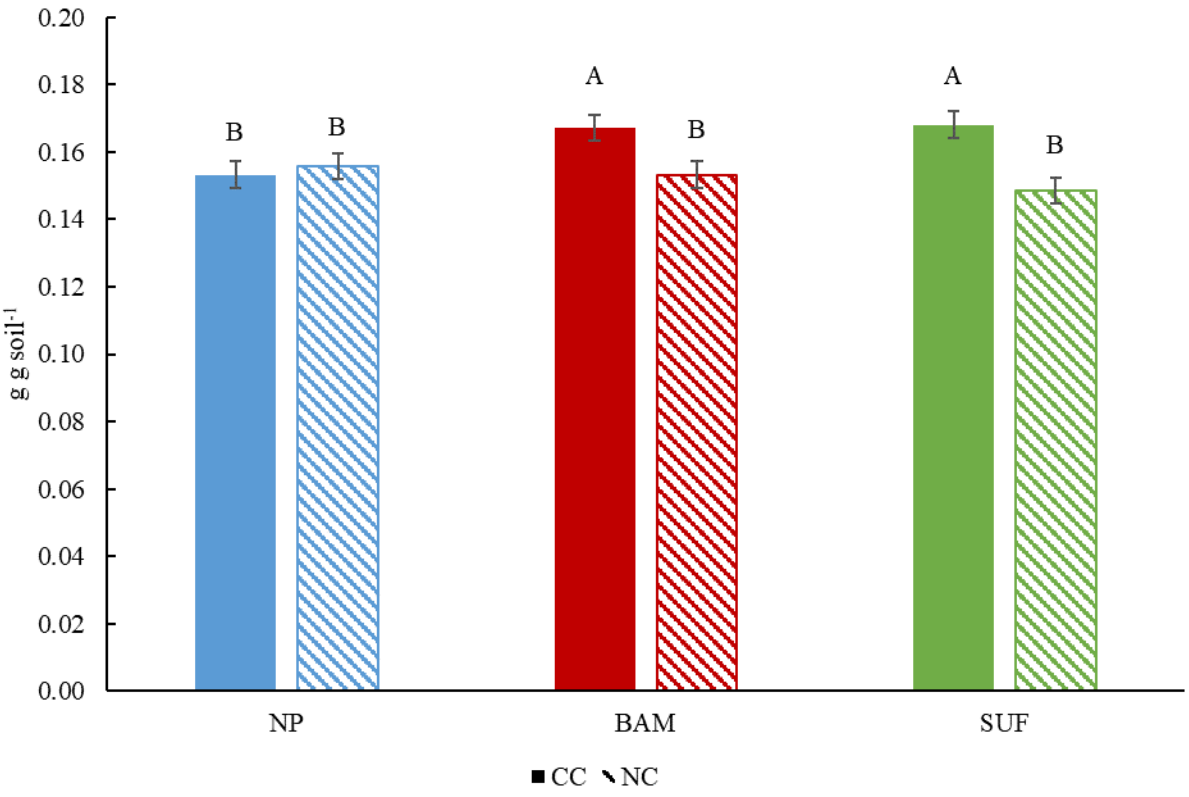

Figure 2.3 Phosphorus (P) fertilizer treatment by cover crop interaction for soil moisture for 2021. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments, and presence of a cover crop (CC) and absence of a cover crop (NC).

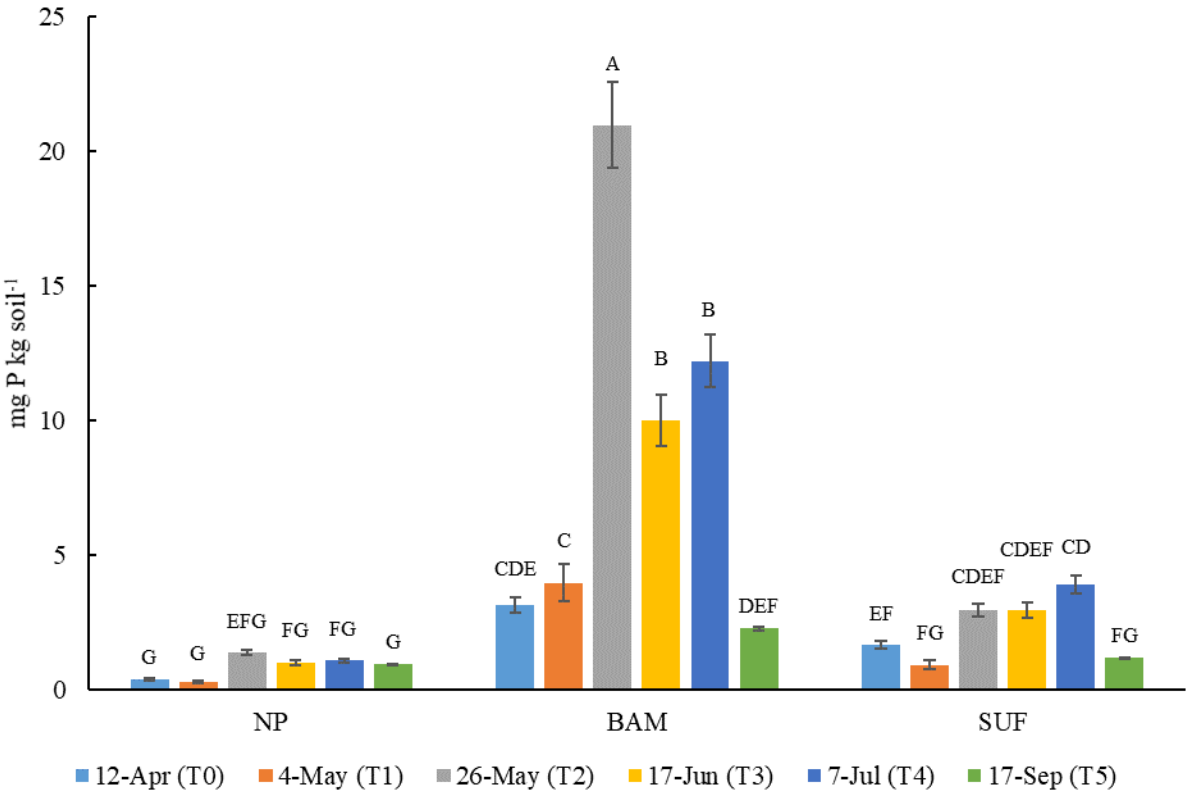

Figure 2.4 Phosphorus (P) fertilizer treatment by sampling time interaction for citrate-extractable P for 2021. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

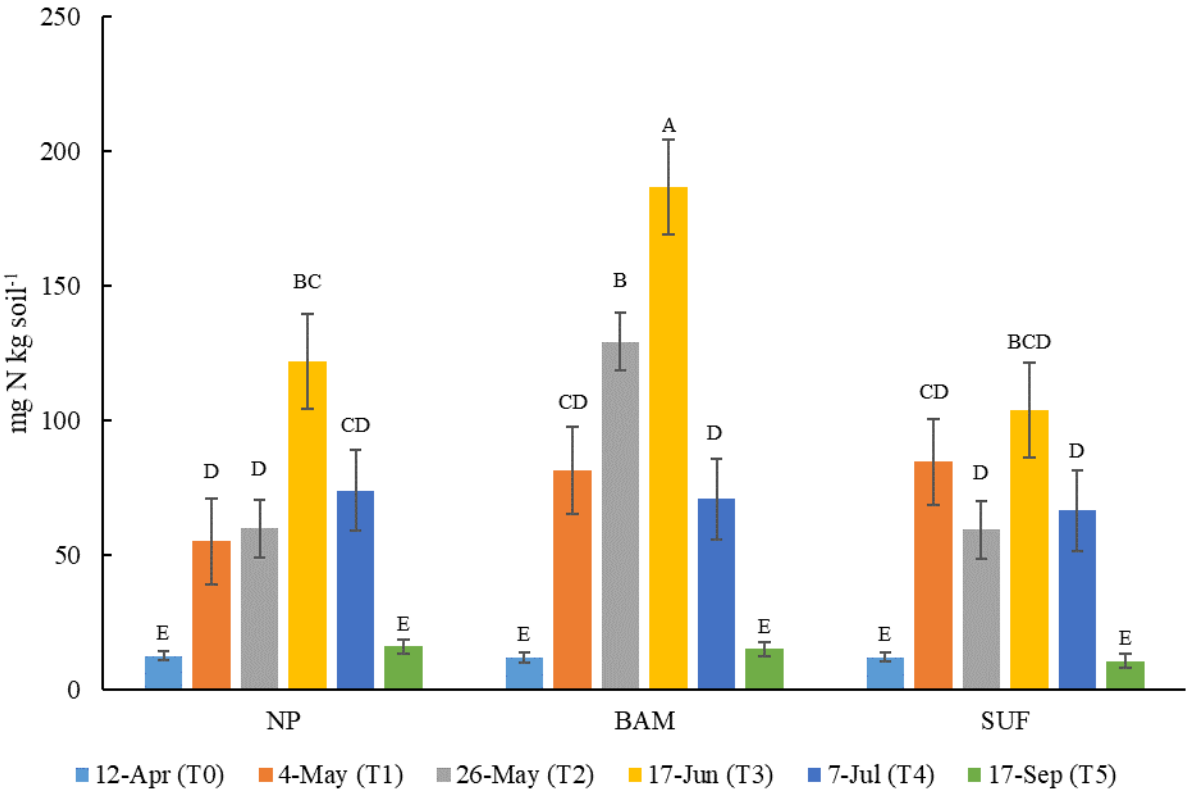

Figure 2.5 Phosphorus (P) fertilizer treatment by sampling time interaction for dissolved total N for 2021. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

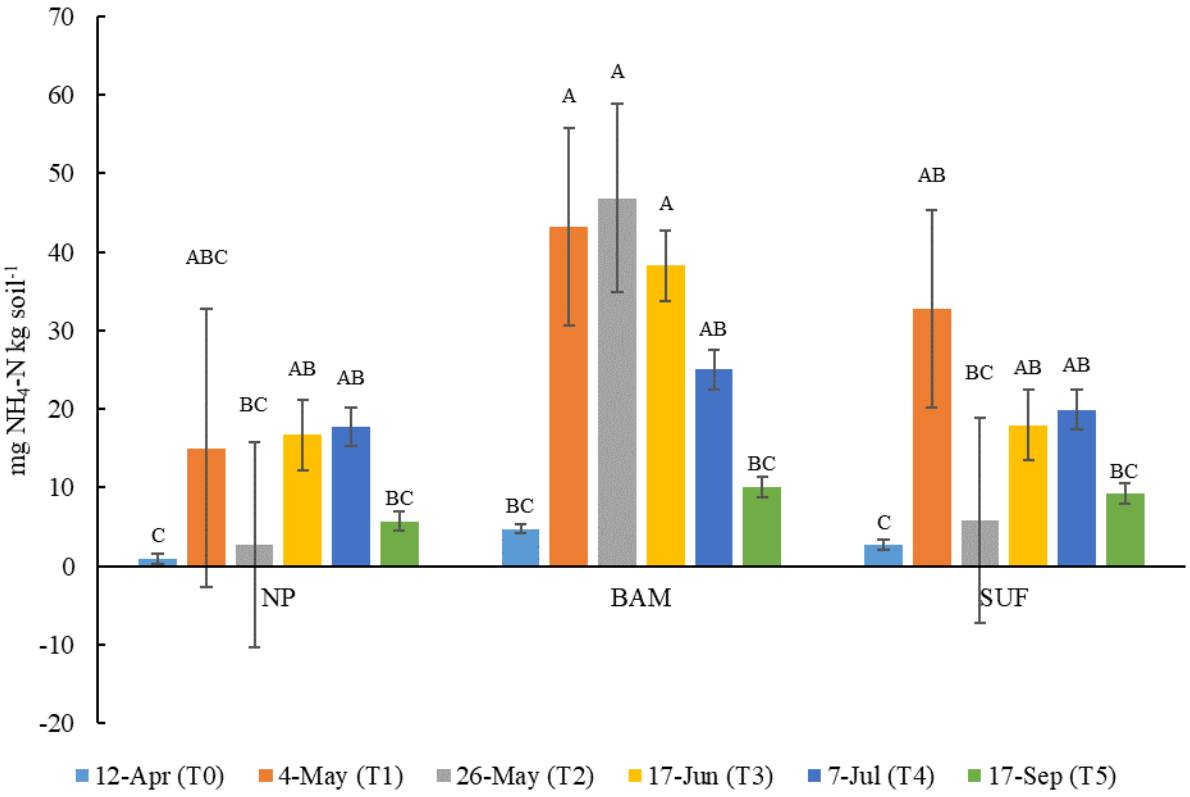

Figure 2.6 Phosphorus (P) fertilizer treatment by sampling time interaction for NH_4-N for 2021. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

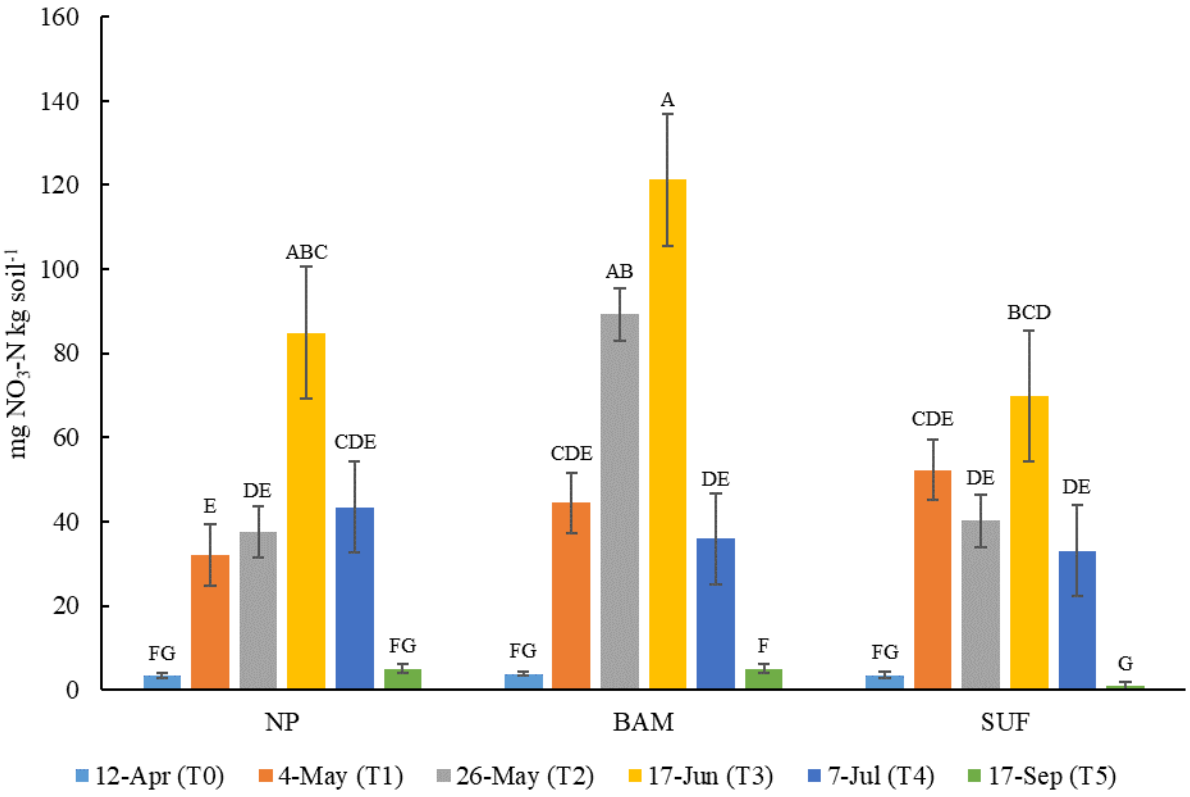

Figure 2.7 Phosphorus (P) fertilizer treatment by sampling time interaction for NO_3-N for 2021. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

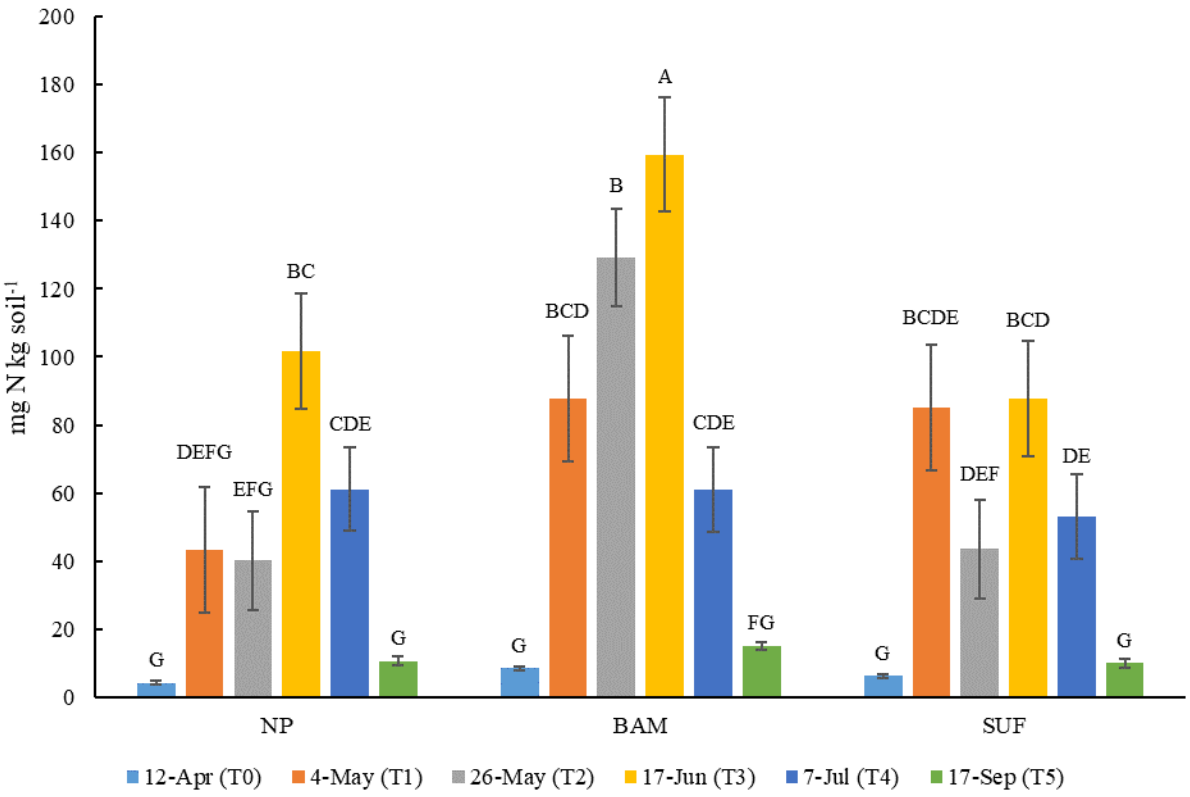

Figure 2.8 Phosphorus (P) fertilizer treatment by sampling time interaction for total inorganic N for 2021. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

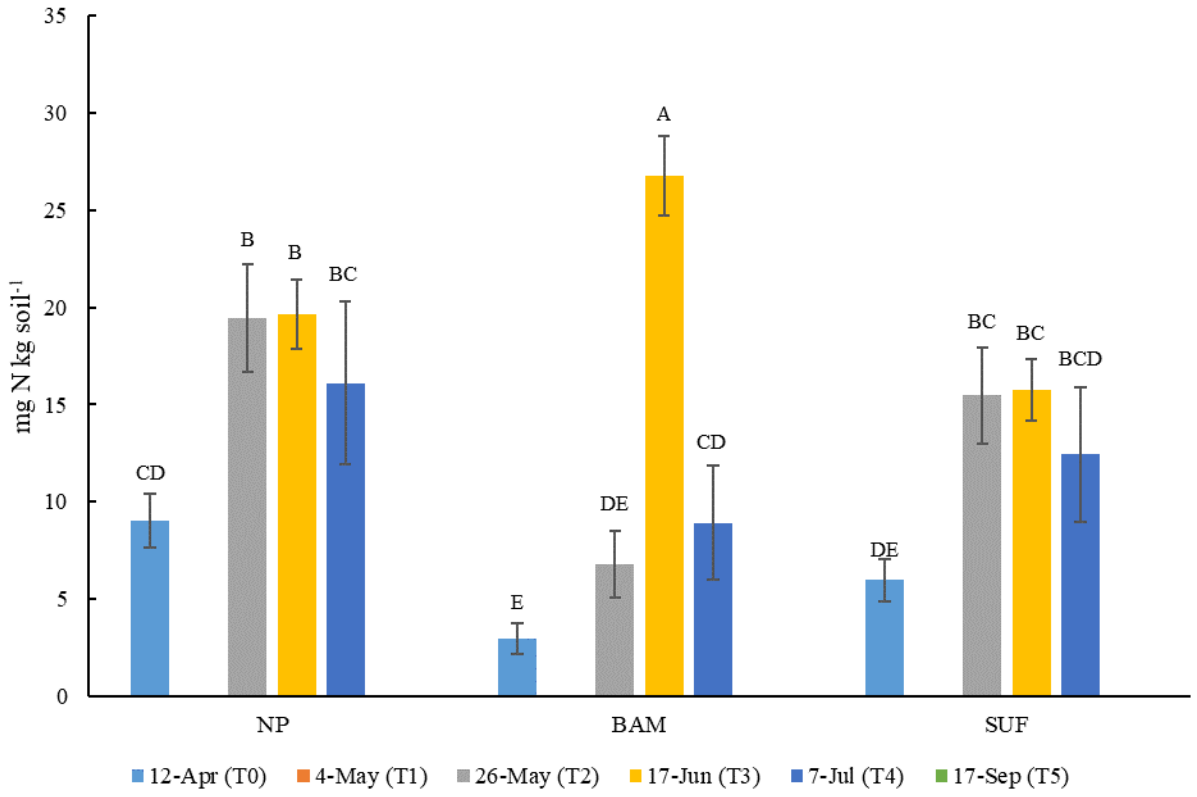

Figure 2.9 Phosphorus (P) fertilizer treatment by sampling time interaction for dissolved organic N for 2021. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Data at 4-May (T1) and 17-Sep (T5) is not reported due to methodological difficulties. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

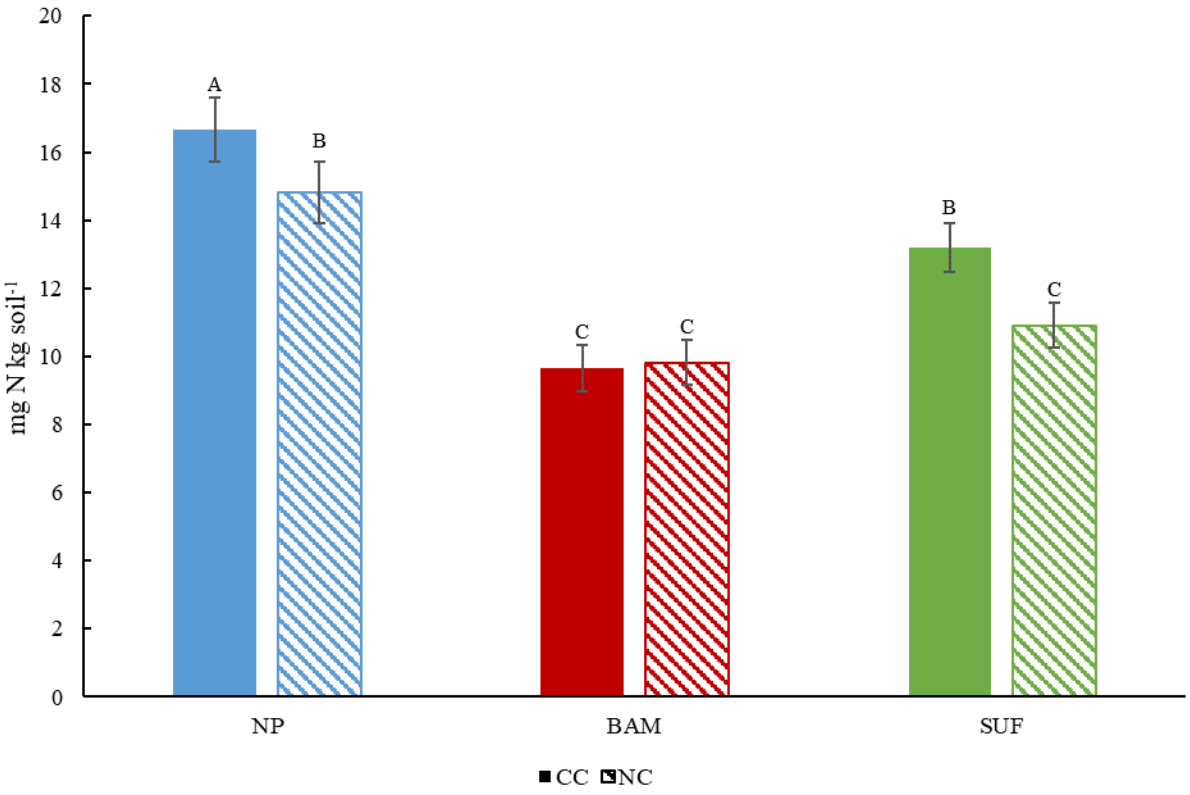

Figure 2.10 Phosphorus (P) fertilizer treatment by cover crop interaction for dissolved organic N for 2021. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments, and presence of a cover crop (CC) and absence of a cover crop (NC).

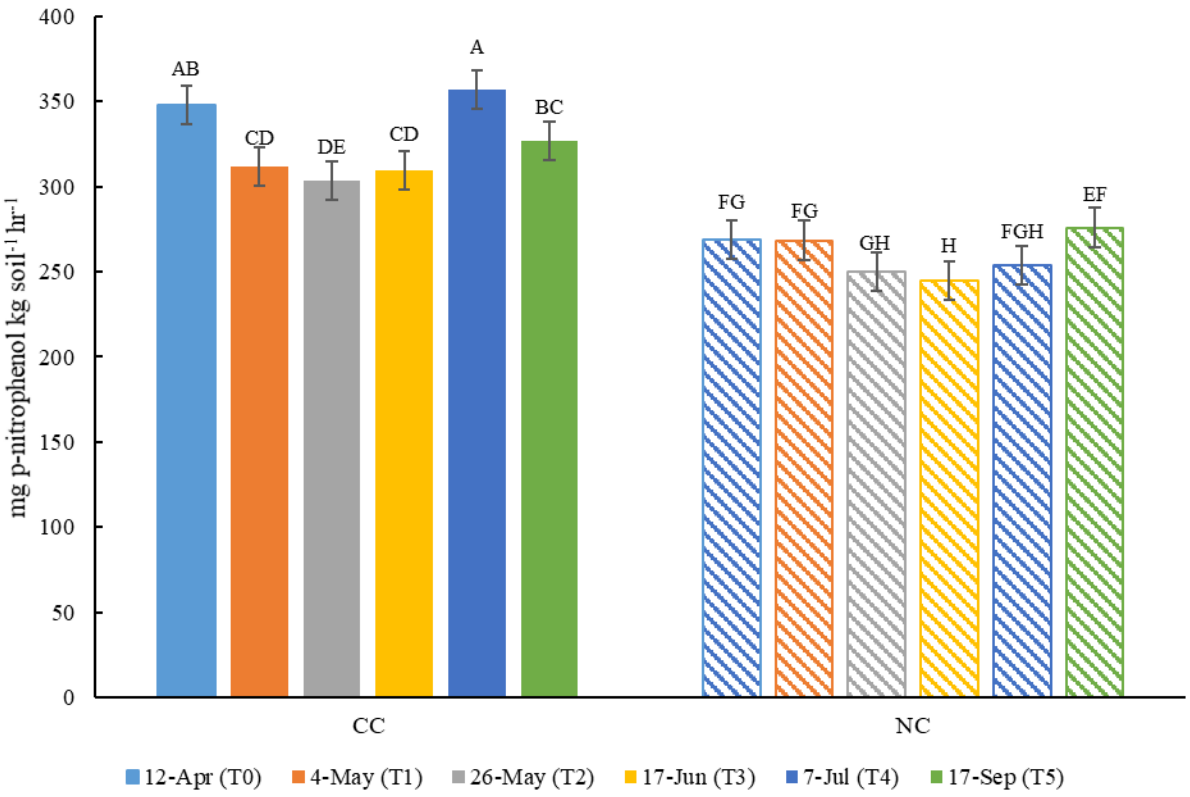

Figure 2.11 Cover crop by sampling time interaction for acid phosphatase for 2021 for 2021. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: presence of a cover crop (CC) and absence of a cover crop (NC).

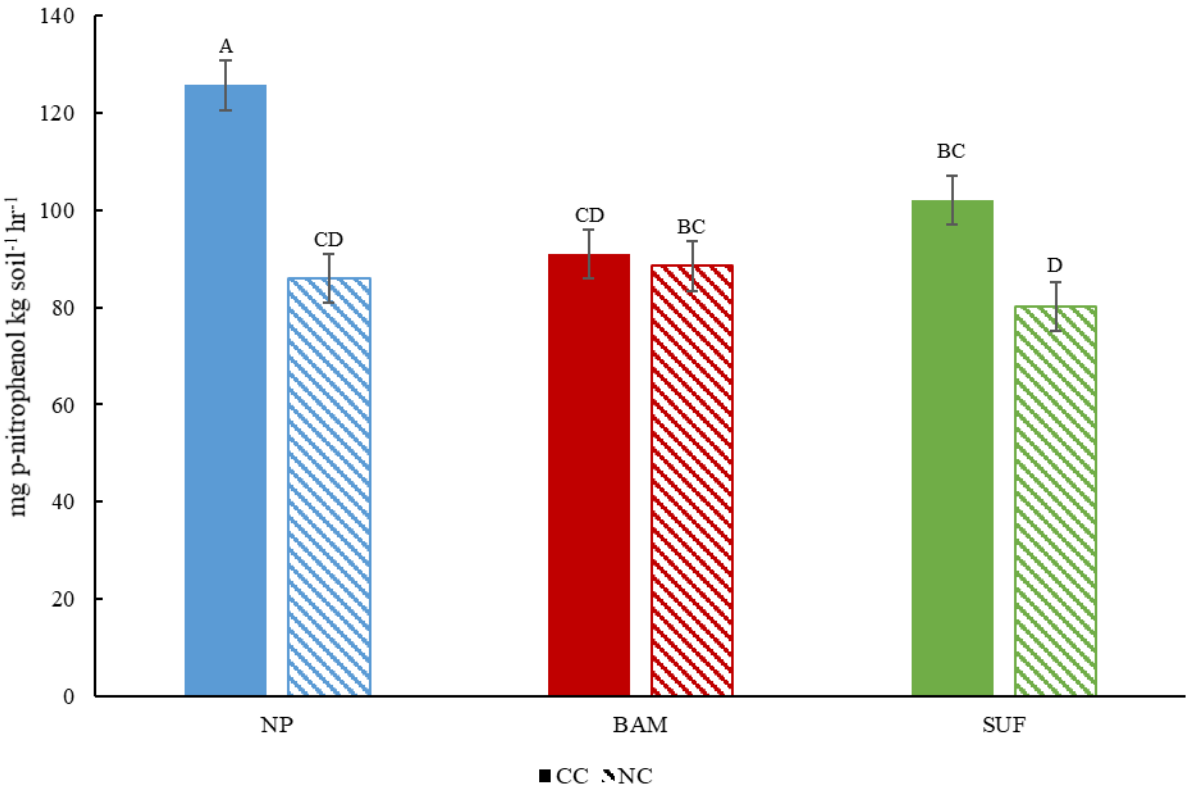

Figure 2.12 Phosphorus (P) fertilizer treatment by cover crop for alkaline phosphatase for 2021. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments, and presence of a cover crop (CC), and absence of a cover crop (NC).

Chapter 3 - Seasonal dynamics and effect of phosphorus fertilizer and cover crops on biological soil health indicators during a soybean year in a no-till corn-soybean rotation

Abstract

Climatic factors, nitrogen (N) and phosphorus (P) fertilizer applications, and cover crops can affect biological soil health in various ways, leading to inconsistent seasonal trends. A study was initiated in 2020 to determine the effects of three P fertilizer management approaches (no P (NP, build and maintain (BAM), and sufficiency (SUF)), as well as the presence/absence of a cereal rye (*Secale cereale*) cover crop, on selected chemical and biological soil health indicators. Chemical/biochemical soil health indicators measured included measures of carbon (C) (active C and dissolved organic C), N (dissolved total N, inorganic N, dissolved organic N, and autoclaved citrate extractable (ACE) protein), and P (citrate-extractable P) pools. Measures of microbial activity and function were also assessed, including microbial biomass C, N, and P, soil respiration, and four soil enzyme activities (β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatases). Composite soil samples were collected (0-5 cm) six times during the 2022 soybean growing season at the Kansas Agricultural Watershed Research Facility (KAW) near Manhattan, KS. Sampling times included a sampling prior to cover crop termination (T0), at cover crop termination (T1), at planting (T2), twice during the growing season (T3-T4) and one sampling post-harvest (T5). Phosphorus fertilizer was applied to the BAM treatment at soybean planting. As there was no significant yield decline for the 2021 harvest year, P fertilizer was not applied to the SUF treatment. The SUF treatment has not received P fertilizer application since December 2018, indicating that the SUF treatment has been in a draw down P phase since this

initiation of the experiment. Nutrient sufficiency recommendations and build-up recommendations occur when soil test P values are below 20 ppm (using the Bray P-1 extraction), while soil test maintenance recommendations occur when soil test P is between 20 and 30 ppm. Soil testing revealed that soil test P was 14 ppm, and as a result, the SUF treatment was considered to be at the margins of deficiency in 2022. Nitrogen applications were not balanced during the 2022 harvest year, resulting in the BAM treatment receiving 19 kg N ha^{-1}, while the NP and SUF treatment did not receive N. Data was analyzed using a repeated measures analysis of variance, with sampling time treated as the repeated measure. There were significant two-way interactions between cover crop and sampling time and P fertilizer treatment and sampling time for dissolved total N, inorganic N, and dissolved organic N pools. There also was a significant cover crop by sampling time interaction for dissolved total N and NO_3-N that demonstrated the nutrient salvaging ability of cereal rye, as the presence of a cover crop decreased NO_3-N at both T0 (when a living cover crop was still in the field) and T1 (three days following termination). Seasonal trends of soil N pools reflect biological transformations, dynamics of NH_4^+ fixation in soil, fertilizer application to the BAM treatment, crop N uptake, and moisture and temperature variations. The presence of a cover crops increased all measures of C and microbial function and activity. In addition, the presence of a cover crop significantly increased alkaline phosphatase and β-glucosidase activity in NP and SUF treatments, suggesting an increase in nutrient cycling when a system is at the margins of deficiency. Soil protein concentrations were significantly influenced by P availability, and increase P availability increased protein concentrations. Sampling time significantly influenced a majority of soil health indicators. However, there were limited cover crop by sampling time interactions for most soil

health indicators, suggesting that moisture and temperature trends were the primary drivers of seasonal trends.

Introduction

In order to combat adverse effects of phosphorus (P) loss on the environment, soil testing provides a crucial tool as it defines the agronomic need for nutrient applications to support crop yields while reducing risks of nutrient loss due to overapplication (Sims et al., 2000; Osmond et al., 2015). While it is a common recommendation to test soils for nutrient applications post-harvest (Lawrence et al., 2020), debate still remains on the best time to soil samples for biological properties. Weather-related factors such as precipitation and temperature, carbon (c) input, and nutrient availability can all influence biological properties, resulting in seasonal variations (Liebig et al., 2006). Therefore, both pre-plant and post-harvest have been recommended for soil sampling for biological properties (Liebig et al., 2006; Benintende et al., 2015). As there is no clear consensus on soil sampling for biological properties, it is often concluded that, while sampling time is of importance, it is more important that soil sampling occurs at the same time each year (Jones, 2001).

Fertilizer P recommendations from soil testing laboratories are based on two different management approaches; build and maintain (BAM) and sufficiency (SUF). In the BAM approach, soil test P is built up to a critical level then maintained at the critical level with subsequent fertilizer applications that are usually equal to the previous crop removal rate (Macnack et al., 2011). On the other hand, the SUF approach allows for just enough P fertilizer to be added to maximize profitability and is typically developed to provide 90 to 95% of maximum yield; only when a crop response is expected will fertilizer be applied in the sufficiency approach (Leikam et al., 2003). The BAM approach is designed to feed the soil,

while the SUF approach is designed to feed the crop (Zhang et al., 2021). Leikam et al. (2003) reported that nutrient sufficiency recommendations and build-up recommendations occur when soil test P values are below 20 ppm (using the Bray P-1 extraction). Soil test maintenance recommendations occurs when soil test P is between 20 and 30 ppm, while no fertilizer P is recommended for soils testing 30 ppm or greater (Leikam et al., 2003).

In addition to excess nutrient applications, other intense agricultural practices aimed to maximize crop productivity, such as conventional tillage, monocultural rotations, and excess herbicide applications have led to increased rates of soil degradation (Bindraban et al., 2012). With knowledge of the adverse effects of intensive agricultural practices, there are efforts designed to preserve soil quality and health through the principles of regenerative agriculture (Laishram et al., 2012). One practice that has been introduced in efforts to preserve soil health is the adoption of cover crops that replace fallow periods, providing a living crop year-around (Blanco-Canqui et al., 2015). Above- and belowground inputs from cover crops can increase substrate availability for soil microorganisms and stimulate growth and microbial biomass (Blanco-Canqui et al., 2015; Chavarria et al., 2016; Muhammad et al., 2021). Cover crops can also influence nitrogen (N) dynamics in soil as they can scavenge residual soil N, reducing the risks of leaching loss and providing N to a subsequent crop through the decomposition of residues or by biological N fixation in the case of legumes (Wagger et al., 1998).

Nutrient cycling is one of the most important ecosystem services that the soil microbial community provides (Philippot et al., 2013). Increasing nutrient cycling while reducing the annual fertilizer inputs can provide a more sustainable agroecosystem (Adegbeye et al., 2020). Phosphatase enzyme activity decreases with increasing soil P availability (Sinsabaugh and Follstad Shad, 2012). As resource allocation theory suggests that soil enzyme production is

induced when a substrate is present in deficient systems (Sinsabaugh and Follstad Shad, 2012), it is possible that increased nutrient cycling when a system is deficient or at the margins of deficiency can increase overall soil health and P availability.

Understanding the implications of cover crops presence and P fertilizer management on various biological soil health indicators, as well as the seasonal dynamics of biological soil health indicators, can provide insight into P cycling and availability and dynamics of the soil microbial community. Therefore, the objectives for this study were to (1) assess the impacts of P fertilizer management approaches and the presence and absence of cover crops on various nutrient pools and indicators of microbial activity and function, and to (2) assess seasonal dynamics of biological soil health indictors in the soybean phase of a no-till corn and soybean rotation. The hypotheses of the study were (1) that the presence of cover crops would increase measures of labile nutrients as well as measures of microbial activity and function (microbial biomass, soil respiration, and soil enzyme activities), (2) increased nutrient cycling (measured by soil enzyme activities) would occur in systems undergoing P nutrient drawdown, (3) cereal rye decomposition would be gradual and little response would be observed in soil N pools during the growing season due to cover crops, and (4) climatic factors will drive the seasonal dynamics of biological soil health indicators.

Materials and Methods

Experimental Site, Design, and Agricultural Management:

The experimental site was located at the Kansas Agricultural Watershed (KAW) Field Laboratory near Manhattan, Kansas. The site consists of primarily eroded Smolan silty clay loam (fine, smectitic, mesic Pachic Argiustoll) with a 6-8% slope, with soil pH ranging from 6 to 7. The site has a hot, humid continental climate, with a mean annual temperature of 12.9°C and

mean annual precipitation of 889 mm. There are 18 terraced watersheds that are approximately 0.5 ha each. The site has been in a continuous no-till, corn-soybean rotation since 2014, where a previous five-year study evaluating P fertilizer source and cover crop influence on crop response and surface water quality was conducted from 2014 to 2019 (Carver et al., 2022).

The experiment was a 2x3 complete factorial, arranged in a randomized complete block design. The blocks were assigned based on landscape position and all treatments were replicated three times (n = 18). There are three levels of P fertilizer management; no P control (NP), build and maintain (BAM), and sufficiency (SUF), and two levels of cover crop management; presence (CC) or absence (NC) of a cereal rye (*Secale cereale*) cover crop. Ammonium polyphosphate (10-34-0) was applied to BAM plots at an application rate of 54 kg P_2O_5 ha^{-1}. The ammonium polyphosphate used was contaminated with urea ammonium nitrate (~6%), which added approx. 3 kg N ha^{-1} in addition to the 16 kg N ha^{-1} from the ammonium polyphosphate. No P fertilizer has been applied to SUF plots since December 2018, while NP plots have not received P fertilizer since 2014. Cereal rye was planted 25 September 2021 and chemically terminated with Glyphosate and 2,4-D on 20 May 2022. Soybeans were planted on 15 June 2022 and harvested on 20 October 2022.

Soil Sampling:

Composite soil samples were collected at 0-5 cm depth six times during the soybean growing season in 2022 (Table 3.1). Soil samples were collected pre-cover crop termination, immediately following termination, at soybean planting, two times during the soybean growing season, and post-harvest. Sampling dates were 27 April (pre-termination T0), 23 May (T1), 15 June (soybean planting-T2), 8 July (T3), 5 August (T4), which correspond to 3, 26, 49, 77 days

after cover crop termination, respectively, and following soybean harvest on 21 October (T5). 40 cores were taken from each plot, then separated into field-moist and air-dried subsamples.

All field-moist soil was processed through a 2 mm sieve and stored at 4°C until analysis were performed. Moist soil samples were analyzed for microbial biomass C, N, and P, inorganic N, dissolved total N, dissolved organic C and N. Soil moisture was determined by oven-drying a subsample of field-moist soil for 48 hr at 90°C. Air-dried soil samples were dried at room temperature then ground and sieved to pass a 2 mm sieve. Air-dried soils were analyzed for active C, autoclaved citrate extractable (ACE) protein, soil respiration, β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase.

Methodology:

Microbial biomass C and N, dissolved total N, inorganic N, and dissolved organic N was determined on a single extraction (Jones and Willett, 2006). Microbial biomass C and N were determined by the chloroform fumigation extraction method (Brookes et al., 1982; Vance et al., 1987). Briefly, two 8 g (dry weight equivalent) samples of moist soil were weighed into 250-mL glass jars. One of the two jars was fumigated with chloroform in a desiccator for 24 hr. Both samples were then extracted with 40 mL of 0.5 M K_2SO_4 and shaken for 30 min. The samples were then filtered through Ahlstrom 74 filter paper into 40-mL glass vials. Samples were analyzed for non-purgeable organic carbon on a Total Organic Carbon (TOC) analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). Microbial biomass C was determined be calculating the difference between the fumigated and unfumigated samples. Dissolved organic C was determined from the extractant of the unfumigated microbial biomass C/N samples and measured with the TOC for non-purgeable organic carbon (Jones and Willett, 2006). Dissolved total N was determined by oxidizing an aliquot of the fumigated and unfumigated filtrate using

the potassium persulfate oxidation method (Cabrera and Beare, 1993). $K_2S_2O_8$ 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). Microbial biomass N was determined by the difference in dissolved total N in the fumigated and unfumigated samples. Inorganic N (NO_3-N and NH_4-N) was measured by the Kansas State Soil Testing Lab. Dissolved organic N was determined by calculating the difference between dissolved total N and inorganic N in the unfumigated sample.

Microbial biomass P was determined using 24-hr chloroform fumigation followed by citrate extraction (Darch et al., 2016). Briefly, two 8 g (dry weight equivalent) moist soil samples were weighed into two 250-mL glass jars. One jar was fumigated with chloroform for 24 hours, while the other jar was not fumigated. Samples were extracted with 40 mL 2 mM citric acid pH 5. Samples were shaken for 30 mins, centrifuged at 10,000 rpm for 10 mins, and filtered with Ahlstrom 74 filter paper into 40-mL glass vials. The amount of molybdate reactive P was determined colormetrically with a spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) at 880 nm using the Murphy Riley Method (Murphy and Riley, 1962; O'Halloran and Cade-Menun, 2008). Microbial biomass P was determined by the difference in fumigated and unfumigated samples.

Active C was determined using the permanganate-oxidizable C method described by Weil et al. (2003). Briefly, 2.5 g of air-dried soil was weighed into 50-mL Falcon tubes; 18 mL of deionized water was added and 2 mL of 0.2 M $KMnO_4$ was added to the Falcon tube. Samples were then shaken for 2 min, and left to settle for 8 min before 0.2 mL of each sample was added

to 20 mL deionized water to stop the reaction. Samples were analyzed colormetrically on a spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) at 550 nm.

Bioavailable N was determined using the ACE protein method (Wright and Upadhyaya, 1996, 1998; Hurisso et al., 2018). Briefly, two sets of 3 g of soil was weighed out into 50-mL centrifuge tubes. 24 mL of 20 mM sodium citrate pH 7 is added to samples then shaken at 180 rpm for 5 min. Samples are then autoclaved at 121°C for 30 min. Following autoclave cycle, 1.75 mL aliquot from each sample is added to a clean 2-mL microcentrifuge tube, where the aliquots were then centrifuged at 10000 x g for 3 mins. After tubes were removed from centrifuge, 1 mL of the cleared extract was added to a microtiter tube in a 96-well format. For quantification, a heat block was heated to 61.5˚C and Pierce bicinchoninic acid (BCA) working solution was prepared. Using a multichannel pipettor, 10 µL of standard were added to the reaction plate, followed by two replicate columns of each strip of eight samples. 200 µL of working reaction was added into each well of the reaction plate, which was then covered with sealing tape and placed on a heat block for one hour. The tape is then removed then read with a plate reader (BioTek Synergy H1, USA) at 562 nm.

Soil respiration was determined using the alkali trap method described by Haney and Haney (2010). Briefly, 20 g of air-dried soil was weighed into a perforated aluminum weigh boat (diameter 51 mm) that was perforated nine times and placed onto two filter papers (qualitative 413-VWR North America). The alkali trap was place on top of the soil and filled with 9 mL of 0.5 M KOH while 7.5 mL of deionized water was added to the filter papers. Jars were then sealed and incubated for 4 days at room temperature. Electric conductivity of the KOH solution change in proportion to the amount of CO_2 trapped; therefore, the amount of CO_2 trapped is

calculated from the change in conductivity, which is used to estimate the amount of CO_2 evolved from the samples.

Activities of β-glucosidase (Eivazi and Tabatabai, 1988), N-acetyl-β-glucosaminidase (Parham and Deng, 2000), and acid and alkaline phosphatases (Eivazi and Tabatabai, 1977) were determined colormetrically at 400 nm. Briefly, three subsamples of 0.5 g of air-dried soil was weighed out into 20-mL vials. Each sample had two replicates (A and B) and one control (C). 2 mL of start buffer was added to all A, B, and C samples. Start and stop buffers as well as p-nitrophenyl substrates for each soil enzyme assay is listed in Table 3.2. Vials were capped and shaken gently by hand before being incubated at 37˚C for 1 hr. To stop the reaction, 0.5 mL of 0.5 M CaCl2 was added to each vial, followed by 2 mL of stop buffer. Substrate was then added to the control only. Five ml of deionized water was added to all the samples to achieve enough extract to analyzed. Samples were analyzed colormetrically on a spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) at 400 nm.

Statistical Analysis:

The effects of P fertilizer treatment, cover crops, sampling time, and their interactions on various biological soil health indicators were compared using a repeated measured analysis of variance in linear mixed effects model via Statistical Analysis Software (SAS version 9.4; Cary, NC) with option DDFM = KR. Natural logarithm, log10, or square root transformations were performed on certain soil health indicators to satisfy the assumption of normality and homogeneity of variance. Citrate-extractable P and soil respiration required natural logarithm transformations, C:N ratio required log10 transformation, and microbial biomass P required square root transformation. Back transformed least squares (LS) means are reported in tables and figures. Three-way analysis of variance was first conducted for all soil health indicators. There

were no significant three-way interactions observed for any measured indicator. The three-way interaction term was then dropped from the model statement, and two-way analysis of variance was conducted. If no significant two-way interactions were observed, the interaction term was dropped from the model statement and the model was rerun only analyzing main effects. The two-way analysis of variance model was used for soil moisture, microbial biomass N, citrate-extractable P, alkaline phosphatase, glucosidase, NO_3-N, NH_4-N, dissolved total N, inorganic N, soil respiration and dissolved organic N. One-way analysis of variance model was used for microbial biomass C and P, C:N ratio, acid phosphatase, glucosaminidase, dissolved organic C, ACE protein, and active C.

Covariance structures were determined by fit statistics, in which the structure that resulted in the lowest Akaike information criterion and Bayesian information criterion was used. Covariance structures selected for this study included compound symmetry, heterogenous compound symmetry, first-order autoregressive, heterogenous first-order autoregressive, or unstructured.

Results

Weather and Soil Moisture Data:

The average maximum and minimum daily air temperatures recorded during the growing season were within normal range of the 30-year normal for Riley County (Weather Data Library, Kansas Mesonet) (Fig. 3.1). Cumulative precipitation 20 days before T0 and T5 sampling, as well as cumulative precipitation between each sampling time (T1-T4) is reported in Table 3.1. Cumulative precipitation before sampling at T0 was greatly reduced compared to all other sampling times. Precipitation greatly increased between T0 and T1 and between T1 and T2. There was a decrease in cumulative precipitation between sampling times for the reminder of the

growing season. In comparison to 30-year normals, precipitation in May, June, and July was slightly higher than reported normals, while precipitation in April, August, September, and October was lower than 30-year normal (Fig. 3.2).

Minimum and maximum daily air temperatures were at the T1 sampling compared to T0 (Table 3.1). While minimum and maximum daily air temperatures at T2, T3, and T4 were relatively consistent. Minimum daily air temperature was greatly reduced at T5 while the maximum daily air temperature was similar to T2-T4 values.

There was significant cover crop by sampling time interaction for soil moisture (Table 3.3). Soil moisture was significantly lower in the cover crop treatment at T0 compared to all other cover treatments and sampling times. Soil moisture significantly increased between T0 and T1 in both cover crop treatments, with no significant difference between either cover crop treatments. Soil moisture was significantly higher in the cover crop treatment at T2 compared to all other sampling times in the cover crop treatment. At T2, soil moisture was significantly higher in the cover crop treatment compared to the no cover crop treatment. A significant decrease in moisture was observed at T3 followed by another significant decrease at T4 for both cover crop treatments. There was no significant difference in cover crop and no cover crop treatments at T3 and T4. There was a significant increase in soil moisture at T5 for both cover crop treatments (Fig. 3.3).

Extractable Nutrients:

Carbon:

Significant main effects of cover crop and sampling time influenced active C concentrations (Table 3.3). The presence of a cover crop increased active C concentrations by 11% (Table 3.4). Active C concentrations were similar at T0 and T1, followed by a significant

decrease in concentrations at T2. There was no significant difference in concentrations at T2 and T3. Concentrations significantly increased at T4 to levels similar to T0 and T1, followed by another significant increase in concentrations at T5. Active C concentrations were significantly greater at T5 compared to all other sampling times (Table 3.4).

Only sampling time significantly influenced dissolved organic C concentrations (Table 3.3). Due to methodology difficulties as a result of dry conditions, no data for dissolved organic C was reported at T0. Dissolved organic C concentrations significantly decreased from T1 to T2. There was no significant difference between T2 and T3. Dissolved organic C concentrations significantly increased at T4 to levels similar to T1, followed by a significant decrease in concentrations at T5. Dissolved organic C concentrations were not statistically different at T1 and T5 (Table 3.4).

Phosphorus:

There was a significant two-way interaction between P fertilizer management and sampling time for citrate-extractable P (Table 3.3). For the BAM treatment, there was a significant decrease in concentrations from T0 to T1 (Fig. 3.4). There was no significant difference in concentrations between T2 and T3. There was a significant peak in concentrations at T3, followed by a significant decrease in concentrations at T4. There was no significant difference between concentrations at T4 and T5. For the SUF treatment, there was no significant difference between citrate-extractable P concentrations at T0 and T1. There was a significant decrease in concentrations at T2, followed by a significant increase in concentrations at T3 to a concentration similar to T0, T1 and T4. Concentrations for the remainder of the growing season (T3-T5) were not statistically different. In the NP treatment, a significant decrease in citrate-extractable P concentrations was observed at T2. For all other sampling times, no significant

difference was observed in citrate-extractable P concentrations in the NP treatment. Concentrations in the BAM treatment were always significantly higher than the SUF and NP treatments, regardless of sampling time. Concentrations in the SUF treatment were always significantly higher than the NP treatment, except at T2. In general, lowest citrate-extractable P concentrations for each P treatment was observed at T2

Nitrogen:

Main effects of P fertilizer treatment and cover crop both significantly influenced ACE protein concentrations (Table 3.5). Protein concentrations were significantly lower in the NP treatment compared to both BAM and SUF treatments, with no significant difference between BAM and SUF treatments (Table 3.6). In comparison to the NP treatment, there was an 8% and 9% increase in soil protein concentrations for BAM and SUF treatments, respectively. The presence of a cover crop significantly increased ACE protein concentrations by 9% (Table 3.6).

Dissolved total N:

There was a significant cover crop by sampling time interaction for dissolved total N concentrations (Table 3.5). Dissolved total N concentrations were similar at T0 for both cover crop treatments. Concentrations significantly increased at T1 in both cover crop and no cover crop treatments, however, concentrations were significantly higher in the no cover crop treatment. In the cover crop treatment at T2, there was a significant increase in dissolved total N concentrations relative to T1. There was no statistical difference in the no cover crop treatment at T1 or T2. Concentrations at T2 were not significantly different between cover crop treatments. There was a significant increase in concentrations at T3 for both cover crop treatments, with no significant difference observed between cover crop treatments. Concentrations in both cover crop treatments significantly decrease from T3 to T4, with no significant difference observed between

cover crop treatments. There was no significant difference in concentrations at T5 for the cover crop treatment. Concentrations were not significantly different at T4 and T5 for both cover crop treatments. Between each sampling time, only concentrations at T1 were significantly different between the cover and no cover crop treatments as concentrations were significantly higher in the no cover crop treatment compared to the treatment with a cover crop (Fig. 3.5).

There was a significant P fertilizer by sampling time interaction for dissolved total N concentrations (Table 3.5). In all P fertilizer treatments, there was a significant increase in concentrations from T0 to T1. Dissolved total N concentrations were similar at T1 and T2 for all P fertilizer treatments. At T3, while neither the SUF and NP treatments showed any significant difference between T2 and T3, dissolved total N concentrations significantly peaked in the BAM treatment. Following T3, concentrations in the BAM treatment significantly decreased. No statistical difference was observed between T1, T2, T4 and T5 in the BAM treatment. In SUF and NP treatments, no significant change was observed following T3. Overall, concentrations in both the SUF and NP treatments tended to remain stable throughout the growing season following T1. Within each sampling time, only T3 showed a significant difference between P fertilizer treatments (Fig. 3.6).

There was a significant P fertilizer treatment by cover crop interaction for dissolved total N (Table 3.5, Fig. 3.7). The presence of a cover crop significantly decreased dissolved total N concentrations by 35% in the NP treatment. There was no significant difference due to the presence of a cover crop on the BAM or SUF treatments. Dissolved total N concentrations were similar in NPxCC, BAMxCC, BAMxNC, and SUFxCC treatments. Dissolved total N was not significantly different in NPxCC and SUFxCC treatments.

NH_4-N, NO_3-N, and Total Inorganic N

There was significant P fertilizer by sampling time interaction for NH_4-N concentrations (Table 3.5). For all P fertilizer treatments, there was a significant decrease in NH_4-N concentrations from T0 to T1. For the BAM treatment, concentrations significantly increased from T1 to T2 and remained stable through T4. Concentrations then significantly increased from T4 to T5 (Fig. 3.8). In general, for both the SUF and NP treatments, NH_4-N concentrations remained stable throughout the growing season. There was no significant difference in NH_4-N concentrations from T0 to T1. In general, NH_4-N concentrations in the BAM and SUF treatments were significantly higher at T2 and T5 compared to the NP treatment.

There was a significant cover crop by sampling time interaction for NO_3-N concentrations (Table 3.5 & Fig. 3.9). At T0 and T1, NO_3-N concentrations were significantly higher in the no cover crop treatment compared to cover crop treatment. There was a significant increase for both cover and no cover crop treatments from T0 to T1. There was a significant increase in NO_3-N concentrations in the cover crop treatment between T1 and T2 and between T2 and T3. Concentrations peaked at T3 in the cover crop treatment and then declined for the reminder of the growing season. In the no cover crop treatment, NO_3-N concentrations were stable from T1 to T3, followed by a significant decrease in concentrations for the reminder of the growing season. There was no significant difference between the no cover crop treatment at T1 and T3 and the cover crop treatment at T3 (Fig. 3.9).

There was a significant P fertilizer treatment by sampling time interaction for NO_3-N concentrations (Table 3.5). For the BAM treatment, there was no significant difference between concentrations at T0 and T1. There was no significant difference for NO_3-N concentrations between T1 and T2, however, concentrations at T2 were significantly higher compared to T0. There was a significant peak in NO_3-N concentrations at T3, followed by a significant decrease

in concentrations at T4. NO_3-N in the BAM treatment was not significant different between T4 and T5. There was no significant difference between NO_3-N concentrations at T0 and T5 in the BAM treatment. In the SUF treatment, NO_3-N concentrations remained relatively stable throughout the growing season. There was no statistical difference from T0 to T1, while concentrations were significantly higher at T2 compared to T0. There was no significant difference in concentrations from T1 through T4. Concentrations at T5 were significantly lower compared to T0 through T3. There was no significant difference between NO_3-N concentrations at T0 and T5 in the SUF treatment. In the NP treatment, NO_3-N concentrations significantly increased from T0 to T1. Concentrations were similar from T1 through T4. Concentrations at T5 were similar to T4, but were significantly lower compared to T1 through T3. There was no significant difference between NO_3-N concentrations at T0 and T5 in the NP treatment. In comparisons between sampling times, only at T3 was a significant difference between P fertilizer treatments; the BAM treatments was significantly higher than SUF and NP treatments (Fig. 3.10).

There was a significant P fertilizer treatment by sampling time interaction for inorganic N concentrations (Table 3.5). In the BAM treatment, there was no significant difference in inorganic N concentrations from T0 to T1. Concentrations significantly increased from T1 to T2 and from T2 to T3. At T3, inorganic N concentrations peaked in the BAM treatment. Following T3, there was a significant decrease in concentrations at T4. Concentrations at T4 and T5 were not statistically different. For the SUF treatment, concentrations were similar at T0 and T1, followed by a significant increase in concentrations at T2. In the SUF treatment, inorganic N concentrations peaked at T2. Concentrations significantly decreased at T3, while concentrations from T3, T4, and T5 were not statistically different. In the NP treatment, inorganic N

concentrations were relatively stable throughout the growing season. Concentrations at T5 were statistically lower than T1 and T2 (Fig. 3.11).

There was a significant P fertilizer by cover crop interaction for inorganic N concentrations (Table 3.5). The presence of a cover crop in the SUF and NP treatments significantly decreased inorganic N concentrations by 28 and 39% for SUF and NP treatments, respectively. There was no significant difference in the BAM treatment when a cover crop was present (Fig. 3.12).

There was a significant P fertilizer by sampling time interaction for dissolved organic N concentrations (Table 3.5). Due to methodology difficulties as a result of dry conditions, no data for dissolved organic N was reported at T0. For the BAM treatment, dissolved organic N concentrations significantly decreased from T1 to T2, followed by a significant increase in concentrations from at T3. Concentrations in the BAM treatment remained stable following T3. In the SUF treatment, concentrations were relatively stable throughout the growing season. Only concentrations at T1 and T5 were significantly different. In the NP treatment, concentrations also tended to remain stable. There was a significant increase in concentrations between T3 and T4, followed by a significant decrease in concentrations at T5. In comparisons between sampling times, only T2 and T4 resulted in significant differences between P fertilizer treatments. At T2, the BAM treatment was significantly lower compared to the NP treatment, while at T4, the NP treatment was significantly higher compared to the BAM and SUF treatments (Fig. 3.13).

Microbial Activity and Function:

Microbial Biomass C, N, and P:

Both cover crop and sampling time significantly influenced microbial biomass C (Table 3.7). The presence of a cover crop increased microbial biomass C concentrations by 19% (Table

3.8). Due to methodology difficulties as a result of dry soil conditions, no data for microbial biomass C was reported at T0. Microbial biomass C concentrations were similar at T1 and T2, followed by a significant decrease in concentrations at T3. There was a significant increase in biomass C concentrations from T3 to T4 and from T4 to T5. Microbial biomass C was significantly higher at T5 compared to the rest of the growing season (Table 3.8).

There was a significant cover crop by sampling time interaction for microbial biomass N concentrations (Table 3.7). In in the cover crop treatment, microbial biomass N was stable from T0, T1, and T2, followed by a significant decrease in concentrations at T3. Concentrations at T4 and T5 returned to levels similar to T0, T1 and T2. In the no cover crop treatment, there was a significant decrease in microbial biomass N concentration between T0 and T1. Concentrations were similar from T1 to T5. In comparison between cover crop treatments, microbial biomass N concentrations were significantly higher in the cover crop treatment at T1 and T4 compared to the no cover crop treatment (Fig. 3.14)

Microbial biomass C:N ratios were significantly influenced by sampling time (Table 3.7). Due to methodology difficulties as a result of dry conditions, no data for C:N ratio was reported at T0. The C:N ratio was similar at T1 and T2. There was a significant increase in C:N ratios from between T2 and T3, followed by a significant decrease in concentrations at T4. Concentrations then significantly decreased between T4 and T5 (Table 3.8).

Phosphorus fertilizer treatment and sampling time significantly influenced biomass P concentrations (Table 3.7). Microbial biomass P concentrations were significantly higher in the BAM treatment compared to SUF and NP treatments, while concentrations in the SUF treatment were significantly higher than in the NP treatment (Table 3.8). For sampling time, microbial biomass P concentrations were not significantly different at T0, T1, or T2. There was a

significant increase in concentrations at T3. Concentrations between T3 and T4 were similar, while concentrations between T4 and T5 were similar (Table 3.8).

Soil Respiration:

There was a significant two-way interaction between P fertilizer treatment and cover crop for soil respiration (Table 3.7). Regardless of P fertilizer treatment, the presence of a cover crop significantly increased soil respiration. Within the no cover crop treatments, soil respiration in the BAM treatment was significantly higher than respiration in SUF treatment. There were no significant differences between NP and BAM treatments and NP and SUF treatments when a cover crop was not present (Fig. 3.15). Sampling time significant influenced soil respiration (Table 3.7). Respiration significantly decreased from T0 to T1, followed by another significant decrease from T1 to T2. Respiration then significantly increased at T3 and T4, where there was no significant difference between respiration at T3 and T4. At T5, respiration significantly decreased. Overall, soil respiration at T0 was significantly higher compared to all other sampling times, while respiration was significantly lower at T2 compared to all other sampling times (Table 3.8).

Soil Enzyme Activities:

Both cover crop and sampling time significantly influenced acid phosphatase activity (Table 3.9). The presence of a cover crop increased acid phosphatase activity by 25% (Table 3.10). Acid phosphatase activity varied throughout the growing season. There was no significant difference for acid phosphatase activity at T0 and T1. There was no significant difference in acid phosphatase activity from T1 to T2. Activity significantly decreased between T2 and T3, followed by a significant increase in activity at T4. Activity then significantly decreased between

T4 and T5 (Table 3.10). Acid phosphatase activity was significantly lower at T3 and T5 compared to all other sampling times

There was a significant two-way interaction between P fertilizer treatment and cover crop on alkaline phosphatase activity (Table 3.9). The presence of a cover crop significantly increased alkaline phosphatase activity by 29% and 22 in the NP and SUF treatments, respectively. The presence of a cover crop did not significantly increase alkaline phosphatase activity in the BAM treatment (Fig. 3.16a). Sampling time significantly influenced alkaline phosphatase activity (Table 3.9). Alkaline phosphatase activity varied over the growing season, with no clear trend observed. There was no significant difference in activity from T0 to T1. Activity then significantly increased at T2, followed by a significant decrease at T3. At T4, there was a significant increase in activity compared to T3, while at T5, activity significant decreased again (Table 3.10).

There was a significant P fertilizer treatment by cover crop interaction for glucosidase activity (Table 3.9). The presence of a cover crop significantly increased activity in both the NP and SUF treatments by 24 and 26%, respectively. The presence of a cover crop did not increase glucosidase activity in the BAM treatment (Fig. 3.16b). Sampling time significantly influenced glucosidase activity (Table 3.9). Glucosidase activity was similar at T0 and T1. Activity at T1 and T2 was not significantly different. Activity at T2 and T3 was not significantly different. Activity significantly increased between T3 and T4, followed by a significant decrease in activity at T5. Glucosidase activity was significantly lower at T5 compared to all other sampling times (Table 3.10).

For glucosaminidase activity, only the presence of a cover crop significantly influenced activity (Table 3.9). The presence of a cover crop increase activity by 36% (Table 3.10).

Discussion

Impact of cover crops and P fertilizer treatment on soil health indicators

Nutrient pools:

The presence of a cover crop significantly increased active C concentrations in the present study (Table 3.3). The increase in active C with a cover crop (Table 3.4) is likely due to increase above- and belowground inputs (Zhou et al., 2012). The presence of a cover crop also significantly increased ACE protein concentrations in the present study (Table 3.5). This was probably caused by stimulation of microbial activity by increased substrate availability provided by the cover crop (Feng et al., 2021).

Soil protein concentrations in the present study were found to be significantly lower in the NP treatment compared to the BAM and SUF treatments (Table 3.6). This finding was in agreement with several studies that have found that soil protein (measured as glomalin related soil protein) was positively correlated with soil P concentrations (Wu et al., 2010; Guo et al., 2012; Qiu et al., 2021; Wang et al., 2022). A positive response to P availability suggests that the soil protein measured was not solely of arbuscular mycorrhizal fungi (AMF) origin, as it is known that AMF populations decrease with increasing P availability (Staunton et al., 2020; Law and Maherali, 2023), further verifying the conclusions of Hurisso et al., (2018) that the ACE protein procedure extracts a wide range of proteins, not just glomalin. Increased ACE protein concentrations might reflect increased primary productivity due to increased P availability in the BAM and SUF treatments relative to the NP treatment (Singh et al., 2013).

The addition of a cover crop significantly decreased inorganic N concentrations in both the NP and SUF treatments, while there was no difference for the BAM treatment (Fig. 3.11). The cereal rye cover crop also significantly decreased NO_3-N concentrations, while there was no

effect for NH_4-N (Table 3.6, Fig. 3.11). It is also likely that ammonium polyphosphate application offset the decrease in NO_3-N concentrations due to cover crops in the BAM treatment, resulting in no significant difference between the cover crop treatments.

Microbial activity:

Microbial biomass C and P and soil respiration

Increases in C inputs from cover crops are likely to increases in microbial biomass (Hao et al., 2023). Increased root biomass due to cover crops can also increase microbial biomass (Blanco-Canqui et al., 2015). Root exudates that contain labile C compounds can be incorporated into the microbial biomass to stimulate growth (McDaniel et al., 2014). Increased active C concentrations due to the presence of a cover crop can also reflect the contribution of root exudates to labile forms of C (Wang et al., 2017). Significant increases in active C and microbial biomass C due to the presence of a cover crop found in this study (Tables 3.4 & 3.8) suggests that increased above- and belowground inputs stimulated the microbial biomass.

In the present study, microbial biomass P was significantly higher in the BAM treatment, compared to the NP and SUF treatments, indicating the importance of soil P levels on microbial biomass P concentrations. Higher microbial biomass P concentrations in the BAM treatment likely reflecting luxury consumption of (Mooshammer et al., 2014). Increases in microbial biomass P concentrations due to P fertilization have been reported (Chen and Xiao, 2023).

A significant P fertilizer treatment by cover crop interaction was observed for soil respiration in the present study (Table 3.7). Soil respiration was significantly higher in all P treatments when a cover crop was present. Increases in soil respiration due to cover crop implementation have been reported (Nunes et al., 2018; Mitchell et al., 2017; Wood and Bowman, 2021). Cover crops contribute both above- and belowground inputs and the turnover of

the biomass by the soil microbial community can increase levels of soil respiration as the microbial community is decomposing residues (Nilahyane et al., 2020) as the activity of soil heterotrophic organisms is proportionate to the decomposition of soil C (Hanson et al., 2000). Although soil respiration was significantly lower when cover crops were absent in all P fertilizer treatments, the SUF treatment resulted in less soil respiration compared to the BAM treatment. In a meta-analysis of soil respiration response to P additions, Feng and Zhu (2019) reported that P additions increased soil respiration by 31.7% in cropland. The authors suggested that P additions stimulated C cycling processes as increased P can promote plant growth leading to more above- and belowground C inputs to soil. It is likely that the differences observed between BAM and SUF in no cover crop treatments was due to the application of P fertilizer that promoted enhanced biomass growth and C cycling in soil.

Soil enzymes

Increased soil enzyme activity due to cover cropping results from greater C inputs that can stimulate the microbial community (Bandick and Dick, 1999). Increased substrate availability can promote the induction of extracellular enzymes to breakdown above- and belowground residues and enhance nutrient cycling (Sinsabaugh et al., 2008; Sinsabaugh and Follstad Shad, 2012). Other studies have also reported increased soil enzyme activity due to cover cropping (Brennen and Acosta-Martinez, 2019; Tyler 2020).

There was a significant P fertilizer treatment by cover crop interaction for both alkaline phosphatase and glucosidase activity in the present study (Table 3.9). The presence of a cover crop on both the NP and SUF treatments resulted in significantly greater soil enzyme activities, while no significant difference was observed when a cover crop was added to the BAM treatment. Resource allocation suggests that the soil microbial community should not induce soil

enzyme production unless the production of enzymes would alleviate nutrient deficiencies or if a substrate is present (Allison et al., 2011). Enzyme activities reflect cellular economics as the benefits of production (increased availability of assimilable mineral nutrients) must balance the resources that are expended for production (C and nutrients required for energy) versus the benefit of increased availability of (Burns et al., 2013). For alkaline phosphatase, it is possible greater soil enzyme activities in the NP treatment were a response to P deficiency, phosphatase induction is depressed with increasing soil P levels (Sinsabaugh and Follstad Shad, 2012). To increase P cycling in systems that are P-limited, the introduction of cover crops would result in increased substrate availability which can induce phosphatase in these P-limited systems. In the BAM treatment, where P levels are not limiting, resource allocation would suggest that cover crops would not promote enzyme production as there is not a need for increased P (Allison et al., 2011). For glucosidase activity, C and P dynamics were probably responsible for the increases of activity in these P limited systems with the inclusion of a cover crop. The lack of interaction between BAM and the presence or absence of the cover crops suggests that the microbial community was not nutrient limited, so resources would not be allocated to increase enzyme activity when cover crop was present. Some studies have shown a negative correlation between available soil P and glucosidase activity (Pan et al. 2013; Zheng et al., 2015).

Phosphorus fertilizer by sampling time interactions for citrate-extractable P and N pools

Citrate-extractable P concentrations were found to be significantly higher in the BAM treatment compared to the SUF and NP treatments regardless of sampling time in the present study (Fig. 3.4). As the BAM treatment receive annual P fertilizer applications, it is likely that citrate-extractable P reflected soil P status. Citrate-extractable P concentrations have been

reported to be positively correlated with available P (measured as Olsen-P) (DeLuca et al., 2015).

The significantly higher citrate-extractable P concentrations at T3 in the BAM treatment relative to the NP and SUF treatments was likely due to P fertilizer application that occurred at T2 (Fig. 3.4). Regardless of P fertilizer treatment, citrate-extractable P concentrations were significantly lower at T2 compared to all other sampling times, while concentrations significantly increase in at T3 regardless of P fertilizer treatment. Citrate-extractable P represents a readily available inorganic P pool sorbed to clay particles or weakly bound in inorganic precipitates that becomes plant available by the release of organic acids from roots and ectomycorrhizas (DeLuca et al., 2015). Citrate can release inorganic P into solution by competition with sorption sites, by anion exchange with iron- and aluminum-associated phosphates, or by chelation of precipitates to form soluble compounds (Hayes et al., 2000). As soybeans were planted at T2, it is possible that the increases in citrate-extractable P at T3 are a result of increased P demand by soybeans.

The significant P fertilizer treatment by sampling time interaction observed for dissolved total N, inorganic N (NH_4-N and NO_3-N), and dissolved organic N (Table 3.5) in the present study was probably due to a combination of environmental conditions, the application of fertilizer on the BAM treatment at T2, dynamics of the soil microbial community in P-deficient systems, and plant uptake of N. Similar to cover crop impacts, dissolved total N followed NO_3-N trends, suggesting that NO_3-N dynamics were the primary driver of dissolved total N in terms of the P fertilizer by treatment interaction. Soil NO_3-N has been reported to dominate total dissolved N concentrations in more intensive agricultural systems (Christou et al., 2005).

In general, NO_3-N and NH_4-N trends in the P fertilizer treatment by sampling time interaction were opposite of each other (Figs.6 & 8). For total inorganic N (Fig. 3.11), trends were variable in comparison to the individual NO_3-N and NH_4-N pool trends (Fig. 3.8 & 3.10). This suggests that at different sampling times, the main form of inorganic N differed. Accessing the distribution of NH_4-N and NO_3-N in inorganic N at each sampling time, NH_4-N was the dominant form of inorganic N at T0 and T5, while at all other sampling times, NO_3-N was dominant (Table 3.6).

Differences between NO_3-N and NH_4-N trends could be due to soil moisture conditions. Soil moisture was significantly lower at T0 compared to all other sampling times. Gravimetric water content significantly increased from 10% to 14% between T0 and T1, respectively, with cumulative precipitation between these two sampling times of 183 mm (Table 3.1). In comparison to T0, increased concentrations of NO_3-N at T1, with simultaneous decreases in NH_4-N concentrations, suggest that nitrification was potential inhibited at T0 due to low soil moisture conditions and cooler soil temperature. Accumulations of NH_4-N in soil during drought conditions can be attributed to decreased diffusion of NH_4^+ to plant roots and the microbial community (Gao et al., 2020). In turn, limited NH_4^+ diffusion to nitrifiers in drought conditions will depress nitrification (Schimel 2018; Deng et al., 2021). Rewetting of dry soils enhances soil microbial activity that can metabolize the available nutrients that accumulated during dry periods, resulting in increased rates of soil nitrification. Increases in NO_3-N concentrations, with simultaneous decreases in NH_4-N concentrations following rewetting of soil at T1 suggests that nitrification rates were no longer limited (Fig. 3.8 & 3.10).

Following observed peaks in NO_3-N concentrations, NO_3-N decreased linearly throughout the remainder of the growing season. On the other hand, NH_4-N concentrations

tended to increase during this same time period. It is possible that depressed nitrification rates were responsible for the NO_3-N and NH_4-N trends. Cumulative precipitation between sampling times T3 to T5 was 178 mm, which was considerably less than the cumulative precipitation between T0 and T3 (345 mm). In the drier conditions during the latter portion of the growing season, it is possible that NH_4^+ started to accumulate due to decreased diffusion and hindered nitrification, resulting in decreases in NO_3-N concentrations (Gao et al., 2020). Of interest, soil moisture at T5 was statistically higher compared to all other sampling times, except for T2. A rainfall event on October 12, 2022, in which 18.8 mm of precipitation was recorded, coupled with decreasing temperatures probably was responsible for an increase in soil moisture observed at T5. As optimal soil temperature for nitrification occurs between 25 and 35°C (Havlin et al., 2014), it is possible that decreases in soil temperature could also decrease nitrification rates. It was observed that the average maximum soil temperature 20 days before soil sampling was 18°C (Table 3.1), falling below the optimal nitrification range.

Soil moisture content can also influence NH_4^+ fixation in soils, as greater soil moisture content can result in decreased NH_4^+ fixation, while drier soil conditions can increase NH_4^+ fixation as the interlayer space is reduced (Allison et al., 1953; Nieder et al., 2011). Therefore, it is possible that decreased soil moisture at T0 resulted in increased NH_4^+ fixation, and following rewetting, NH_4^+ fixation decreased. The decrease in NH_4^+ fixation could have led to increased NH_4-N in the soil solution that was subjected to nitrification.

In the BAM treatment, NO_3-N concentrations tended to be similar to the NP and SUF treatments at each sampling time. The exception to this trend was at T3, where the BAM treatment had significantly higher NO_3-N concentrations compared to the NP and SUF treatments. However, no such effect was observed for NH_4-N concentrations. The significant

difference between P fertilizer treatments at T3 for NO_3-N was likely due to ammonium polyphosphate application that occurred at soybean planting (T2). Djinadou et al. (1995) reported greatest relative NH_4-N decreases in soils incubated with urea-ammonium nitrate or urea-ammonium nitrate + ammonium polyphosphate from one to four weeks following application. The lack of a significant response in NH_4-N concentrations at T3 in the BAM treatment could indicate that nitrification had occurred before the T3 sampling (Fig. 3.8), resulting in increased NO_3-N concentrations by the time soil samples were collected at T3.

The significant P fertilizer treatment by sampling time interaction that was observed for dissolved organic N (Fig. 3.13) could be contributed to the relative importance of P availability on organic N concentrations. Although not significant, the NP treatment had higher dissolved organic N averaged across each sampling time. In a meta-analysis comparing P additions to N transformations in soil, Wang et al. (2022) found that P additions generally decreased dissolved organic N. Therefore, it is possible that the marked increases in dissolved organic N under the NP treatment is a response to P availability.

Decreases in NO_3-N concentrations after T3 in all P fertilizer treatments could also be attributed to N uptake during soybean growth. Biological N_2 fixation, soil N, or fertilizer N are the main sources to meet the N requirement of soybeans (George and Singleton, 1992). Biological N_2 fixation can supply 50-60% of soybean N demand (Salvagiotti et al., 2008), and generally reaches a peak at early pod fill and declines during the late reproductive stages (George and Singleton, 1992). Nitrate uptake by soybean increases throughout vegetative growth, peaks during the early reproductive stages, and then declines during pod and seed development (Imsande and Touraine, 1994). Therefore, it is possible that increased N uptake by soybean

plants during early reproductive growth resulted in a decrease in soil NO_3-N following sampling at T3.

Cover crop by sampling time interactions for soil moisture, dissolve total N, NO3-N, and microbial biomass N

The presence of a cover crop significantly decreased soil moisture at T0 compared to the no cover crop treatment, while the presence of a cover crop significantly increased soil moisture at T2 compared to no cover crop treatment. The difference in soil moisture at T0 likely reflects the consumption of moisture for cover crop growth, as the cover crop was still living at T0. The difference observed between cover treatments at T2 likely reflects the residue amount on the soil surface and the cumulative precipitation that occurred between T1 and T2. Between T1 and T2, there was 182.87 mm of precipitation that was received. It is possible that the residue biomass retained more moisture than the no cover crop treatment.

In the present study, a significant cover crop by sampling time interaction was observed for both dissolved total N and NO_3-N. For both pools, the cover crop by sampling time trend was the same. As dissolved total N consists of both organic and inorganic forms of N, and no significant cover crop effect was observed for dissolved organic N or NH_4-N, it is likely that NO_3-N was primary driver of dissolved total N dynamics in the present study. Therefore, only NO_3-N trends will be discussed. At sampling times T0 and T1, NO_3-N concentrations in the cover crop treatment were significantly lower compared to the no cover crop treatment. Cereal rye is an example of a catch cover crop that can assimilate soil N during crop growth, thereby reducing the amount of N that is lost due to leaching or denitrification (Dabney et al., 2010; Adetunji et al., 2020). Soil N that is assimilated during cover crop growth can be recycled back into the soil following cover crop decomposition (Adetunji et al., 2020). It is likely that cereal

rye assimilated residual soil NO_3-N during crop growth, resulting in significantly lower NO_3-N concentrations under the cover crop treatment in the present study during the early portion of the growing season.

At the T2 sampling time, there was no significant difference observed between the cover and no cover crop treatments for dissolved total N and NO_3-N; a trend that would continue throughout the remainder of the growing season. Several litterbag studies have determined that N release from cereal rye residues is gradual compared to other cover crop species with narrower C:N ratios (Wagger 1989; Lacey et al., 2020). Jahanzad et al. (2016) observed that the percentage of N remaining in surface applied cereal rye residues was 50%, 12 weeks after litterbag placement. Other studies have demonstrated that decomposition of cereal rye residues initially immobilize N early in the decomposition process before gradually releasing N (Sievers and Cook, 2018; Nevins et al., 2020; Singh et al., 2020). Immobilization of N during decomposition of cereal rye residues suggests that the soil microbial community utilizes plant available N in soil for continual decomposition of rye residues, which can lead to decrease on N present in soil solution that is needed for the subsequent cash crop (Nevins et al., 2018; 2020). As no significant difference was observed after T2 between the cover crop treatments, it is likely that limited immobilization of N was occurring during cover crop decomposition, causing no significant decreases on soil N concentrations. The lack of difference between cover crop treatments was probably attributed to limited N release from cover crop residues during the soybean growing season.

Biochemical composition of the crop (such as initial C:N ratio, lignin content, and fiber content) soil factors (such as texture and organic matter content), management factors (i.e. tillage), and climatic factors can all influence decomposition rates and subsequent N release from

cover crop residues (Dabney et al., 2010; Adetunji et al., 2020). Temperature and precipitation can play an important role in residue decomposition and nutrient release as both can influence microbial activities, biochemical processes, and solute transport (Singh et al., 2020). It is likely that both the initial C:N ratio (38:1, averaged over all P fertilizer treatments) and climatic factors contributed to the lack of significant N release during decomposition in the present study. Even though N release from cereal rye is gradual throughout the subsequent growing season, it is possible that the drier conditions towards the latter part of the growing season further decreased the amount of N released from cover crop residues due to decreased microbial activity.

A significant cover crop by sampling time interaction was observed for microbial biomass N in the present study. Although not always significant, the cover crop treatment tended to have higher levels of microbial biomass N in all sampling times, except at T0. Only at sampling times T1 and T4 was microbial biomass N greater in the cover crop treatment compared to no cover crop treatment. At all other sampling times, there was no significant difference observed between cover treatments. The slight difference between cover treatments in regard to microbial biomass N concentrations at T0 was likely due to N uptake during cover crop growth that decrease N availability for the soil microbial community (Kuzyakov and Xu, 2013). Elevated concentrations of microbial biomass in cover treatments at T1 and T2 relative to no cover crop treatment, might be due to changes in the microbial community following cover crop termination, as changes in the microbial community during cover crop decomposition have been reported (Nevins et al., 2018). Overall, there was a significant increase in microbial biomass N concentrations when cover crops were present. Muhammad et al. (2021) reported that cover crops increased microbial biomass N concentrations in a meta-analysis possibly due to enhanced N inputs from above- and below-ground inputs.

Overall effects of sampling time

Other than dissolved total N, NO_3-N, and microbial biomass N, no cover crop by sampling time interactions were observed. This suggests that the sampling time effects were primarily driven by soil moisture and temperature dynamics instead of cover crop decomposition. Dynamics in the soluble nutrient pools of dissolved organic C and N were likely due to precipitation trends. Highest concentrations of dissolved organic C concentrations in the present study was observed at T1 and T4 (Table 3.4). Greater concentrations of dissolved organic C at T1 likely reflect the effects of rewetted following a long period of dry conditions, and although soil moisture levels were decreased at T4 (Aug 5) compared to T3, a significant precipitation event was observed on July 24 and 25. Dissolved organic C concentrations are known to increased following rewetting as the reduced rates of microbial decomposition in dry soils allows for microbial products to accumulate (Kalbitz et al., 2000). Sampling time significantly affected active C concentrations.

In addition, highest dissolved organic N concentrations coincided with lowest soil moisture in the present study. It is likely that dissolved organic N accumulated under drier conditions, and quickly mineralized when soils were rewetted, resulting in decreases in dissolved organic N in sampling times with increased soil moisture (Schaeffer et al., 2017). It is also possible that a small quantity of dissolved organic N might have been lost thorough leaching follow periods of rewetting (Kalbitz et al., 2000; Gao et al., 2020).

Sampling time greatly influenced acid and alkaline phosphatase activity as well as glucosidase activity in the present study. Soil moisture and temperature were likely responsible for the seasonal dynamics of soil enzyme activities. In general, activities increased with increasing soil moisture from T0 to T2, followed by a decrease in activity that coincided with

decreasing levels of soil moisture at T3. Increased moisture allows for the diffusion of soil enzymes to pair with substrates (Steinweg et al., 2012; Wang et al., 2013). At T4, there was an increase in enzyme activities that appeared not to be related to soil moisture, as soil moisture levels had declined between these sampling times. However, a rain event on July 24 and 25, that resulted in 70 mm of precipitation, could have resulted in increased levels of soil enzyme activity by increasing the diffusion of enzymes. There was a sharp decrease in activities at T5, which coincided with increased levels of soil moisture. It is possible that the drier conditions that persisted between T4 and T5 hindered soil enzyme activities which had not recovered by the time of sample collection (Baldrian et al., 2010; Kotroczo et al., 2014).

Soil respiration was significantly higher at T0 compared to all other sampling times in the present study, which coincided with lowest soil moisture levels during the study. This finding was surprising as drought stress reduces the activity of the soil microbial community and reduces substrate availability to the microbial community due to limited diffusion (Manzoni et al., 2012). A reduction in microbial activity can lead to reduced levels of soil respiration in drought conditions (Wang et al., 2014; Schimel 2018). After rewetting of soil following drought conditions, C is mobilized from physically protected soil aggregates, intercellular osmolytes are released, and enhancement of metabolic and enzyme activities is observed; all leading to increased soil respiration and nutrient mineralization (Manzoni et al., 2012). However, the precipitation received between sampling times T0 and T1 (113 mm) or between T1 and T2 (183 mm) did not result in a pulse of CO_2 (the Birch effect) (Schimel 2018). In fact, soil respiration was significantly lower at T2 compared to all other sampling times, which coincided with highest soil moisture levels found in the present study.

Temperature also plays a key role in soil respiration as increasing temperature can increase microbial activity, resulting in increased rates soil respiration (Wang et al., 2013). However, the coupling of soil temperature and respiration is broken down under drought stress (Wang et al., 2013). An increase in soil temperature did not result in an increase in soil respiration (as seen by decreasing respiration with increasing temperature between T0 and T2) in the present study. However, increases in soil respiration from T2 and T3, where afterward, soil respiration remained stable, might be contributed to increasing temperature conditions (as well as adequate soil moisture levels that would promote microbial activity).

Therefore, soil moisture and temperature were likely not responsible for the trends observed for soil respiration during the early portion of the growing season (T0-T2). It is possible that the drier conditions observed around T0 promoted changes in the microbial community structure, with fungi and actinomycetes becoming the dominant community (Wang et al., 2013). Fungi are considered more tolerant than bacteria in low moisture conditions as they can accumulate osmoregulatory solutes and the filamentous structure allows fungi to reach substrates even at low moisture conditions (Manzoni et al., 2012).

Active C and microbial biomass C and P concentrations were significantly higher at T5 compared to all other sampling times (Tables 4 and 8). It is possible that increases in active C concentrations at harvest is due to the release of labile C substrates from root exudation (Wang et al., 2017). The release of simple C substrates and secondary metabolites through root exudation from the soybean plants might explain the significantly higher levels of microbial biomass C post-soybean harvest compared to all other sampling times (Broeckling et al., 2008). It is possible that increases in C during the growing season from above and belowground inputs

resulted in increased P cycling within the microbial biomass as microbial biomass P can acts as a sink or source of P (Katsalirou et al., 2016).

Differences in soil health indicators in preplant and post-harvest – When is the best time to sample?

There was no significant difference between results at T0 and T5 for N and P pools in the present study (Table 3.4 & 3.6). Active C and microbial biomass C and P concentrations were significantly higher post-harvest compared to T0 sampling, while soil respiration, and enzyme activities were significantly higher at T0 compared to T5. In regards to nutrient applications, the lack of significant difference between T0 and T5 suggests that either spring or fall would be appropriate opportunities to soil test (Liebig et al., 2006). Increased in active C and microbial biomass relative to the spring sampling might suggest the decomposition of above- and belowground inputs of the cover crop. While enzyme activities were significantly higher during the spring sampling compared to post-harvest, this was not the case in 2021 (Chapter 2), reflecting the importance of moisture and temperature on microbial activity. Therefore, these results suggest that either sampling time is appropriate as long as the sampling time is consistent each year and the moisture and temperature dynamics are considered (Jones, 2001).

Conclusion

In the present study, it was found that cover crops, P fertilizer treatment, and sampling time influence measures of soil health. The presence of a cover crop significantly increased all C measures and measures of microbial activity and function, supporting the first hypothesis. Both alkaline phosphatase and glucosidase activity significantly increased in NP and SUF treatments in the presence of a cover crop. Increased enzyme activity in P-limited systems indicates that the system has the capacity for increased nutrient cycling, supporting the second hypothesis the

microbial community can enhance activity in systems undergoing P nutrient drawdown. Dissolved total N, inorganic N, and dissolved organic N were influenced by P fertilizer treatments and sampling time. Cover crops and sampling time also influenced soil NO_3-N and dissolved total N concentrations. Although the main effect of cover crop influenced NO_3-N and dissolved total N, a difference was not observed between cover treatments following termination. This suggests that cereal rye decomposition was gradual and N released from decomposition was not in plant available form, supporting the third hypothesis of this study. Finally, sampling time greatly influenced a majority of soil health indicators. It is likely that soil moisture and temperature are primary drives of seasonal trends in biological soil health indicators as very few cover crop by sampling time interactions were observed. For microbial biomass N, dissolved total N, and NO_3-N, once the cover crop was termination, no other differences were observed throughout the remained of the growing season.

References

Adegbeye, M.J., Reddy, P.R.K., Obaisi, A.I., Elghandour, M.M.M.Y., Oyebamiji, K.J., Salem, A.Z.M., Morakinyo-Fasipe, O.T., Cipriano-Salazar, M., Camacho-Díaz, L.M., 2020. Sustainable agriculture options for production, greenhouse gasses and pollution alleviation, and nutrient recycling in emerging and transitional nations-An overview. J. Clean. Prod. 242, 118319.

Adetunji, A.T., Ncube, B., Mulidzi, R., Lewu, F.B., 2020. Management impact and benefit of cover crops on soil quality: a review. Soil Tillage Res. 204, 104717.

Allison, F.E., Doetsch, J.H., Roller, E.M., 1953. Availability of fixed ammonium in soil containing different clay minerals. Soil Sci. 75, 373-381.

Allison, S.D., Weintraub, P.M., Gartner, T.B., Waldrop, M.P., 2011. Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. Soil Enzymology, Springer, pp. 229-243.

Baldrian, P., Merhautova, V., Petrankova, M., Cajthaml, T., Snajdr, J., 2010. Distribution of microbial biomass and activity on extracellular enzymes in a hardwood forest reflect soil moisture content. Appl. Soil Ecol. 46, 177-182.

Bandick, A.K., and Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biol. Biochem. 31, 1471-1479.

Benintende, S., Benintende, M., Sterren, M., Saluzzio, M., Barbagelata, P., 2015. Biological variables as soil quality indicators: Effect of sampling time and ability to classify soils by their suitability. Ecol. Indic. 52, 147-152.

Bindraban, P.S., van der Velde, M., Ye, L., Van den Berg, M., Materechera, S., Kiba, D.I., Tamene, L., Ragnarsdóttir, K.V., Jongschaap, R., Hoogmoed, M., Hoogmoed, W., 2012. Assessing the impact of soil degradation on food production. Curr. Opin. Environ. Sustain. 4, 478-488.

Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A., Hergert, G.W., 2015. Cover crops and ecosystem services: insights from studies in temperate soils. Agron. J. 107, 2449-2474.

Brennan, E.B., and Acosta-Martinez, V., 2019. Cover crops and compost influence soil enzymes during six years of tillage-intensive organic vegetable production. Soil Sci. Soc. Am. J. 83, 624-637.

Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K., Vivanco, J.M., 2008. Root Exudates Regulate Soil Fungal Community Composition and Diversity. Appl. Environ. Microbiol. 74, 738-744.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S. 1982. Measurement of microbial biomass phosphorus in soil. Soil Biol. Biochem. 14, 319-329.

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol. Biochem. 58, 216-234.

Cabrera, M.L., and Beare, M.H., 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. Am. J. 57, 1007-1012.

Carver, R.E., Nelson, N.O., Roozeboom, K.L., Kluitenberg, G.J., Tomlinson, P.J., Kang, Q., Abel, D.S., 2022. Cover crop and phosphorus fertilizer management impacts on surface water quality from a no-till corn-soybean rotation. J. Environ. Manage. 301, 113818.

Chavarría, D.N., Verdenelli, R.A., Serri, D.L., Restovich, S.B., Andriulo, A.E., Meriles, J.M., Vargas-Gil, S., 2016. Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability. Eur. J. Soil Biol. 76, 74-82.

Chen, C., and Xiao, W., 2023. The global positive effect of phosphorus addition on soil microbial biomass. Soil Biol. Biochem. 176, 108882.

Christou, M., Avramides, E.J., Roberts, J.P., Jones, D.L., 2005. Dissolved organic nitrogen in contrasting agricultural ecosystems. Soil Biol. Biochem. 37, 1560-1563.

Dabney, S.M., Delgado, J.A., Meisinger, J.J., Schomberg, H.H., Liebig, M.A., Kaspar, T., Mitchell, J., Reeves, W., 2010. Using cover crops and cropping systems for nitrogen management. In: Delgado, J.A., Follett, R., (Eds.), Advances in Nitrogen Management for Water Quality. Soil Water Conservation Society, Ankeny, Iowa.

Darch, T., Blackwell, M.S.A., Chadwick, D., Haygarth, P.M., Hawkins, J.M.B., Turner, B.L., 2016. Assessment of bioavailable organic phosphorus in tropical forest soils by organic acid extraction and phosphatase hydrolysis. Geoderma 284, 93-102.

DeLuca, T.H., Glanville, H.C., Harris, M., Emmett, B.A., Pingree, M.R., de Sosa, L.L., Cerdá-Moreno, C., Jones, D.L., 2015. A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. Soil Biol. Biochem. 88, 110-119.

Deng, L., Peng, C., Kim, D-G., Li, J., Liu, Y., Hai, X., Liu, Q., Huang, C., Shangguan, Z., Kuzyakov, Y., 2021. Drought effects on soil carbon and nitrogen dynamics in global natural ecosystems. Earth-Sci. Rev. 214, 103501.

Djinadou, K.A., Pierzynski, G.M., Havlin, J.L., 1995. Phosphorus and micronutrient availability from dual application of nitrogen and phosphorus using liquid fertilizers. Soil Sci. 159, 49-58.

Eivazi, F., and Tabatabai, M.A., 1977. Phosphatases in soils. Soil Biol. Biochem. 9, 167–172.

Eivazi, F., and Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606.

Feng, H., Sekaran, U., Wang, T., Kumar, S., 2021. On-farm assessment of cover cropping effects on soil C and N pools, enzyme activities, and microbial community structure. J. Agric. Sci. 159, 216–226.

Feng, J., and Zhu, B., 2019. A global meta-analysis of soil respiration and its components in response to phosphorus addition. Soil Biol. Biochem. 135, 38-47.

Gao, D., Bai, E., Li, M., Zhao, C., Yu, K., Hagedorn, F., 2020. Responses of soil nitrogen and phosphorus cycling to drying and rewetting cycles: a meta-analysis. Soil Biol. Biochem. 148, 107896

George, T., and Singleton, P.W., 1992. Nitrogen assimilation traits and dinitrogen fixation in soybean and common bean. Agron. J. 84, 1020-1028.

Guo, H. He, X., Li, Y., 2012. Spatial distribution of arbuscular mycorrhiza and glomalin in the rhizosphere of Caragana korshinskii Kom. in the Otindag sandy land, China. Afr. J. Microbiol. Res. 6, 5745-5753.

Haney, R.L., and Haney, E.B., 2010. Simple and rapid laboratory method for rewetting dry soil for incubations. Commun. Soil Sci, Plant Anal. 41, 1493–1501.

Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Separating root and soil microbial contributions to soil respirations: a review of methods and observations. Biogeochemistry 48, 115-146.

Hao, X., Najm, M.A., Steenwerth, K.L., Nocco, M.A., Basset, C., Daccache, A., 2023. Are there universal soil responses to cover cropping? A systematic review. Sci. Total Environ. 861, 160600

Havlin, J.L., Tisdale, S.L. Nelson, W.L., Beaton, J.D., 2014. Soil Fertility and Fertilizers: An Introduction to Nutrient Management. 8th ed., Pearson, Boston, MA.

Hayes, J.E., Richardson, A.E., Simpson, R.J., 2000. Components of organic phosphorus in soil extracts that are hydrolysed by phytase and acid phosphatase. Biol. Fertil. Soils 32, 279-286.

Hurisso, T.T., Moebius-Clune, D.J., Culman, S.W., Moebius-Clune, B.N., Thies, J.E.,van Es, H.M., 2018. Soil protein as a rapid soil health indicator of potentially available organic nitrogen. Agric. Environ. Lett. 3, 180006.

Imsande, J., and Touraine, B., 1994. N demand and the regulation of nitrate uptake. Plant Physiol. 105, 3.

Jahanzad, E., Barker, A.V., Hashemi, M., Eaton, T., Sadeghpour, A., Weis, S.A., 2016. Nitrogen release dynamics and decomposition of buried and surface cover crop residues. Agron. J. 108, 1735-1741.

Jones Jr, J.B., 2001. Laboratory guide for conducting soil tests and plant analysis. CRC Press, New York.

Jones, D. L., Willett, V. B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem., 38, 991–999.

Kalbitz, K., Solinger, S., Park, J.H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: a review. Soil Sci. 165, 277-304.

Katsalirou, E., Deng, S., Derakis, A., Nofziger, D.L., 2016. Long-term management effects on soil P, microbial biomass P, and phosphatase activities in prairie soils. Eur. J. Soil Biol. 76, 61-69.

Kotroczo, Z., Veres, Z., Fekete, I., Krakomperger, Z., Attila Toth, J., Lajtha, K., Tothmeresz, B., 2014. Soil enzyme activity in response to long-term organic matter manipulation. Soil Biol. Biochem. 70, 237-243.

Kuzyakov, Y. and Xu, X., 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytol. 198, 656-669.

Lacey, C., Nevins, C., Camberato, J., Kladivko, E., Sadeghpour, A., Armstrong, S., 2020. Carbon and nitrogen release from cover crop residues and implications for cropping systems management. J. Soil Water Conserv. 75, 505-514.

Laishram, J., Saxena, K.G., Maikhuri, R.K., Rao, K.S., 2012. Soil quality and soil health: a review. Int. J. Ecol. Environ. Sci. 38, 19-37.

Law, S.M., and Maherali, H., 2023. Variation in glomalin-related soil protein and plant growth response to arbuscular mycorrhizal fungi along a nutrient gradient in temperate grasslands. Plant Soil 487, 623-637.

Lawrence, P.G., Roper, W., Morris, T.F., Guillard, K., 2020. Guiding soil sampling strategies using classical and spatial statistics: A review. Agron. J. 112, 493-510.

Leikam, D.F., Lamond, R.E., Mengel, D.B., 2003. Providing flexibility in phosphorus and potassium fertilizer recommendations. Better Crops 87, 6-10.

Macnack, N., Khin Chim, B., Amedy, B., Arnall, B., 2011. Fertilization based on sufficiency, build-up and maintenance concept. Oklahoma Cooperative Extension Service Publication, PSS-2266.

Manzoni, S., Schimel, J.P., Porporatem A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930-938.

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecol. Appl. 24, 560-570.

Mitchell, J.P., Shrestha, A., Mathesius, K., Scow, K.M., Southard, R.J., Haney, R.L., Schmidt, R., Munk, D.S., Horwath, W.R., 2017. Cover cropping and no-tillage improve soil health in an arid irrigated cropping system in California's San Joaquin Valley, USA. Soil Tillage Res. 165, 325-335.

Mooshammer, M., Wanek, W., Zechmesiter-Boltenstern, S., Richter, A., 2014. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Front. Microbiol. 22.

Muhammad, I., Wang, J., Sainju, U.M., Zhang, S., Zhao, F., Khan, A., 2021. Cover cropping enhances soil microbial biomass and affects microbial community structure: A meta-analysis. Geoderma 381, 114696.

Murphy, J., and Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta. 27, 31-36.

Nevins, C.J., Lacey, C., Armstrong, S., 2020. The synchrony of cover crop decomposition, enzyme activity, and nitrogen availability in a corn agroecosystem in the Midwest United States. Soil Tillage Res. 197, 104518.

Nevins, C.J., Nakatsu, C., Armstrong, S., 2018. Characterization of microbial community response to cover crop residue decomposition. Soil Biol. Biochem. 127, 39-49.

Nieder, R., Benbi, D.K., Scherer, H.W., 2011. Fixation and defixation of ammonium in soils: a review. Biol. Fertil. Soils 47, 1-14.

Nilahyane, A., Ghimire, R., Thapa, V.R., Sainju, U.M., 2020. Cover crop effects on soil carbon dioxide emissions in a semiarid cropping system. Agricul. Ecosys. Environ. 3, e20012.

Nunes, M.R., van Es, H.M., Schindelbeck, R., Ristow, A.J.., Ryan, M., 2018. No-till and cropping system diversification improve soil health and crop yield. Geoderma 328, 30-43.

O'Halloran, I. P., & Cade-Menun, B. J. 2008. Chapter 24 Total and organic phosphorus. In Carter, M.R., and Gregorich, E.G. (Eds), Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, Taylor & Francis Group, pp. 279-280.

Osmond, D.L., Hoag, D.L., Luloff, A.E., Meals, D.W., Neas, K., 2015. Farmers' use of nutrient management: Lessons from watershed case studies. J. Environ. Qual. 44, 382-390.

Pan, C., Liu, C., Zhao, H., Wang, Y., 2013. Changes of soil physico-chemical properties and enzyme activities in relation to grassland salinization. Eur. J. Soil Biol. 55, 13-19.

Parham, J. A., and Deng, S.P., 2000. Detection, quantification and characterization of β-glucosaminidase activity in soil. Soil Biol. Biochem. 32, 1183–1190.

Philippot, L., Spor, A., Henault, C., Bru, D., Bizouard, F., Jones C.M., Sarr, A., Maron, P-A., 2013. Loss in microbial diversity affects nitrogen cycling in soil. ISME J. 7, 1609-1619.

Qiu, L., Lin, H., Song, B., Kong, T., Sun, W., Sun, X., Zhang, Y., Li, B., 2021. Glomalin-related soil protein (GRSP) in metal sequestration at Pb/Zn-contaminated sites. J. Soils Sed. 22, 577-593.

Ros, G.H., Hanegraaf, M.C., Hoffland, E., van Riemsdijk, W.H., 2011. Predicting soil N mineralization: Relevance of organic matter fractions and soil properties. Soil Biol. Biochem. 43, 1714-1722.

Salvagiotti, F., Cassman, K.G., Specht, J.E., Walters, D.T., Weiss, A., Dobermann, A., 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. Field Crops Res. 108, 1-13.

Schaeffer, S.M., Homyak, P.M., Boot, C.M., Roux-Michollet, D., Schimel, J.P., 2017. Soil carbon and nitrogen dynamics throughout the summer drought in a California annual grassland. Soil Biol. Biochem. 115, 54-62.

Schimel, J.P., 2018. Life in dry soils: effects of drought on soil microbial communities and processes. Annu. Rev. Ecol. Evol. Syst. 49, 409-432.

Sievers, T., and Cook, R.L., 2018. Aboveground and root decomposition of cereal rye and hairy vetch cover crops. Soil. Soc. Sci. Am. J. 82, 147-155.

Sims, J.T., Edwards, A.C., Schoumans, O.F., Simard, R.R., 2000. Integrating soil phosphorus testing into environmentally based agricultural management practices. J. Environ. Qual. 29, 60-71.

Singh, G., Dhakal, M., Yang, L., Kaur, G., Williard, K.W., Schoonover, J.E., Sadeghpour, A., 2020. Decomposition and nitrogen release of cover crops in reduced-and no-tillage systems. Agron. J. 112, 3605-3618.

Singh, P.K., Singh, M., Tripathi, B.N., 2013. Glomalin: an arbuscular mycorrhizal fungal soil protein. Protoplasm 250, 663-669.

Sinsabaugh, R.L., and Follstad Shah, J.J., 2012. Ecoenzymatic stoichiometry and ecological theory. Annu. Rev. Ecol. Evol. Syst. 43, 313-343.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252e1264.

Staunton, S., Saby, N.P.A., Arrouays, D., Quiquampoxi, H., 2020. Can soil properties and land use explain glomalin-related soil protein (GRSP) accumulation? A nationwide survey in France. Catena 193, 104620.

Steinweg, J.M., Dukes, J.S., Paul, E.A., Wallenstein, M.D., 2013. Microbial response to multi-factor climate change: effects of soil enzymes. Front. Microbiol. 4, 146.

Tyler, H.L., 2020. Winter cover crops and no till management enhance enzyme activities in soybean field soils. Pedobiologia 81-82, 150666.

Vance, E.D., Brookes, P.C., Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703-707.

Wagger, M.G., 1989. Cover crop management and nitrogen rate in relation to growth and yield of no-till corn. Agron. J. 81, 533-538.

Wagger, M.G., Cabrera, M.L., Ranells, N.N., 1998. Nitrogen and carbon cycling in relation to cover crop residue quality. J. Soil Water Conserv. 53, 214-218.

Wang, F., Weil, R.R., Nan, X., 2017. Total and permanganate-oxidizable organic carbon in the corn rooting zone of US Coastal Plain soils as affected by forage radish cover crops and N fertilizer. Soil Tillage Res. 165, 247-257.

Wang, Q., Xiao, F., He, T., Wang, S., 2013. Responses of labile soil organic carbon and enzyme activity in mineral soils to forest conversion in the subtropics. Annals of Forest Sci. 70, 579-587.

Wang, R., Bicharanloo, B., Hou, E., Jiang, Y. and Dijkstra, F.A., 2022a. Phosphorus supply increases nitrogen transformation rates and retention in soil: a global meta-analysis. Earth's Future, 10, e2021EF002479.

Wang, X., Cao, Q., Yang, W., Zhu, X., 2022b. Spatial changes in glomalin-related soil protein and their correlation with soil properties in the black soil region of Northeast China. Agronomy 12, .2165.

Wang, Y., Hao, Y., Cui, X.Y., Zhao, H., Xu, C., Zhou, X., Xu, Z., 2014. Responses of soil respiration and its components to drought stress. J. Soils Sed. 14, 99-109.

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. Am. J. Alter. Agric. 18, 3-17.

Wood, S.A., and Bowman, M., 2021. Large-scale farmer-led experiment demonstrates positive impact of cover crops on multiple soil health indicators. Nat. Food, 2, 97-103.

Wright, S.F., and Upadhyaya, A., 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci. 161, 575–586.

Wu, F., Dong, M., Liu, Y., Ma, X., An, L., Young, J.P.W., Feng, H., 2011. Effects of long-term fertilization on AM fungal community structure and Glomalin-related soil protein in the Loess Plateau of China. Plant Soil 342, 233-247.

Zhang, H., Antonangelo, J., Grove, J., Osmond, D., Slaton, N.A., Alford, S., Florence, R., Huluka, G., Hardy, D.H., Lessl, J., Maguire, R., 2021. Variation in soil-test-based

phosphorus and potassium rate recommendations across the southern USA. Soil Sci. Soc. Am. J. 85, 975-988.

Zheng, M., Huang, J., Chen, H., Wang, H., Mo, J., 2015. Responses of soil acid phosphatase and beta-glucosidase to nitrogen and phosphorus addition in two subtropical forests in southern China. Eur. J. Soil Biol. 68, 77-84.

Zhou, X., Chen, C., Lu, S., Rui, Y., Wu, H., Xu, Z., 2012. The short-term cover crops increase soil labile organic carbon in southeastern Australia. Biol. Fertil. Soils 48, 239-244.

Table 3.1 Sampling dates, soil moisture, and weather conditions at time of sample collection.

Time	Sampling Date	Days after cover crop termination	Cumulative Precip. (20-d) (mm)	Soil Moisture[a] ($g\ g^{-1}$)	Minimum Daily Air Temperature (°C)	Maximum Daily Air Temperature (°C)
T0	27 April	-	5.33	0.102d	17.7	27.0
T1	23 May	3	113.02	0.145c	9.6	19.0
T2	15 June	26	182.87	0.187a	21.4	31.6
T3	8 July	49	162.31	0.160b	21.8	30.8
T4	5 August	77	76.20	0.145c	21.9	34.8
T5	21 October	154	36.58	0.181a	5.9	31.5

[a]Letters indicate significant differences in soil moisture at $p < 0.05$; Cumulative precipitation, minimum and maximum daily air temperature were revived from the Kansas Mesonet station near the field site in Ashland Bottoms. Cumulative precipitation reported at T0 and T5 represents precipitation received 20-days before sample collection. Cumulative precipitation reported at T1 through T4 represents precipitation received between the sampling times.

Table 3.2 p-nitrophenol substrates and start and stop buffers for glucosidase, glucosaminidase, acid and alkaline phosphatase enzyme assays.

Enzyme	Substrate	Start Buffer	Stop Buffer
β-glucosidase	0.05 M p-nitrophenyl-β-D glucopyranoside	MUB pH 6	0.1 M THAM pH 12
N-acetyl-β-glucosaminidase	0.01 M p-nitrophenyl-N-acetyl-β-D-glucosaminide	0.1 M Acetate Buffer	0.5 M NaOH
Acid Phosphatase	0.05 M p-nitrophenyl phosphate	MUB pH 6.5	0.5 M NaOH
Alkaline Phosphatase	0.05 M p-nitrophenyl phosphate	MUB pH 11	0.5 M NaOH

Table 3.3 Analysis of variance (ANOVA) table for soil moisture, active carbon (C), dissolved organic C, and citrate-extractable phosphorus (P). Table abbreviations include block (BLOC), P fertilizer treatments (TRT), cover crop (CC), and sampling time (TIME). Bolded values represent statistical significance at $p < 0.05$.

	Moisture	**Active C**	**Dissolved Organic C**	**Citrate-Extractable P**
BLOC[a]	0.0008	0.0086	0.1208	0.3616
TRT	0.1185	0.0596	0.5084	**<.0001**
CC	0.2231	**0.0043**	0.8665	0.0763
TIME	**<.0001**	**<.0001**	**<.0001**	**<.0001**
TRT*CC	0.676	-	-	0.4046
TRT*TIME	0.3132	-	-	**0.0010**
CC*TIME	**<.0001**	-	-	0.0590
Covariance Structure[b]	CS	CS	CS	CSH

[a]p value presented in table represent two-way ANOVA (soil moisture and citrate-extractable P) and one-way ANOVA results (active C and dissolved organic C). If no significant two-way interactions were observed, the interaction terms were removed from the model and only main effects were analyzed.

[b]Covariance structure abbreviations: compound symmetry (CS) and heterogeneous compound symmetry (CSH)

Table 3.4 Least squares (LS) means table for soil moisture, active carbon (C), dissolved organic C, and citrate-extractable phosphorus (P). Table abbreviations include, P fertilizer treatments (TRT), no P (NP), build and maintain (BAM), sufficiency (SUF) presence of cover crop (CC), absence of cover crop (NC). Letters indicate significant difference at $p < 0.05$.

Effect	TRT	Cover	Time	Moisture	Active C	Dissolved Organic C	Citrate-Extractable P
				g g soil^{-1}	mg C kg soil^{-1}	mg C kg soil^{-1}	mg P kg soil^{-1}
TRT	**NP**			0.150	211.31	46.07	1.10C
TRT	**BAM**			0.154	218.36	43.2	7.03A
TRT	**SUF**			0.156	233.06	44.56	2.19B
CC		**CC**		0.152	232.79A	44.47	2.35
CC		**NC**		0.155	209.03B	44.74	2.80
TIME			**T0**[a]	0.102D	226.21B	-	3.09AB
TIME			**T1**	0.145C	235.76B	47.36AB	2.53C
TIME			**T2**	0.190A	188.09C	37.9D	1.23D
TIME			**T3**	0.160B	185.54C	41.47CD	3.56A
TIME			**T4**	0.145C	214.43B	51.85A	2.85BC
TIME			**T5**	0.181A	275.41A	44.46BC	2.92BC

[a]Sampling times: 27-Apr (T0), 23-May (T1), 15-Jun (T2), 8-Jul (T3), 5-Aug (T4), and 21-Oct (T5)

Table 3.5 Analysis of variance (ANOVA) table for autoclaved citrate extractable (ACE) protein, dissolved total nitrogen (N), NO_3-N, NH_4-N, inorganic N, and dissolved total N. Table abbreviations include block (BLOC), phosphorus (P) fertilizer treatments (TRT), cover crop (CC), and sampling time (TIME). Bolded values represent statistical significance at $p < 0.05$.

	ACE Protein	**Dissolved Total N**	**NH_4-N**	**NO_3-N**	**Inorganic N**	**Dissolved Organic N**
BLOC[a]	0.7374	0.1044	0.2893	0.3498	0.3677	0.6112
TRT	**0.0108**	**0.0204**	**0.0006**	**0.0003**	**0.0002**	0.0822
CC	**0.0017**	**0.0385**	0.6138	**0.0451**	**0.0213**	0.9892
TIME	0.0643	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**0.0190**
TRT*CC	-	**0.0137**	0.0616	0.2774	**0.0241**	0.0905
TRT*TIME	-	**<.0001**	**0.0446**	**0.0014**	**<.0001**	**0.0063**
CC*TIME	-	**0.0168**	0.9178	**0.0238**	0.1108	0.2138
Covariance[b] Structure	CS	CS	ARH(1)	ARH(1)	ARH(1)	ARH(1)

[a]p value presented in table represent two-way ANOVA (dissolved total N, NO_3-N, NH_4-N, and dissolved organic N) and one-way ANOVA results (ACE protein). If no significant two-way interactions were observed, the interaction terms were removed from the model and only main effects were analyzed.

[b]Covariance structure abbreviations: compound symmetry (CS) and first-order autoregressive (ARH(1))

Table 3.6 Least squares (LS) means table for autoclaved citrate extractable (ACE) protein, dissolved total nitrogen (N), NO_3-N, NH_4-N, inorganic N, and dissolved total N. Table abbreviations include, phosphorus (P) fertilizer treatments (TRT), no P (NP), build and maintain (BAM), sufficiency (SUF) presence of cover crop (CC), absence of cover crop (NC). Letters indicate significant difference at $p < 0.05$.

Effect	TRT	Cover	Time	ACE Protein	Dissolved Total N	NH_4-N	NO_3-N	Inorganic N	Dissolved Organic N
				mg protein kg $soil^{-1}$	mg N kg $soil^{-1}$	mg NH4-N kg $soil^{-1}$	mg NO3-N kg $soil^{-1}$	mg N kg $soil^{-1}$	mg N kg $soil^{-1}$
TRT	**NP**			4257.07B	11.94B	2.1B	4.86B	6.87C	6.09
TRT	**BAM**			4615.50A	14.65A	4.32A	8.31A	11.32A	4.71
TRT	**SUF**			4646.37A	11.32B	3.84A	5.63A	8.84B	3.88
CC		**CC**		4699.85A	11.64A	3.50	5.53B	8.13B	4.94
CC		**NC**		4312.78B	13.64B	3.34	7.00A	9.89A	4.85
TIME			**T0**[a]	4570.27	6.04C	5.58A	3.09CD	6.52B	-
TIME			**T1**	4511.22	14.19B	1.63C	7.03B	8.42B	6.12A
TIME			**T2**	4579.86	14.77B	3.37B	8.65B	12.02A	3.65B
TIME			**T3**	4483.63	19.96A	1.91BC	13.53A	13.95A	5.02AB
TIME			**T4**	4540.75	12.33B	2.83B	4.15C	6.8B	5.53AB
TIME			**T5**	4352.16	8.56C	5.19A	1.16D	6.35B	4.15B

[a]Sampling times: 27-Apr (T0), 23-May (T1), 15-Jun (T2), 8-Jul (T3), 5-Aug (T4), and 21-Oct (T5)

Table 3.7 Analysis of variance (ANOVA) table for microbial biomass carbon (C), nitrogen (N), phosphorus (P), biomass C:N ratios and soil respiration. Table abbreviations include block ((BLOC), P fertilizer treatments (TRT), cover crop (CC), and sampling time (TIME). Bolded values represent statistical significance at $p < 0.05$.

	Microbial Biomass C	**Microbial Biomass N**	**Microbial Biomass P**	**C:N Ratio**	**Soil Respiration**
BLOC[a]	0.0128	0.743	0.1749	0.0452	0.1775
TRT	0.3007	0.7485	**<.0001**	0.8604	0.7891
CC	**0.0005**	**0.0323**	0.426	0.1808	**<.0001**
TIME	**<.0001**	**0.0077**	**<.0001**	**0.0188**	**<.0001**
TRT*CC	-	0.7441	-	-	**0.0248**
TRT*TIME	-	0.1296	-	-	0.9812
CC*TIME	-	**0.0166**	-	-	0.3257
Covariance[b] Structure	ARH(1)	ARH(1)	CS	CSH	ARH(1)

[a]p value presented in table represent two-way ANOVA (microbial biomass N and soil respiration) and one-way ANOVA results (microbial biomass C and P and C:N). If no significant two-way interactions were observed, the interaction terms were removed from the model and only main effects were analyzed.

[b]Covariance structure abbreviations: first-order autoregressive (ARH(1)), compound symmetry (CS) and heterogeneous compound symmetry (CSH)

Table 3.8 Least squares (LS) means table for microbial biomass carbon (C), nitrogen (N), phosphorus (P), biomass C:N ratios and soil respiration. Table abbreviations include, P fertilizer treatments (TRT), no P (NP), build and maintain (BAM), sufficiency (SUF) presence of cover crop (CC), absence of cover crop (NC). Letters indicate significant difference at $p < 0.05$.

Effect	TRT	Cover	Time	Microbial Biomass C	Microbial Biomass N	Microbial Biomass P	C:N Ratio	Soil Respiration
				mg C kg soil^{-1}	mg N kg soil^{-1}	mg P kg soil^{-1}		mg CO2 g soil^{-1}
TRT	**NP**			117.77	16.52	0.39C	7.34	11.38
TRT	**BAM**			121.19	15.53	2.68A	8.93	11.48
TRT	**SUF**			120.38	16.34	1.55C	8.20	11.31
CC		**CC**		130.10A	17.4A	1.30	7.61	12.25A
CC		**NC**		109.46B	14.86B	1.43	8.70	10.59B
TIME			**T0**[a]	-	18.85A	1.04C	-	19.20A
TIME			**T1**	124.98B	15.55A	0.72C	8.61ABC	17.68B
TIME			**T2**	113.47B	17.16A	0.81C	7.15BC	7.23E
TIME			**T3**	102.21C	11.85B	1.67B	9.37A	9.37CD
TIME			**T4**	114.24B	16.2A	1.94AB	7.08C	10.27C
TIME			**T5**	143.99A	17.17A	2.40A	8.71AB	9.25D

[a]Sampling times: 27-Apr (T0), 23-May (T1), 15-Jun (T2), 8-Jul (T3), 5-Aug (T4), and 21-Oct (T5)

Table 3.9 Analysis of variance (ANOVA) table for acid phosphatase, alkaline phosphatase, glucosidase, and glucosaminidase. Table abbreviations include block (BLOC), P fertilizer treatments (TRT), cover crop (CC), and sampling time (TIME). Bolded values represent statistical significance at $p < 0.05$.

	Acid Phosphatase	**Alkaline Phosphatase**	**Glucosidase**	**Glucosaminidase**
BLOC[a]	0.0081	0.0203	0.585	0.0696
TRT	0.1185	0.1281	0.2304	0.8781
CC	**<.0001**	**<.0001**	**0.0016**	**<.0001**
TIME	**<.0001**	**<.0001**	**<.0001**	0.2033
TRT*CC	-	**0.0378**	**0.0203**	-
TRT*TIME	-	0.7135	0.7014	-
CC*TIME	-	0.9059	0.2415	-
Covariance[b] Structure	CS	AR(1)	CS	CS

[a]p value presented in table represent two-way ANOVA (alkaline phosphatase and glucosidase) and one-way ANOVA results (acid phosphatase and glucosaminidase). If no significant two-way interactions were observed, the interaction terms were removed from the model and only main effects were analyzed.

[b]Covariance structure abbreviations: first-order autoregressive (ARH(1)), compound symmetry (CS) and heterogeneous compound symmetry (CSH)

Table 3.10 Least squares (LS) means table for acid phosphatase, alkaline phosphatase, β-glucosidase, and β-glucosaminidase. Table abbreviations include, phosphorus (P) fertilizer treatments (TRT), no P (NP), build and maintain (BAM), sufficiency (SUF) presence of cover crop (CC), absence of cover crop (NC). Letters indicate significant difference at $p < 0.05$.

Effect	TRT	Cover	Time	Acid Phosphatase	Alkaline Phosphatase	Glucosidase	Glucosaminidase
TRT	**NP**			308.03	107.05	116.34	35.24
TRT	**BAM**			326.69	100.83	123.87	34.34
TRT	**SUF**			336.12	98.04	124.42	35.00
CC		**CC**		358.94A	110.23A	130.11A	40.19A
CC		**NC**		288.29B	93.72B	112.98B	29.53B
TIME			**T0**[a]	327.00B	104.58BC	114.62C	33.93
TIME			**T1**	344.92AB	94.81CD	122.10BC	34.60
TIME			**T2**	358.08A	112.35AB	133.40AB	34.14
TIME			**T3**	288.34C	99.65C	122.71BC	33.66
TIME			**T4**	343.26AB	116.08A	138.96A	35.67
TIME			**T5**	280.08C	84.38D	97.48D	37.15

[a]Sampling times: 27-Apr (T0), 23-May (T1), 15-Jun (T2), 8-Jul (T3), 5-Aug (T4), and 21-Oct (T5)

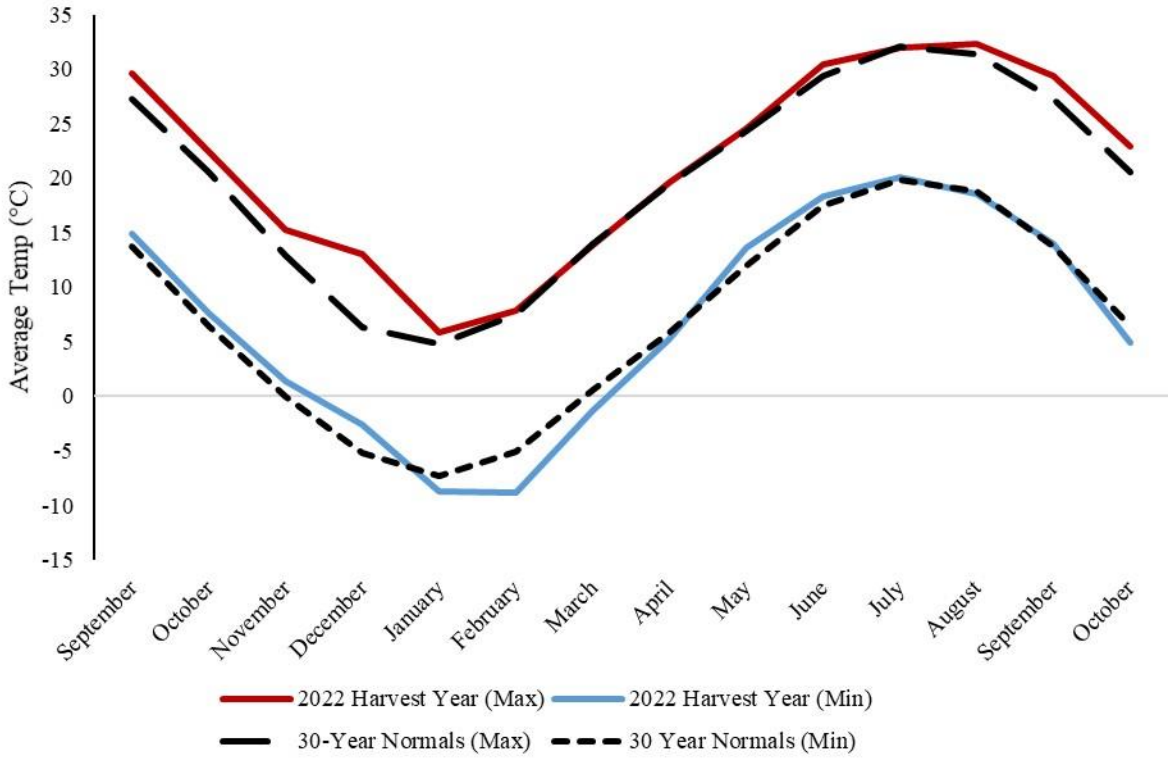

Figure 3.1 Monthly maximum and minimum daily air temperatures for 2022 harvest year as recorded by Kansas Mesonet station nearby field site in Ashland Bottoms (operated by Kansas State University). 30-years minimum and maximum daily air temperatures represent averages for Riley County, KS.

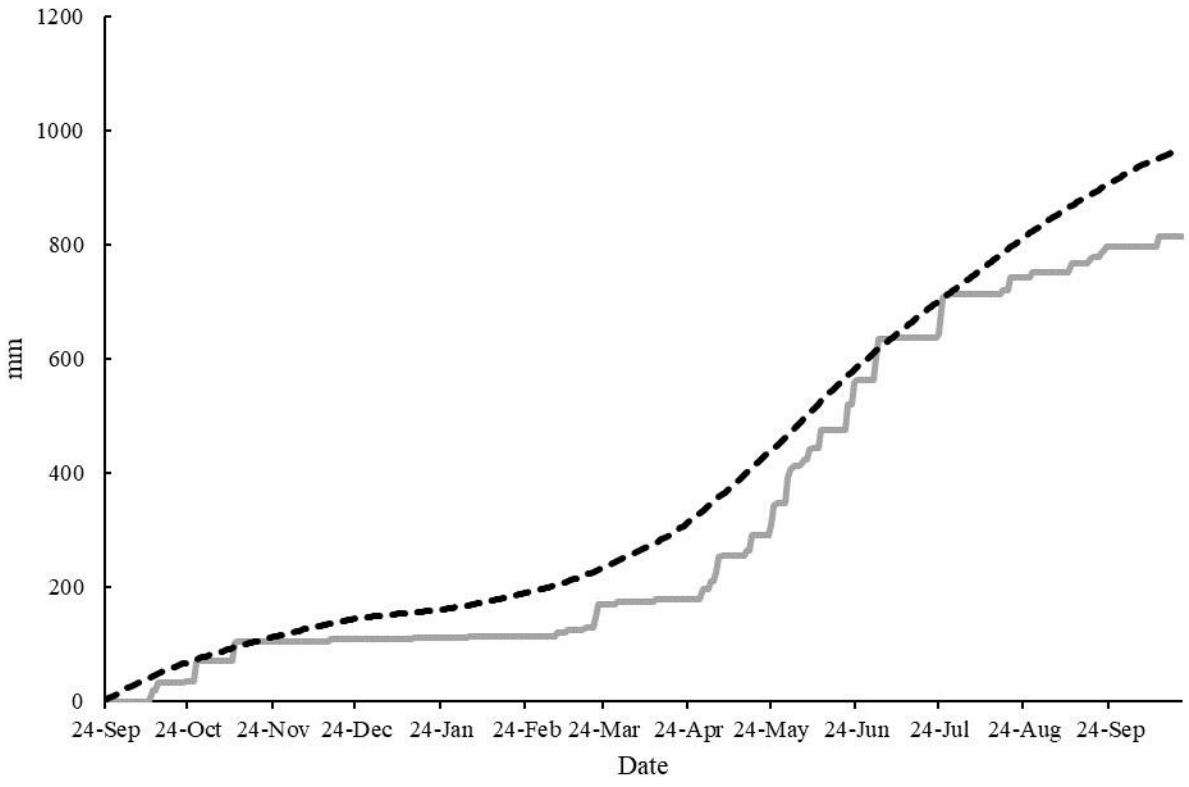

Figure 3.2 Cumulative precipitation during 2022 harvest year as reported by the Kansas Mesonet station nearby field site in Ashland Bottoms (operated by Kansas State University). 30-years minimum and maximum daily air temperatures represent averages for Riley County, KS.

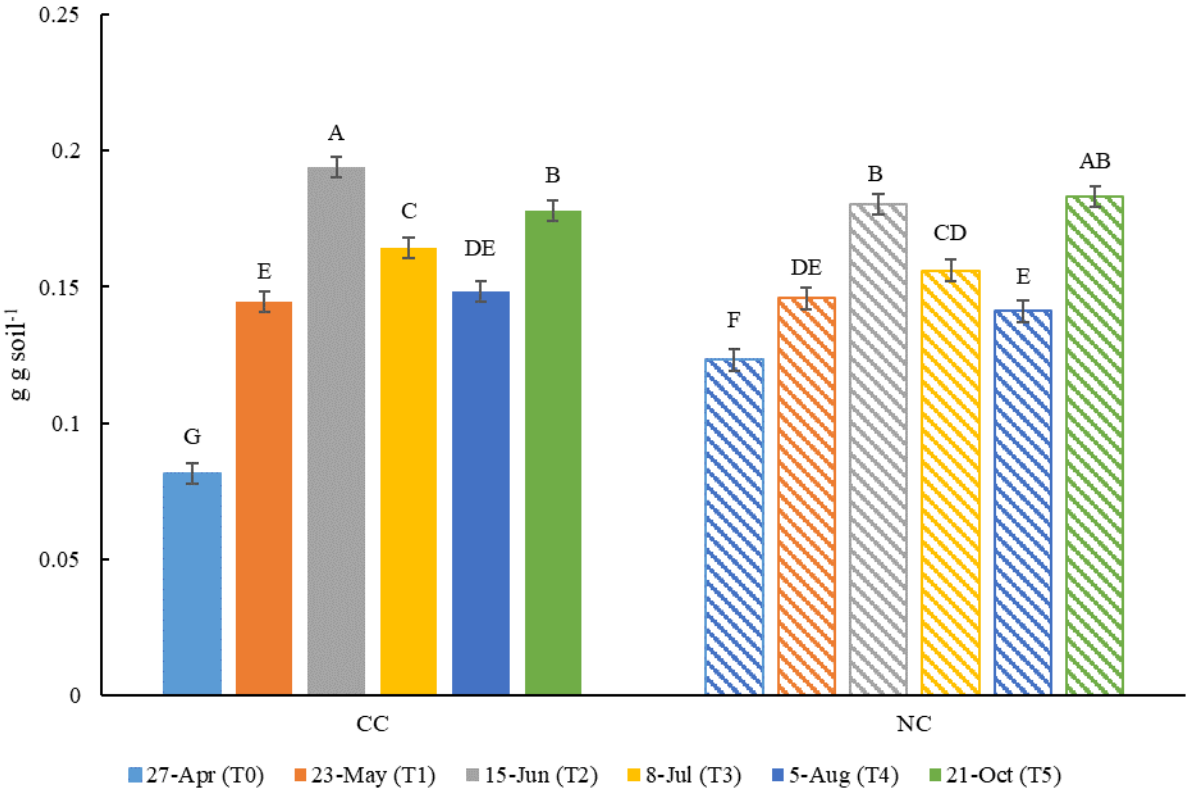

Figure 3.3 Cover crop by sampling time interactions for soil moisture in 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: presence of a cover crop (CC) and absence of a cover crop (NC).

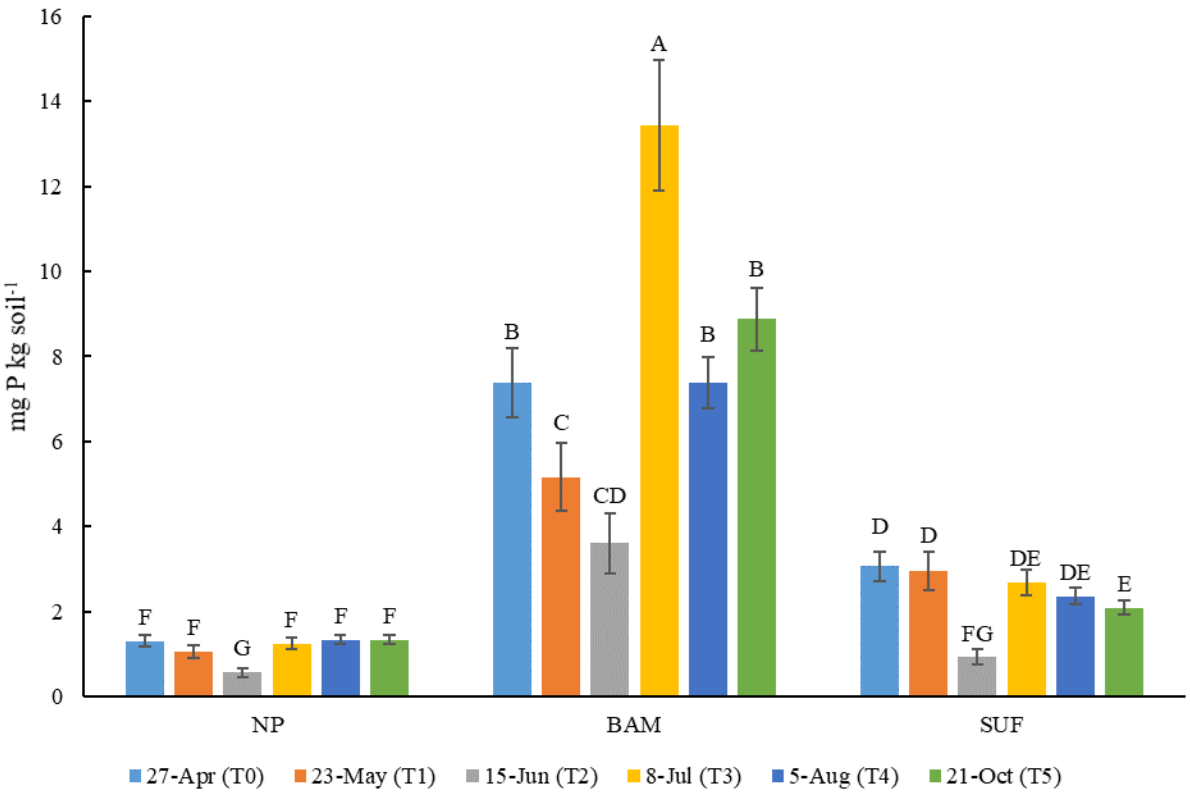

Figure 3.4 Phosphorus (P) fertilizer treatment by sampling time interactions for citrate-extractable P in 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

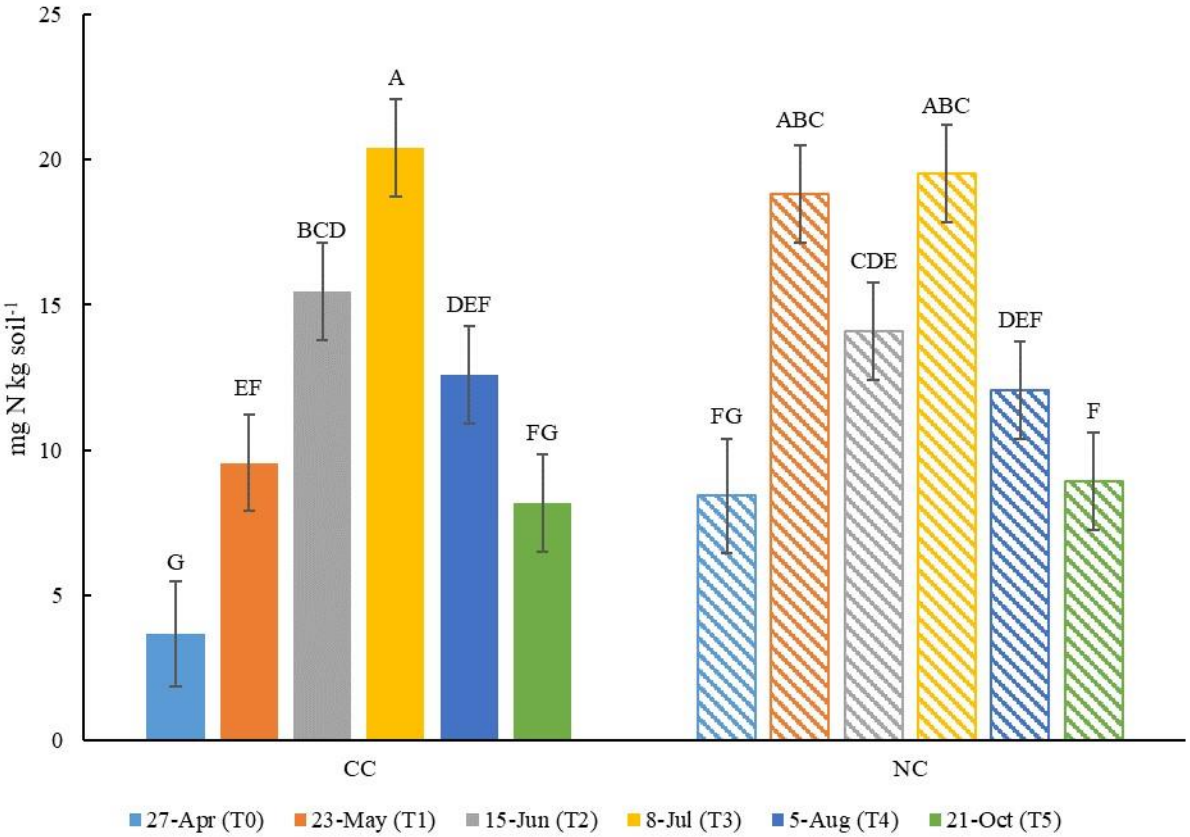

Figure 3.5 Cover crop by sampling time interaction for dissolved total N for 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: presence of a cover crop (CC) and absence of a cover crop (NC).

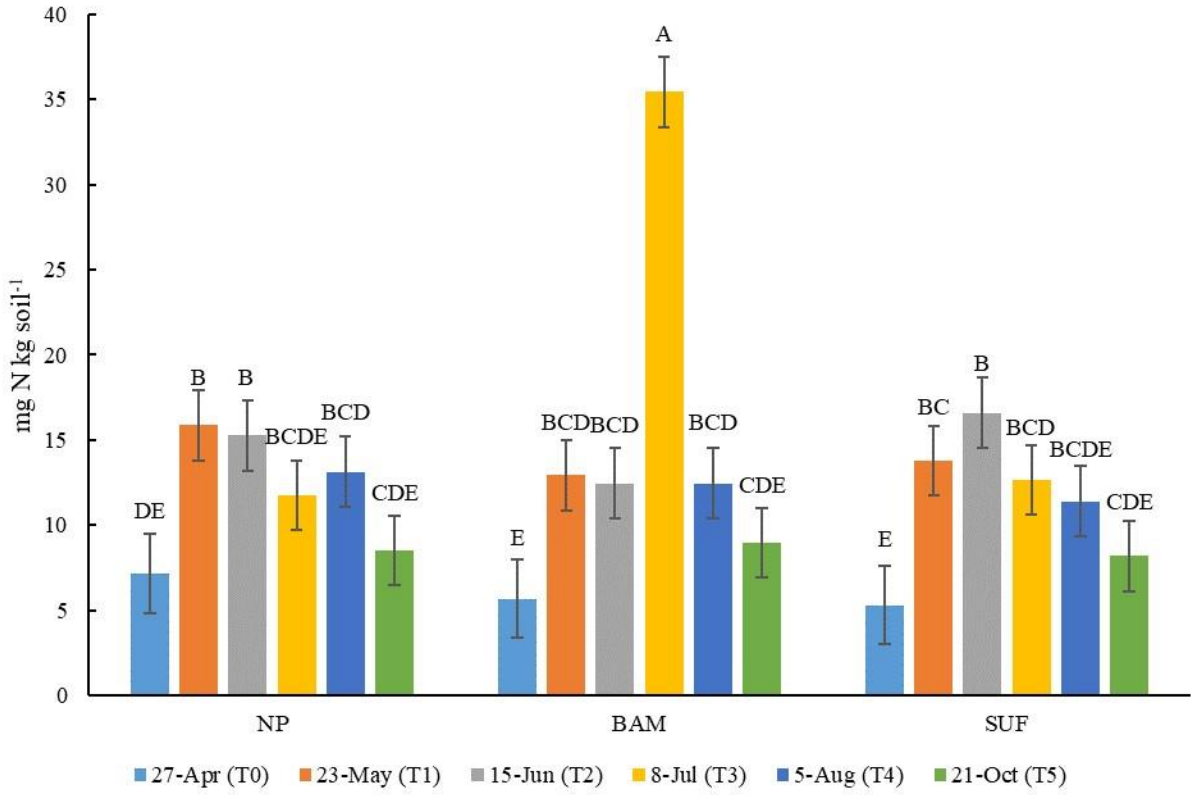

Figure 3.6 Phosphorus (P) fertilizer treatment by sampling time interaction for dissolved total N for 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

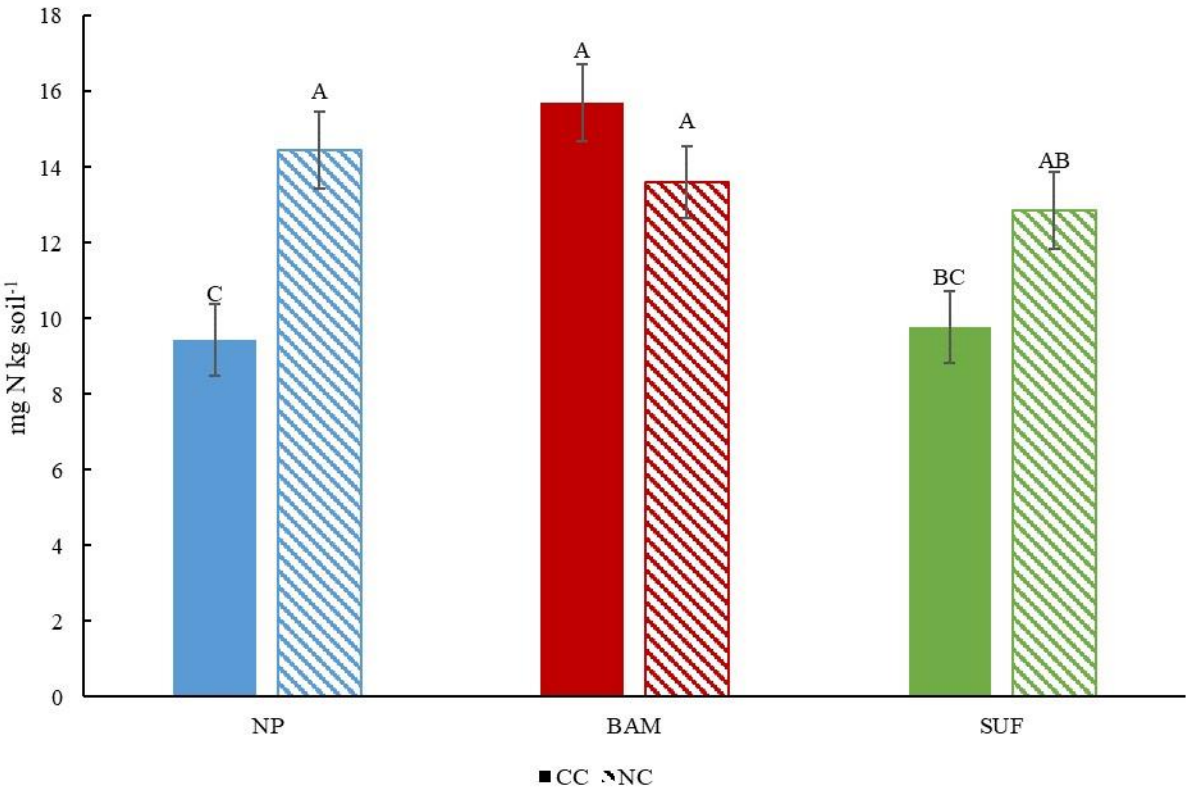

Figure 3.7 Phosphorus (P) fertilizer treatment by cover crop interaction for dissolved total N for 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments and presence of a cover crop (CC) and absence of a cover crop (NC).

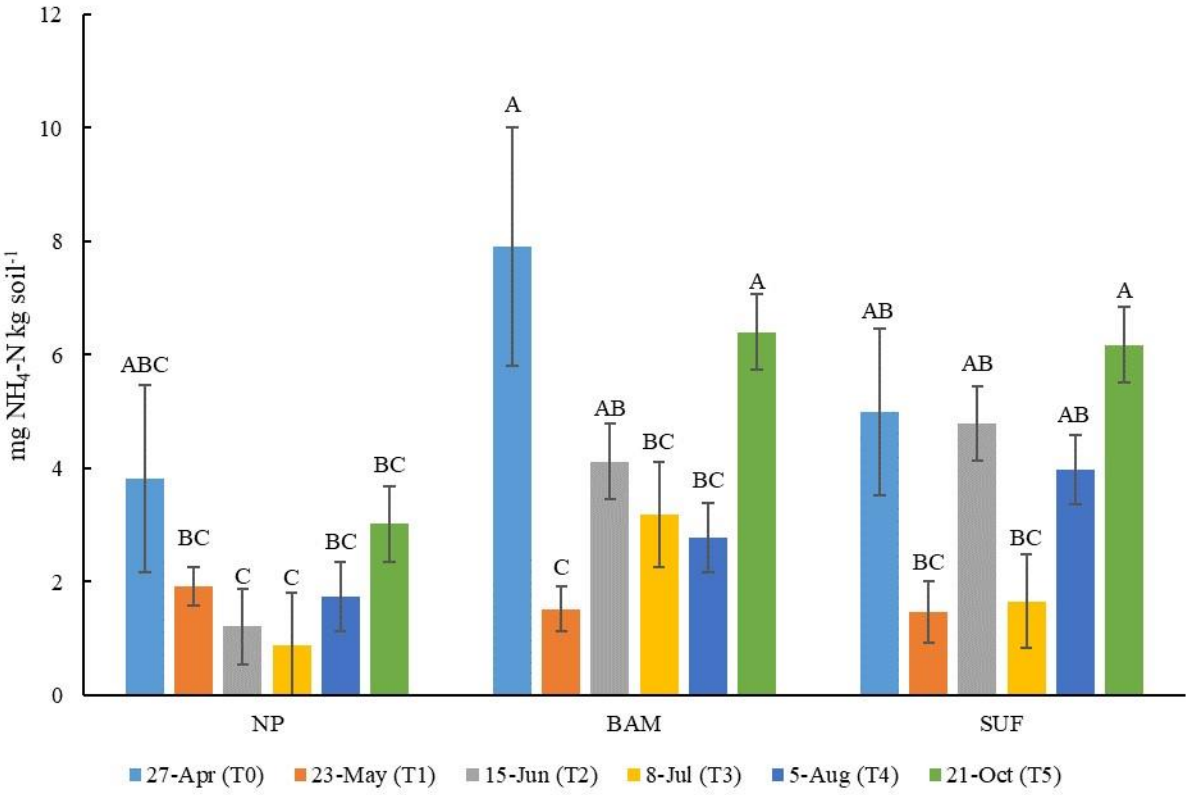

Figure 3.8 Phosphorus (P) fertilizer treatment by sampling time interaction for NH_4-N for 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

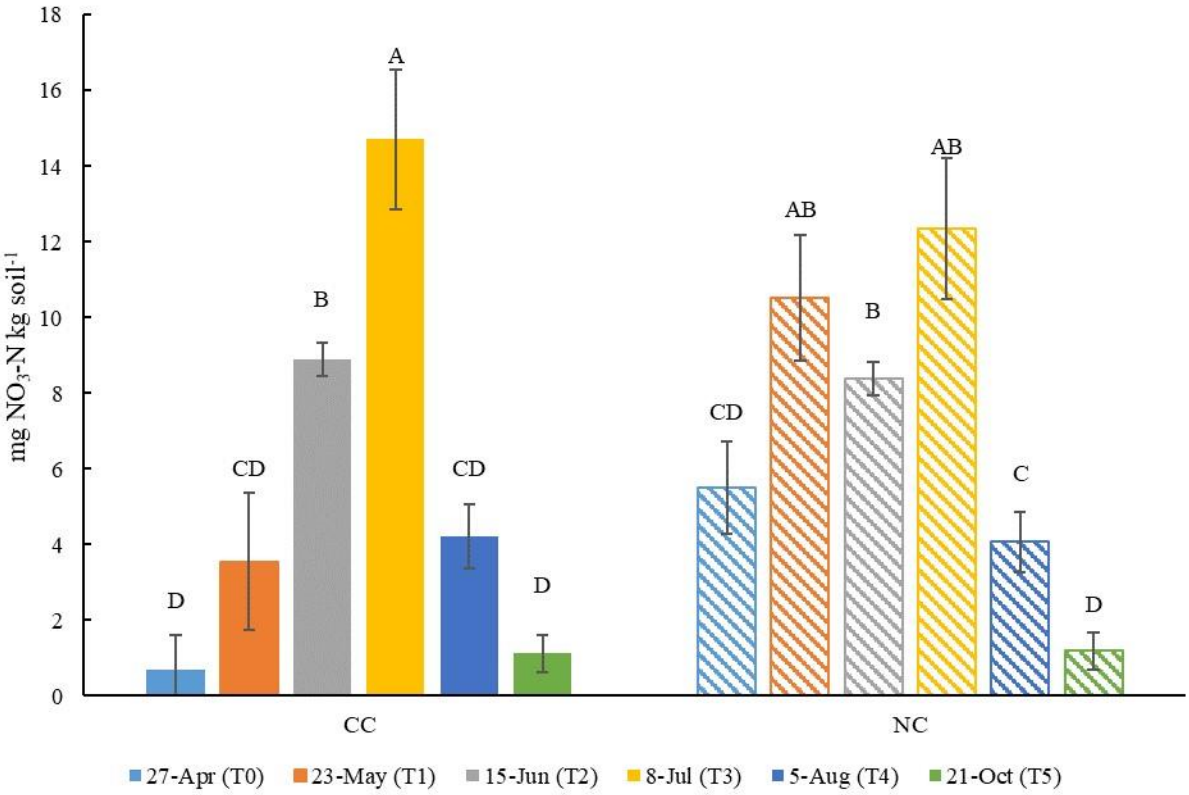

Figure 3.9 Cover crop by sampling time interaction for NO_3-N for 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: presence of a cover crop (CC) and absence of a cover crop (NC).

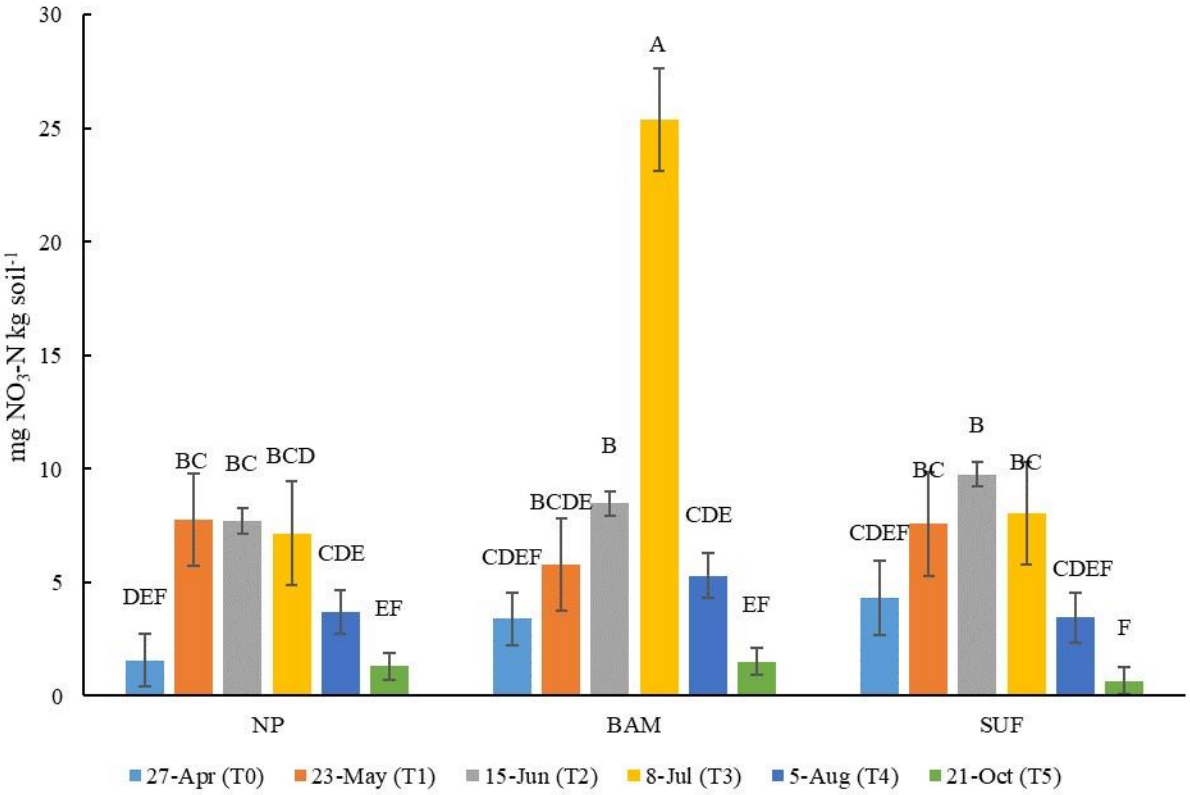

Figure 3.10 Phosphorus (P) fertilizer treatment by sampling time interaction for NO_3-N for 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

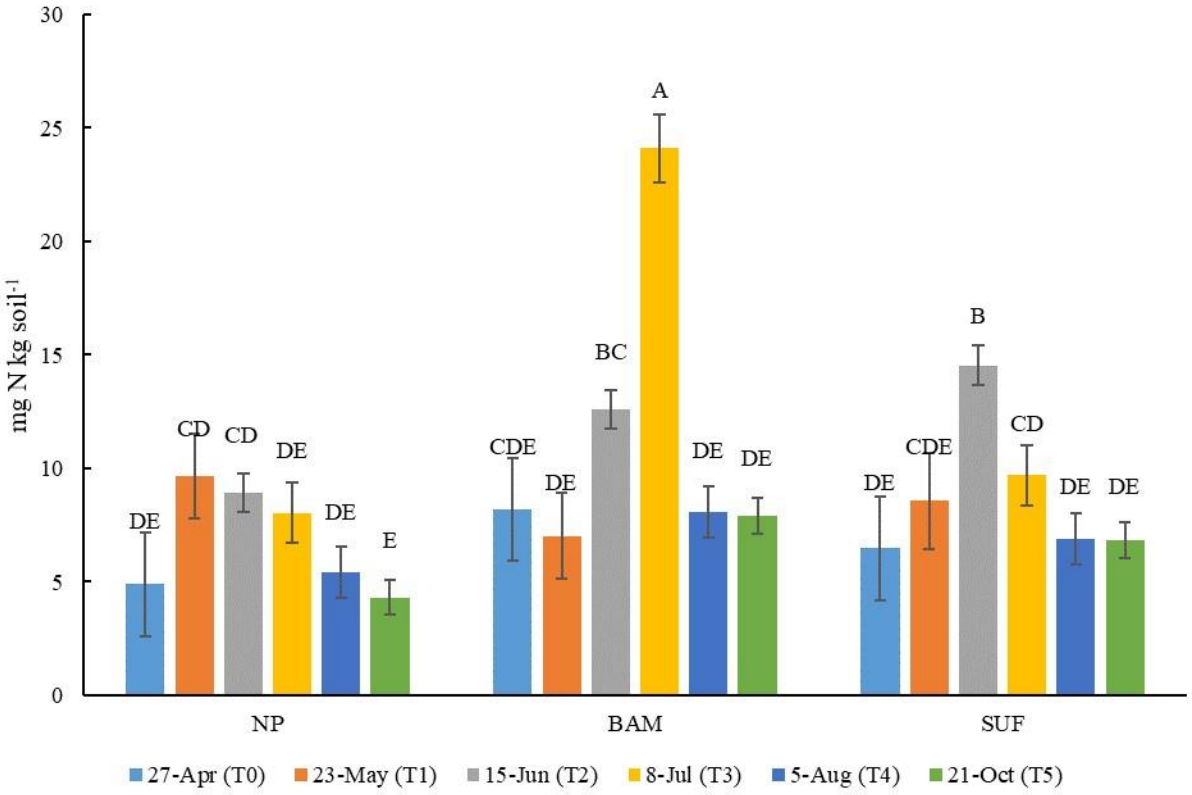

Figure 3.11 Phosphorus (P) fertilizer treatment by sampling time interaction for inorganic N for 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

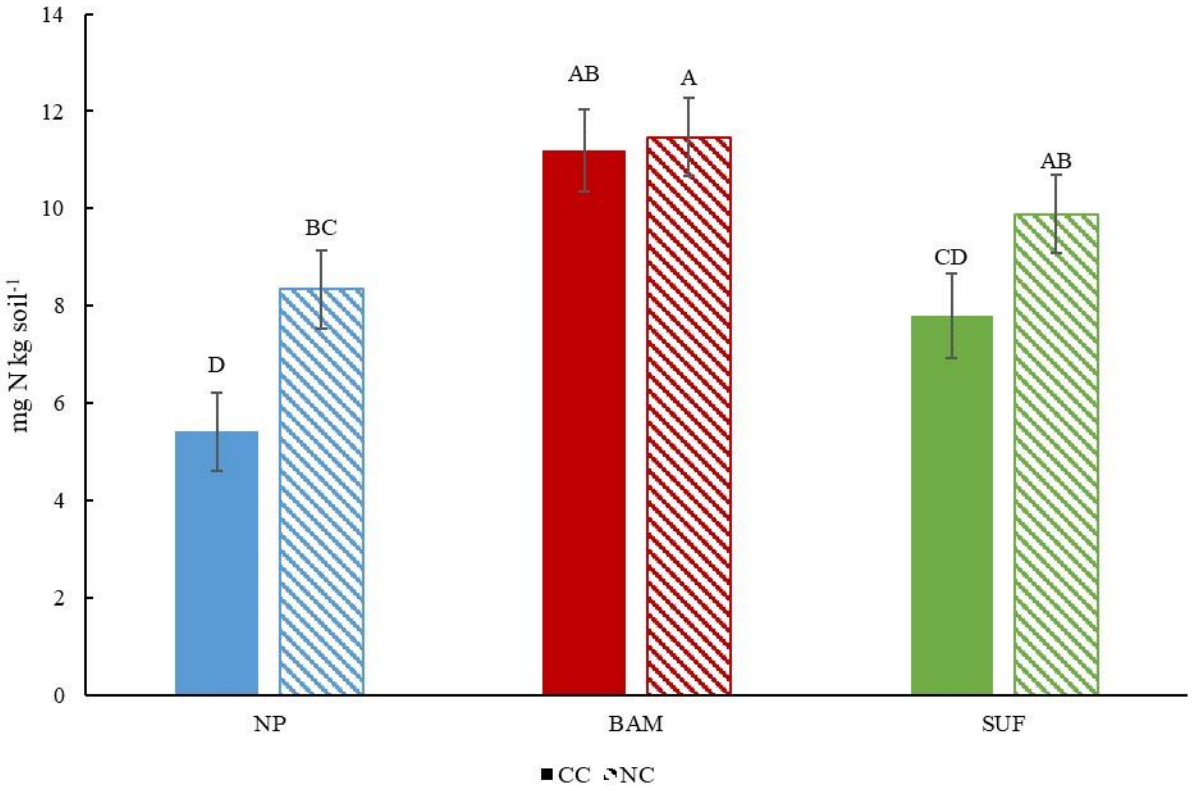

Figure 3.12 Phosphorus (P) fertilizer treatment by cover crop interaction for inorganic N for 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments and presence of a cover crop (CC) and absence of a cover crop (NC).

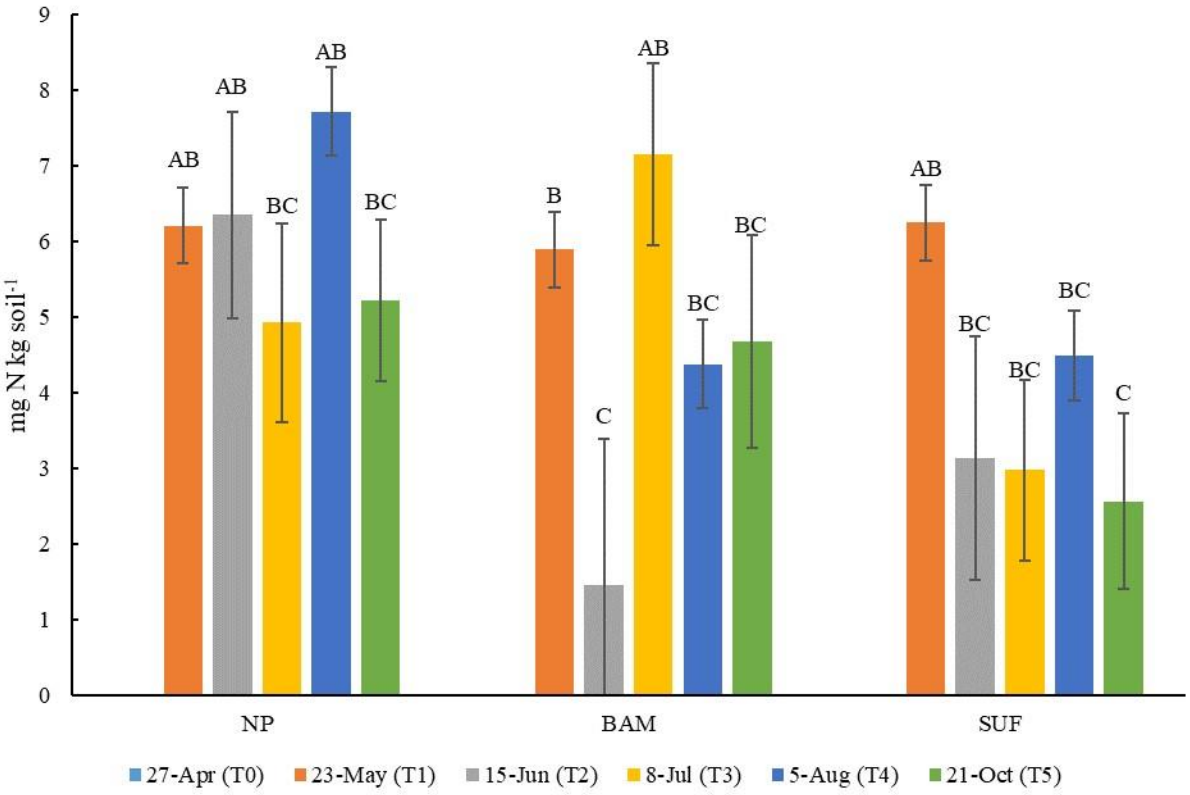

Figure 3.13 Phosphorus (P) fertilizer treatment by sampling time interaction for dissolved organic nitrogen for 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Data at 27-Apr (T0) is not reported due to methodological difficulties due to dry soil conditions. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments.

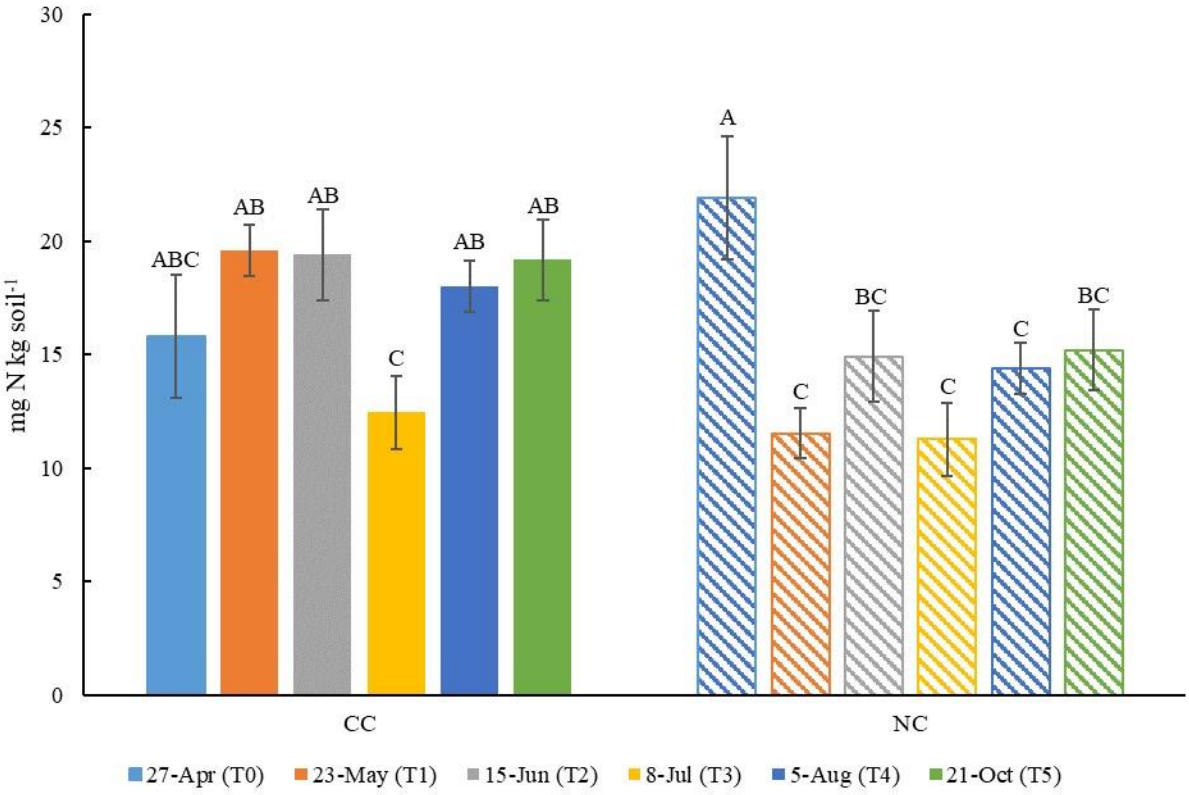

Figure 3.14 Cover crop by sampling time interaction for microbial biomass nitrogen for 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: presence of a cover crop (CC) and absence of a cover crop (NC).

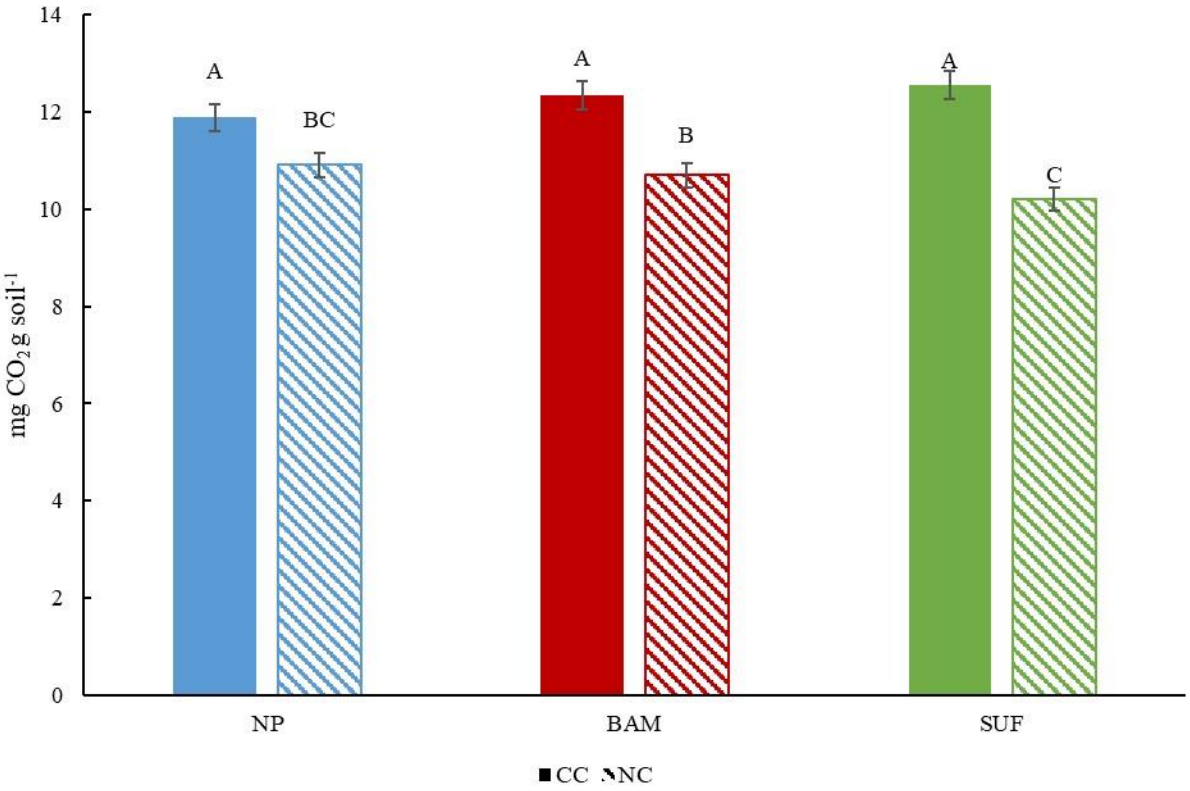

Figure 3.15 Phosphorus (P) fertilizer treatment by cover crop interaction for soil respiration for 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments and presence of a cover crop (CC) and absence of a cover crop (NC).

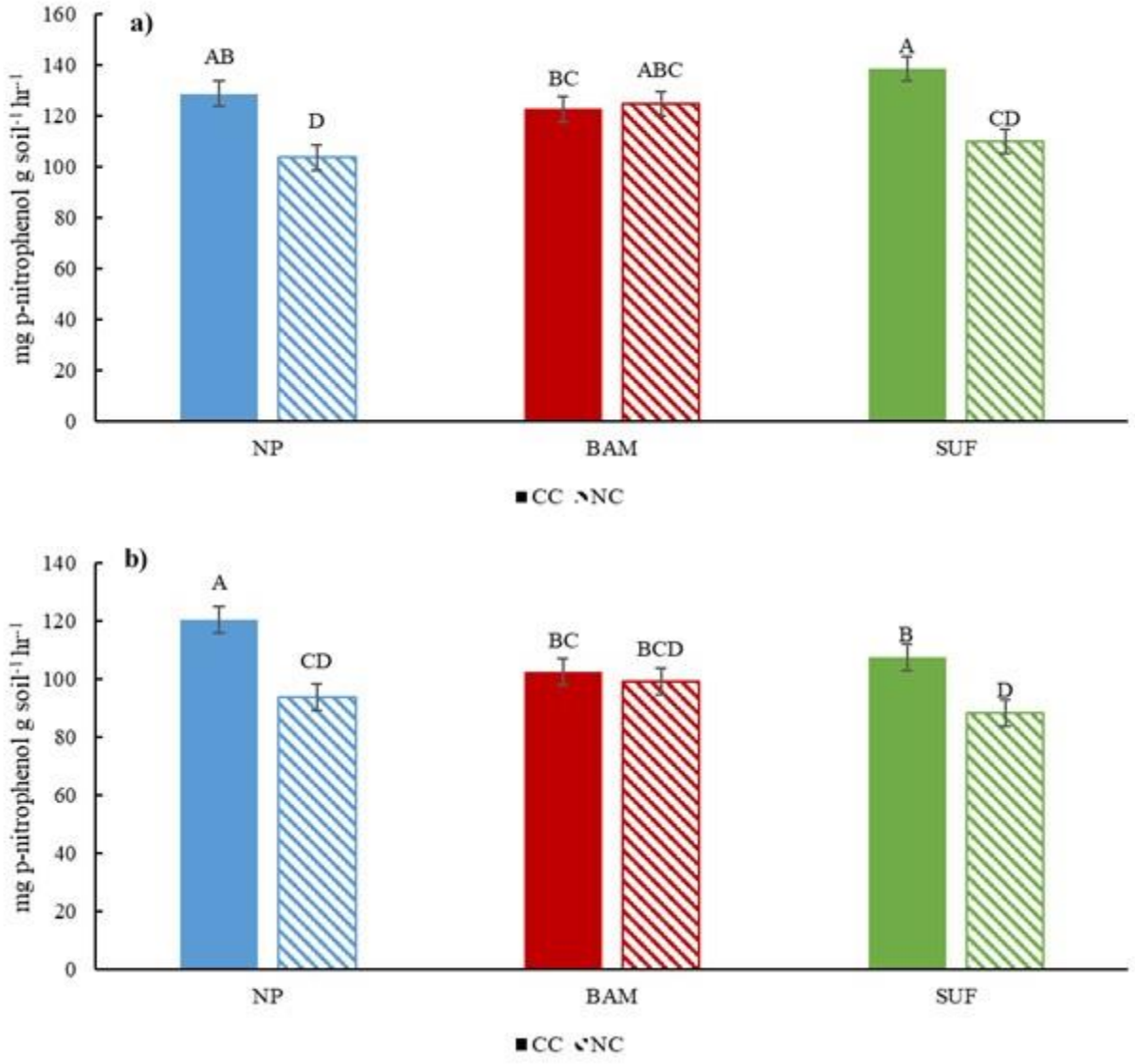

Figure 3.16 Phosphorus (P) fertilizer treatment by cover crop interaction for a) alkaline phosphatase and b) β-glucosidase activity in 2022. Letters indicate significant difference at $p < 0.05$ and error bars represent standard error of the mean. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments and presence of a cover crop (CC) and absence of a cover crop (NC).

Chapter 4 - Changes in dynamic biological soil health indicators over time in response to different phosphorus fertilizer managements and cover crops

Abstract

Conservation practices such as no-till and the implementation of cover crops can improve soil health. However, no-till practices can increase nutrient stratification, which results in less organic inputs in subsurface soils, thereby reducing microbial activity. Cover crops provide organic inputs that provide increased substrate availability to the microbial community. A study was initiated in 2020 to determine the effects of three phosphorus (P) fertilizer management approaches; (no P (NP), build and maintain (BAM), and sufficiency (SUF)), as well as the presence (CC)/absence (NC) of a cereal rye (*Secale cereale*) cover crop, on different dynamic soil health indicators in a no-till corn-soybean rotation. Ammonium polyphosphate was applied to the BAM treatment at 17, 31, and 54 kg P_2O_5 ha^{-1} in 2020, 2021, and 2022, respectively. For the three-year duration of the experiment, no P was applied to the SUF treatment, resulting in the SUF treatment undergoing a P drawn down phase. Composite soil samples were collected at 0-5, 5-10, and 10-15 cm post-harvest in 2020, 2021, and 2022. Soil health indicators measured included active carbon, autoclaved citrate extractable (ACE) protein, four soil enzyme activities (β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase), and soil respiration. Due to a different crop present each year, year was analyzed separately. Sampling depth influenced all measures of soil health in all three years. Response to cover crop presence varied annually for most indicators. Decreases in availability of P resulted in less soil protein in all years.

Additionally, significant two-way interactions were observed for some soil health indicators throughout the three-year study. In some cases, soil enzyme activity was increased due to presence of a cover crop in systems deficient in P or at the margins of P deficiency. Soil respiration was consistently higher in the cover crop treatments, but effects of P fertilizer treatments varied between years. Variation in cover crop impacts on soil health indicators was likely due to precipitation differences between years, as precipitation affected annual biomass production and possibly decomposition.

Introduction

In order to meet global food demands for a growing population, increased production will require additional inputs to meet crop nutrient demand (Chowdhury et al., 2017). Phosphorus (P) is an essential nutrient, but can be one of the most limiting nutrients for crop growth. Phosphorus is relatively immobile in soil as P is generally found in non-available forms (Hansen et al., 2002). Phosphorus can be sorbed onto mineral surface or precipitate as secondary minerals; both of which are unavailable for crop uptake. Crops utilize P from the soil solution as orthophosphates ions, however, less than 0.01% of total P required for crop growth is found in the soil solution at any specific time due to absorption and precipitation reactions (Hansen et al., 2000). The application of P fertilizer results in an increase in soil solution P during crop P demand, but will eventually become unavailable following P reactions in soil (Mardamootoo et al., 2021). However, the overapplication of P fertilizers can lead to adverse environmental effects, namely eutrophication (Sharpley et al., 1994). Soil testing is crucial to reduce the risks of overapplication of P (Mardamootoo et al., 2021). Fertilizer recommendations from soil testing laboratories are based on two approaches: build and maintain (BAM) or sufficiency (SUF). The BAM approach requires the buildup of soil test P to a critical level, then maintaining soil test P

levels with subsequent fertilizer applications that will generally equal crop removal rates (Macnack et al., 2011). The SUF approach allows for just enough P fertilizer to be added to maximize profitability and are typically developed to provide 90 to 95% of maximum yield (Leikam et al., 2003).

Soil degradation due to intense agricultural practices in efforts to maximize crop productivity have led to efforts designed to preserve soil quality and health through principals of regenerative agriculture (Laishram et al., 2012). One strategy that can improve soil health is the adoption of no-till practices (Lehman et al., 2015). No-till practices result in increased residue retention on soil surface that will increase organic inputs in the soil (Malobane et al., 2020), thus increasing the amount of substrate that is available to the microbial community (Balota et al., 2004). The accumulation of crop residues at the soil surface in no-till systems can result in isolation of crop residues in the surface and leads to nutrient stratification (Franzluebbers 2002). Stratification of dynamic soil health indicators have been reported in no-till systems (Green et al., 2007). Another strategy to improve soil health is the adoption of cover crops in replacement of a fallow period (Brennen and Acosta-Martinez, 2019). Cover crops provide increased above- and belowground inputs that provide nutrients and energy to the soil microbial community (Blanco-Canqui et al., 2015). Positive impacts of cover crops on dynamic soil health indicators have been reported (Nunes et al., 2018; Brennan and Acosta-Martinez, 2019). However, potential benefits from cover crops can be dependent on cover crop species, precipitation during cover crop growth, planting and termination dates, soil texture, initial soil carbon amounts, and the number of years since cover crop establishment can all influence the accumulation of soil carbon (C) (Blanco-Canqui 2022).

Therefore, the objectives of this study were to 1) assess P fertilizer treatment and cover crop impacts on dynamic soil health indicators and 2) assess effect of sampling depth on dynamic soil health indicators. It was hypothesized that 1) cover crops will significantly influence all dynamic soil health indicators each year, 2) observed increased nutrient cycling when a system is at the margins of deficiency, and 3) sampling depth will significantly influence soil health indicators due to nutrient stratification.

Materials and Methods

Experimental Site, Design, and Agricultural Management:

The experimental site was located at the Kansas Agricultural Watershed (KAW) Field Laboratory near Manhattan, Kansas. The site consists of primarily erode Smolan silty clay loam (fine, smectitic, mesic Pachic Argiustoll) with a 6-8% slope, with soil pH ranging from 6 to 7. The site has a hot, humid continental climate, with a mean annual temperature of 12.9°C and mean annual precipitation of 889 mm. There are 18 terraced watersheds that are approximately 0.5 ha each. The site has been in a continuous no-till, corn-soybean rotation since 2014, where a previous five-year study evaluating P fertilizer source and timing and the influence of a cover crop on crop response and surface water quality was conducted from 2014 to 2019 (Carver et al., 2022).

The experiment is a 2x3 complete factorial, arranged in a randomized complete block design. The blocks were assigned based on landscape position and all treatments are replicated three times (n = 18). There were three levels of P fertilizer management; no P control (NP), BAM, and SUF, and two levels of cover crop management; presence (CC) or absence (NC) of a cereal rye (*Secale cereale*) cover crop.

Cereal rye was planted following corn harvest on 27 September 2019, following soybean harvest on 13 October 2020, and following corn harvest on 24-25 September 2021. Rye was chemically terminated with glyphosate and Liberty on 19 May 2020, with glyphosate and Diacamba on 13 April 2021, and with glyphosate and 2,4-D on 20 May 2022. Soybeans were planted on 18-19 May 2020 and harvested on 7 October 2020. Corn was planted on 26-29 April 2021 and harvested on 16-17 September 2021. Soybeans were planted on 15 June 2022 and harvested on 20 October 2022. Cumulative precipitation for all harvest years is shown in Fig. 4.1. Cumulative precipitation was 988, 734, and 816 mm for harvest years 2020, 2021, and 2022, respectively.

Ammonium polyphosphate (10-34-0) was applied to BAM plots at an application rate of 39 kg P_2O_5 ha^{-1} on 18 May 2020, a rate of 71 kg P_2O_5 ha^{-1} on 29 April 2021, and a rate of 54 kg P_2O_5 ha^{-1} on 15 June 2022. P fertilizer has not been applied to SUF plots since December 2018, while NP plots have not received P fertilizer since 2014. In 2021, nitrogen (N) fertilizer applications were balanced between all treatments with 28% urea-ammonium nitrate to achieve a total N application rate of 145.7 kg N ha^{-1} on 22 April 2021. The ammonium polyphosphate used in 2022 was contaminated with urea ammonium nitrate (~6%), which added approx. 3 kg N ha^{-1} in addition to the 16 kg N ha^{-1} from the ammonium polyphosphate to the BAM treatment. No other N amendments were applied for the 2020 and 2022 harvest year.

Soil Sampling:

Composite soil samples were collected at 0-5, 5-10, and 10-15 cm depths following harvest of the main crop on 16 October 2020, 17 September 2021, and 21 October 2022. 20 cores were taken from each plot and then air-dried. Air-dried soil samples were dried at room temperature then ground and sieved to pass a 2 mm sieve. Air-dried soils were analyzed for

active C, autoclaved citrate extractable (ACE) protein, soil respiration, β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase.

Methodology:

Active C was determined using the permanganate-oxidizable C method described by Weil et al. (2003). Briefly, 2.5 g of air-dried soil was weighed into 50-mL Falcon tubes; 18 mL of deionized water was added and 2 mL of 0.2 M $KMnO_4$ was added to the Falcon tube. Samples were then shaken for 2 min, and left to settle for 8 min before 0.2 mL of each sample was added to 20 mL deionized water to stop the reaction. Samples are analyzed colormetrically on a spectrophotometer at 550 nm. Soil protein (a measure of bioavailable N) was determined using the autoclaved citrate extractable (ACE) protein method (Wright and Upadhyaya, 1996, 1998; Hurisso et al., 2018). Briefly, 3 g of soil was weighed out into 50-mL centrifuge tubes and extracted with 20 mM sodium citrate pH 7. Samples were then autoclaved at 121°C and 15 psi for 30 min. The samples were then allowed to cool, shaken for one min and a 1.75 mL aliquot from each sample was transferred added to a clean 2-mL microcentrifuge tube. The aliquot was centrifuged at 10000 x g for 3 mins and then 1 mL of the cleared extract was transferred to a 1.5-ml microcentrifuge tube. For quantification, a dry heat block (VWR heat block, 97043-610, USA) was heated to 61.5˚C and Pierce bicinchoninic acid (BCA) working solution was prepared. Using a multichannel pipettor, 10 µL of standard were added to reaction plate, followed by two replicate columns of each strip of eight sample tubes into plate wells. 200 µL of working reaction was added into each well of the reaction plate, which is then sealed with tape seal and placed on heat block for one hour. Tape was then removed, and the plate was allowed to cool for 10 min. The plate was then read with a plate reader (BioTek Synergy H1, USA) at 562 nm.

Soil respiration was determined using the alkali trap method described by Haney and Haney (2010). Briefly, 20 g of air-dried soil was weighed into an aluminum weigh boat (diameter 51 mm) that was perforated nine times (three by three array) and place onto two filter papers (qualitative 413-VWR North America). The alkali trap was then supported above the soil surface on a plastic pizza stool and filled with 9 mL of 0.5 M KOH and 7.5 mL of deionized water was added to the inside edge of the jar to wet the filter papers and soil. Jars were then incubated for 4 days at room temperature. Electrical conductivity of the KOH solution change in proportion to the amount of CO_2 trapped; therefore, the amount of CO_2 trapped is calculated from change in conductivity and used to estimate the amount of CO_2 evolved from the samples. All analyzes were performed in duplicate.

Activities of β-glucosidase (Eivazi and Tabatabai, 1988), N-acetyl-β-glucosaminidase (Parham and Deng, 2000), and acid and alkaline phosphatases (Eivazi and Tabatabai, 1977) were determined colormetrically at 400 nm. Briefly, three subsamples of 0.5 g of air-dried soil was weighed out into 20-mL vials. Each sample had two replicates (A and B) and one control (C). 2 mL of start buffer was added to all A, B, and C samples. Start buffers included modified universal buffer adjusted for pH 6, 6.5, and 11 for glucosidase, acid and alkaline phosphatase, respectively. For glucosaminidase, 0.1 M acetate buffer was used. 0.5 mL of substrate was then added at A and B only. Substrates were 0.05 M p-nitrophenyl-β-D-glucopyranoside, 0.01 M p-nitrophenyl-N-acetyl-β-D-glucosaminide, and 0.05 M p-nitrophenol phosphate for glucosidase, glucosaminidase, and phosphatases, respectively. Vials were capped and shaken gently by hand before being incubated at 37˚C for 1 hr. To stop the reaction, 0.5 mL of 0.5 M CaCl2 was added to each vial, followed by 2 mL of stop buffer. Substrate was then added to the control only.

Statistical Analysis:

The effect of P fertilizer treatment, cover crops, sampling time, and all interactions on biological soil health indicators was conducted using SAS version 9.4 (Cary, NC, U.S.A). Fixed effects of the model were P fertilizer treatment, cover crop, and sampling time while the blocking factor was considered a random effect. Data was analyzed using PROC GLIMMIX with option DDFM = SATTERTH. Normality of all data was tested using PROC UNIVARIANTE. Logarithm transformations were used for data that was not normally distributed. Back transformations of mean and standard errors were used to create all figures and table. Years were analyzed separately and not compared statistically. Significant differences were detected using $p < 0.05$.

Results

There was a significant main effect of sampling depth in all three years for active C concentrations (Table 4.1). In all years, active C concentrations were significantly higher at 0-5 cm depth compared to 5-10 and 10-15 cm (Table 4.2). Concentrations at 5-10 cm were significantly higher than concentrations at 10-15 cm in all three years (Table 4.2). The presence of a cover crop significant influenced active C concentrations in fall 2020 only (Table 4.1). Cover crops significantly increased active C concentrations by 14% (Table 4.2).

There was a main effect of P fertilizer treatment in all three years for ACE protein concentrations (Table 4.1). For all three years, ACE protein concentrations were significantly lower in the NP treatment compared to the BAM and SUF treatments; with no significant difference between concentrations in BAM and SUF treatments (Table 4.2). Cover crops significantly influenced ACE protein concentrations in fall 2021 and 2022 (Table 4.2). Protein concentrations were 8 and 7% higher in covered treatments in fall 2021 and 2022, respectively.

Sampling depth significantly influenced ACE protein concentrations in all three years (Table 4.1). In all three years, protein concentrations were significantly higher at 0-5 cm compared to 5-10 and 10-15 cm, while concentrations were significantly higher at 5-10 cm compared to 10-15 cm in all three years (Table 4.2).

There was a significant P fertilizer by cover crop interaction in fall 2021 and fall 2022 for soil respiration (Table 4.1). In both the NP and BAM treatments, no cover crop treatments resulted in significantly less soil respiration in fall 2021 compared to the SUF treatment (Fig. 4.2a). Soil respiration was significantly higher in BAMxCC treatments compared to all other P fertilizer and cover crop treatments. In fall 2022, no cover crop treatment resulted in less soil respiration in both BAM and SUF treatments (Fig. 4.2b). Both cover crop and sampling depth significantly influenced respiration in all three years (Table 4.1). The presence of a cover crop s increased soil respiration by 9% in fall 2020 and 11% in fall 2021 and 2022 (Table 4.2). In all three years, respiration was significantly higher at 0-5 cm compared to 5-10 and 10-15 cm (Table 4.2). Respiration was significantly higher at 5-10 cm compared to 10-15 cm.

There was a significant P fertilizer treatment by cover crop interaction in fall 2020 for glucosidase activity (Table 4.3). The presence of a cover crop significantly increased glucosidase activity by 34% in the NP treatment, and by 32% in the SUF treatment (Fig. 4.3). There was no significant increase in the BAM treatment due to the presence of a cover crop. Both cover crop and sampling depth significantly influenced glucosidase activity (Table 4.3). The presence of a cover crop significantly increased activity by 17, 14, and 20% in fall 2020, 2021, and 2022, respectively (Table 4.4). Glucosidase activity was significantly higher at 0-5 cm compared to 5-10 and 10-15 cm in all three years (Table 4.4). Activity was significantly higher at 5-10 cm compared to 10-15 cm in all three years.

There was a significant cover crop by sampling depth interaction for glucosaminidase activity in fall 2022 (Table 4.3). Activity was significantly higher in the cover crop treatment at 0-5 cm compared to all other sampling depths and cover crop treatments (Fig. 4.4). Activity in the no cover crop treatment at 0-5 cm was significantly higher compared to the cover and no cover crop treatment at 5-10 and 10-15 cm. Activity in the cover crop treatment at 5-10 cm was significantly higher compared to no cover crop treatment at 5-10 cm and cover and no cover crop treatments at 10-15 cm. There was no significant difference between the cover crop treatments at 10-15 cm. Cover crops significantly increased glucosaminidase activity in all three years (Table 4.3). Activity significantly increased due to the presence of a cover crop by 28% in all three years (Table 4.4). There was a significant main effect of sampling depth in all three years (Table 4.3). For all three years, activity was significantly higher at the 0-5 cm depth compared to 5-10 and 10-15 cm (Table 4.4). Activity was significantly higher at 5-10 cm compared to 10-15 cm in all three years (Table 4.4). P fertilizer treatment did not significantly influence activity in any year.

In fall 2020, there was a significant P fertilizer treatment by cover crop interaction for acid phosphatase activity (Table 4.5). With the presence of a cover crop, acid phosphatase activity significantly increased by 38% (Fig. 4.5). The presence of a cover crop did not significantly increase acid phosphatase activity in either the BAM or SUF treatments. Both cover crop and sampling depth significantly influenced acid phosphatase activity in all three years (Table 4.5). The presence of a cover crop significantly increased acid phosphatase activity by 17, 20, and 15% in fall 2020, 2021, and 2022, respectively (Table 4.6). Acid phosphatase activity in fall 2020 and fall 2021 was significantly higher at 0-5 cm compared to 5-10 and 10-15 cm. Activity at 5-10 cm was significantly higher compared to 10-15 cm for fall 2020 and 2021. In

fall 2022, activity was significantly higher at 0-5 cm compared to 5-10 and 10-15 cm, however, there was no significant difference between activities at 5-10 and 10-15 cm.

There was a significant P fertilizer treatment by cover crop interaction in fall 2020 and 2021 for alkaline phosphatase activity (Fig. 4.6 a&b). Alkaline phosphatase activity was significantly higher in NPxCC treatments compared to all other treatments. There was no significant difference between NPxNC, BAMxCC, BAMxNC, SUFxCC, and SUFxNC in fall 2020 (Fig. 4.6a). In fall 2021, the presence of a cover crop significantly increased phosphatase activity in the NP treatment by 39% (Fig. 4.6b). There was no significant difference due to the presence of a cover crop in either the BAM and SUF treatment. Activity was similar in BAM (regardless of cover crop treatment) and SUF (regardless of cover crop treatment). There was a significant main effect of cover crop in fall 2022 (Table 4.5), as the presence of a cover crop increased alkaline phosphatase activity by 19% (Table 4.6). Sampling depth significantly influenced alkaline phosphatase activity in all three years (Table 4.5). In both fall 2020 and fall 2022, activity was significantly higher at 0-5 cm compared to 5-10 and 10-15 cm, with no significant difference between 5-10 and 10-15 cm. In fall 2021, activity was significantly higher at 0-5 cm compared to 5-10 and 10-15 cm. Activity was significantly higher at 5-10 cm compared to 10-15 cm (Table 4.6).

Discussion

Effect of P fertilizer treatment and cover crops on active C, ACE protein, and soil respiration

Active C concentrations were significantly increased by the presence of a cover crop in fall 2020 only. The general lack of cover crop effect on active C concentrations during the fall sampling is consistent with previous research at this study site (Stewart, 2020). Cover crops

provide additional above- and belowground inputs that can increase labile C concentrations and stimulate microbial activity (Zhou et al., 2012). The lack of cover crop effect on active C concentrations in fall 2021 and 2022 is likely due to the amount of cover crop biomass produced relative to fall 2020 (Table 4.1). Cover crop biomass was considerably greater for the 2020 harvest year compared to 2021 and 2022 (3605 kg ha^{-1} vs 462 and 1408 kg ha^{-1}, averaged over P fertilizer treatments for fall 2020, 2021 and 2022, respectively) (Nelson-Personal Communication). Biomass production was regulated by the amount of precipitation that was received and the temperature during the cover crop growth periods. Average maximum and minimum daily air temperatures were within normal range of the 30-year normal average for Riley county for the duration of the study (Weather Data Library, Kansas Mesonet). Precipitation during cover crop growth was 25% less for the 2021 harvest year in comparison to harvest year 2020 (256 mm vs 340 mm), and 14% less for the 2022 harvest year in comparison to harvest year 2020 (293 mm compared to 340 mm). Importance of precipitation to cover crop growth was reported by Duval et al. (2016), who found significant relationships between precipitation during cover crop growth and C-inputs to soil in a long-term cover crop experiment. Likewise, differences in biomass production due to precipitation in relation to active C was also reported by Steele et al. (2012), who found inconsistent results of cover crop impacts on labile C concentrations in a two-year study on Coastal Plain and Piedmont soils in Maryland that were under long-term cover crop management (>12 years). The authors contributed drought-like conditions as the reason for low biomass production that decreased the amount of labile C returned to the soil in year two (Steele et al., 2012).

The presence of a cover crop significantly increased ACE protein concentrations in fall 2021 and 2022 only (Table 4.1). Inconsistencies in ACE protein response to cover cropping have

been reported, as studies have found both positive effects (Balota et al., 2014; Nunes et al., 2018; Chahal and Van Eerd, 2019; Marshall et al. 2021; Feng et al., 2021) and neutral (Pokhrel et al., 2021; Wood and Bowman, 2021) effects. It is possible that the differences in cumulative precipitation during the period of cover crop decomposition influenced ACE protein concentrations. In comparison to the 2020 harvest year, there was 169.96 and 124.46 mm less precipitation during the cover crop decomposition period in 2021 and 2022, respectively (Fig. 1). Cover crop decomposition rates can be influenced by precipitation amounts, temperature, number of rainfall days, and relative humidity (Thapa et al., 2022). Before N from cover crop residues can be released into the soil, it must be mineralized. Mineralization of N from cover crop residues is dependent of biochemical composition and environmental conditions (O'Connell et al., 2015). It is possible that the reduced precipitation during cover crop decomposition in 2021 and 2022 resulted in decreased N mineralization compared to 2020 in the present study. Therefore, it is possible that residual cover crop N remained mainly in an organic form that was highlighted in the ACE protein results.

For all three years, the presence of a cover crop significantly increased soil respiration (Table 4.1). This is contradictory of previous research at the study site that observed no cover crop effect on soil respiration during fall samplings (Stewart, 2020). The increase in soil respiration in fall 2020, 2021, and 2022 relative to previous work from 2018 and 2019 might reflect the contribution of cover crops to total C (Carver 2022). Greater C mineralization rates can indicate more bioavailable C and a more active soil microbial community (Fierer et al., 2021). Significant increases in respiration from cover crops is likely due to the decomposition of both above- and belowground inputs as well as the turnover of the biomass by the soil microbial community that the microbial community can utilize, resulting in increased carbon dioxide

production (Nilahyane et al., 2020). Kim et al. (2020) reported that increased CO_2 respiration is likely associated with increased cellulose decomposition in cover crops compared to no soil cover between cash crops. Discussed below, glucosidase hydrolyzes oligosaccharides and releases monosaccharides and catalyzes the final step in the breakdown of cellulose (Luo et al., 2017). Increases in glucosidase activity due to cover cropping support the assumption that respiration increases with increased cellulose decomposition in cover crops (Kim et a., 2020).

Significantly less ACE protein concentrations were found in the NP treatment compared to the BAM and SUF treatments in all three years (Table 4.1). The positive response to soil protein with increasing P availability suggests that arbuscular mycorrhizal fungi (AMF) might not solely contribute to soil protein production as AMF abundance decreases with increasing P availability (Hurisso et al., 2018; Law and Maherali, 2023). The effect of P availability on soil protein concentrations is inconsistent in literature, however, positive correlations between soil protein (measured as glomalin related soil protein) and P availability have been reported in previous studies (Wu et al., 2010; Guo et al., 2012; Qiu et al., 2021; Wang et al., 2022).

For soil respiration, differences between NP and SUF treatments due to the presence or absence of cover crops in 2021 and 2022 are difficult to interpret (Fig. 4.2 a&b). In both years, the presence of a cover crop significantly increased soil respiration. This finding agrees with a meta-analysis conducted by Feng and Zhu (2019), who found that P additions to cropland increased soil respiration due to the stimulation of microbial biomass and enhanced C cycling. The response of the BAM treatment to the presence of a cover crop might reflect the in importance of P availability to increased primary productivity, as P limitation has been reported to reduce net primary productivity (Wang et al., 2010). Biomass production in the SUF treatment

for the 2021 harvest year was similar to the BAM treatment, however, only the BAM treatment resulted in significantly higher soil respiration when a cover crop was present in both years.

Effect of P fertilizer treatment and cover crops on soil enzyme activities:

There was a significant P fertilizer by cover crop interaction observed for glucosidase activity in fall 2020 (Table 4.3). The presence of a cover crop on both the NP and SUF treatments resulted in significantly greater enzyme activity in comparison to the no cover crop treatment (Fig. 4.3). Cover crop biomass production would have resulted in increased C inputs into the soil. As glucosidase activity is involved in cellulose degradation, it is possible that increased biomass production resulted in greater amounts of cellulose, prompting the induction of glucosidase production (Nevins et al., 2020). It is also possible that greater availability of P in the BAM treatment depressed the production of glucosidase in cover crop treatment, and the microbial demand was met without the need of additional enzyme activity. Greater cover crop biomass production in 2020 could have resulted in greater enzyme activity in the NP and SUF treatment, while reduced biomass production in 2021 and 2022 did not attribute to greater enzyme activity in the NP and SUF treatments.

The presence of a cover crop in fall 2020 significantly increased acid phosphatase activity in the NP treatment only; there was no significant difference between cover and no cover crop treatments in either the SUF or BAM treatments (Fig. 4.5). As P was deficient in the NP treatment, resource allocation suggests that increased substrate availability due to the inclusion of cover crop promoted increased acid phosphatase activity to meet microbial P demand (Allison et al., 2011). It is surprising that increased activity due to the presence of cover crops in P-limited systems was not seen in fall 2021 or fall 2022 in the NP treatment. It would have also been expected to see a response in the SUF treatment as SUF has been in a draw down soil test P

phase during the duration of the present study. It is possible that the increased biomass production in the 2020 harvest year resulted in increased enzyme activity in the NP treatment due to increased substrate availability relative to harvest years 2021 and 2022. Although, it is possible that the increased biomass production in 2020 resulted in increased extracellular enzyme activities that were released from plants, as acid phosphatase is both microbially and plant-derived, plant-derived enzymes are generally short-lived in soil due to the stabilization of enzymes in mineral complexes, the degradation of enzymes by the microbial populations, and the possible inhibition of enzyme activities due to organic and inorganic compounds in the soil solution (Dick et al., 1983). Due to increased biomass production in 2020, it is possible that increased levels of organic P due to cover crop decomposition, relative to 2021 and 2022, was the primary reason for the lack of response in 2021 and 2022. In a meta-analysis on phosphatase response to P deficiency, Janes-Bassett et al. (2022) found that, while phosphatase production was depressed due to increased inorganic P availability, phosphatase induction was enhanced due to increased levels of organic P, suggesting the organic P, not inorganic P, maybe responsible for phosphatase dynamics in P-deficient systems.

In both fall 2020 and 2021, the presence of a cover crop significantly increased alkaline phosphatase activity in the NP treatment only (Fig. 4.6a&b). This would suggest that phosphatase production was induced due to declining P availability (Sinsabaugh and Follstad Shad, 2012). However, the lack of response of phosphatase in 2022 to the inclusion of a cover crop on the NP treatment was surprising as it would be expected that cover crop presence on P deficient systems would induce enzyme production to alleviate P deficiencies due to resource allocation (Allison et al., 2011). Enzyme activities reflect cellular economics as production must balance the resources that are expended during production (C and nutrients required for energy)

versus the benefit of increased availability of assimilable mineral nutrients (Burns et al., 2013). In addition, it would be expected that the cover crops on the SUF treatment would increase enzyme production as soil test P would fall at the margins of deficiency since P was being drawn down though the duration of this experiment. Decreased P availability in the SUF treatment would suggest an increase in phosphatase availability (Sinsabaugh and Follstad Shad, 2012. It is possible that drier conditions towards the latter portion of the 2022 growing season resulted in less soil enzyme activity, regardless of treatment. Results from the seasonal dynamics during 2022 (chapter 3) showed that phosphatase activity was significantly lower post-harvest compared to all other sampling times. The 20-day cumulative precipitation before soil sampling in 2022 was 36.58 mm, compared to 95.76 mm in 2021 (chapters 2 and 3). As alkaline phosphatase is only excreted by soil microorganisms (Tabatabai, 1994), it is possible that decreased microbial activity due to drier conditions resulted in less production of alkaline phosphatase.

Main effects of P fertilizer treatment and cover crops on soil enzyme activities:

Overall, the presence of a cover crop increased soil enzyme activities in all three years (Tables 4.4 & 4.6). The only exception to this trend was in fall 2020 where the increase in alkaline phosphatase activity was not significant at $p < 0.05$ ($p = 0.0737$). Above- and belowground inputs of cover crops can increase substrate availability and induce the production of extracellular enzymes, leading to increases in microbial activity (Bandick and Dick, 1999; Allison et al., 2011). Increases in enzyme activity due to the inclusion of a cover crop have been reported (Chavarria et al., 2016; Brennen and Acosta-Martinez, 2019; Feng et al., 2021; Hallama et al., 2021). Increases in soil enzyme activity due to cover cropping at this study site were reported for the earlier phase of the project (Starr, 2020; Stewart, 2020).

There was an overall lack of response of soil enzyme activities to P fertilizer treatments in all three years (Tables 4.4 & 4.6). Only alkaline phosphatase in fall 2020 was significantly influenced by P fertilizer treatment. In fall 2020, alkaline phosphatase activity was significantly higher in the NP treatment compared to the BAM and SUF treatments, with no significant difference observed between the BAM and SUF treatments. It has been reported that the signaling for production of phosphatases is dependent on P availability, as increased P availability generally leads to a depression of phosphatase production (Sinsabaugh and Follstad Shad, 2012). It is surprising that only in 2020 was a significant response seen for the NP treatment. Additionally, during the duration of this study, the SUF treatment has been in a drawn down soil test P phase, suggesting that P availability should decrease until a critical soil test P level is reached, prompting P fertilizer application. Therefore, it would be expected that phosphatase activity should increase in the SUF treatment relative to the BAM treatment. Results from previous chapters (chapters 2 and 3) indicate the importance of sampling conditions on enzyme activities. Cumulative precipitation differences between the three years might explain the lack of response of alkaline phosphatase activity in fall 2021 and 2022. Towards the latter portion of the growing season, cumulative precipitation was greater in 2020 relative to 2021 and 2022. As phosphatase activity has been reported to be positively associated with higher precipitation and temperatures (Margalef et al., 2016), it is likely that precipitation was responsible for the differences in observed phosphatase activity between the three years.

Effect of sampling depth on dynamic soil health indicators:

Sampling depth significantly influenced all measures of dynamics soil health in all three years of the present study (Tables 4.1, 4.3, & 4.5). Decreasing nutrient pools and activity in subsurface samples highlight the potential nutrient stratification that has been linked to no-till management

(Blanco-Cancui and Ruis, 2018). The accumulation of labile nutrients at the soil surface results in decreased substrate availability for the microbial community in subsurface soils (Balota et al., 2004; Malobane et al., 2020). The accumulation of residues at the surface has increased organic C on the soil surface (Carver, 2022). This accumulation of residues likely resulted in significantly higher soil enzyme activity and soil respiration compared to the subsurface, as decreased substrate availability at subsurface depths is likely responsible for the decreases in soil enzyme activity and soil respiration found at 5-10 and 10-15 cm in the present study.

Additionally, in fall 2022, a significant cover crop by sampling depth interaction was observed for glucosaminidase activity (Table 4.5). At both 0-5 cm and 5-10 cm depths, the presence of a cover crop significantly increased glucosaminidase activity, while no statistical difference was observed for 10-15 cm (Fig. 4.4). This suggests that benefits of cover crops on glucosaminidase activity were only seen in the surface 0-10 cm of soil. It is possible that diffusion of enzymes or substrates from cover crops was limited below 10 cm due to drier conditions that occurred during the latter portion of the 2022 harvest year (Steinweg et al., 2012; Wang et al., 2013).

Conclusion

It was found that cover crop impacts on dynamic soil health indicators varied annually, likely due to differences in precipitation during cover crop growth, resulting in variations in biomass production, precipitation during the growing season, and cover crop decomposition. The lack of consistent impacts of cover crops detail the importance of sampling conditions and suggest that soil health indicators can vary annually in response to environmental conditions. As cover crop response of soil health indicators was variable, the first hypothesis that cover crops would impact all soil health indicators was disproven. Soil enzyme activity and interactions

between P fertilizer treatment and cover crops varied between years. Response of phosphatases and glucosidase during higher cover crop biomass harvest years resulted in increased nutrient cycling in P deficient systems. However, increased activity in the SUF treatment when a cover crop was present was not observed when P was at the margins of deficiency in 2022, disproving the second hypothesis that increased nutrient cycling would be observed when as system is at the margins of deficiency. The lack of response in the SUF treatment in 2022 reflects the importance of precipitation and temperature at the point of sampling. It was observed that sampling depth significantly influenced all soil health indicators in all three years. This was likely due to increased nutrient stratification do to no-till practices that results in accumulation of organic matter on the soil surface, aligning with the third hypothesis of this study.

References

Allison, S.D., Weintraub, P.M., Gartner, T.B., Waldrop, M.P., 2011. Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. Soil Enzymology, Springer, pp. 229-243.

Balota, E.L., Calegari, A., Nakatani, A.S., Coyne, M.S., 2014. Benefits of winter cover crops and no-tillage for microbial parameters in a Brazilian Oxisol: A long-term study. Agric. Ecosyst. Environ. 197, 31-40.

Balota, E.L., Filho, A.C., Andrade, D.S., Dick, R.P., 2004. Long-term tillage and crop rotation effects on microbial biomass and C and N mineralization in a Brazilian Oxisol. Soil Tillage Res. 77, 137-145.

Bandick, A.K., and Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biol. Biochem. 31, 1471-1479.

Blanco-Canqui, H., 2022. Cover crops and carbon sequestration: Lessons from U.S. studies. Soil Sci. Soc. Am. J. 86, 501-519.

Blanco-Canqui, H., and Ruis, S.J., 2018. No-tillage and soil physical environment. Geoderma 326, 164-200.

Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A., Hergert, G.W., 2015. Cover crops and ecosystem services: insights from studies in temperate soils. Agron. J. 107, 2449-2474.

Brennan, E.B., and Acosta-Martinez, V., 2019. Cover crops and compost influence soil enzymes during six years of tillage-intensive organic vegetable production. Soil Sci. Soc. Am. J. 83, 624-637.

Carver, R.E., 2022. Cover crop and phosphorus management implications for water quality in a no-till corn-soybean rotation. (Doctoral dissertation, Kansas State University).

Carver, R.E., Nelson, N.O., Roozeboom, K.L., Kluitenberg, G.J., Tomlinson, P.J., Kang, Q., Abel, D.S., 2022. Cover crop and phosphorus fertilizer management impacts on surface water quality from a no-till corn-soybean rotation. J. Environ. Manage. 301, 113818.

Chahal, I., and Van Eerd, L.L., 2019. Quantifying soil quality in a horticultural-cover cropping system. Geoderma 352, 38-48.

Chavarría, D.N., Verdenelli, R.A., Serri, D.L., Restovich, S.B., Andriulo, A.E., Meriles, J.M., Vargas-Gil, S., 2016. Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability. Eur. J. Soil Biol. 76, 74-82.

Chowdhury, R.B., Moore, G.A., Weatherley, A.J., Arora, M., 2017. Key sustainability challenges for the global phosphorus resource, their implications for global food security, and options for mitigation. J. Clean. Prod. 140, 945-936.

Dick, W.A., Juma, N.G., Tabatabai, M.A., 1983. Effects of soils on acid phosphatase and inorganic pyrophosphatase of corn roots. Soil Sci. 136, 19-25.

Duval, M.E., Galantini, J.A., Capurro, J.E., Martinez, J.M., 2016. Winter cover crops in soybean monoculture: effects on soil organic carbon and its fractions. Soil Tillage Res. 161, 95-105.

Eivazi, F., and Tabatabai, M.A., 1977. Phosphatases in soils. Soil Biol. Biochem. 9, 167–172.

Eivazi, F., and Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606.

Feng, H., Sekaran, U., Wang, T., Kumar, S., 2021. On-farm assessment of cover cropping effects on soil C and N pools, enzyme activities, and microbial community structure. J. Agric. Sci. 159, 216–226.

Feng, J., and Zhu, B., 2019. A global meta-analysis of soil respiration and its components in response to phosphorus addition. Soil Biol. Biochem. 135, 38-47.

Fierer, N., Wood, S.A., de Mesquita, C.P., 2021. How microbes can, and cannot, be used to assess soil health. Soil Biol. Biochem. 153, 108111.

Franzluebbers, A.J., 2002. Soil organic matter stratification ratios as an indicator of soil quality. Soil Tillage Res. 66, 95-106.

Green, V.S., Stott, D.E., Cruz, J.C., Curi, N., 2007. Tillage impacts of soil biological activity and aggregation in a Brazilian Cerrado Oxisol. Soil Tillage Res. 92, 144-121.

Guo, H. He, X., Li, Y., 2012. Spatial distribution of arbuscular mycorrhiza and glomalin in the rhizosphere of Caragana korshinskii Kom. in the Otindag sandy land, China. Afr. J. Microbiol. Res. 6, 5745-5753.

Hallama, M., Pekrun, C., Lambers, H., Kandeler, E., 2019. Hidden miners-the roles of cover crops and soil microorganisms in phosphorus cycling. Plant Soil 434, 7-45.

Haney, R.L., and Haney, E.B., 2010. Simple and rapid laboratory method for rewetting dry soil for incubations. Commun. Soil Sci, Plant Anal. 41, 1493–1501.

Hansen, N.C., Daniel, T.C., Sharpley, A.N., Lemunyon, J.L., 2002. The fate and transport of phosphorus in agricultural systems. J. Soil Water Conserv. 57, 408-417.

Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Separating root and soil microbial contributions to soil respirations: a review of methods and observations. Biogeochemistry 48, 115-146.

Hurisso, T.T., Moebius-Clune, D.J., Culman, S.W., Moebius-Clune, B.N., Thies, J.E., van Es, H.M., 2018. Soil protein as a rapid soil health indicator of potentially available organic nitrogen. Agricul. Environ. Letters, 3, 180006.

Janes-Bassett, V., Blackwell, M.S.A., Blair, G., Davies, J., Haygarth, P.M., Mezeli, M.M., Stewart, G., 2022. A meta-analysis of phosphatase activity in agricultural settings in response to phosphorus deficiency. Soil Biol. Biochem. 165, 108537.

Kim, N., Zabaloy, M.C., Guan, K., Villamil, M.B., 2020. Do cover crops benefit soil microbiome? A meta-analysis of current research. Soil Biol. Biochem. 142, 107701.

Law, S.M. and Maherali, H., 2023. Variation in glomalin-related soil protein and plant growth response to arbuscular mycorrhizal fungi along a nutrient gradient in temperate grasslands. Plant Soil 487, 623-637.

Laishram, J., Saxena, K.G., Maikhuri, R.K., Rao, K.S., 2012. Soil quality and soil health: a review. Int. J. Ecol. Environ. Sci. 38, 19-37.

Lehman, R.M., Cambardella, C.A., Stott, D.E., Acosta-Martinez, V., Manter, D.K., Buyer, J.S., Maul, J.E., Smith, J.L., Collins, H.P., Halvorson, J.J., Kremer, R.J., Lundgren, J.G., Ducey, T.F., Jin, V.L., Karlen. D.L., 2015.Understanding and enhancing soil biological health: The solution for reversing soil degradation. Sustainability 7, 988-1027.

Leikam, D.F., Lamond, R.E., Mengel, D.B., 2003. Providing flexibility in phosphorus and potassium fertilizer recommendations. Better Crops 87, 6-10.

Luo, L., Meng, H., Gu, J-D., 2017. Microbial extracellular enzymes in biogeochemical cycling of ecosystems. J. Environ. Manag. 197, 539-549.

Macnack, N., Khin Chim, B., Amedy, B., Arnall, B., 2011. Fertilization based on sufficiency, build-up and maintenance concept. Oklahoma Cooperative Extension Service Publication, PSS-2266.

Malobane, M.E., Nciizah, A.D., Nyambo, P., Mudau, F.N., Wakindiki, I.I.C., 2020. Microbial biomass carbon and enzyme activities as influenced by tillage, crop rotation and residue management in a sweet sorghum cropping system in marginal soils of South Africa. Heliyon 6, e05513.

Mardamootoo, T., du Preez, C.C., Barnard, J.H., 2021. Phosphorus management issues for crop production: a review. Afr. J. Agric. Res. 17, 939-952.

Marshall, C.B., Burton, D.L., Lynch, D.H., 2021. Cover crops improve some, but not all, soil health indicators in horticultural rotations. Can. J. Plant Sci. 102, 1-10.

Margalef, O., Sardans, J., Fernandez-Martinez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., Goll, D., Aichter, A., Obersteiner, M., Asensio, D., Penuelas, J., 2017. Global patterns of phosphatase activity in natural soils. Sci. Rep. 7, 1337.

Nevins, C.J., Lacey, C., Armstrong, S., 2020. The synchrony of cover crop decomposition, enzyme activity, and nitrogen availability in a corn agroecosystem in the Midwest United States. Soil Tillage Res. 197, 104518.

Nilahyane, A., Ghimire, R., Thapa, V.R., Sainju, U.M., 2020. Cover crop effects on soil carbon dioxide emissions in a semiarid cropping system. Agric. Ecosyst. Environ. 3, 20012.

Nunes, M.R., van Es, H.M., Schindelbeck, R., Ristow, A.J.., Ryan, M., 2018. No-till and cropping system diversification improve soil health and crop yield. Geoderma 328, 30-43.

Parham, J.A., and Deng, S.P., 2000. Detection, quantification and characterization of β-glucosaminidase activity in soil. Soil Biol. Biochem. 32, 1183–1190.

Pokhrel, S., Kingery, W.L., Cox, M.S., Shankle, M.W., Shanmugam, S.G., 2021. Impact of cover crops and poultry litter on selected soil properties and yield in dryland soybean production. Agronomy 11, 119.

O'Connell, S., Shi, W., Grossman, J.M., Hoyt, G.D., Fager, K.L., Creamer, N.G., 2015. Short-term nitrogen mineralization from warm-season cover crops in organic farming systems. Plant Soil 396, 353-367.

Qiu, L., Lin, H., Song, B., Kong, T., Sun, W., Sun, X., Zhang, Y., Li, B., 2021. Glomalin-related soil protein (GRSP) in metal sequestration at Pb/Zn-contaminated sites. J. Soils Sed. 22, 577-593.

Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., Reddy, K.R., 1994. Managing agricultural phosphorus for protection of surface waters: issues and options. J. Environ. Qual. 23, 437-451.

Sinsabaugh, R.L., and Follstad Shah, J.J., 2012. Ecoenzymatic stoichiometry and ecological theory. Annu. Rev. Ecol. Evol. Syst. 43, 313-343.

Starr, L., 2020. Linkages between cover crops, phosphorus fertilizer management, soil health, and phosphorus bioavailability in replicated research watersheds. (Doctoral dissertation, Kansas State University).

Steele, M.K., Coale, F.J., Hill, R.H., 2012. Winter annual cover crop impacts on no-till soil physical properties and organic matter. Soil Sci. Soc. Am. J. 76, 2164-2173.

Steinweg, J.M., Dukes, J.S., Paul, E.A., Wallenstein, M.D., 2013. Microbial response to multi-factor climate change: effects of soil enzymes. Front. Microbiol. 4, 146.

Stewart, C., 2020. Soil microbial community dynamics in response to cover crop implementation and P fertilizer management. (Doctoral dissertation, Kansas State University).

Tabatabai, M. A., 1994. Soil Enzymes. In: Weaver, R., Angle, J., Bottomley, P. (eds.), Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 775-833.

Thapa, R., Tully, K.L., Reberg-Horton, C., Cabrera, M., Davis, B.W., Fleisher, D., Gaskin, J., Hitchcock, R., Poncet, A., Schomberg, H.H., Seehaver, S.A., 2022. Cover crop residue

decomposition in no-till cropping systems: Insights from multi-state on-farm litter bag studies. Agric. Ecosys. Environ. 326, 107823.

Wang, Q., Xiao, F., He, T., Wang, S., 2013. Responses of labile soil organic carbon and enzyme activity in mineral soils to forest conversion in the subtropics. Annals of Forest Sci. 70, 579-587.

Wang, X., Cao, Q., Yang, W., Zhu, X., 2022. Spatial changes in glomalin-related soil protein and their correlation with soil properties in the black soil region of northeast China. Agronomy 12, 2165.

Wang, Y.P., Law, R.M., Pak, B., 2010. A global model of carbon, nitrogen and phosphorus cycles for the terrestrial biosphere. Biogeosciences 7, 2261-2282.

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. Am. J. Alter. Agric. 18, 3-17.

Wood, S.A., and Bowman, M., 2021. Large-scale farmer-led experiment demonstrates positive impact of cover crops on multiple soil health indicators. Nat. Food, 2, 97-103.

Wright, S.F., and Upadhyaya, A., 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci. 161, 575–586.

Wu, F., Dong, M., Liu, Y., Ma, X., An, L., Young, J.P.W., Feng, H., 2011. Effects of long-term fertilization on AM fungal community structure and Glomalin-related soil protein in the Loess Plateau of China. Plant Soil, 342, 233-247.

Zhou, X., Chem, C., Wu, H., Xu, Z., 2012. Dynamics of soil extractable carbon and nitrogen under difference cover crop residues. J. Soils Sediments. 12, 844-853.

Table 4.1 ANOVA table analyzing the effect of phosphorus (P) fertilizer treatment, cover crop, sampling depth and all interactions for active carbon, autoclaved citrate extractable protein, and soil respiration measured in fall 2020, 2021, and 2022. Years were analyzed separately. Table abbreviations include P fertilizer treatment (TRT) and cover crop treatment (CC). Bolded values indicate significance at $p < 0.05$.

Effect	Fall 2020	Fall 2021	Fall 2022
	Active Carbon		
TRT	0.3460	0.3464	0.4392
CC	**0.0010**	0.1199	0.1815
TRT*CC	0.5564	0.2280	0.8198
DEPTH	**<0.0001**	**<.0001**	**<.0001**
DEPTH*TRT	0.1911	0.7187	0.6681
DEPTH*CC	0.1779	0.5146	0.7125
DEPTH*TRT*CC	0.9610	0.0794	0.9430
	Autoclaved Citrate Extractable Protein		
TRT	**0.0160**	**0.0324**	**0.0355**
CC	0.0967	**0.0228**	**0.0316**
TRT*CC	0.5913	0.7003	0.9291
DEPTH	**<.0001**	**<.0001**	**<.0001**
DEPTH*TRT	0.5273	0.9929	0.9990
DEPTH*CC	0.8177	0.7544	0.9072
DEPTH*TRT*CC	0.9912	0.8983	0.9154
	Soil Respiration		
TRT	0.9711	0.1765	0.3679
CC	**0.0026**	**0.0001**	**0.0002**
TRT*CC	0.3136	**0.0445**	**0.0474**
DEPTH	**<.0001**	**<.0001**	**<.0001**
DEPTH*TRT	0.7673	0.2895	0.9584
DEPTH*CC	0.3339	0.7325	0.4827
DEPTH*TRT*CC	0.8075	0.4552	0.8232

Table 4.2 Least Squares (LS) means for active carbon, autoclaved citrate extractable (ACE) protein, and soil respiration in fall 2020, 2021, and 2022. Years were analyzed separately. Letters indicate significant difference at $p < 0.05$. Table abbreviations: no phosphorus (P) (NP), build and maintain (BAM), and sufficiency (SUF), presence of a cover crop (CC) and absence of cover crop (NC).

	Fall 2020	**Fall 2021**	**Fall 2022**
		Active Carbon	
		mg C kg soil^{-1}	
NP	321.72	176.69	137.50
BAM	335.10	197.51	146.35
SUF	313.57	197.51	156.06
CC	345.13**A**	201.05	154.74
NC	301.79**B**	180.14	138.61
0-5cm	453.65**A**	368.72**a**	272.65**AA**
5-10 cm	326.20**B**	232.06**b**	119.76**BB**
10-15cm	190.54**C**	80.56**c**	96.21**CC**
		ACE Protein	
		mg protein kg soil^{-1}	
NP	3551**B**	3257**b**	3394**BB**
BAM	3901**A**	3603**a**	3746**AA**
SUF	4003**A**	3557**a**	3849**AA**
CC	3926	3600**a**	3780**AA**
NC	3710	3343**b**	3546**BB**
0-5cm	4696**A**	4365**a**	4352**AA**
5-10 cm	3596**B**	3273**b**	3436**BB**
10-15cm	3163**C**	2921**c**	3201**BB**
		Soil Respiration	
		mg CO_2 g soil^{-1}	
NP	16.45	18.38	7.28
BAM	16.55	19.37	6.98
SUF	16.55	18.86	7.06
CC	17.20**A**	19.82**a**	7.48**AA**
NC	15.86**B**	17.96**b**	6.75**BB**
0-5cm	19.37**A**	21.38**a**	9.24**AA**
5-10 cm	15.99**B**	18.67**b**	7.13**BB**
10-15cm	14.55**C**	16.83**c**	5.45**CC**

Table 4.3 ANOVA table analyzing the effect of phosphorus (P) fertilizer treatment, cover crop, sampling depth and all interactions for β-glucosidase and β-glucosaminidase measured in fall 2020, 2021, and 2022. Years were analyzed separately. Table abbreviations include P fertilizer treatment (TRT) and cover crop treatment (CC). Bolded values indicate significance at $p < 0.05$.

Effect	Fall 2020	Fall 2021	Fall 2022
		Glucosidase	
TRT	0.1316	0.4705	0.2019
CC	**0.0234**	**0.0432**	**0.0030**
TRT*CC	**0.0086**	0.4812	0.9558
DEPTH	**<.0001**	**<.0001**	**<.0001**
DEPTH*TRT	0.1055	0.9569	0.9334
DEPTH*CC	0.6857	0.6852	0.2950
DEPTH*TRT*CC	0.9774	0.4713	0.7009
		Glucosaminidase	
TRT	0.6612	0.6325	0.2832
CC	**<.0001**	**<.0001**	**<.0001**
TRT*CC	0.2765	0.4036	0.2941
DEPTH	**<.0001**	**<.0001**	**<.0001**
DEPTH*TRT	0.1699	0.8369	0.9320
DEPTH*CC	0.1676	0.8362	**0.0303**
DEPTH*TRT*CC	0.6603	0.5783	0.4503

Table 4.4 Least Squares (LS) means for β-glucosidase and β-glucosaminidase in fall 2020, 2021, and 2022. Years were analyzed separately. Letters indicate significant difference at $p < 0.05$. Table abbreviations: no phosphorus (P) (NP), build and maintain (BAM), and sufficiency (SUF), presence of a cover crop (CC) and absence of a cover crop (NC).

	Fall 2020	**Fall 2021**	**Fall 2022**
		Glucosidase	
		mg p-nitrophenol kg soil^{-1} hr^{-1}	
NP	54.11	56.60	46.77
BAM	62.06	62.78	47.38
SUF	59.80	61.97	51.36
CC	62.56**A**	65.28**a**	52.08**AA**
NC	54.82**B**	55.85**b**	45.09**BB**
0-5cm	127.85**A**	135.43**a**	96.23**AA**
5-10 cm	48.85**B**	50.77**b**	39.87**BB**
10-15cm	32.15**C**	32.03**c**	29.66**CC**
		Glucosaminidase	
		mg p-nitrophenol kg soil^{-1} hr^{-1}	
NP	23.44	23.87	21.43
BAM	22.62	22.85	20.81
SUF	23.50	24.05	22.27
CC	25.79**A**	26.57**a**	23.87**AA**
NC	20.84**B**	20.93**b**	19.36**BB**
0-5cm	39.71**A**	41.21**a**	36.31**AA**
5-10 cm	22.16**B**	20.10**b**	18.86**BB**
10-15cm	14.16**C**	15.83**c**	14.51**CC**

Table 4.5 ANOVA table analyzing the effect of phosphorus (P) fertilizer treatment, cover crops, sampling depth and all interactions for acid and alkaline phosphatase measured in fall 2020, 2021, and 2022. Years were analyzed separately. Table abbreviations include P fertilizer treatment (TRT) and cover crop (CC). Bolded values indicate significance at $p < 0.05$.

Effect	Fall 2020	Fall 2021	Fall 2022
	Acid Phosphatase		
TRT	0.6037	0.5608	0.0980
CC	**0.0256**	**0.0022**	**0.0013**
TRT*CC	**0.0442**	0.7089	0.2867
DEPTH	**<.0001**	**<.0001**	**<.0001**
DEPTH*TRT	0.6851	0.9599	0.9688
DEPTH*CC	0.5009	0.9755	0.5133
DEPTH*TRT*CC	0.8553	0.8039	0.9962
	Alkaline Phosphatase		
TRT	**0.0087**	0.9465	0.3067
CC	0.0737	**0.0015**	**0.0026**
TRT*CC	**0.0257**	**0.0058**	0.4230
DEPTH	**<.0001**	**<.0001**	**<.0001**
DEPTH*TRT	0.4710	0.9311	0.9920
DEPTH*CC	0.5570	0.5628	0.1910
DEPTH*TRT*CC	0.3080	0.3008	0.8714

Table 4.6 Least Squares (LS) means for acid and alkaline phosphatase in fall 2020, 2021, and 2022. Years were analyzed separately. Letters indicate significant difference at $p < 0.05$. Table abbreviations: no phosphorus (P) (NP), build and maintain (BAM), and sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

	Fall 2020	**Fall 2021**	**Fall 2022**
	Acid Phosphatase		
	mg p-nitrophenol kg soil^{-1} hr^{-1}		
NP	211.49	199.48	187.76
BAM	222.74	213.85	200.59
SUF	206.92	205.35	208.69
CC	229.35**A**	224.96**a**	212.86**AA**
NC	198.98**B**	188.93**b**	185.69**BB**
0-5cm	281.71**A**	298.19**a**	276.95**AA**
5-10 cm	220.39**B**	191.16**b**	172.27**BB**
10-15cm	157.00**C**	153.71**c**	164.74**BB**
	Alkaline Phosphatase		
	mg p-nitrophenol kg soil^{-1} hr^{-1}		
NP	76.71**A**	72.33	68.32
BAM	65.31**B**	70.55	63.78
SUF	65.83**B**	70.22	62.05
CC	65.83	70.02**a**	70.20**AA**
NC	72.36	59.29**b**	59.23**BB**
0-5cm	66.21**A**	64.97**a**	84.38**AA**
5-10 cm	57.23**B**	59.79**b**	55.08**BB**
10-15cm	50.97**C**	43.52**c**	54.68**BB**

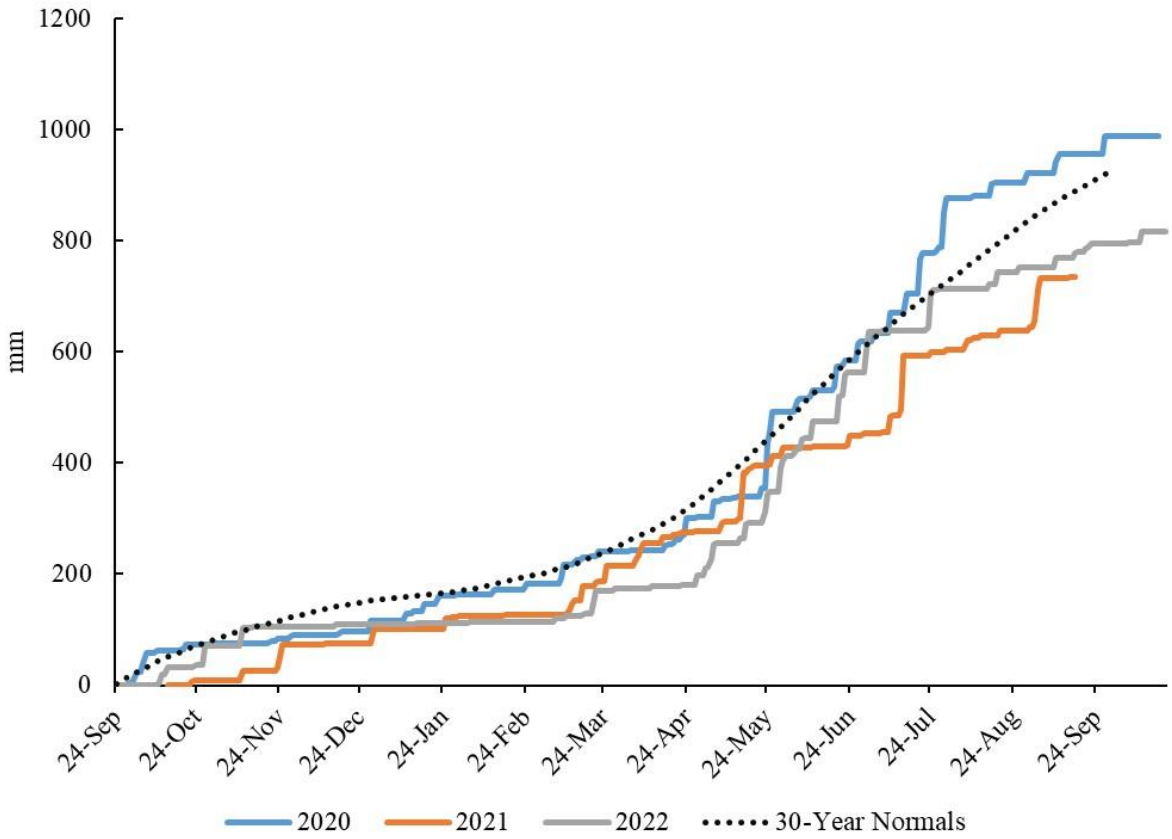

Figure 4.1 Cumulative precipitation for harvest years 2020, 2021, and 2022 as reported by Kansas Mesonet Station near field site in Ashland Bottoms (operated by Kansas State University). 30-year normals for Riley County are also reported.

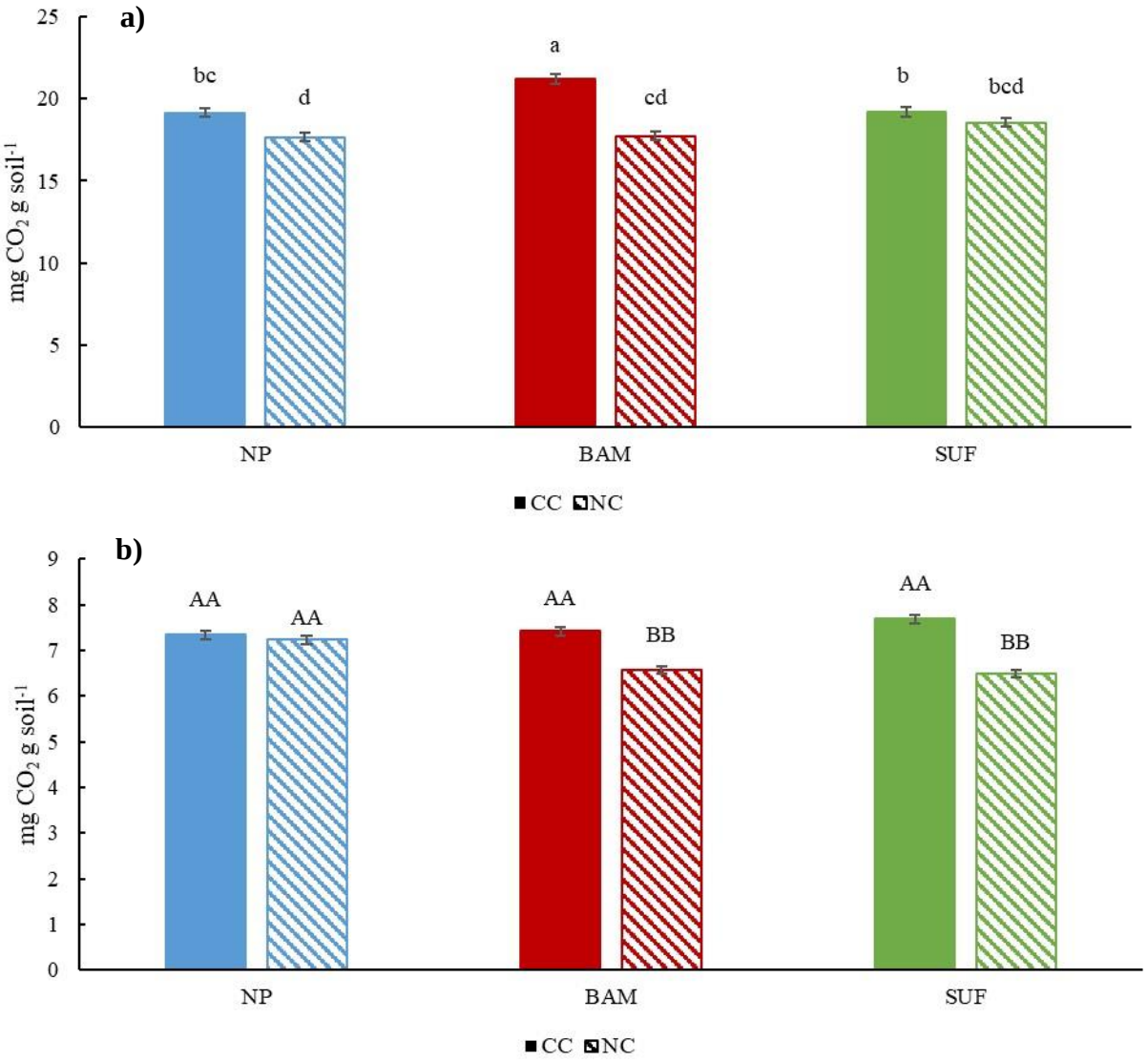

Figure 4.2 Phosphorus (P) fertilizer treatment by cover crop interaction for soil respiration in a) fall 2021 and b) fall 2022. Error bars represent standard error or the mean and letters indicate significant difference at $p < 0.05$. Years were analyzed separately. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments, and presence of a cover crop (CC) and absence of a cover crop (NC).

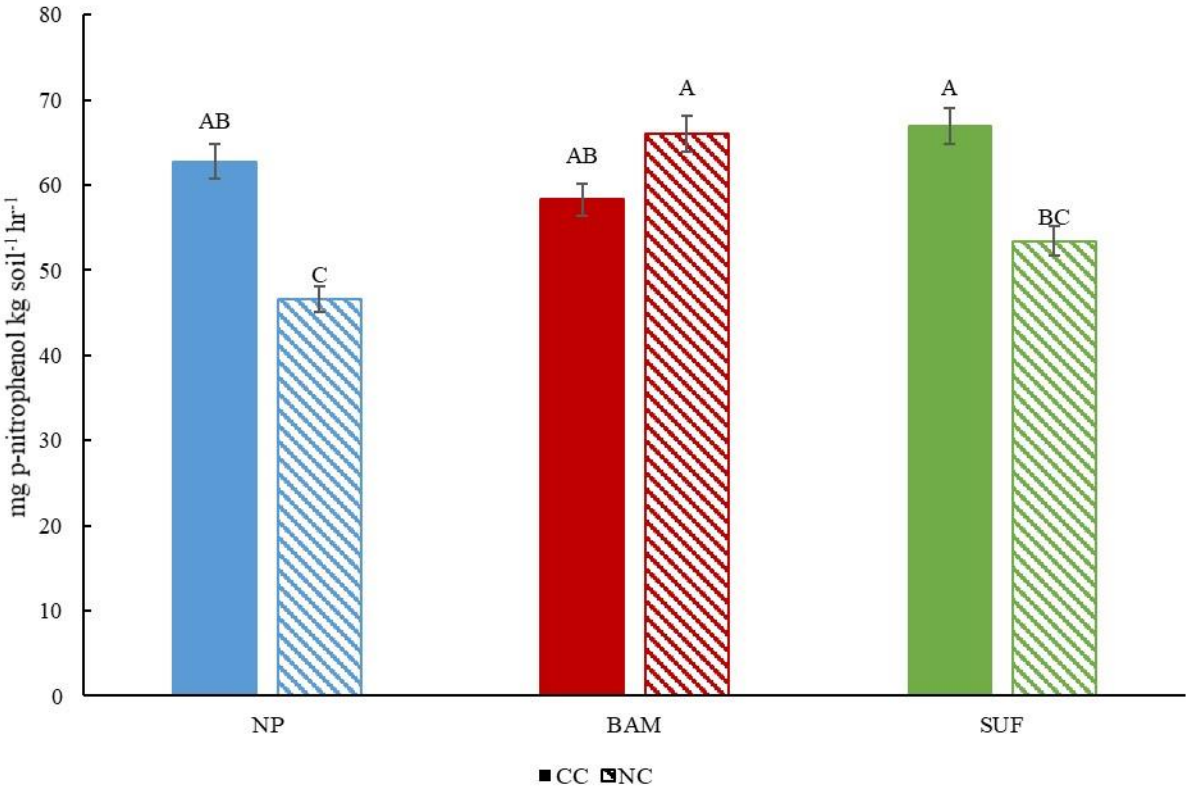

Figure 4.3 Phosphorus (P) fertilizer treatment by cover crop interaction for β-glucosidase activity in fall 2020. Error bars represent standard error of the mean and letters indicate significant difference at $p < 0.05$. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments, and presence of a cover crop (CC) and absence of a cover crop (NC).

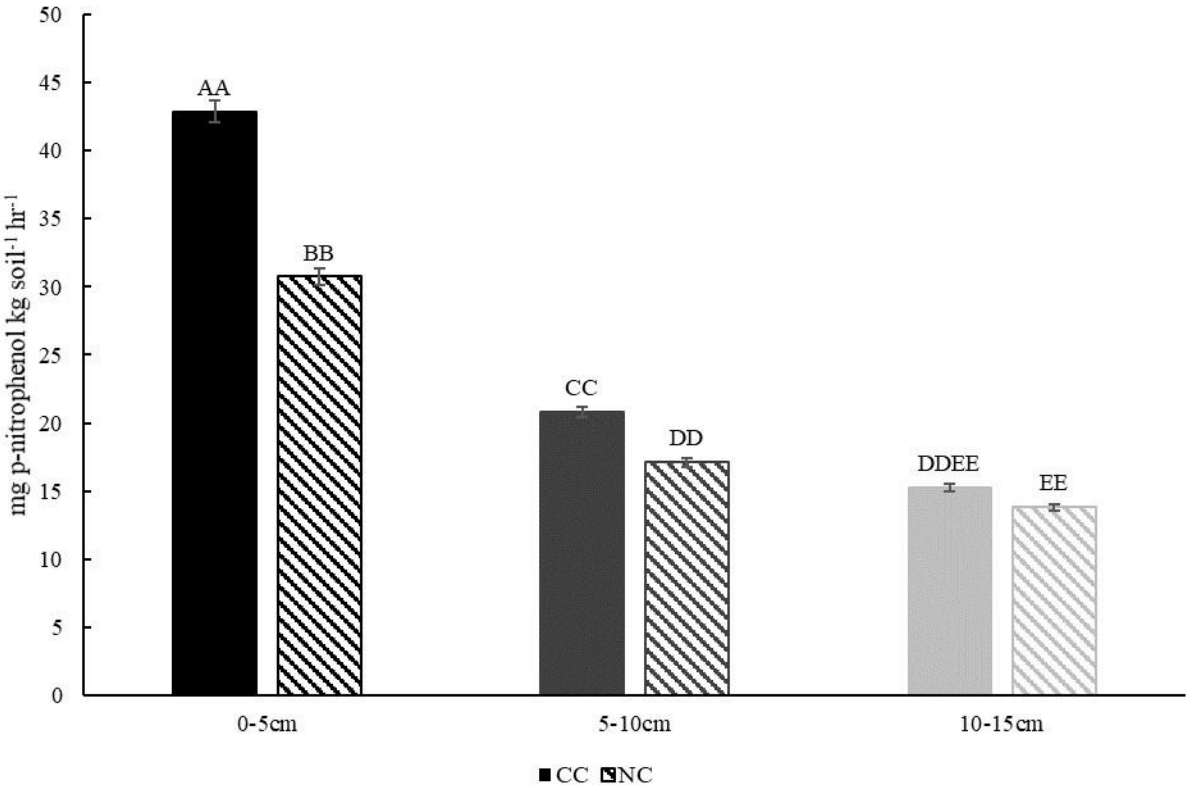

Figure 4.4 Cover crop by sampling depth interaction for glucosaminidase activity in fall 2022. Error bars represent standard error of the mean and letters indicate significant difference at $p < 0.05$. Figure abbreviations: presence of a cover crop (CC) and absence of a cover crop (NC).

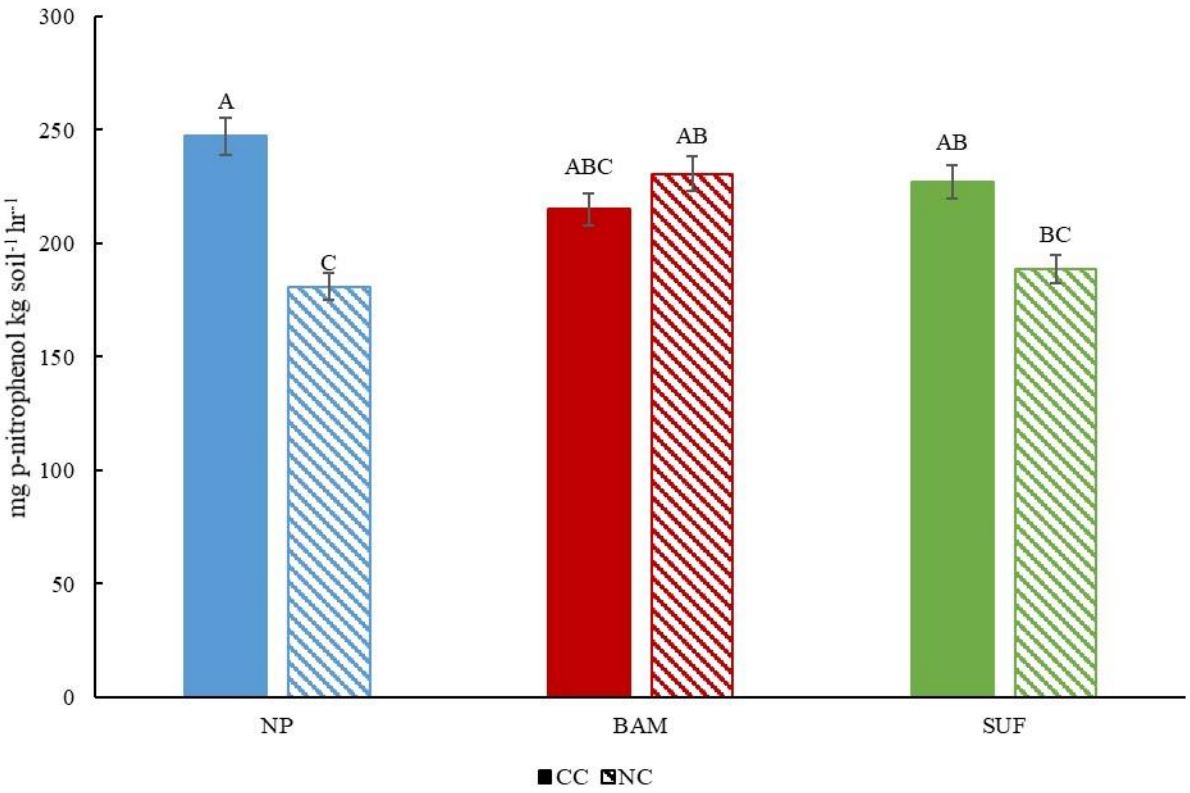

Figure 4.5 Phosphorus (P) fertilizer treatment by cover crop interaction for acid phosphatase in fall 2020. Error bars represent standard error of the mean and letters indicate significant difference at $p < 0.05$. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments, and presence of a cover crop (CC), and absence of a cover crop (NC).

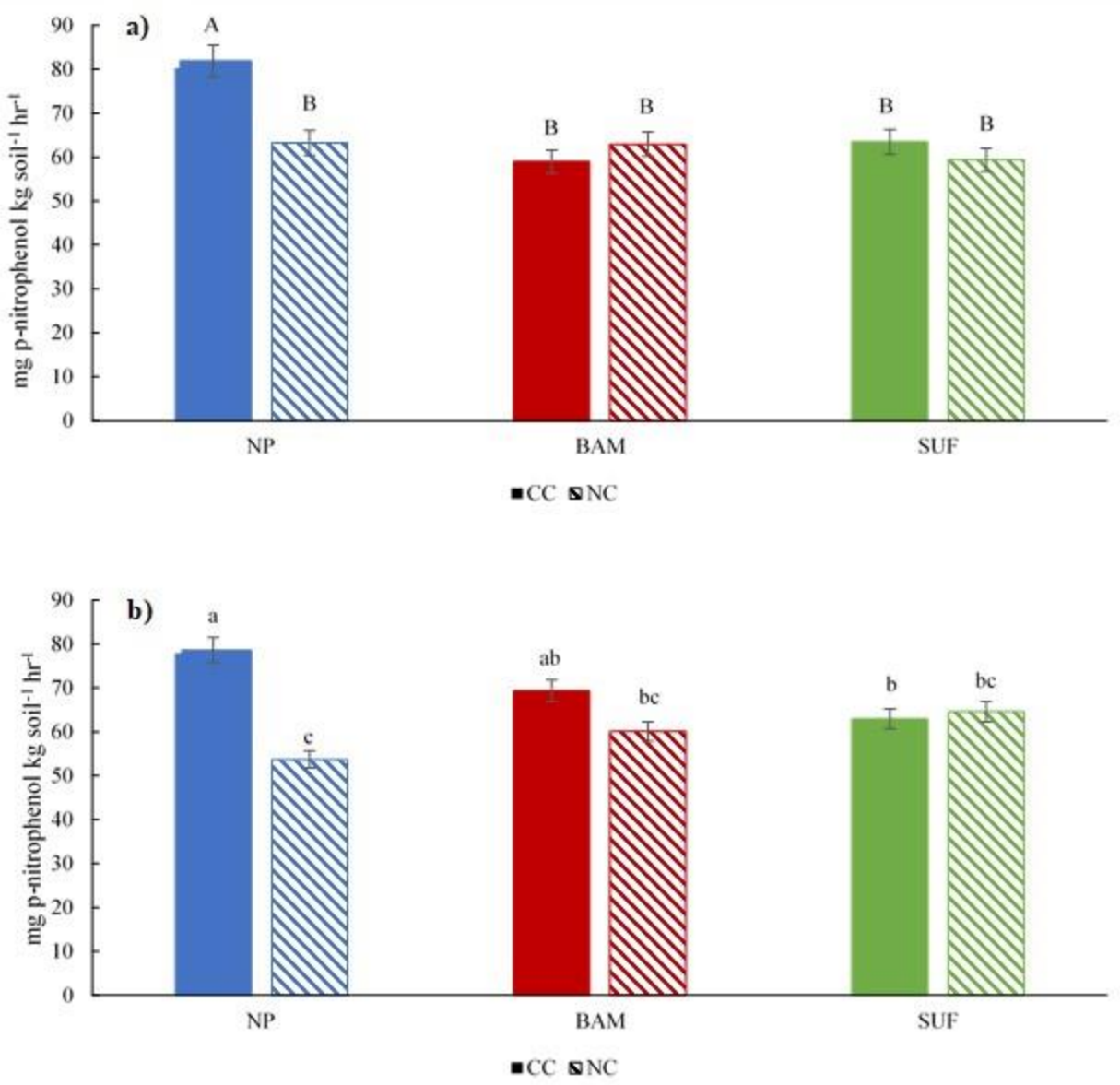

Figure 4.6 Phosphorus fertilizer treatment by cover crop interaction for alkaline phosphatase for a) fall 2020 and b) fall 2021. Error bars represent standard error of the mean and letters indicate significant difference at $p < 0.05$. Years were analyzed separately. Figure abbreviations: no P (NP), build and maintain (BAM), and sufficiency (SUF) P fertilizer treatments, and presence of a cover crop (CC) and absence of a cover crop (NC).

Chapter 5 - Summary

Increased agricultural production to meet the needs of an increasing world population will require additional fertilizer inputs to meet nutrient crop demand. However, overapplication of synthetic phosphorus (P) fertilizer and/or animal manure can lead to adverse effects on the environment, namely water quality issues. Phosphorus losses through surface runoff can contribute to eutrophication, resulting in harmful algae blooms that can deplete oxygen when algae die and decompose, resulting in fish kills. Utilizing soil testing allows for crop nutrient demand to be met while reducing the risks of nutrient loss. Additionally, conservation practices such as the inclusion of cover crops in a cropping system and no-till management can increase soil health. It is possible that cover crops can promote nutrient cycling in systems with low nutrient availability, however the response of the soil microbial community to increased additions of cover crop residues can vary due to differences in precipitation and temperature and crop residue amounts. Seasonal trends in biological soil health indicators can yield contrasting results, making interpretation difficult. Therefore, while sampling time greatly influences biological soil health indicators, it might be more important to ensure that soil sampling occurs annually during a consistent time point.

The purpose of this research was to assess the impacts of P fertilizer management practices and presence of a cover crop on various indicators of soil health in a no-till corn-soybean rotation. Soil health indicators assessed in this study included nutrient pools of carbon (C) - active C and dissolved organic C, citrate-extractable P, and nitrogen (N) - dissolved total N, inorganic N (NH_4-N and NO_3-N), dissolved organic N, and autoclaved citrate extractable (ACE) protein as well as measures of microbial activity and function including microbial biomass C, N, and P, soil respiration, and four soil enzyme activities that are responsible for C (β-glucosidase),

C and N (β-glucosaminidase), and P (acid and alkaline phosphatase) cycling. In addition, the seasonal dynamics of these soil health indicators were assessed during the 2021 and 2022 growing season. Soil samples were taken from 0-5 cm six times during the corn and soybean growing season, including a sampling pre-cover crop termination, samplings that occurred four times during the growing season, and one sampling immediately following crop harvest.

The presence of a cover crop significantly increased active C and ACE protein concentrations as well as all measures of microbial activity and function in both years of this study (Table 5.5). This finding highlights the contribution of above- and belowground inputs from cover crops that increase substrate availability for the soil microbial community. Phosphorus fertilizer treatment significantly influenced ACE protein concentrations, as ACE protein was significantly lower in the control (NP) treatments compared to treatments that received P fertilizer in both years of this study, highlighting the importance of P availability on the activity of the soil microbial community. Microbial biomass P and citrate-extractable P were found to be significantly larger in the build and maintain (BAM) treatment that received annual applications of P fertilizer, reflecting the soil P status. Significant P fertilizer by cover crop interactions were observed for alkaline phosphatase activity in both years of this study. The presence of a cover crop on NP and sufficiency (SUF) treatments significantly increased activity, while no increase in activity was observed in the BAM treatment. This finding supports resource allocation models that suggest that the induction of enzyme production by the soil microbial community can be enhanced due to the presence of a substrate, but increased enzyme production will only occur if nutrient availability is low, in order to alleviate nutrient deficiencies.

However, there were some soil health indicators for which P fertilizer management and the presence or absence of a cover crop did not result in similar trends between both years of this

study. In 2021, cover crops significantly increased dissolved organic C and N concentrations, however, no effect of cover crops was observed in 2022 (Table 5.5). In 2021, cover crops did not significantly influence microbial biomass N, dissolved total N, inorganic N, or NO_3-N concentrations. However, it was observed that cover crops significantly increased microbial biomass N concentrations, while reducing inorganic N and NO_3-N concentrations in 2022 (Table 5.5). The reduction of NO_3-N concentrations in 2022 by the cereal rye cover crop highlights the nutrient scavenging ability of the crop to reduce NO_3-N losses. Differences in NO_3-N concentrations due to presence of a cover crop in 2021 and 2022 could be due to the timing of soil sampling (in regards to cover crop termination) and differences in precipitation and temperature that resulted in different amounts of cover crop biomass production for the 2021 and 2022 harvest year.

Sampling time greatly influenced most measures of soil health in both years of this study. However, the overall lack of cover crop by sampling time interactions in either year of this study indicate that climatic conditions (especially moisture and temperature) are the primary drivers of seasonal dynamics of soil health indicators instead of cover crops. Significant P fertilizer treatment by sampling time interactions were observed in both years of this study. Overall, it appeared that N and P fertilizer applications, crop N uptake, and differences in moisture and temperature were responsible for these dynamics in both years. In comparing results from the spring sampling and post-harvest sampling, differences were observed between these samplings (Table 5.2), highlighting the difficultly of determining a recommendation for timing of soil sampling for biological soil health indicators. Therefore, it is suggested that soil sampling occurs annually at the same time each year to assess trends.

In addition to seasonal dynamics, this study also looked at the influence of sampling depth, P fertilizer treatment, and cover crops on dynamic soil health indicators (active C, ACE protein, soil respiration, and soil enzyme activities) during post-harvest fall samplings. Soil samples were collected from 0-5, 5-10, and 10-15 cm in 2020, 2021, and 2022. Nutrient stratification was evident as sampling depth significantly influenced all dynamic soil health measures in all years. Soil microbial activity decreases in subsurface soil samples as nutrient pools were reduced. Differences in the response of soil health indictors to the presence of a cover crop between all three years was most likely due to differences in cover crop biomass production and climatic factors that influence microbial activity.

The overall results of this study, highlight the potential to improve nutrient cycling by cover crop implementation and promote the soil microbial community to meeting the challenges of meeting crop nutrient demand while minimizing nutrient loss by managing a nutrient such as P closer to the margins of deficiency. The differences observed for both the seasonal trends and cover crop and P fertilizer response between years highlights the overall dynamics of the soil microbial community that is influenced by numerous factors. Revising the hypotheses of microbial response to P fertilization approaches and cover crops of this study: it was proposed that (1) cover crops will result in increased microbial activity and labile nutrient concentrations, (2) the sufficiency approach will enhance nutrient cycling when a system is at the margins of deficiency, and (3) in addition, the presence of cover crops will promote enhanced nutrient cycling when a system is that the margins of deficiency. Results from this study demonstrated that cover crops have the potential to increase microbial activity and labile nutrient concentrations due to increased above- and belowground inputs. Increased alkaline phosphatase and glucosidase activity due to the presence of a cover crop of P deficient systems or systems at

the margins of deficiency suggests that nutrient cycling can be enhanced when a system is at the margins of deficiency. Revisiting the hypotheses for seasonal trends of various soil health indicators during cover crop decomposition, it was proposed that (1) sampling time will greatly influence all measures of soil health, and (2) cover crop decomposition and variations in soil temperature and moisture throughout the growing season will influence seasonal trends. The results of this study revealed that sampling time greatly influenced most measures of soil health. However, the lack of cover crop by sampling time interactions that were observe suggests that precipitation and temperature trends are the primary drivers of seasonal dynamics of soil health indicators. Finally, it was hypothesized that microbial activity will decrease in subsurface soils due to nutrient stratification as a result of no-till management. Results from chapter 4 of this study demonstrated that decreased microbial activity as well as active nutrient pools in subsurface was likely due to nutrient stratification.

Table 5.1 Summary of main effect of cover crop response to microbial biomass carbon (C), nitrogen (N), and phosphorus (P), active C, autoclaved citrate extractable (ACE) protein, soil NO_3-N, soil enzyme activities (β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase), and soil respiration in 2021 and 2022.

Indicator	2021-Corn	2022-Soybean
Microbial Biomass C	+	+
Microbial Biomass N	0	+
Microbial Biomass P	0	0
Dissolved Organic C	+	0
Dissolved Organic N	+	0
Active C	+	+
ACE Protein	+	+
Soil NO_3-N	0	-
Soil Enzyme Activity[a]	+	+
Soil Respiration	+	+

+ indicated positive response to presence of a cover crop; 0 indicated no response to the presence of a cover crop; - indicated a negative response to the presence of a cover crop

[a] β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase response to the presence of a cover crop was similar, so all enzyme activities are represented in the soil enzyme activity grouping

Table 5.2 Differences in response of microbial biomass carbon (C), nitrogen (N), and phosphorus (P), dissolved organic C, active C, autoclaved citrate extractable (ACE) protein, soil NH_4-N and NO_3-N, citrate extractable P, β-glucosidase, β-glucosaminidase, and acid and alkaline phosphatase, and soil respiration at spring (T0) and fall (T5) sampling.

Indicator	2021-Corn	2022-Soybean
Microbial Biomass C	Fall	Fall
Microbial Biomass N	-	-
Microbial Biomass P	Fall	Fall
Dissolved Organic C	-	-
Active C	Fall	Fall
ACE Protein	Fall	No Effect
Soil NH_4-N	Fall	-
Soil NO_3-N	-	-
Citrate-Extractable P	-	-
β-glucosidase	Fall	Spring
β-glucosaminidase	Fall	No Effect
Acid Phosphatase	Spring	Spring
Alkaline Phosphatase	Spring	Spring
Soil Respiration	Fall	Spring

Fall represents significantly greater response in the fall while Spring represents significantly greater response in the spring. – indicates no significant difference between spring and fall sampling, while No Effect represents no significant effect of sampling time on the soil health indicator.

Appendix A - 2021 KAW: Supplemental Material

Table A.1. Three-way analysis of variance (ANOVA) table for soil moisture, active carbon (C), dissolved organic C, and citrate-extractable phosphorus (P). Table abbreviations include block (BLOC), P fertilizer treatments (TRT), cover crop treatment (CC), and sampling time (TIME).

	Soil Moisture	Active C	Dissolved Organic C	Citrate-Extractable P
BLOC	0.0007	0.0643	0.0422	0.5018
TRT	0.3514	0.4505	0.8704	<.0001
CC	0.0045	0.0011	0.0408	0.5795
TRT*CC	0.0300	0.7790	0.3798	0.9231
TIME	<.0001	<.0001	<.0001	<.0001
TRT*TIME	0.6201	0.8530	0.5687	<.0001
CC*TIME	0.0867	0.4611	0.6532	0.8236
TRT*CC*TIME	0.3957	0.6430	0.2432	0.8765
Covariance Structure[a]	CSH	CSH	AR(1)	ARH(1)

[a]Covariance structure abbreviations: heterogeneous compound symmetry (CSH), autoregressive (AR(1)), and first-order autoregressive (ARH(1))

Table A.2. Three-way analysis of variance (ANOVA) table for autoclaved citrate extractable (ACE) protein, dissolved total nitrogen (N), NO_3-N, NH_4-N, inorganic N, and dissolved total N. Table abbreviations include block (BLOC), phosphorus (P) fertilizer treatments (TRT), cover crop treatments (CC), and sampling time (TIME).

	ACE Protein	Dissolved Total N	NH4-N	NO3-N	Inorganic N	Dissolved Organic N
BLOC	0.4705	0.6767	0.2212	0.1456	0.0276	0.0005
TRT	0.0364	<.0001	0.0009	0.0007	<.0001	<.0001
CC	0.0031	0.5297	0.9413	0.7736	0.6439	0.0714
TRT*CC	0.8462	0.1703	0.6098	0.1652	0.0949	0.2556
TIME	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
TRT*TIME	0.9555	0.0001	0.0424	<.0001	0.0001	0.0006
CC*TIME	0.3921	0.9386	0.6299	0.9274	0.8671	0.4873
TRT*CC*TIME	0.9970	0.6474	0.4132	0.2094	0.1773	0.7782
Covariance Structure[a]	CS	ARH(1)	ARH(1)	ARH(1)	ARH(1)	CSH

[a]Covariance structure abbreviations: compound symmetry (CS), first-order autoregressive (ARH(1)), and heterogenous compound symmetry

Table A.3. Three-way analysis of variance (ANOVA) table for microbial biomass carbon (C), nitrogen (N), phosphorus (P), biomass C:N, N:P, and C:P ratios and soil respiration. Table abbreviations include block (BLOC), P fertilizer treatments (TRT), cover crop treatments (CC), and sampling time (TIME).

	Microbial Biomass C	Microbial Biomass N	Microbial Biomass P	C:N Ratio	N:P Ratio	C:P Ratio	Soil Respiration
BLOC	0.0211	0.5968	0.7847	0.2339	0.6639	0.3549	0.0030
TRT	0.1924	0.7116	0.0009	0.4857	0.0048	0.0003	<.0001
CC	0.0119	0.3846	0.6928	0.7966	0.853	0.9649	<.0001
TRT*CC	0.0899	0.9412	0.6937	0.2642	0.9677	0.3338	0.9582
TIME	<.0001	0.0001	0.0009	0.1273	0.8783	0.5722	<.0001
TRT*TIME	0.5005	0.7896	0.4422	0.8521	0.7911	0.261	0.1557
CC*TIME	0.7189	0.7843	0.2573	0.2511	0.9085	0.3278	0.2054
TRT*CC*TIME	0.7154	0.9013	0.3473	0.8467	0.7703	0.0824	0.5819
Covariance Structure[a]	CS	CSH	CSH	ARH(1)	ARH(1)	CSH	AR(1)

[a]Covariance structure abbreviations: compound symmetry (CS), heterogeneous compound symmetry (CSH), and first-order autoregressive (ARH(1))

Table A.4. Three-way analysis of variance (ANOVA) table for acid phosphatase, alkaline phosphatase, β-glucosidase, and β-glucosaminidase. Table abbreviations include block (BLOC), phosphorus (P) fertilizer treatments (TRT), cover crop treatments (CC), and sampling time (TIME).

	Acid Phosphatase	Alkaline Phosphatase	Glucosidase	Glucosaminidase
BLOC	0.0035	0.2229	0.0738	0.0289
TRT	0.7848	0.0165	0.4064	0.5635
CC	0.0003	0.0003	0.0059	<.0001
TRT*CC	0.3057	0.0126	0.0974	0.6508
TIME	0.0001	<.0001	<.0001	<.0001
TRT*TIME	0.5391	0.5710	0.2148	0.8625
CC*TIME	0.0044	0.2937	0.0773	0.1665
TRT*CC*TIME	0.5827	0.5721	0.9630	0.9965
Covariance Structure[a]	CS	CSH	CSH	CS

[a]Covariance structure abbreviations: compound symmetry (CS) and heterogeneous compound symmetry (CSH)

Table A.5. Least squares (LS) means table for active carbon (C), dissolved organic C, and citrate-extractable phosphorus (P). Table abbreviations include block (BLOC), P fertilizer treatments (P TRT), build and maintain (BAM), no P (NP), sufficiency (SUF), presence of cover crop (CC), absence of cover crop (NC), and sampling time (TIME). Sampling times: 12-Apr (T0), 4-May (T1), 26-May (T2), 17-Jun (T3), 7-Jul (T4), and 17-Sept (T5).

Effect	P TRT	Cover	Time	Active C	Dissolved Organic C	Citrate-Extractable P
				mg C kg soil^{-1}	mg C kg soil^{-1}	mg P kg soil^{-1}
TRT	BAM			329.54	51.47	10.07
TRT	NP			320.90	50.05	0.87
TRT	SUF			336.64	49.42	2.46
CC		CC		350.58	53.22	4.29
CC		NC		307.48	47.41	4.65
TRT*CC	BAM	CC		347.57	57.26	9.82
TRT*CC	BAM	NC		311.50	45.68	10.32
TRT*CC	NP	CC		347.25	52.64	0.91
TRT*CC	NP	NC		294.56	47.45	0.84
TRT*CC	SUF	CC		356.92	49.75	2.13
TRT*CC	SUF	NC		316.37	49.09	2.79
TIME			T0	344.36	41.88	1.87
TIME			T1	282.37	55.56	2.13
TIME			T2	252.32	43.02	9.28
TIME			T3	365.15	-	5.55
TIME			T4	359.11	63.33	6.44
TIME			T5	370.86	47.78	1.54
TRT*TIME	BAM		T0	335.09	39.40	3.35
TRT*TIME	BAM		T1	282.26	58.35	4.56
TRT*TIME	BAM		T2	247.34	48.86	23.36
TRT*TIME	BAM		T3	381.57	-	12.66
TRT*TIME	BAM		T4	361.76	63.76	14.02
TRT*TIME	BAM		T5	369.21	46.99	2.48

TRT*TIME	NP		T0	337.93	44.40	0.44
TRT*TIME	NP		T1	277.04	58.65	0.37
TRT*TIME	NP		T2	243.48	39.86	1.39
TRT*TIME	NP		T3	343.26	-	1.00
TRT*TIME	NP		T4	347.52	59.86	1.10
TRT*TIME	NP		T5	376.19	47.47	0.94
TRT*TIME	SUF		T0	360.07	41.83	1.82
TRT*TIME	SUF		T1	287.80	49.67	1.48
TRT*TIME	SUF		T2	266.16	40.34	3.08
TRT*TIME	SUF		T3	370.61	-	2.99
TRT*TIME	SUF		T4	368.04	66.38	4.19
TRT*TIME	SUF		T5	367.17	48.88	1.19
CC*TIME		CC	T0	376.00	46.24	1.66
CC*TIME		CC	T1	304.23	59.48	2.57
CC*TIME		CC	T2	273.38	47.22	8.16
CC*TIME		CC	T3	376.93	-	6.30
CC*TIME		CC	T4	390.85	63.38	5.80
CC*TIME		CC	T5	382.08	49.77	1.23
CC*TIME		NC	T0	312.73	37.51	2.08
CC*TIME		NC	T1	260.51	51.64	1.70
CC*TIME		NC	T2	231.27	38.82	10.40
CC*TIME		NC	T3	353.36	-	4.80
CC*TIME		NC	T4	327.37	63.29	7.07
CC*TIME		NC	T5	359.63	45.79	1.85
TRT*CC*TIME	BAM	CC	T0	354.43	42.73	2.82
TRT*CC*TIME	BAM	CC	T1	302.15	69.98	5.56
TRT*CC*TIME	BAM	CC	T2	265.76	53.97	20.40
TRT*CC*TIME	BAM	CC	T3	397.40	-	15.29
TRT*CC*TIME	BAM	CC	T4	395.59	72.01	13.14

TRT*CC*TIME	BAM	CC	T5	370.10	47.61	1.72
TRT*CC*TIME	BAM	NC	T0	315.75	36.06	3.88
TRT*CC*TIME	BAM	NC	T1	262.37	46.71	3.55
TRT*CC*TIME	BAM	NC	T2	228.91	43.74	26.31
TRT*CC*TIME	BAM	NC	T3	365.75	-	10.02
TRT*CC*TIME	BAM	NC	T4	327.92	55.51	14.89
TRT*CC*TIME	BAM	NC	T5	368.32	46.36	3.24
TRT*CC*TIME	NP	CC	T0	373.48	49.27	0.50
TRT*CC*TIME	NP	CC	T1	313.27	56.93	0.45
TRT*CC*TIME	NP	CC	T2	262.25	44.09	1.50
TRT*CC*TIME	NP	CC	T3	361.39	-	1.00
TRT*CC*TIME	NP	CC	T4	384.58	57.03	1.08
TRT*CC*TIME	NP	CC	T5	388.52	55.90	0.92
TRT*CC*TIME	NP	NC	T0	302.37	39.54	0.39
TRT*CC*TIME	NP	NC	T1	240.82	60.37	0.29
TRT*CC*TIME	NP	NC	T2	224.71	35.62	1.28
TRT*CC*TIME	NP	NC	T3	325.14	-	1.00
TRT*CC*TIME	NP	NC	T4	310.47	62.70	1.11
TRT*CC*TIME	NP	NC	T5	363.87	39.05	0.96
TRT*CC*TIME	SUF	CC	T0	400.08	46.72	1.66
TRT*CC*TIME	SUF	CC	T1	297.27	51.52	1.70
TRT*CC*TIME	SUF	CC	T2	292.13	43.60	2.56
TRT*CC*TIME	SUF	CC	T3	372.02	-	2.62
TRT*CC*TIME	SUF	CC	T4	392.37	61.11	3.19
TRT*CC*TIME	SUF	CC	T5	387.63	45.81	1.03
TRT*CC*TIME	SUF	NC	T0	320.07	36.93	1.97
TRT*CC*TIME	SUF	NC	T1	278.33	47.83	1.25
TRT*CC*TIME	SUF	NC	T2	240.18	37.09	3.60
TRT*CC*TIME	SUF	NC	T3	369.20	-	3.37

TRT*CC*TIME	SUF	NC	T4	343.72	71.65	5.20
TRT*CC*TIME	SUF	NC	T5	346.70	51.95	1.35

Table A.6. Least squares (LS) means table for autoclaved citrate extractable (ACE) protein, dissolved total nitrogen (N), NO_3-N, NH_4-N, inorganic N, and dissolved total N. Table abbreviations include block (BLOC), phosphorus (P) fertilizer treatments (P TRT), build and maintain (BAM), no P (NP), sufficiency (SUF), presence of cover crop (CC), absence of cover crop (NC), and sampling time (TIME). Sampling times: 12-Apr (T0), 4-May (T1), 26-May (T2), 17-Jun (T3), 7-Jul (T4), and 17-Sept (T5).

Effect	P TRT	Cover	Time	ACE Protein	Dissolved Total N	Inorganic N	NH4-N	NO3-N	Dissolved Organic N
				mg protein kg soil^{-1}	mg N kg soil^{-1}		mg NH4-N kg soil^{-1}	mg NO3-N kg soil^{-1}	mg N kg soil^{-1}
TRT	BAM			4338.74	82.4	76.8	28.04	49.91	12.15
TRT	NP			3956.13	56.45	43.55	9.82	34.34	15.81
TRT	SUF			4330.4	56.08	47.6	14.74	33.28	12.9
CC		CC		4442.81	66.44	57.03	17.41	39.66	14.46
CC		NC		3974.04	63.51	54.94	17.66	38.69	12.78
TRT*CC	BAM	CC		4621.42	86.04	80.68	29.39	51.29	12.11
TRT*CC	BAM	NC		4056.06	78.75	72.93	26.69	48.54	12.18
TRT*CC	NP	CC		4131.95	61.98	48.84	10.42	38.34	17.49
TRT*CC	NP	NC		3780.31	50.93	38.26	9.22	30.35	14.12
TRT*CC	SUF	CC		4575.06	51.31	41.57	12.41	29.36	13.77
TRT*CC	SUF	NC		4085.73	60.85	53.64	17.07	37.2	12.03
TIME			T0	3995.21	12.11	6.35	2.78	3.56	6.34
TIME			T1	3521.52	73.63	72.04	30.37	42.88	-
TIME			T2	3804.72	82.67	70.93	18.49	55.61	14.6
TIME			T3	4917.07	137.32	116.28	24.33	91.95	21.03
TIME			T4	4596.16	70.31	58.37	20.9	37.47	12.49
TIME			T5	4415.86	13.84	11.94	8.34	3.6	-
TRT*TIME	BAM		T0	4010.16	11.92	8.54	4.7	3.83	3.38
TRT*TIME	BAM		T1	3683.95	81.35	87.66	43.28	44.39	-
TRT*TIME	BAM		T2	3802.34	128.98	129.16	46.87	89.17	8.43
TRT*TIME	BAM		T3	5221.59	186.44	159.48	38.3	121.18	26.97
TRT*TIME	BAM		T4	4717.67	70.7	60.9	25.02	35.88	9.8
TRT*TIME	BAM		T5	4596.74	15	15.1	10.09	5.01	-

TRT*TIME	NP		T0	3821.79	12.42	4.28	0.94	3.32	9.26
TRT*TIME	NP		T1	3337.65	55	43.39	15.03	31.99	-
TRT*TIME	NP		T2	3625.65	59.74	40.14	2.73	37.5	19.6
TRT*TIME	NP		T3	4428.88	121.69	101.62	16.76	84.86	20.06
TRT*TIME	NP		T4	4367.14	73.84	61.19	17.74	43.45	14.3
TRT*TIME	NP		T5	4155.66	16.03	10.68	5.73	4.95	-
TRT*TIME	SUF		T0	4153.66	11.98	6.23	2.69	3.54	6.38
TRT*TIME	SUF		T1	3542.95	84.53	85.07	32.81	52.26	-
TRT*TIME	SUF		T2	3986.16	59.28	43.5	5.86	40.15	15.78
TRT*TIME	SUF		T3	5100.75	103.82	87.75	17.93	69.82	16.06
TRT*TIME	SUF		T4	4703.67	66.38	53.01	19.94	33.07	13.37
TRT*TIME	SUF		T5	4495.18	10.51	10.05	9.21	0.84	-
CC*TIME		CC	T0	4237.08	13.89	6.82	3.23	3.57	7.82
CC*TIME		CC	T1	3613.28	81.75	81.52	35.73	45.78	-
CC*TIME		CC	T2	3972.15	85.51	71.25	13.9	57.63	16.11
CC*TIME		CC	T3	5181.11	132.7	111.78	23.38	88.4	20.92
CC*TIME		CC	T4	5041.91	69.45	57.56	19.12	38.44	12.98
CC*TIME		CC	T5	4611.32	15.35	13.23	9.08	4.15	-
CC*TIME		NC	T0	3753.33	10.32	5.88	2.32	3.55	4.86
CC*TIME		NC	T1	3429.76	65.5	62.56	25.01	39.98	-
CC*TIME		NC	T2	3637.28	79.82	70.61	23.08	53.59	13.1
CC*TIME		NC	T3	4653.04	141.93	120.78	25.27	95.51	21.15
CC*TIME		NC	T4	4150.4	71.17	59.17	22.68	36.49	12
CC*TIME		NC	T5	4220.4	12.33	10.66	7.61	3.05	-
TRT*CC*TIME	BAM	CC	T0	4287.01	11.11	6.99	4.68	2.31	4.12
TRT*CC*TIME	BAM	CC	T1	3846.04	108.29	118.53	62.67	55.86	-
TRT*CC*TIME	BAM	CC	T2	3882.27	128.52	126.62	34.23	92.39	7.46
TRT*CC*TIME	BAM	CC	T3	5560.04	184.99	158	38	120	27
TRT*CC*TIME	BAM	CC	T4	5232.71	65.92	56.04	25.58	30.46	9.87
TRT*CC*TIME	BAM	CC	T5	4920.45	17.4	17.88	11.19	6.69	-

TRT*CC*TIME	BAM	NC	T0	3733.32	12.72	10.08	4.73	5.36	2.64
TRT*CC*TIME	BAM	NC	T1	3521.86	54.4	56.79	23.88	32.91	-
TRT*CC*TIME	BAM	NC	T2	3722.41	129.44	131.69	59.5	85.95	9.41
TRT*CC*TIME	BAM	NC	T3	4883.15	187.89	160.96	38.59	122.36	26.94
TRT*CC*TIME	BAM	NC	T4	4202.62	75.48	65.75	24.45	41.29	9.72
TRT*CC*TIME	BAM	NC	T5	4273.04	12.6	12.31	8.98	3.34	-
TRT*CC*TIME	NP	CC	T0	4035.15	14.76	6.2	0.91	5.23	10.81
TRT*CC*TIME	NP	CC	T1	3400.31	56.31	48.06	15.69	32.37	-
TRT*CC*TIME	NP	CC	T2	3826.4	64.96	42.83	3.38	39.04	22.12
TRT*CC*TIME	NP	CC	T3	4559.52	136.54	114.95	17.87	97.08	21.59
TRT*CC*TIME	NP	CC	T4	4782.48	82.15	70	17.92	52.08	15.43
TRT*CC*TIME	NP	CC	T5	4187.85	17.14	10.99	6.75	4.24	-
TRT*CC*TIME	NP	NC	T0	3608.43	10.07	2.37	0.96	1.41	7.71
TRT*CC*TIME	NP	NC	T1	3275	53.7	38.71	14.36	31.61	-
TRT*CC*TIME	NP	NC	T2	3424.9	54.51	37.45	2.08	35.96	17.08
TRT*CC*TIME	NP	NC	T3	4298.25	106.83	88.28	15.64	72.64	18.54
TRT*CC*TIME	NP	NC	T4	3951.8	65.53	52.38	17.56	34.82	13.17
TRT*CC*TIME	NP	NC	T5	4123.48	14.91	10.37	4.71	5.66	-
TRT*CC*TIME	SUF	CC	T0	4389.09	15.8	7.28	4.11	3.17	8.53
TRT*CC*TIME	SUF	CC	T1	3593.48	80.67	77.96	28.84	49.11	-
TRT*CC*TIME	SUF	CC	T2	4207.79	63.05	44.3	4.08	41.46	18.75
TRT*CC*TIME	SUF	CC	T3	5423.77	76.56	62.4	14.27	48.12	14.16
TRT*CC*TIME	SUF	CC	T4	5110.55	60.27	46.64	13.86	32.78	13.64
TRT*CC*TIME	SUF	CC	T5	4725.67	11.52	10.81	9.29	1.52	-
TRT*CC*TIME	SUF	NC	T0	3918.24	8.16	5.18	1.27	3.9	4.23
TRT*CC*TIME	SUF	NC	T1	3492.42	88.39	92.19	36.77	55.42	-
TRT*CC*TIME	SUF	NC	T2	3764.53	55.51	42.7	7.65	38.84	12.8
TRT*CC*TIME	SUF	NC	T3	4777.73	131.08	113.09	21.58	91.51	17.97
TRT*CC*TIME	SUF	NC	T4	4296.8	72.49	59.38	26.02	33.36	13.1
TRT*CC*TIME	SUF	NC	T5	4264.69	9.49	9.29	9.13	0.16	-

Table A.7. Least squares (LS) means table for microbial biomass carbon (C), nitrogen (N), phosphorus (P), biomass C:N, N:P, and C:P ratios and soil respiration. Table abbreviations include block (BLOC), P fertilizer treatments (P TRT), build and maintain (BAM), no P (NP), sufficiency (SUF), presence of cover crop (CC), absence of cover crop (NC), and sampling time (TIME). Sampling times: 12-Apr (T0), 4-May (T1), 26-May (T2), 17-Jun (T3), 7-Jul (T4), and 17-Sept (T5).

Effect	P TRT	Cover	Time	Microbial Biomass C	Microbial Biomass N	Microbial Biomass P	C:N	N:P	C:P	Soil Respiration
				ug C g soil^{-1}	ug N g soil^{-1}	ug P g soil^{-1}				mg CO2 g soil^{-1}
TRT	BAM			104.67	12.06	2.98	10.28	7.83	71.44	15.1
TRT	NP			94.05	14.86	0.46	10.12	21.38	202.87	13.51
TRT	SUF			108.09	13.03	1.51	14.77	9.02	111.96	15.29
CC		CC		111.65	14.58	1.59	12.49	11.24	123.29	16.16
CC		NC		92.89	12.05	1.71	10.95	14.25	134.22	13.11
TRT*CC	BAM	CC		104.24	12.84	2.74	8.72	3.99	33.23	16.84
TRT*CC	BAM	NC		105.1	11.27	3.21	11.84	11.68	109.66	13.37
TRT*CC	NP	CC		104.81	15.96	0.4	10.66	20.76	253.53	14.78
TRT*CC	NP	NC		83.29	13.77	0.52	9.57	22	152.21	12.24
TRT*CC	SUF	CC		125.91	14.95	1.62	18.09	8.98	83.12	16.86
TRT*CC	SUF	NC		90.27	11.11	1.41	11.45	9.06	140.8	13.73
TIME			T0	116.33	17.57	0.97	7.73	-	-	14.56
TIME			T1	55.16	6.63	1.47	10.1	8.4	140.14	15.26
TIME			T2	95.82	11.8	1.55	10.35	11.54	116.04	10.9
TIME			T3	-	-	1.52	-	-	-	11.55
TIME			T4	106.98	14.19	2.02	20.37	19.33	169.54	13.95
TIME			T5	137.06	16.39	2.37	10.06	11.71	89.31	21.59
TRT*TIME	BAM		T0	126.4	18.22	1.24	7.57	-	-	14.05
TRT*TIME	BAM		T1	44.61	6.48	2.56	9.15	5.7	49.38	14.75
TRT*TIME	BAM		T2	106.26	9.65	2.89	12.04	3.2	32.56	11.59
TRT*TIME	BAM		T3	-	-	3.37	-	-	-	12.81
TRT*TIME	BAM		T4	111.28	12.03	3.69	12.7	19.05	170.48	14.32
TRT*TIME	BAM		T5	134.81	13.91	4.11	9.94	3.39	33.35	23.11
TRT*TIME	NP		T0	106.55	16.26	0.3	8.62	-	-	13.76
TRT*TIME	NP		T1	53.49	6.97	0.29	9.04	10.36	287.37	13.66
TRT*TIME	NP		T2	82.32	10.97	0.69	8.36	17.32	127.76	9.89
TRT*TIME	NP		T3	-	-	0.21	-	-	-	9.64
TRT*TIME	NP		T4	100.17	21.99	0.5	15.13	34	225.35	13.6
TRT*TIME	NP		T5	127.74	18.11	0.78	9.44	23.85	171	20.49
TRT*TIME	SUF		T0	116.03	18.23	1.38	7.01	-	-	15.87
TRT*TIME	SUF		T1	67.39	6.45	1.56	12.13	9.13	83.67	17.36
TRT*TIME	SUF		T2	98.89	14.8	1.06	10.66	14.12	187.79	11.21
TRT*TIME	SUF		T3	-	-	0.99	-	-	-	12.22
TRT*TIME	SUF		T4	109.5	8.55	1.87	33.27	4.95	112.8	13.93
TRT*TIME	SUF		T5	148.64	17.14	2.21	10.79	7.89	63.59	21.16
CC*TIME		CC	T0	125.74	17.44	0.78	9.29	-	-	15.94
CC*TIME		CC	T1	61.45	7.7	1.06	10.03	8.84	182.38	17.76
CC*TIME		CC	T2	101.41	13.24	1.53	8.51	12.32	86.15	11.66
CC*TIME		CC	T3	-	-	1.11	-	-	-	13.15
CC*TIME		CC	T4	120.25	14.76	2.63	25.23	11.19	135.96	15.7
CC*TIME		CC	T5	149.42	19.78	2.41	9.4	12.63	88.68	22.73
CC*TIME		NC	T0	106.92	17.7	1.17	6.17	-	-	13.18

CC*TIME		NC	T1	48.87	5.57	1.88	10.18	7.95	97.9	12.75
CC*TIME		NC	T2	90.24	10.36	1.57	12.2	10.77	145.93	10.13
CC*TIME		NC	T3	-	-	1.93	-	-	-	9.95
CC*TIME		NC	T4	93.71	13.62	1.41	15.51	27.48	203.12	12.21
CC*TIME		NC	T5	124.7	13	2.32	10.71	10.79	89.95	20.45
TRT*CC*TIME	BAM	CC	T0	133.14	19.49	0.94	8.1	-	-	15.4
TRT*CC*TIME	BAM	CC	T1	33.05	6.56	1.35	6.22	5.95	45.78	17.29
TRT*CC*TIME	BAM	CC	T2	107.03	12.03	2.34	9.11	3.87	30.87	12.24
TRT*CC*TIME	BAM	CC	T3	-	-	2.36	-	-	-	14.54
TRT*CC*TIME	BAM	CC	T4	112.22	11.69	5.28	10.73	2.65	23.45	15.81
TRT*CC*TIME	BAM	CC	T5	135.77	14.42	4.18	9.43	3.48	32.81	25.77
TRT*CC*TIME	BAM	NC	T0	119.66	16.95	1.54	7.04	-	-	12.7
TRT*CC*TIME	BAM	NC	T1	56.16	6.41	3.78	12.07	5.45	52.98	12.21
TRT*CC*TIME	BAM	NC	T2	105.5	7.26	3.45	14.96	2.53	34.25	10.94
TRT*CC*TIME	BAM	NC	T3	-	-	4.38	-	-	-	11.08
TRT*CC*TIME	BAM	NC	T4	110.33	12.36	2.09	14.67	35.44	317.51	12.83
TRT*CC*TIME	BAM	NC	T5	133.85	13.4	4.03	10.45	3.3	33.89	20.44
TRT*CC*TIME	NP	CC	T0	112.18	14.57	0.07	11.46	-	-	14.19
TRT*CC*TIME	NP	CC	T1	71.89	9.98	0.16	8.69	13.3	430.9	15.2
TRT*CC*TIME	NP	CC	T2	82.13	11.15	0.56	8.9	19.93	147.66	10.69
TRT*CC*TIME	NP	CC	T3	-	-	0.21	-	-	-	11.42
TRT*CC*TIME	NP	CC	T4	114.51	21.42	0.47	13.72	25.64	282.9	15.86
TRT*CC*TIME	NP	CC	T5	143.34	22.66	0.94	10.52	24.18	152.68	21.3
TRT*CC*TIME	NP	NC	T0	100.93	17.95	0.53	5.78	-	-	13.33
TRT*CC*TIME	NP	NC	T1	35.08	3.96	0.42	9.39	7.42	143.85	12.12
TRT*CC*TIME	NP	NC	T2	82.5	10.79	0.82	7.82	14.7	107.86	9.09
TRT*CC*TIME	NP	NC	T3	-	-	0.2	-	-	-	7.86
TRT*CC*TIME	NP	NC	T4	85.83	22.57	0.54	16.53	42.35	167.81	11.34
TRT*CC*TIME	NP	NC	T5	112.14	13.56	0.63	8.36	23.52	189.32	19.69
TRT*CC*TIME	SUF	CC	T0	131.89	18.25	1.34	8.32	-	-	18.23
TRT*CC*TIME	SUF	CC	T1	79.4	6.55	1.66	15.16	7.27	70.47	20.8
TRT*CC*TIME	SUF	CC	T2	115.06	16.55	1.68	7.5	13.14	79.91	12.05
TRT*CC*TIME	SUF	CC	T3	-	-	0.77	-	-	-	13.51
TRT*CC*TIME	SUF	CC	T4	134.01	11.16	2.15	51.22	5.27	101.55	15.42
TRT*CC*TIME	SUF	CC	T5	169.16	22.25	2.11	8.25	10.24	80.55	21.12
TRT*CC*TIME	SUF	NC	T0	100.17	18.21	1.43	5.69	-	-	13.51
TRT*CC*TIME	SUF	NC	T1	55.37	6.34	1.46	9.09	10.98	96.87	13.92
TRT*CC*TIME	SUF	NC	T2	82.72	13.04	0.45	13.81	15.09	295.68	10.36
TRT*CC*TIME	SUF	NC	T3	-	-	1.2	-	-	-	10.92
TRT*CC*TIME	SUF	NC	T4	84.98	5.94	1.59	15.31	4.63	124.05	12.45
TRT*CC*TIME	SUF	NC	T5	128.12	12.03	2.31	13.33	5.55	46.62	21.20

Table A.8. Least squares (LS) means table for acid phosphatase, alkaline phosphatase, β-glucosidase, and β-glucosaminidase. Table abbreviations include block (BLOC), phosphorus (P) fertilizer treatments (P TRT), build and maintain (BAM), no P (NP), sufficiency (SUF), presence of cover crop (CC), absence of cover crop (NC), and sampling time (TIME). Sampling times: 12-Apr (T0), 4-May (T1), 26-May (T2), 17-Jun (T3), 7-Jul (T4), and 17-Sept (T5).

Effect	P TRT	Cover	Time	Acid Phosphatase	Alkaline Phosphatase	Glucosidase	Glucosaminidase
				mg p-nitrophenol kg soil^{-1} hr^{-1}			
TRT	BAM			289.21	89.71	116.47	33.86
TRT	NP			291.27	105.79	112.27	35.31
TRT	SUF			299.22	91.09	122.52	35.56
CC		CC		326.14	106.21	128.15	40.58
CC		NC		260.31	84.84	106.02	29.25
TRT*CC	BAM	CC		308.3	90.94	117.23	38.62
TRT*CC	BAM	NC		270.11	88.48	115.71	29.11
TRT*CC	NP	CC		333.77	125.66	130.42	41.37
TRT*CC	NP	NC		248.76	85.92	94.12	29.25
TRT*CC	SUF	CC		336.37	102.05	136.82	41.74
TRT*CC	SUF	NC		262.07	80.12	108.22	29.38
TIME			T0	308.41	104.28	113.44	36.99
TIME			T1	290.02	92.22	97.02	33.32
TIME			T2	276.78	78.37	98.35	27.94
TIME			T3	276.98	95.53	124.79	34.66
TIME			T4	305.68	92.92	131.06	34.74
TIME			T5	301.5	109.83	137.86	41.83
TRT*TIME	BAM		T0	308.98	99.34	106	33.99
TRT*TIME	BAM		T1	292.8	88.93	101.49	33.21
TRT*TIME	BAM		T2	262.44	65.81	90.01	26.53
TRT*TIME	BAM		T3	262.27	95.68	138.1	33.5
TRT*TIME	BAM		T4	303.99	81.36	125.96	34.39
TRT*TIME	BAM		T5	304.75	107.13	137.27	41.58
TRT*TIME	NP		T0	305.9	115.32	109.59	39.03
TRT*TIME	NP		T1	290.39	99.97	89.45	33.11
TRT*TIME	NP		T2	280.73	95.88	100.02	28.7
TRT*TIME	NP		T3	279.35	104.24	108.57	33.66
TRT*TIME	NP		T4	291.56	107.2	135.75	35.94
TRT*TIME	NP		T5	299.65	112.12	130.23	41.44
TRT*TIME	SUF		T0	310.36	98.19	124.74	37.94
TRT*TIME	SUF		T1	286.88	87.77	100.13	33.64
TRT*TIME	SUF		T2	287.18	73.43	105.02	28.61
TRT*TIME	SUF		T3	289.31	86.66	127.69	36.81
TRT*TIME	SUF		T4	321.47	90.22	131.47	33.89

TRT*TIME	SUF		T5	300.09	110.25	146.06	42.49
CC*TIME		CC	T0	347.99	119.94	133.83	45.31
CC*TIME		CC	T1	311.57	103.81	104.79	38.39
CC*TIME		CC	T2	303.64	86.37	110.67	32.76
CC*TIME		CC	T3	309.39	104.44	130.47	39.67
CC*TIME		CC	T4	357.28	103.51	146.05	40.13
CC*TIME		CC	T5	326.99	119.21	143.11	47.22
CC*TIME		NC	T0	268.84	88.62	93.04	28.66
CC*TIME		NC	T1	268.48	80.64	89.25	28.26
CC*TIME		NC	T2	249.93	70.38	86.03	23.13
CC*TIME		NC	T3	244.56	86.61	119.1	29.64
CC*TIME		NC	T4	254.07	82.33	116.06	29.35
CC*TIME		NC	T5	276.01	100.46	132.6	36.45
TRT*CC*TIME	BAM	CC	T0	332.87	100.17	116.3	41.19
TRT*CC*TIME	BAM	CC	T1	303.01	90.05	101.72	37.16
TRT*CC*TIME	BAM	CC	T2	267.22	64.15	93.66	30.8
TRT*CC*TIME	BAM	CC	T3	276.02	94.94	129.32	37.1
TRT*CC*TIME	BAM	CC	T4	351.79	82.61	132.73	40.04
TRT*CC*TIME	BAM	CC	T5	318.88	113.69	129.63	45.44
TRT*CC*TIME	BAM	NC	T0	285.09	98.5	95.69	26.79
TRT*CC*TIME	BAM	NC	T1	282.59	87.82	101.26	29.25
TRT*CC*TIME	BAM	NC	T2	257.67	67.47	86.35	22.26
TRT*CC*TIME	BAM	NC	T3	248.52	96.42	146.88	29.89
TRT*CC*TIME	BAM	NC	T4	256.2	80.1	119.19	28.73
TRT*CC*TIME	BAM	NC	T5	290.62	100.57	144.91	37.71
TRT*CC*TIME	NP	CC	T0	347.88	143.01	135.78	48.67
TRT*CC*TIME	NP	CC	T1	325.72	123.13	104.53	38.61
TRT*CC*TIME	NP	CC	T2	320.4	110.91	118.03	33.44
TRT*CC*TIME	NP	CC	T3	325.22	127.2	130.4	39.23
TRT*CC*TIME	NP	CC	T4	357.55	123.03	158.12	41.48
TRT*CC*TIME	NP	CC	T5	325.87	126.66	135.64	46.8
TRT*CC*TIME	NP	NC	T0	263.93	87.62	83.39	29.39
TRT*CC*TIME	NP	NC	T1	255.06	76.82	74.37	27.62
TRT*CC*TIME	NP	NC	T2	241.07	80.85	82	23.95
TRT*CC*TIME	NP	NC	T3	233.48	81.27	86.74	28.08
TRT*CC*TIME	NP	NC	T4	225.58	91.37	113.38	30.4
TRT*CC*TIME	NP	NC	T5	273.44	97.59	124.83	36.07
TRT*CC*TIME	SUF	CC	T0	363.24	116.64	149.42	46.08
TRT*CC*TIME	SUF	CC	T1	305.98	98.25	108.13	39.39
TRT*CC*TIME	SUF	CC	T2	323.3	84.04	120.3	34.04
TRT*CC*TIME	SUF	CC	T3	326.94	91.17	131.69	42.67
TRT*CC*TIME	SUF	CC	T4	362.51	104.9	147.31	38.86

TRT*CC*TIME	SUF	CC	T5	336.21	117.28	164.04	49.41
TRT*CC*TIME	SUF	NC	T0	257.48	79.75	100.05	29.8
TRT*CC*TIME	SUF	NC	T1	267.78	77.28	92.13	27.9
TRT*CC*TIME	SUF	NC	T2	251.05	62.82	89.73	23.17
TRT*CC*TIME	SUF	NC	T3	251.68	82.15	123.68	30.95
TRT*CC*TIME	SUF	NC	T4	280.44	75.53	115.62	28.92
TRT*CC*TIME	SUF	NC	T4	263.98	103.21	128.08	35.57

Appendix B - 2022 KAW: Supplemental Data

Table B.1. Three-way analysis of variance (ANOVA) table for soil moisture, active carbon (C), dissolved organic C, and citrate-extractable phosphorus (P). Table abbreviations include block (BLOC), P fertilizer treatments (TRT), cover crop treatments (CC), and sampling time (TIME)

	Soil Moisture	Active C	Dissolved Organic C	Citrate-Extractable P
BLOC	0.0008	0.0144	0.1300	0.3702
TRT	0.1185	0.0787	0.5273	<.0001
CC	0.2231	0.0071	0.8952	0.0708
TRT*CC	0.6760	0.6084	0.4561	0.3149
TIME	<.0001	<.0001	<.0001	<.0001
TRT*TIME	0.3177	0.0930	0.1358	0.0023
CC*TIME	<.0001	0.6514	0.3780	0.0619
TRT*CC*TIME	0.4642	0.8876	0.1883	0.5363
Covariance Structure[a]	CS	CS	CS	CSH

[a]Covariance structure abbreviations: compound symmetry (CS) and heterogeneous compound symmetry (CSH)

Table B.2. Three-way analysis of variance (ANOVA) table for autoclaved citrate extractable (ACE) protein, dissolved total nitrogen (N), NO_3-N, NH_4-N, inorganic N, and dissolved total N. Table abbreviations include block (BLOC), phosphorus (P) fertilizer treatments (TRT), cover crop treatment (CC), and sampling time (TIME).

	ACE Protein	Dissolved Total N	NH4-N	NO3-N	Inorganic N	Dissolved Organic N
BLOC	0.7086	0.0761	0.2198	0.2372	0.3121	0.7103
TRT	0.0094	0.0175	0.0008	0.0002	<.0001	0.0625
CC	0.0016	0.0342	0.6895	0.0287	0.0147	0.9003
TRT*CC	0.2078	0.0142	0.1147	0.0749	0.2729	0.7720
TIME	0.0811	<.0001	<.0001	<.0001	<.0001	0.0437
TRT*TIME	0.6583	<.0001	0.0338	0.0011	0.0001	0.0108
CC*TIME	0.3145	0.0224	0.9578	0.0117	0.1164	0.2753
TRT*CC*TIME	0.6932	0.6206	0.4249	0.0843	0.0784	0.4138
Covariance Structure[a]	CS	CS	ARH(1)	ARH(1)	ARH(1)	ARH(1)

[a]Covariance structure abbreviations: compound symmetry (CS) and first-order autoregressive (ARH(1))

Table B.3. Three-way analysis of variance (ANOVA) table for microbial biomass carbon (C), nitrogen (N), phosphorus (P), biomass C:N, ratios and soil respiration. Table abbreviations include block ((BLOC), P fertilizer treatments (TRT), cover crop treatment (CC), and sampling time (TIME). Bolded values represent statistical significance at p < 0.05

	Microbial Biomass C	Microbial Biomass N	Microbial Biomass P	C:N Ratio	Soil Respiration
BLOC	0.0116	0.7793	0.2015	0.0311	0.2380
TRT	0.8499	0.7562	<.0001	0.1644	0.7923
CC	0.0008	0.0354	0.6763	0.1365	<.0001
TRT*CC	0.2064	0.5804	0.9392	0.7057	<.0001
TIME	<.0001	0.0140	<.0001	0.0394	0.0487
TRT*TIME	0.7969	0.1549	0.1913	0.2620	0.9818
CC*TIME	0.6618	0.0238	0.1168	0.5646	0.3274
TRT*CC*TIME	0.5610	0.7569	0.6693	0.4858	0.3726
Covariance Structure[a]	ARH(1)	ARH(1)	CS	CSH	ARH(1)

[a]Covariance structure abbreviations: first-order autoregressive (ARH(1)), compound symmetry (CS), and heterogeneous compound symmetry (CSH)

Table B.4. Three-way analysis of variance (ANOVA) table for acid phosphatase, alkaline phosphatase, β-glucosidase, and β-glucosaminidase. Table abbreviations include block (BLOC), phosphorus (P) fertilizer treatments (TRT), cover crop treatment (CC), and sampling time (TIME). Bolded values represent statistical significance at p < 0.05

	Acid Phosphatase	Alkaline Phosphatase	Glucosidase	Glucosaminidase
BLOC	0.0048	0.0278	0.5850	0.0458
TRT	0.0794	0.1524	0.2304	0.8485
CC	<.0001	0.0002	0.0016	<.0001
TRT*CC	0.1134	0.0498	0.0203	0.1210
TIME	<.0001	<.0001	<.0001	0.2055
TRT*TIME	0.7411	0.7376	0.6477	0.1815
CC*TIME	0.3036	0.9127	0.2054	0.9497
TRT*CC*TIME	0.7871	0.7306	0.1402	0.4652
Covariance Structure[a]	CS	AR(1)	CS	CS

[a]Covariance structure abbreviations: compound symmetry (CS) and autoregressive (AR(1))

Table B.5. Least squares (LS) means table for active carbon (C), dissolved organic C, and citrate-extractable phosphorus (P). Table abbreviations include block (BLOC), P fertilizer treatments (P TRT), build and maintain (BAM), no P (NP), sufficiency (SUF), presence of cover crop (CC), absence of cover crop (NC), and sampling time (TIME). Sampling times: 27-Apr (T0), 23-May (T1), 15-Jun (T2), 8-Jul (T3), 5-Aug (T4), and 21-Oct (T5).

Effect	P TRT	Cover	Time	Active C	Dissolved Organic C	Citrate-Extractable P
				mg C kg soil^{-1}	mg C kg soil^{-1}	mg P kg soil^{-1}
TRT	BAM			218.36	43.20	2.76
TRT	NP			211.31	46.07	1.06
TRT	SUF			233.06	44.56	1.53
CC		CC		232.79	44.47	1.72
CC		NC		209.03	44.74	1.85
TRT*CC	BAM	CC		226.35	44.27	2.68
TRT*CC	BAM	NC		210.37	42.13	2.84
TRT*CC	NP	CC		222.28	44.11	1.06
TRT*CC	NP	NC		200.33	48.02	1.06
TRT*CC	SUF	CC		249.73	45.04	1.42
TRT*CC	SUF	NC		216.40	44.08	1.65
TIME			T0	226.21		1.89
TIME			T1	235.76	47.36	1.71
TIME			T2	188.09	37.90	1.26
TIME			T3	185.54	41.47	2.17
TIME			T4	214.43	51.85	1.81
TIME			T5	275.41	44.46	1.87
TRT*TIME	BAM		T0	225.16		2.75
TRT*TIME	BAM		T1	245.67	44.37	2.32
TRT*TIME	BAM		T2	176.95	34.53	2.06
TRT*TIME	BAM		T3	173.09	46.14	3.72
TRT*TIME	BAM		T4	228.94	46.26	2.72
TRT*TIME	BAM		T5	260.33	44.70	3.00

TRT*TIME	NP		T0	225.23		1.15
TRT*TIME	NP		T1	204.76	50.83	1.03
TRT*TIME	NP		T2	180.94	40.76	0.76
TRT*TIME	NP		T3	201.21	38.71	1.12
TRT*TIME	NP		T4	202.28	54.50	1.16
TRT*TIME	NP		T5	253.42	45.55	1.16
TRT*TIME	SUF		T0	228.25		1.77
TRT*TIME	SUF		T1	256.86	46.87	1.79
TRT*TIME	SUF		T2	206.39	38.42	0.97
TRT*TIME	SUF		T3	182.33	39.57	1.67
TRT*TIME	SUF		T4	212.07	54.80	1.55
TRT*TIME	SUF		T5	312.49	43.14	1.45
CC*TIME		CC	T0	248.75		1.99
CC*TIME		CC	T1	248.53	46.70	1.59
CC*TIME		CC	T2	196.54	37.42	1.07
CC*TIME		CC	T3	193.98	39.87	2.10
CC*TIME		CC	T4	229.80	54.61	1.74
CC*TIME		CC	T5	279.11	43.75	1.83
CC*TIME		NC	T0	203.67		1.79
CC*TIME		NC	T1	222.99	48.01	1.83
CC*TIME		NC	T2	179.64	38.38	1.45
CC*TIME		NC	T3	177.10	43.07	2.23
CC*TIME		NC	T4	199.06	49.09	1.88
CC*TIME		NC	T5	271.71	45.17	1.90
TRT*CC*TIME	BAM	CC	T0	235.02		2.95
TRT*CC*TIME	BAM	CC	T1	254.08	41.98	2.20
TRT*CC*TIME	BAM	CC	T2	178.26	39.41	1.60
TRT*CC*TIME	BAM	CC	T3	172.97	47.70	3.74
TRT*CC*TIME	BAM	CC	T4	241.71	47.51	2.60
TRT*CC*TIME	BAM	CC	T5	276.05	44.74	3.01

TRT*CC*TIME	BAM	NC	T0	215.30		2.54
TRT*CC*TIME	BAM	NC	T1	237.26	46.77	2.43
TRT*CC*TIME	BAM	NC	T2	175.64	29.64	2.52
TRT*CC*TIME	BAM	NC	T3	173.20	44.57	3.70
TRT*CC*TIME	BAM	NC	T4	216.18	45.01	2.85
TRT*CC*TIME	BAM	NC	T5	244.61	44.66	2.98
TRT*CC*TIME	NP	CC	T0	249.68		1.18
TRT*CC*TIME	NP	CC	T1	217.11	48.52	1.03
TRT*CC*TIME	NP	CC	T2	187.34	38.48	0.76
TRT*CC*TIME	NP	CC	T3	220.35	36.79	1.11
TRT*CC*TIME	NP	CC	T4	206.44	54.23	1.13
TRT*CC*TIME	NP	CC	T5	252.77	42.54	1.15
TRT*CC*TIME	NP	NC	T0	200.78		1.11
TRT*CC*TIME	NP	NC	T1	192.42	53.13	1.02
TRT*CC*TIME	NP	NC	T2	174.54	43.03	0.76
TRT*CC*TIME	NP	NC	T3	182.07	40.63	1.13
TRT*CC*TIME	NP	NC	T4	198.12	54.76	1.18
TRT*CC*TIME	NP	NC	T5	254.06	48.56	1.17
TRT*CC*TIME	SUF	CC	T0	261.55		1.82
TRT*CC*TIME	SUF	CC	T1	274.41	49.61	1.53
TRT*CC*TIME	SUF	CC	T2	224.03	34.38	0.86
TRT*CC*TIME	SUF	CC	T3	188.62	35.13	1.47
TRT*CC*TIME	SUF	CC	T4	241.25	62.10	1.49
TRT*CC*TIME	SUF	CC	T5	308.52	43.97	1.34
TRT*CC*TIME	SUF	NC	T0	194.94		1.72
TRT*CC*TIME	SUF	NC	T1	239.31	44.13	2.04
TRT*CC*TIME	SUF	NC	T2	188.74	42.47	1.09
TRT*CC*TIME	SUF	NC	T3	176.03	44.02	1.87
TRT*CC*TIME	SUF	NC	T4	182.89	47.50	1.61
TRT*CC*TIME	SUF	NC	T5	316.46	42.30	1.56

Table B.6. Least squares (LS) means table for autoclaved citrate extractable (ACE) protein, dissolved total nitrogen (N), NO_3-N, NH_4-N, inorganic N, and dissolved total N. Table abbreviations include block (BLOC), phosphorus (P) fertilizer treatments (P TRT), build and maintain (BAM), no P (NP), sufficiency (SUF), presence of cover crop (CC), absence of cover crop (NC), and sampling time (TIME). Sampling times: 27-Apr (T0), 23-May (T1), 15-Jun (T2), 8-Jul (T3), 5-Aug (T4), and 21-Oct (T5).

Effect	P TRT	Cover	Sampling Time	ACE Protein	Dissolved Total N	Inorganic N	NH4-N	NO3-N	Dissolved Organic N
				mg protein kg soil^{-1}	mg N kg soil^{-1}		mg NH4-N kg soil^{-1}	mg NO3-N kg soil^{-1}	mg N kg soil^{-1}
TRT	BAM			4615.50	14.65	11.32	4.32	8.31	4.71
TRT	NP			4257.07	11.94	6.87	2.10	4.86	6.08
TRT	SUF			4646.37	11.32	8.84	3.84	5.63	3.88
CC		CC		4699.85	11.64	8.13	3.50	5.53	4.94
CC		NC		4312.78	13.64	9.89	3.34	7.00	4.85
TRT*CC	BAM	CC		4789.78	15.71	11.18	5.09	8.62	5.11
TRT*CC	BAM	NC		4441.22	13.60	11.46	3.55	8.01	4.32
TRT*CC	NP	CC		4355.65	9.44	5.41	1.86	3.54	6.10
TRT*CC	NP	NC		4158.50	14.45	8.34	2.33	6.17	6.06
TRT*CC	SUF	CC		4954.11	9.78	7.79	3.56	4.44	3.61
TRT*CC	SUF	NC		4338.63	12.86	9.89	4.13	6.82	4.16
TIME			T0	4570.27	6.04	6.52	5.58	3.09	
TIME			T1	4511.22	14.19	8.42	1.63	7.03	6.12
TIME			T2	4579.86	14.77	12.02	3.37	8.65	3.65
TIME			T3	4483.63	19.96	13.95	1.91	13.53	5.02
TIME			T4	4540.75	12.33	6.80	2.83	4.15	5.53
TIME			T5	4352.16	8.56	6.35	5.19	1.16	4.15
TRT*TIME	BAM		T0	4609.30	5.68	8.22	7.91	3.40	
TRT*TIME	BAM		T1	4631.56	12.92	7.03	1.52	5.78	5.89
TRT*TIME	BAM		T2	4582.46	12.46	12.61	4.12	8.49	1.46
TRT*TIME	BAM		T3	4653.43	35.45	24.11	3.19	25.39	7.15
TRT*TIME	BAM		T4	4775.50	12.45	8.07	2.78	5.29	4.38
TRT*TIME	BAM		T5	4440.75	8.97	7.91	6.40	1.52	4.68
TRT*TIME	NP		T0	4349.52	7.14	4.89	3.82	1.57	
TRT*TIME	NP		T1	4182.41	15.86	9.65	1.91	7.74	6.21
TRT*TIME	NP		T2	4417.19	15.27	8.91	1.21	7.71	6.35
TRT*TIME	NP		T3	4261.69	11.76	8.05	0.88	7.16	4.93
TRT*TIME	NP		T4	4252.45	13.14	5.42	1.73	3.69	7.72
TRT*TIME	NP		T5	4079.19	8.51	4.31	3.02	1.30	5.21
TRT*TIME	SUF		T0	4751.99	5.31	6.47	4.99	4.30	
TRT*TIME	SUF		T1	4719.69	13.78	8.58	1.47	7.58	6.25

TRT*TIME	SUF		T2	4739.93	16.59	14.54	4.79	9.76	3.14
TRT*TIME	SUF		T3	4535.77	12.67	9.69	1.66	8.04	2.98
TRT*TIME	SUF		T4	4594.29	11.40	6.91	3.98	3.46	4.49
TRT*TIME	SUF		T5	4536.55	8.19	6.83	6.17	0.66	2.56
CC*TIME		CC	T0	4777.79	3.66	4.93	6.31	0.69	
CC*TIME		CC	T1	4758.87	9.56	4.87	1.56	3.55	5.39
CC*TIME		CC	T2	4787.92	15.47	12.25	3.35	8.91	3.94
CC*TIME		CC	T3	4558.83	20.39	13.54	1.61	14.71	4.88
CC*TIME		CC	T4	4787.26	12.59	6.89	3.02	4.22	5.71
CC*TIME		CC	T5	4528.40	8.18	6.29	5.17	1.13	4.78
CC*TIME		NC	T0	4362.76	8.42	8.12	4.84	5.49	
CC*TIME		NC	T1	4263.56	18.81	11.97	1.71	10.51	6.84
CC*TIME		NC	T2	4371.80	14.08	11.79	3.40	8.39	3.36
CC*TIME		NC	T3	4408.43	19.52	14.36	2.21	12.34	5.16
CC*TIME		NC	T4	4294.23	12.06	6.71	2.64	4.08	5.35
CC*TIME		NC	T5	4175.93	8.93	6.41	5.22	1.19	3.52
TRT*CC*TIME	BAM	CC	T0	4740.71	2.62	2.86	8.92	0.14	
TRT*CC*TIME	BAM	CC	T1	4919.10	11.52	5.87	1.77	4.65	5.65
TRT*CC*TIME	BAM	CC	T2	4679.62	16.23	15.25	6.05	9.20	1.94
TRT*CC*TIME	BAM	CC	T3	4780.58	40.22	25.72	4.03	30.05	6.14
TRT*CC*TIME	BAM	CC	T4	4963.83	15.21	9.89	3.25	6.65	5.31
TRT*CC*TIME	BAM	CC	T5	4654.88	8.44	7.51	6.51	1.01	6.49
TRT*CC*TIME	BAM	NC	T0	4477.90	8.73	13.57	6.91	6.66	
TRT*CC*TIME	BAM	NC	T1	4344.02	14.32	8.18	1.28	6.90	6.13
TRT*CC*TIME	BAM	NC	T2	4485.31	8.69	9.96	2.19	7.77	0.99
TRT*CC*TIME	BAM	NC	T3	4526.29	30.67	22.50	2.34	20.73	8.17
TRT*CC*TIME	BAM	NC	T4	4587.18	9.69	6.24	2.31	3.93	3.45
TRT*CC*TIME	BAM	NC	T5	4226.63	9.50	8.31	6.28	2.03	2.86
TRT*CC*TIME	NP	CC	T0	4512.64	4.18	6.03	4.55	1.47	
TRT*CC*TIME	NP	CC	T1	4355.78	7.76	2.84	1.13	1.71	4.92
TRT*CC*TIME	NP	CC	T2	4505.46	14.31	7.52	0.37	7.15	6.79
TRT*CC*TIME	NP	CC	T3	4242.43	10.54	6.57	0.41	6.14	6.41
TRT*CC*TIME	NP	CC	T4	4340.63	11.18	4.36	1.82	2.54	6.82
TRT*CC*TIME	NP	CC	T5	4176.95	8.67	5.13	2.88	2.26	5.59
TRT*CC*TIME	NP	NC	T0	4186.41	10.09	3.74	3.10	1.66	
TRT*CC*TIME	NP	NC	T1	4009.03	23.95	16.46	2.69	13.77	7.49
TRT*CC*TIME	NP	NC	T2	4328.93	16.22	10.31	2.05	8.26	5.92
TRT*CC*TIME	NP	NC	T3	4280.94	12.97	9.52	1.34	8.18	3.45
TRT*CC*TIME	NP	NC	T4	4164.27	15.09	6.48	1.64	4.84	8.61
TRT*CC*TIME	NP	NC	T5	3981.42	8.35	3.50	3.16	0.34	4.84
TRT*CC*TIME	SUF	CC	T0	5080.02	4.17	5.89	5.46	0.44	
TRT*CC*TIME	SUF	CC	T1	5001.74	9.40	5.89	1.79	4.30	5.60

TRT*CC*TIME	SUF	CC	T2	5178.69	15.86	13.99	3.62	10.36	3.10
TRT*CC*TIME	SUF	CC	T3	4653.47	10.41	8.32	0.38	7.95	2.09
TRT*CC*TIME	SUF	CC	T4	5057.33	11.39	6.40	4.00	3.46	4.99
TRT*CC*TIME	SUF	CC	T5	4753.37	7.45	6.25	6.12	0.13	2.27
TRT*CC*TIME	SUF	NC	T0	4423.96	6.44	7.05	4.52	8.16	
TRT*CC*TIME	SUF	NC	T1	4437.63	18.16	11.27	1.15	10.87	6.89
TRT*CC*TIME	SUF	NC	T2	4301.16	17.32	15.10	5.96	9.15	3.18
TRT*CC*TIME	SUF	NC	T3	4418.07	14.92	11.06	2.94	8.12	3.86
TRT*CC*TIME	SUF	NC	T4	4131.25	11.41	7.42	3.96	3.46	3.99
TRT*CC*TIME	SUF	NC	T5	4319.73	8.93	7.42	6.22	1.19	2.86

Table B.7. Least squares (LS) means table for microbial biomass carbon (C), nitrogen (N), phosphorus (P), biomass C:N ratios, and soil respiration. Table abbreviations include block (BLOC), P fertilizer treatments (P TRT), build and maintain (BAM), no P (NP), sufficiency (SUF), presence of cover crop (CC), absence of cover crop (NC), and sampling time (TIME). Sampling times: 27-Apr (T0), 23-May (T1), 15-Jun (T2), 8-Jul (T3), 5-Aug (T4), and 21-Oct (T5).

Effect	P TRT	Cover	Time	Microbial Biomass C	Microbial Biomass N	Microbial Biomass P	C:N	Soil Respiration
				ug C g soil^{-1}	ug N g soil^{-1}	ug P g soil^{-1}		mg CO2 g soil^{-1}
TRT	BAM			121.19	15.53	2.85	10.15	12.30
TRT	NP			117.77	16.52	0.48	7.51	12.25
TRT	SUF			120.38	16.34	1.70	9.08	12.24
CC		CC		130.10	17.40	1.64	8.07	13.12
CC		NC		109.46	14.86	1.72	9.76	11.41
TRT*CC	BAM	CC		126.10	16.19	2.81	8.45	13.14
TRT*CC	BAM	NC		116.27	14.87	2.88	11.86	11.47
TRT*CC	NP	CC		127.11	17.58	0.49	7.18	12.88
TRT*CC	NP	NC		108.42	15.45	0.48	7.85	11.63
TRT*CC	SUF	CC		137.08	18.43	1.63	8.57	13.35
TRT*CC	SUF	NC		103.69	14.25	1.78	9.59	11.12
TIME			T0	-	18.85	1.17	-	19.32
TIME			T1	124.98	15.55	1.03	10.22	17.74
TIME			T2	113.47	17.16	1.17	7.71	7.36
TIME			T3	102.21	11.85	1.94	10.38	9.53
TIME			T4	114.24	16.20	2.12	7.14	10.32
TIME			T5	143.99	17.17	2.64	9.12	9.31
TRT*TIME	BAM		T0	-	19.66	1.86	-	19.08
TRT*TIME	BAM		T1	117.54	12.62	1.92	15.46	18.10
TRT*TIME	BAM		T2	111.93	17.32	2.28	6.90	7.63
TRT*TIME	BAM		T3	107.62	9.96	3.45	10.90	9.30
TRT*TIME	BAM		T4	123.73	18.01	3.47	6.83	10.47
TRT*TIME	BAM		T5	145.13	15.64	4.12	10.68	9.23
TRT*TIME	NP		T0	-	17.31	0.54	-	19.71
TRT*TIME	NP		T1	133.24	18.32	0.04	7.25	17.32
TRT*TIME	NP		T2	111.46	16.74	0.05	7.58	7.22
TRT*TIME	NP		T3	100.36	14.77	0.56	7.08	9.78
TRT*TIME	NP		T4	106.97	14.52	0.82	7.53	10.19
TRT*TIME	NP		T5	136.80	17.45	0.89	8.14	9.31
TRT*TIME	SUF		T0	-	19.57	1.12	-	19.16
TRT*TIME	SUF		T1	124.16	15.72	1.12	7.96	17.81
TRT*TIME	SUF		T2	117.01	17.42	1.17	8.64	7.22
TRT*TIME	SUF		T3	98.65	10.81	1.81	13.17	9.52
TRT*TIME	SUF		T4	112.04	16.08	2.08	7.07	10.31
TRT*TIME	SUF		T5	150.05	18.44	2.91	8.56	9.39
CC*TIME		CC	T0	-	15.81	0.85	-	20.97
CC*TIME		CC	T1	143.33	19.58	0.90	7.35	18.86
CC*TIME		CC	T2	122.78	19.39	0.94	6.59	8.37

CC*TIME		CC	T3	106.46	12.44	2.14	10.91	9.59
CC*TIME		CC	T4	124.16	18.02	2.07	6.94	11.00
CC*TIME		CC	T5	153.76	19.16	2.94	8.53	9.94
CC*TIME		NC	T0	-	21.88	1.50	-	17.66
CC*TIME		NC	T1	106.62	11.52	1.15	13.09	16.63
CC*TIME		NC	T2	104.15	14.92	1.40	8.82	6.34
CC*TIME		NC	T3	97.97	11.25	1.74	9.85	9.48
CC*TIME		NC	T4	104.33	14.38	2.17	7.34	9.65
CC*TIME		NC	T5	134.22	15.19	2.33	9.72	8.69
TRT*CC*TIME	BAM	CC	T0	-	17.04	1.37	-	20.44
TRT*CC*TIME	BAM	CC	T1	126.38	18.19	1.50	6.98	19.24
TRT*CC*TIME	BAM	CC	T2	112.07	16.25	1.97	7.63	8.59
TRT*CC*TIME	BAM	CC	T3	107.10	9.65	3.99	11.23	10.34
TRT*CC*TIME	BAM	CC	T4	127.61	18.84	3.23	6.73	10.50
TRT*CC*TIME	BAM	CC	T5	157.35	17.19	4.82	9.69	9.73
TRT*CC*TIME	BAM	NC	T0	-	22.28	2.35	-	17.71
TRT*CC*TIME	BAM	NC	T1	108.69	7.04	2.33	23.94	16.97
TRT*CC*TIME	BAM	NC	T2	111.78	18.39	2.59	6.18	6.67
TRT*CC*TIME	BAM	NC	T3	108.14	10.27	2.91	10.56	8.26
TRT*CC*TIME	BAM	NC	T4	119.84	17.18	3.70	6.94	10.45
TRT*CC*TIME	BAM	NC	T5	132.90	14.08	3.42	11.67	8.74
TRT*CC*TIME	NP	CC	T0	-	14.51	0.49	-	21.27
TRT*CC*TIME	NP	CC	T1	156.93	21.39	0.06	7.33	18.61
TRT*CC*TIME	NP	CC	T2	116.18	19.25	-0.02	6.03	7.77
TRT*CC*TIME	NP	CC	T3	101.64	14.25	0.53	7.69	9.37
TRT*CC*TIME	NP	CC	T4	113.81	16.34	0.85	7.06	10.66
TRT*CC*TIME	NP	CC	T5	147.00	19.73	1.01	7.78	9.56
TRT*CC*TIME	NP	NC	T0	-	20.10	0.59	-	18.14
TRT*CC*TIME	NP	NC	T1	109.55	15.24	0.01	7.17	16.03
TRT*CC*TIME	NP	NC	T2	106.74	14.23	0.13	9.13	6.67
TRT*CC*TIME	NP	NC	T3	99.08	15.29	0.59	6.46	10.18
TRT*CC*TIME	NP	NC	T4	100.12	12.69	0.79	7.99	9.72
TRT*CC*TIME	NP	NC	T5	126.60	15.17	0.77	8.49	9.07
TRT*CC*TIME	SUF	CC	T0	-	15.88	0.68	-	21.21
TRT*CC*TIME	SUF	CC	T1	146.68	19.16	1.15	7.75	18.74
TRT*CC*TIME	SUF	CC	T2	140.09	22.68	0.87	6.13	8.76
TRT*CC*TIME	SUF	CC	T3	110.62	13.43	1.90	13.82	9.06
TRT*CC*TIME	SUF	CC	T4	131.06	18.89	2.14	7.04	11.83
TRT*CC*TIME	SUF	CC	T5	156.93	20.56	3.01	8.14	10.52
TRT*CC*TIME	SUF	NC	T0	-	23.27	1.55	-	17.12
TRT*CC*TIME	SUF	NC	T1	101.63	12.29	1.10	8.17	16.88
TRT*CC*TIME	SUF	NC	T2	93.93	12.16	1.47	11.16	5.69
TRT*CC*TIME	SUF	NC	T3	86.68	8.20	1.73	12.53	9.99
TRT*CC*TIME	SUF	NC	T4	93.02	13.28	2.02	7.09	8.79
TRT*CC*TIME	SUF	NC	T5	143.17	16.32	2.81	8.99	8.27

Table B.8. Least squares (LS) means table for acid phosphatase, alkaline phosphatase, β-glucosidase, and β-glucosaminidase. Table abbreviations include block (BLOC), phosphorus (P) fertilizer treatments (P TRT), build and maintain (BAM), no P (NP), sufficiency (SUF), presence of cover crop (CC), absence of cover crop (NC), and sampling time (TIME). Sampling times: 27-Apr (T0), 23-May (T1), 15-Jun (T2), 8-Jul (T3), 5-Aug (T4), and 21-Oct (T5).

Effect	P TRT	Cover	Time	Acid Phosphatase	Alkaline Phosphatase	Glucosidase	Glucosaminidase
				ug p-nitrophenol g soil^{-1} hr^{-1}			
TRT	BAM			326.69	100.83	123.87	34.34
TRT	NP			308.03	107.05	116.34	35.24
TRT	SUF			336.12	98.04	124.42	35.00
CC		CC		358.94	110.23	130.11	40.19
CC		NC		288.29	93.72	112.98	29.53
TRT*CC	BAM	CC		348.32	102.56	122.79	38.22
TRT*CC	BAM	NC		305.06	99.10	124.96	30.46
TRT*CC	NP	CC		344.86	120.49	128.89	39.95
TRT*CC	NP	NC		271.20	93.61	103.79	30.53
TRT*CC	SUF	CC		383.63	107.65	138.65	42.40
TRT*CC	SUF	NC		288.62	88.43	110.19	27.59
TIME			T0	327.00	104.58	114.62	33.93
TIME			T1	344.92	94.81	122.10	34.60
TIME			T2	358.08	112.35	133.40	34.14
TIME			T3	288.34	99.65	122.71	33.66
TIME			T4	343.26	116.08	138.96	35.67
TIME			T5	280.08	84.38	97.48	37.15
TRT*TIME	BAM		T0	323.83	95.72	113.45	30.94
TRT*TIME	BAM		T1	353.99	93.83	122.46	34.01
TRT*TIME	BAM		T2	354.54	114.57	138.82	34.45
TRT*TIME	BAM		T3	290.50	101.33	130.27	33.30
TRT*TIME	BAM		T4	353.59	114.55	142.24	37.36
TRT*TIME	BAM		T5	283.69	85.00	96.01	35.99
TRT*TIME	NP		T0	324.29	116.68	116.75	37.16
TRT*TIME	NP		T1	315.35	92.27	111.93	32.84
TRT*TIME	NP		T2	339.73	112.90	121.69	33.96
TRT*TIME	NP		T3	277.64	108.43	118.45	36.11
TRT*TIME	NP		T4	332.66	124.88	137.09	35.55
TRT*TIME	NP		T5	258.49	87.15	92.12	35.82
TRT*TIME	SUF		T0	332.89	101.35	113.67	33.71
TRT*TIME	SUF		T1	365.42	98.32	131.92	36.96
TRT*TIME	SUF		T2	379.95	109.59	139.69	34.02
TRT*TIME	SUF		T3	296.89	89.18	119.39	31.57
TRT*TIME	SUF		T4	343.53	108.81	137.54	34.10
TRT*TIME	SUF		T5	298.06	81.00	104.30	39.63
CC*TIME		CC	T0	364.29	116.46	127.76	39.81
CC*TIME		CC	T1	388.55	101.42	134.64	39.76
CC*TIME		CC	T2	390.89	119.07	139.46	38.95

CC*TIME		CC	T3	314.39	105.58	123.83	38.70
CC*TIME		CC	T4	387.80	125.05	145.79	40.68
CC*TIME		CC	T5	307.71	93.82	109.16	43.22
CC*TIME		NC	T0	289.72	92.71	101.48	28.05
CC*TIME		NC	T1	301.29	88.19	109.57	29.44
CC*TIME		NC	T2	325.26	105.63	127.34	29.33
CC*TIME		NC	T3	262.29	93.72	121.58	28.61
CC*TIME		NC	T4	298.72	107.11	132.12	30.65
CC*TIME		NC	T5	252.46	74.94	85.79	31.08
TRT*CC*TIME	BAM	CC	T0	343.30	95.20	111.07	33.98
TRT*CC*TIME	BAM	CC	T1	388.74	95.59	122.84	37.40
TRT*CC*TIME	BAM	CC	T2	369.54	112.65	131.14	36.88
TRT*CC*TIME	BAM	CC	T3	312.87	106.98	129.03	37.81
TRT*CC*TIME	BAM	CC	T4	372.43	112.03	131.56	40.26
TRT*CC*TIME	BAM	CC	T5	303.05	92.91	111.07	42.98
TRT*CC*TIME	BAM	NC	T0	304.36	96.23	115.83	27.90
TRT*CC*TIME	BAM	NC	T1	319.24	92.06	122.08	30.61
TRT*CC*TIME	BAM	NC	T2	339.54	116.49	146.50	32.01
TRT*CC*TIME	BAM	NC	T3	268.14	95.67	131.50	28.78
TRT*CC*TIME	BAM	NC	T4	334.74	117.07	152.92	34.45
TRT*CC*TIME	BAM	NC	T5	264.33	77.08	80.94	29.00
TRT*CC*TIME	NP	CC	T0	361.07	137.57	139.17	43.73
TRT*CC*TIME	NP	CC	T1	356.96	98.12	126.43	36.26
TRT*CC*TIME	NP	CC	T2	369.09	128.91	131.88	39.85
TRT*CC*TIME	NP	CC	T3	309.55	119.55	126.62	41.93
TRT*CC*TIME	NP	CC	T4	380.58	142.89	146.77	39.12
TRT*CC*TIME	NP	CC	T5	291.90	95.89	102.49	38.79
TRT*CC*TIME	NP	NC	T0	287.51	95.79	94.33	30.58
TRT*CC*TIME	NP	NC	T1	273.74	86.42	97.44	29.41
TRT*CC*TIME	NP	NC	T2	310.38	96.88	111.51	28.06
TRT*CC*TIME	NP	NC	T3	245.72	97.32	110.29	30.28
TRT*CC*TIME	NP	NC	T4	284.75	106.87	127.40	31.98
TRT*CC*TIME	NP	NC	T5	225.07	78.40	81.76	32.85
TRT*CC*TIME	SUF	CC	T0	388.51	116.60	133.06	41.74
TRT*CC*TIME	SUF	CC	T1	419.95	110.55	154.65	45.63
TRT*CC*TIME	SUF	CC	T2	434.03	115.66	155.37	40.11
TRT*CC*TIME	SUF	CC	T3	320.76	90.20	115.83	36.36
TRT*CC*TIME	SUF	CC	T4	410.38	120.25	159.05	42.67
TRT*CC*TIME	SUF	CC	T5	328.16	92.66	113.92	47.88
TRT*CC*TIME	SUF	NC	T0	277.28	86.10	94.27	25.68
TRT*CC*TIME	SUF	NC	T1	310.89	86.09	109.19	28.29
TRT*CC*TIME	SUF	NC	T2	325.88	103.53	124.02	27.92
TRT*CC*TIME	SUF	NC	T3	273.01	88.17	122.96	26.77
TRT*CC*TIME	SUF	NC	T4	276.67	97.38	116.04	25.52
TRT*CC*TIME	SUF	NC	T5	267.97	69.33	94.67	31.38

Appendix C - KAW Fall Comparisons: Supplemental Data

Table C.1 Least squares (LS) means for active carbon (C), autoclaved citrate extractable (ACE) protein, and soil respiration for fall 2020, 2021, and 2022 for all main effects and interactions. Table abbreviations include no phosphorus (P) (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

			Fall 2020	Fall 2021	Fall 2022	Fall 2020	Fall 2021	Fall 2022	Fall 2020	Fall 2021	Fall 2022
			Active C			ACE Protein			Soil Respiration		
			mg C kg soil^{-1}			mg protein kg soil^{-1}			mg CO_2 g soil^{-1}		
NP			321.72	225.69	162.10	3551.48	3333.25	3393.84	16.64	18.47	7.47
BAM			335.10	236.21	158.48	3900.69	3685.42	3746.07	16.76	19.75	7.19
SUF			313.57	232.18	180.84	4002.77	3627.77	3848.52	16.76	18.98	7.28
CC			345.13	238.74	172.99	3926.38	3682.22	3779.59	17.43	20.06	7.70
NC			301.79	223.99	161.28	3710.24	3415.40	3546.03	16.00	18.08	6.93
NP	**CC**		350.98	234.23	165.02	3689.53	3411.64	3472.11	17.20	19.23	7.55
NP	**NC**		292.47	217.16	159.18	3413.42	3254.85	3315.57	16.07	17.72	7.39
BAM	**CC**		348.37	242.85	169.33	3917.96	3879.66	3843.00	17.15	21.63	7.62
BAM	**NC**		321.82	229.58	147.63	3883.41	3491.17	3649.13	16.37	17.86	6.76
SUF	**CC**		336.06	239.13	184.64	4171.66	3755.36	4023.65	17.95	19.32	7.93
SUF	**NC**		291.08	225.23	177.03	3833.87	3500.19	3673.38	15.56	18.65	6.63
0-5cm			453.65	371.22	275.16	4696.12	4399.87	4352.16	19.52	21.56	9.31
5-10cm			326.20	236.29	125.02	3596.10	3289.91	3435.71	16.08	18.71	7.16
10-15cm			190.54	86.57	101.24	3162.71	2956.66	3200.55	14.56	16.94	5.47
0-5cm	**NP**		465.17	376.39	252.44	4493.72	4135.89	4079.19	19.33	20.45	9.36
5-10cm	**NP**		340.94	219.51	121.36	3318.92	3104.13	3183.02	15.95	18.15	7.41
10-15cm	**NP**		159.06	81.18	112.50	2841.79	2759.72	2919.32	14.63	16.82	5.63
0-5cm	**BAM**		456.12	370.18	260.75	4580.88	4581.27	4440.75	20.13	23.08	9.20
5-10cm	**BAM**		335.43	251.17	128.52	3844.06	3408.15	3529.63	15.74	18.51	7.05
10-15cm	**BAM**		213.74	87.29	86.17	3277.12	3066.83	3267.82	14.40	17.65	5.32
0-5cm	**SUF**		439.67	367.10	312.28	5013.77	4482.44	4536.55	19.11	21.15	9.36
5-10cm	**SUF**		302.23	238.19	125.17	3625.31	3357.46	3594.48	16.53	19.46	7.03
10-15cm	**SUF**		198.82	91.24	105.06	3369.22	3043.43	3414.52	14.63	16.35	5.46
0-5cm	**CC**		488.76	383.40	278.22	4809.53	4590.11	4528.40	20.54	22.73	9.96
0-5cm	**NC**		418.54	359.04	272.10	4582.72	4209.62	4175.93	18.51	20.39	8.65
5-10cm	**CC**		348.93	239.49	130.24	3750.65	3379.22	3503.53	17.00	19.43	7.39
5-10cm	**NC**		303.47	233.10	119.79	3441.54	3200.61	3367.89	15.15	17.98	6.94
10-15cm	**CC**		197.71	93.32	110.53	3218.97	3077.33	3306.83	14.76	18.02	5.75
10-15cm	**NC**		183.37	79.82	91.96	3106.45	2835.99	3094.27	14.35	15.86	5.19
0-5cm	**NP**	**CC**	510.84	389.93	251.76	4580.81	4163.63	4176.95	19.81	21.26	9.66
0-5cm	**NP**	**NC**	419.50	362.85	253.12	4406.63	4108.16	3981.42	18.84	19.65	9.06

5-10cm	**NP**	**CC**	364.97	209.70	122.80	3519.42	3162.84	3197.85	16.76	18.77	7.22
5-10cm	**NP**	**NC**	316.91	229.32	119.92	3118.43	3045.42	3168.18	15.14	17.52	7.59
10-15cm	**NP**	**CC**	177.12	103.05	120.50	2968.37	2908.46	3041.53	15.02	17.65	5.76
10-15cm	**NP**	**NC**	141.00	59.32	104.49	2715.20	2610.98	2797.12	14.24	15.98	5.50
0-5cm	**BAM**	**CC**	486.69	370.18	275.72	4608.14	4882.66	4654.88	21.11	25.88	9.71
0-5cm	**BAM**	**NC**	425.55	370.18	245.77	4553.62	4279.87	4226.63	19.16	20.28	8.68
5-10cm	**BAM**	**CC**	352.54	261.58	141.27	3923.49	3530.12	3593.15	15.99	19.22	7.52
5-10cm	**BAM**	**NC**	318.31	240.76	115.77	3764.62	3286.19	3466.11	15.50	17.80	6.58
10-15cm	**BAM**	**CC**	205.88	96.80	90.99	3222.25	3226.20	3280.97	14.34	19.80	5.63
10-15cm	**BAM**	**NC**	221.61	77.79	81.35	3332.00	2907.45	3254.67	14.46	15.49	5.01
0-5cm	**SUF**	**CC**	468.76	390.10	307.18	5239.64	4724.05	4753.37	20.69	21.06	10.50
0-5cm	**SUF**	**NC**	410.58	344.11	317.39	4787.90	4240.82	4319.73	17.53	21.23	8.21
5-10cm	**SUF**	**CC**	329.27	247.18	126.65	3809.05	3444.70	3719.58	18.25	20.29	7.43
5-10cm	**SUF**	**NC**	275.18	229.21	123.69	3441.56	3270.22	3469.39	14.81	18.62	6.64
10-15cm	**SUF**	**CC**	210.14	80.11	120.09	3466.29	3097.32	3598.00	14.92	16.60	5.85
10-15cm	**SUF**	**NC**	187.49	102.36	90.03	3272.16	2989.54	3231.03	14.34	16.10	5.06

Table C.2 Least squares (LS) means for acid and alkaline phosphatase activity for fall 2020, 2021, and 2022 for all main effects and interactions. Table abbreviations include no phosphorus (P) (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

			Fall 2020	Fall 2021	Fall 2022	Fall 2020	Fall 2021	Fall 2022
			Acid Phosphatase			Alkaline Phosphatase		
			mg p-nitrophenol kg soil^{-1} hr^{-1}					
NP			228.71	211.23	196.18	76.61	76.71	72.33
BAM			229.30	225.97	207.84	65.12	65.31	70.55
SUF			218.19	217.02	217.00	65.74	65.83	70.22
CC			243.15	237.76	221.83	72.18	72.36	77.10
NC			207.65	198.38	192.18	66.13	66.21	64.97
NP	**CC**		265.24	234.26	216.45	85.21	85.44	84.25
NP	**NC**		192.19	188.20	175.91	68.01	67.99	60.41
BAM	**CC**		222.41	245.29	214.38	62.00	62.22	75.63
BAM	**NC**		236.20	206.66	201.30	68.23	68.39	65.47
SUF	**CC**		241.79	233.74	234.67	69.33	69.42	71.42
SUF	**NC**		194.58	200.29	199.34	62.14	62.25	69.03
0-5cm			288.13	301.47	280.38	99.42	99.65	109.80
5-10cm			228.25	195.47	173.87	57.21	57.23	59.79
10-15cm			159.82	157.28	166.76	50.84	50.97	43.52
0-5cm	**NP**		312.78	298.83	258.39	108.61	108.88	111.90
5-10cm	**NP**		219.30	182.93	169.83	60.96	60.88	59.75
10-15cm	**NP**		154.06	151.93	160.30	60.27	60.38	45.35
0-5cm	**BAM**		273.49	304.97	283.97	98.07	98.33	107.02
5-10cm	**BAM**		239.70	203.32	172.60	52.34	52.44	61.51
10-15cm	**BAM**		174.72	169.63	166.95	44.94	45.15	43.13
0-5cm	**SUF**		278.12	300.61	298.79	91.56	91.74	110.49
5-10cm	**SUF**		225.75	200.16	179.18	58.34	58.37	58.11
10-15cm	**SUF**		150.69	150.27	173.05	47.31	47.39	42.07
0-5cm	**CC**		315.24	327.43	308.28	100.08	100.64	119.25
0-5cm	**NC**		261.01	275.51	252.49	98.75	98.67	100.35
5-10cm	**CC**		251.12	212.15	183.29	61.79	61.67	66.19
5-10cm	**NC**		205.38	178.79	164.45	52.63	52.79	53.39
10-15cm	**CC**		163.08	173.71	173.93	54.68	54.78	45.87
10-15cm	**NC**		156.57	140.84	159.60	47.00	47.17	41.16
0-5cm	**NP**	**CC**	360.31	324.98	292.64	112.76	113.83	127.05

0-5cm	**NP**	**NC**	265.25	272.69	224.15	104.47	103.94	96.75
5-10cm	**NP**	**CC**	260.70	200.19	183.55	72.58	72.15	71.34
5-10cm	**NP**	**NC**	177.91	165.67	156.12	49.33	49.62	48.16
10-15cm	**NP**	**CC**	174.72	177.61	173.14	70.30	70.35	54.37
10-15cm	**NP**	**NC**	133.41	126.25	147.46	50.24	50.40	36.33
0-5cm	**BAM**	**CC**	263.89	320.08	302.64	89.68	90.14	113.15
0-5cm	**BAM**	**NC**	283.09	289.86	265.31	106.46	106.52	100.89
5-10cm	**BAM**	**CC**	237.41	229.72	175.32	49.30	49.34	66.58
5-10cm	**BAM**	**NC**	241.99	176.92	169.88	55.38	55.54	56.44
10-15cm	**BAM**	**CC**	165.92	186.07	165.19	47.03	47.18	47.16
10-15cm	**BAM**	**NC**	183.51	153.19	168.71	42.85	43.11	39.09
0-5cm	**SUF**	**CC**	321.53	337.23	329.56	97.80	97.94	117.56
0-5cm	**SUF**	**NC**	234.71	263.99	268.01	85.32	85.54	103.42
5-10cm	**SUF**	**CC**	255.25	206.55	190.99	63.50	63.51	60.64
5-10cm	**SUF**	**NC**	196.25	193.78	167.36	53.18	53.22	55.59
10-15cm	**SUF**	**CC**	148.60	157.45	183.45	46.70	46.79	36.06
10-15cm	**SUF**	**NC**	152.78	143.09	162.64	47.91	47.99	48.07

Table C.3 Least squares (LS) means for glucosidase and glucosaminidase activity for fall 2020, 2021, and 2022 for all main effects and interactions. Table abbreviations include no P (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

			Fall 2020	Fall 2021	Fall 2022	Fall 2020	Fall 2021	Fall 2022
			Glucosidase			Glucosaminidase		
			mg p-nitrophenol kg soil^{-1} hr^{-1}					
NP			69.35	69.90	53.69	27.04	26.27	23.25
BAM			73.76	77.64	54.93	24.91	25.69	22.82
SUF			71.02	77.09	59.39	25.66	26.61	24.77
CC			76.84	79.73	61.00	29.10	29.43	26.51
NC			65.92	70.03	51.00	22.64	22.95	20.71
NP	**CC**		79.42	76.48	58.11	31.18	30.11	24.89
NP	**NC**		59.28	63.33	49.27	22.91	22.43	21.61
BAM	**CC**		70.37	80.23	60.61	26.61	28.84	25.90
BAM	**NC**		77.16	75.05	49.25	23.20	22.55	19.73
SUF	**CC**		80.73	82.48	64.29	29.50	29.35	28.75
SUF	**NC**		61.32	71.70	54.49	21.82	23.87	20.79
0-5cm			131.30	138.01	97.53	40.86	41.87	37.18
5-10cm			49.65	52.59	40.32	22.45	20.41	19.03
10-15cm			33.20	34.03	30.15	14.30	16.30	14.62
0-5cm	**NP**		133.23	130.43	92.01	44.85	41.34	35.93
5-10cm	**NP**		48.24	48.00	40.27	22.81	20.28	19.34
10-15cm	**NP**		26.58	31.28	28.79	13.47	17.20	14.49
0-5cm	**BAM**		131.71	137.71	96.06	39.61	41.57	36.02
5-10cm	**BAM**		53.70	56.59	39.69	20.95	19.43	18.11
10-15cm	**BAM**		35.88	38.62	29.04	14.16	16.08	14.32
0-5cm	**SUF**		128.94	145.90	104.52	38.11	42.69	39.61
5-10cm	**SUF**		47.00	53.18	41.00	23.59	21.52	19.63
10-15cm	**SUF**		37.13	32.19	32.64	15.27	15.63	15.07
0-5cm	**CC**		143.54	142.97	109.27	47.39	47.15	43.25
0-5cm	**NC**		119.06	133.05	85.78	34.32	36.59	31.12
5-10cm	**CC**		53.19	57.61	42.20	24.63	22.47	20.88
5-10cm	**NC**		46.10	47.57	38.45	20.27	18.35	17.18
10-15cm	**CC**		33.79	38.61	31.53	15.26	18.68	15.41
10-15cm	**NC**		32.60	29.45	28.77	13.34	13.92	13.83
0-5cm	**NP**	**CC**	152.90	135.47	102.56	52.86	46.69	38.77
0-5cm	**NP**	**NC**	113.56	125.38	81.45	36.84	35.99	33.09
5-10cm	**NP**	**CC**	55.77	55.11	41.53	25.58	22.97	20.34
5-10cm	**NP**	**NC**	40.71	40.89	39.01	20.04	17.58	18.34

10-15cm	**NP**	**CC**	29.59	38.86	30.22	15.09	20.67	15.57
10-15cm	**NP**	**NC**	23.57	23.71	27.35	11.86	13.73	13.40
0-5cm	**BAM**	**CC**	126.96	129.50	110.98	42.86	45.21	43.07
0-5cm	**BAM**	**NC**	136.46	145.91	81.13	36.36	37.92	28.97
5-10cm	**BAM**	**CC**	51.96	67.48	42.35	22.07	22.25	20.28
5-10cm	**BAM**	**NC**	55.44	45.70	37.04	19.82	16.61	15.95
10-15cm	**BAM**	**CC**	32.18	43.70	28.50	14.91	19.05	14.36
10-15cm	**BAM**	**NC**	39.57	33.54	29.58	13.41	13.10	14.28
0-5cm	**SUF**	**CC**	150.74	163.94	114.27	46.46	49.54	47.91
0-5cm	**SUF**	**NC**	107.14	127.87	94.77	29.77	35.84	31.30
5-10cm	**SUF**	**CC**	51.84	50.24	42.71	26.24	22.18	22.02
5-10cm	**SUF**	**NC**	42.15	56.13	39.29	20.95	20.85	17.25
10-15cm	**SUF**	**CC**	39.60	33.27	35.88	15.80	16.33	16.31
10-15cm	**SUF**	**NC**	34.67	31.11	29.39	14.74	14.93	13.82

Table C.4 Least squares (LS) means microbial biomass carbon (C), nitrogen (N), and phosphorus (P) for fall 2020, 2021, and 2022 for all main effects and interactions. Table abbreviations include no P (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

			Fall 2020	Fall 2021	Fall 2022	Fall 2020	Fall 2021	Fall 2022	Fall 2020	Fall 2021	Fall 2022
			Microbial Biomass C			Microbial Biomass N			Microbial Biomass P		
			mg C kg soil^{-1}			mg N kg soil^{-1}			mg P kg soil^{-1}		
NP			83.01	87.91	102.67	-	12.56	11.06	0.13	0.53	0.50
BAM			79.51	88.59	105.54	-	12.61	10.47	0.61	1.88	1.93
SUF			90.06	91.17	109.74	-	11.39	13.10	0.37	1.14	1.29
CC			91.30	96.47	114.00	-	13.91	12.33	0.32	1.17	1.29
NC			77.09	81.97	97.96	-	10.46	10.76	0.42	1.19	1.18
NP	**CC**		90.18	95.40	107.55	-	14.11	11.10	0.13	0.59	0.50
NP	**NC**		75.85	80.41	97.79	-	11.01	11.02	0.13	0.47	0.50
BAM	**CC**		76.75	91.30	116.44	-	13.43	10.88	0.47	1.77	2.04
BAM	**NC**		82.27	85.87	94.64	-	11.79	10.05	0.75	1.98	1.82
SUF	**CC**		106.99	102.71	118.02	-	14.21	15.01	0.36	1.16	1.34
SUF	**NC**		73.14	79.64	101.47	-	8.57	11.19	0.38	1.12	1.23
0-5cm			119.67	138.36	143.99	-	16.39	17.17	0.54	2.37	2.64
5-10cm			77.09	83.02	67.97	-	11.85	10.76	0.23	0.52	0.37
10-15cm			55.83	46.29	-	-	8.32	6.69	0.33	0.66	0.71
0-5cm	**NP**		119.36	127.74	136.80	-	18.11	17.45	0.13	0.78	0.89
0-5cm	**BAM**		104.47	134.81	145.13	-	13.91	15.64	0.93	4.11	4.11
0-5cm	**SUF**		135.19	152.54	150.05	-	17.14	18.44	0.57	2.21	2.91
5-10cm	**NP**		74.21	86.90	68.54	-	12.31	9.66	0.17	0.35	0.34
5-10cm	**BAM**		73.24	82.29	65.95	-	12.16	9.88	0.46	0.92	1.23
5-10cm	**SUF**		83.82	79.87	69.44	-	11.07	12.75	0.38	0.70	0.57
10-15cm	**NP**		55.47	49.08	-	-	7.26	6.09	0.10	0.45	0.27
10-15cm	**BAM**		60.82	48.66	-	-	11.75	5.88	0.43	0.60	0.44
10-15cm	**SUF**		51.19	41.12	-	-	5.95	8.11	0.16	0.51	0.39
0-5cm	**CC**		137.46	149.42	153.76	-	19.78	19.16	0.44	2.41	2.94
0-5cm	**NC**		101.88	127.30	134.22	-	13.00	15.19	0.65	2.32	2.33
5-10cm	**CC**		78.16	88.31	74.24	-	12.28	11.22	0.39	0.60	0.60
5-10cm	**NC**		76.02	77.73	61.71	-	11.41	10.30	0.28	0.71	0.82
10-15cm	**CC**		58.29	51.68	-	-	9.68	6.61	0.13	0.51	0.34
10-15cm	**NC**		53.36	40.89	-	-	6.96	6.78	0.33	0.53	0.39
0-5cm	**NP**	**CC**	140.30	143.34	147.00	-	22.66	19.73	0.15	0.94	1.00
0-5cm	**NP**	**NC**	98.42	112.14	126.60	.	13.56	15.17	0.11	0.63	0.78
0-5cm	**BAM**	**CC**	102.72	135.77	157.35	-	14.42	17.19	0.60	4.18	4.81
0-5cm	**BAM**	**NC**	106.22	133.85	132.90	-	13.40	14.08	1.25	4.04	3.40
0-5cm	**SUF**	**CC**	169.35	169.16	156.93	-	22.25	20.56	0.57	2.12	3.00
0-5cm	**SUF**	**NC**	101.02	135.92	143.17	-	12.03	16.32	0.57	2.30	2.81

5-10cm	**NP**	**CC**	72.55	87.23	68.09	-	10.80	8.70	0.20	0.38	0.25
5-10cm	**NP**	**NC**	75.86	86.57	68.99	-	13.82	10.61	0.13	0.31	0.43
5-10cm	**BAM**	**CC**	65.45	83.82	75.52	-	10.64	9.96	0.65	0.62	0.92
5-10cm	**BAM**	**NC**	81.03	80.77	56.38	-	13.67	9.80	0.26	1.22	1.55
5-10cm	**SUF**	**CC**	96.48	93.89	79.11	-	15.40	15.01	0.30	0.80	0.64
5-10cm	**SUF**	**NC**	71.16	65.84	59.76	-	6.73	10.49	0.45	0.60	0.49
10-15cm	**NP**	**CC**	57.67	55.63	-	-	8.86	4.88	0.04	0.45	0.25
10-15cm	**NP**	**NC**	53.27	42.53	-	-	5.65	7.29	0.15	0.46	0.28
10-15cm	**BAM**	**CC**	62.08	54.32	-	-	15.21	5.49	0.14	0.52	0.39
10-15cm	**BAM**	**NC**	59.56	43.00	-	-	8.30	6.28	0.72	0.69	0.50
10-15cm	**SUF**	**CC**	55.13	45.08	-	-	4.97	9.47	0.20	0.55	0.39
10-15cm	**SUF**	**NC**	47.25	37.15	-	-	6.94	6.76	0.12	0.46	0.39

Table C.5 Least squares (LS) means for microbial biomass carbon (C), nitrogen (N), and phosphorus (P) ratios for fall 2020, 2021, and 2022 for all main effects and interactions. Table abbreviations include no P (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

			Fall 2020	Fall 2021	Fall 2022	Fall 2020	Fall 2021	Fall 2022	Fall 2020	Fall 2021	Fall 2022
			C:N Ratio			N:P Ratio			C:P Ratio		
NP			-	7.00	9.28	-	23.82	22.23	632.72	166.71	206.25
BAM			-	7.03	10.08	-	6.71	5.43	131.14	47.16	54.71
SUF			-	8.01	8.38	-	10.00	10.18	244.34	80.10	85.31
CC			-	6.93	9.24	-	11.85	9.53	286.85	82.20	88.08
NC			-	7.84	9.11	-	8.79	9.11	183.94	68.94	82.94
NP	**CC**		-	6.76	9.69	-	23.96	22.26	684.19	162.00	215.62
NP	**NC**		-	7.30	8.87	-	23.64	22.19	580.34	172.63	196.84
BAM	**CC**		-	6.80	10.70	-	7.57	5.33	164.56	51.49	57.05
BAM	**NC**		-	7.28	9.41	-	5.94	5.53	110.24	43.29	52.08
SUF	**CC**		-	7.23	7.86	-	12.26	11.18	299.86	88.63	87.86
SUF	**NC**		-	9.29	9.07	-	7.67	9.10	192.32	71.25	82.54
0-5cm			-	8.44	8.38	-	6.92	6.52	220.27	58.46	54.64
5-10cm			-	7.01	6.32	-	22.75	29.40	336.34	159.44	185.67
10-15cm			-	5.56	-	-	12.67	9.40	167.30	70.49	.
0-5cm	**NP**		-	7.05	7.84	-	23.16	19.58	906.30	163.39	153.50
0-5cm	**BAM**		-	9.69	9.28	-	3.39	3.81	112.66	32.82	35.32
0-5cm	**SUF**		-	8.90	8.14	-	7.75	6.35	236.84	68.99	51.65
5-10cm	**NP**		-	7.06	7.10	-	35.38	28.73	448.65	249.70	203.86
5-10cm	**BAM**		-	6.77	6.67	-	13.16	8.01	160.09	89.11	53.46
5-10cm	**SUF**		-	7.22	5.45	-	15.85	22.54	221.57	114.39	122.72
10-15cm	**NP**		-	6.76	-	-	16.04	22.88	573.83	108.54	-
10-15cm	**BAM**		-	4.14	-	-	19.44	13.24	140.05	80.50	-
10-15cm	**SUF**		-	6.91	-	-	11.78	20.90	326.87	81.37	-
0-5cm	**CC**		-	7.55	8.02	-	8.20	6.52	311.49	61.95	52.31
0-5cm	**NC**		-	9.79	8.84	-	5.60	6.52	157.90	54.83	57.59
5-10cm	**CC**		-	7.19	6.61	-	20.40	18.67	202.49	146.70	123.49
5-10cm	**NC**		-	6.81	5.99	-	16.04	12.52	270.04	109.30	75.01
10-15cm	**CC**		-	5.34	-	-	19.09	19.32	456.49	101.95	-
10-15cm	**NC**		-	5.87	-	-	13.03	17.38	161.36	76.52	-
0-5cm	**NP**	**CC**	-	6.33	7.45	-	24.22	19.71	927.30	153.19	146.85
0-5cm	**NP**	**NC**	-	8.27	8.35	-	21.59	19.41	878.72	178.57	162.04
0-5cm	**BAM**	**CC**	-	9.41	9.15	-	3.45	3.57	170.94	32.49	32.69
0-5cm	**BAM**	**NC**	-	9.99	9.44	-	3.32	4.14	84.73	33.17	39.03
0-5cm	**SUF**	**CC**	-	7.60	7.63	-	10.49	6.84	296.22	79.74	52.25
0-5cm	**SUF**	**NC**	-	11.30	8.77	-	5.23	5.81	177.23	59.07	51.02
5-10cm	**NP**	**CC**	-	8.07	7.82	-	28.28	35.19	357.93	228.34	275.33

5-10cm	**NP**	**NC**	**-**	6.26	6.50	**-**	44.02	24.97	592.21	275.69	162.32
5-10cm	**BAM**	**CC**	**-**	7.88	7.58	**-**	17.08	10.83	100.18	134.48	82.15
5-10cm	**BAM**	**NC**	**-**	5.91	5.75	**-**	11.17	6.33	309.63	66.00	36.42
5-10cm	**SUF**	**CC**	**-**	6.10	5.27	**-**	19.23	23.56	319.47	117.26	124.20
5-10cm	**SUF**	**NC**	**-**	9.78	5.69	**-**	11.30	21.21	156.49	110.53	120.79
10-15cm	**NP**	**CC**	**-**	6.28	-	**-**	19.73	19.68	-	123.90	-
10-15cm	**NP**	**NC**	**-**	7.53	-	**-**	12.41	25.68	350.48	93.41	-
10-15cm	**BAM**	**CC**	**-**	3.57	-	**-**	29.40	14.08	428.43	105.01	-
10-15cm	**BAM**	**NC**	**-**	5.18	-	**-**	11.99	12.59	82.30	62.17	-
10-15cm	**SUF**	**CC**	**-**	9.08	-	**-**	8.96	24.34	280.13	81.33	-
10-15cm	**SUF**	**NC**	**-**	5.35	-	**-**	15.21	17.46	406.25	81.42	-

Table C.6 Least squares (LS) means for dissolved organic carbon (C), and citrate-extractable phosphorus (P) for fall 2020, 2021, and 2022 for all main effects and interactions. Table abbreviations include no P (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

			Fall 2020	Fall 2021	Fall 2022	Fall 2020	Fall 2021	Fall 2022
			Dissolved Organic C			Citrate-Extractable P		
			mg C kg soil^{-1}			mg P kg soil^{-1}		
NP			37.22	39.33	39.40	0.81	1.05	1.23
BAM			37.83	38.90	41.52	1.46	2.07	4.09
SUF			37.84	42.11	38.96	0.96	1.29	1.52
CC			36.80	41.30	38.36	1.06	1.28	2.16
NC			38.46	38.93	41.56	1.09	1.66	2.40
NP	**CC**		36.81	42.00	37.82	0.79	1.05	1.23
NP	**NC**		37.64	36.66	40.98	0.84	1.04	1.24
BAM	**CC**		33.89	42.01	39.30	1.34	1.53	3.85
BAM	**NC**		41.77	35.79	43.74	1.57	2.61	4.32
SUF	**CC**		39.71	39.88	37.95	1.05	1.25	1.39
SUF	**NC**		35.97	44.35	39.97	0.86	1.33	1.64
0-5cm			28.06	47.72	44.46	1.44	1.54	4.20
5-10cm			45.16	41.21	35.46	0.97	1.59	1.29
10-15cm			39.67	31.41	-	0.82	1.28	1.34
0-5cm	**NP**		28.29	47.46	45.55	0.90	0.94	1.34
0-5cm	**BAM**		25.66	47.00	44.70	2.15	2.48	9.15
0-5cm	**SUF**		30.23	48.70	43.14	1.25	1.19	2.12
5-10cm	**NP**		39.36	30.66	33.26	0.62	1.19	1.02
5-10cm	**BAM**		42.90	32.02	38.34	1.47	2.24	1.77
5-10cm	**SUF**		36.77	31.53	34.79	0.80	1.34	1.09
10-15cm	**NP**		44.02	39.87	-	0.91	1.01	1.34
10-15cm	**BAM**		44.94	37.68	-	0.74	1.48	1.35
10-15cm	**SUF**		46.52	46.10	-	0.82	1.35	1.34
0-5cm	**CC**		28.65	49.78	43.75	1.33	1.23	4.09
0-5cm	**NC**		27.47	45.66	45.17	1.54	1.85	4.31
5-10cm	**CC**		37.07	33.09	32.97	0.98	1.33	1.04
5-10cm	**NC**		42.28	29.72	37.95	0.95	1.84	1.55
10-15cm	**CC**		44.69	41.01	-	0.87	1.27	1.34
10-15cm	**NC**		45.63	41.42	-	0.77	1.29	1.34
0-5cm	**NP**	**CC**	26.25	55.89	42.54	0.91	0.92	1.32
0-5cm	**NP**	**NC**	30.33	39.03	48.56	0.90	0.96	1.35
0-5cm	**BAM**	**CC**	23.24	47.63	44.74	1.59	1.72	9.14
0-5cm	**BAM**	**NC**	28.08	46.37	44.66	2.71	3.24	9.16
0-5cm	**SUF**	**CC**	36.47	45.83	43.97	1.48	1.03	1.81
0-5cm	**SUF**	**NC**	24.00	51.57	42.30	1.02	1.35	2.43

5-10cm	**NP**	**CC**	40.29	30.79	33.11	0.59	1.21	1.00
5-10cm	**NP**	**NC**	38.43	30.54	33.40	0.65	1.17	1.04
5-10cm	**BAM**	**CC**	35.71	37.32	33.86	1.55	1.51	1.07
5-10cm	**BAM**	**NC**	50.08	26.72	42.81	1.40	2.96	2.46
5-10cm	**SUF**	**CC**	35.21	31.17	31.93	0.80	1.28	1.03
5-10cm	**SUF**	**NC**	38.32	31.90	37.65	0.80	1.40	1.14
10-15cm	**NP**	**CC**	43.90	39.32	-	0.87	1.02	1.36
10-15cm	**NP**	**NC**	44.14	40.41	-	0.95	1.00	1.32
10-15cm	**BAM**	**CC**	42.73	41.08	-	0.87	1.34	1.35
10-15cm	**BAM**	**NC**	47.15	34.27	-	0.61	1.62	1.35
10-15cm	**SUF**	**CC**	47.44	42.64	-	0.88	1.45	1.32
10-15cm	**SUF**	**NC**	45.60	49.57	-	0.76	1.25	1.36

Table C.7 Least squares (LS) means for dissolved total nitrogen (N), NH_4-N, NO_3-N, and inorganic N for fall 2021 and 2022 for all main effects and interactions. No data is reported for fall 2020. Table abbreviations include no phosphorus (P) (NP), build and maintain (BAM), sufficiency (SUF), presence of a cover crop (CC), and absence of a cover crop (NC).

			Fall 2021	Fall 2022	Fall 2021	Fall 2022	Fall 2021	Fall 2022	Fall 2021	Fall 2022
			Dissolved Total N		NH_4-N		NO_3-N		Inorganic N	
			mg N kg soil^{-1}		mg NH_4 kg soil^{-1}		mg NO_3 kg soil^{-1}		mg N kg soil^{-1}	
NP			15.28	8.41	3.68	1.48	5.08	4.34	8.03	2.57
BAM			13.63	9.12	5.92	3.04	5.30	3.69	9.14	4.53
SUF			11.75	7.96	5.83	2.56	5.17	3.01	8.85	4.00
CC			13.39	8.29	5.58	2.25	5.42	4.00	9.41	3.74
NC			13.71	8.70	4.71	2.47	4.96	3.37	7.94	3.66
NP	**CC**		13.51	8.90	3.80	1.51	5.43	4.23	8.03	3.21
NP	**NC**		17.05	7.91	3.56	1.45	4.73	4.46	8.02	1.93
BAM	**CC**		13.76	8.91	6.54	2.73	5.35	3.99	10.01	4.49
BAM	**NC**		13.49	9.32	5.29	3.34	5.25	3.40	8.28	4.56
SUF	**CC**		12.91	7.06	6.40	2.50	5.46	3.78	10.18	3.53
SUF	**NC**		10.59	8.86	5.27	2.63	4.88	2.24	7.51	4.47
0-5cm			13.84	8.56	8.34	5.19	6.22	3.60	11.94	6.35
5-10cm			14.85	6.90	3.08	0.92	5.67	4.17	6.37	2.26
10-15cm			11.96	10.02	4.01	0.96	3.66	3.29	7.71	2.49
0-5cm	**NP**		16.03	8.51	5.73	3.02	5.69	4.95	10.68	4.31
0-5cm	**BAM**		15.00	8.97	10.09	6.40	5.55	5.01	15.10	7.91
0-5cm	**SUF**		10.51	8.19	9.21	6.17	7.42	0.84	10.05	6.83
5-10cm	**NP**		18.89	7.86	3.13	0.77	5.95	4.82	7.95	1.51
5-10cm	**BAM**		15.16	6.47	4.79	1.53	6.31	2.89	6.28	2.79
5-10cm	**SUF**		10.50	6.38	4.11	0.59	4.77	4.78	8.89	3.16
10-15cm	**NP**		10.92	8.85	2.18	0.66	3.61	3.27	5.45	1.90
10-15cm	**BAM**		10.72	11.90	2.88	1.19	4.05	3.17	6.05	2.88
10-15cm	**SUF**		14.25	9.30	4.18	0.93	3.33	3.41	7.60	2.00
0-5cm	**CC**		15.35	8.18	9.08	5.17	6.58	4.15	13.23	6.29
0-5cm	**NC**		12.33	8.93	7.61	5.22	5.86	3.05	10.66	6.41
5-10cm	**CC**		12.28	7.25	4.37	0.73	6.52	4.37	8.22	2.73
5-10cm	**NC**		17.42	6.56	3.65	1.19	4.83	3.96	7.20	2.25
10-15cm	**CC**		12.55	9.43	3.29	0.84	3.15	3.48	6.77	2.20
10-15cm	**NC**		11.38	10.60	2.87	1.01	4.18	3.09	5.96	2.31
0-5cm	**NP**	**CC**	17.14	8.67	6.75	2.88	6.32	4.24	10.99	5.13
0-5cm	**NP**	**NC**	14.91	8.35	4.71	3.16	5.06	5.66	10.37	3.50
0-5cm	**BAM**	**CC**	17.40	8.44	11.19	6.51	5.48	6.69	17.88	7.51
0-5cm	**BAM**	**NC**	12.60	9.50	8.98	6.28	5.61	3.34	12.31	8.31
0-5cm	**SUF**	**CC**	11.52	7.45	9.29	6.12	7.93	1.52	10.81	6.25
0-5cm	**SUF**	**NC**	9.49	8.93	9.13	6.22	6.90	0.16	9.29	7.42

5-10cm	**NP**	**CC**	11.97	9.65	2.64	0.87	6.30	4.97	7.61	2.34
5-10cm	**NP**	**NC**	25.81	6.07	3.62	0.67	5.59	4.67	8.29	0.68
5-10cm	**BAM**	**CC**	12.12	6.70	5.47	0.75	7.70	2.58	6.48	3.18
5-10cm	**BAM**	**NC**	18.19	6.24	4.11	2.30	4.92	3.20	6.09	2.40
5-10cm	**SUF**	**CC**	12.74	5.40	5.00	0.57	5.55	5.57	10.57	2.66
5-10cm	**SUF**	**NC**	8.25	7.37	3.22	0.61	3.99	4.00	7.22	3.67
10-15cm	**NP**	**CC**	11.41	8.38	2.00	0.78	3.68	3.49	5.50	2.17
10-15cm	**NP**	**NC**	10.42	9.32	2.36	0.53	3.54	3.04	5.40	1.62
10-15cm	**BAM**	**CC**	11.77	11.59	2.97	0.94	2.87	2.70	5.66	2.77
10-15cm	**BAM**	**NC**	9.68	12.22	2.79	1.43	5.23	3.65	6.44	2.98
10-15cm	**SUF**	**CC**	14.46	8.33	4.91	0.80	2.90	4.26	9.16	1.67
10-15cm	**SUF**	**NC**	14.03	10.27	3.46	1.05	3.76	2.57	6.03	2.32

Appendix D - SAS Codes

2021

```
DATA SET1;
INPUT PLOT BLOC TRT $ CC $ T0-T5;
CARDS;
;
RUN;
PROC SORT DATA=SET1;BY PLOT BLOC TRT CC;
PROC TRANSPOSE DATA=SET1 OUT=SET2(RENAME=(COL1=RESP)) NAME=TIME;BY PLOT BLOC
TRT CC;
RUN;
PROC FORMAT;
VALUE $SD 'T0'='APR 27' 'T1'='MAY 4' 'T2'='MAY 26' 'T3'='JUN 17' 'T4'='JUL 7'
'T5' = 'SEPT 17';
RUN;
PROC TABULATE DATA=SET2 STYLE=[JUST=C];
FORMAT TIME $SD.;
CLASS TRT CC TIME;
VAR RESP;
TABLE TIME=' '*(TRT=' ' ALL='ACROSS'),(CC=' ' ALL='ACROSS')*RESP=' '*(N MEAN
STD MIN MAX);
RUN;
%MACRO MODEL_FIT(TYPE);
TITLE "&TYPE";
PROC MIXED DATA=SET2;
CLASS BLOC TRT CC TIME PLOT;
MODEL RESP=BLOC TRT|CC|TIME/DDFM=KR;
REPEATED  TIME/SUBJECT=PLOT TYPE=&TYPE;
ODS SELECT FITSTATISTICS;
RUN;
%MEND;
%MODEL_FIT(TYPE=CS);
%MODEL_FIT(TYPE=CSH);
%MODEL_FIT(TYPE=AR(1));
%MODEL_FIT(TYPE=ARH(1));
%MODEL_FIT(TYPE=UN);
/*PICK THE TYPE WITH THE SMALLEST AIC/BIC/AICC*/
ODS GRAPHICS/ RESET;
TITLE;
PROC MIXED DATA=SET2 PLOTS=(STUDENTPANEL);
CLASS BLOC TRT CC TIME PLOT;
MODEL RESP=BLOC TRT|CC|TIME/DDFM=KR;
REPEATED TIME/SUBJECT=PLOT TYPE=CSH;
LSMEANS  CC TIME TRT/pdiff;
*SLICE CC*TIME/SLICEBY=CC PDIFF LINES;
ODS EXCLUDE DIFFPLOT;
ODS OUTPUT LSMEANS=LSM ;
RUN;
```

2022

```
DATA SET1;
INPUT PLOT BLOC TRT $ CC $ T0-T5;
CARDS;
```

```
;
RUN;
PROC SORT DATA=SET1;BY PLOT BLOC TRT CC;
PROC TRANSPOSE DATA=SET1 OUT=SET2(RENAME=(COL1=RESP)) NAME=TIME;BY PLOT BLOC
TRT CC;
RUN;
PROC FORMAT;
VALUE $SD 'T0'='APRIL 27' 'T1'='MAY 23' 'T2'='JUNE 15' 'T3'='JULY 8'
'T4'='AUGUST 5' 'T5' = 'OCTOBER 21';
RUN;
PROC TABULATE DATA=SET2 STYLE=[JUST=C];
FORMAT TIME $SD.;
CLASS TRT CC TIME;
VAR RESP;
TABLE TIME=' '*(TRT=' ' ALL='ACROSS'),(CC=' ' ALL='ACROSS')*RESP=' '*(N MEAN
STD MIN MAX);
RUN;
%MACRO MODEL_FIT(TYPE);
TITLE "&TYPE";
PROC MIXED DATA=SET2;
CLASS BLOC TRT CC TIME PLOT;
MODEL RESP=BLOC TRT|CC|TIME/DDFM=KR;
REPEATED  TIME/SUBJECT=PLOT TYPE=&TYPE;
ODS SELECT FITSTATISTICS;
RUN;
%MEND;
%MODEL_FIT(TYPE=CS);
%MODEL_FIT(TYPE=CSH);
%MODEL_FIT(TYPE=AR(1));
%MODEL_FIT(TYPE=ARH(1));
%MODEL_FIT(TYPE=UN);
/*PICK THE TYPE WITH THE SMALLEST AIC/BIC/AICC*/
ODS GRAPHICS/ RESET;
TITLE;
PROC MIXED DATA=SET2 PLOTS=(STUDENTPANEL);
CLASS BLOC TRT CC TIME PLOT;
MODEL RESP=BLOC TRT|CC|TIME/DDFM=KR;
REPEATED TIME/SUBJECT=PLOT TYPE=ARH(1);
LSMEANS TRT|CC|TIME;
*SLICE TRT*CC*TIME/SLICEBY=TIME PDIFF LINES;
ODS EXCLUDE DIFFPLOT;
ODS OUTPUT LSMEANS=LSM ;
RUN;
```

Multi Depth for Fall

```
TITLE "Fall2020";
data MultiDepth20;
input plot rep fert $ cover $ depth$ response;
datalines;
PROC Print DATA= MultiDepth20;
;
proc glimmix data = MultiDepth20;
class depth rep fert cover;
model response = fert|cover|depth/ddfm = satterth;
random rep ;
lsmeans fert|cover|depth/lines cl;
```

```
Run;

TITLE "Fall2021";
data MultiDepth21;
input plot rep fert $ cover $ depth$ response;
datalines;
PROC Print DATA= MultiDepth21;
;
proc glimmix data = MultiDepth21;
class depth rep fert cover;
model response = fert|cover|depth/ddfm = satterth;
random rep ;
lsmeans fert|cover|depth/lines cl;
Run;

TITLE "Fall2022";
data MultiDepth22;
input plot rep fert $ cover $ depth$ response;
datalines;
PROC Print DATA= MultiDepth22;
;
proc glimmix data = MultiDepth22;
class depth rep fert cover;
model response = fert|cover|depth/ddfm = satterth;
random rep ;
lsmeans fert|cover|depth/lines cl;
Run;
```