Linkages between cover crops, phosphorus fertilizer management, soil health, and phosphorus bioavailability in replicated research watersheds

by

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AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

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Abstract

Phosphorus (P) pollution from agricultural remains a persistent and complex problem that negatively affects freshwater quality, causing harmful algal blooms and eutrophication. Phosphorus can be lost from fields as sediment bound solids and dissolved in leachate or runoff. Phosphorus is cycled through the soil ecosystem via biotic and abiotic interactions as organic or inorganic compounds. Conservation practices such as no-till and cover cropping have been promoted as ways to promote soil health and reduce sediment loss from cropping systems. A growing body of research has documented increased dissolved reactive P in runoff from cover crops. It is not clear how conservation management interacts with P fertilizer management, nor what their impact is on the biogeochemical cycling of P and its potential for loss. The objective of this study was to document the impact of cover crops and P fertilizer management on P bioavailability and stratification, as well as investigate changing nutrient status on microbial biomass P (MB-P) and the activity of P cycling enzymes. In 2014, a field scale experiment was established in a no-till, corn-soybean cropping system, at the Kansas Agricultural Watershed in NE Kansas. The experiment was organized as a 2*3 full factorial with eighteen, 0.5 ha watersheds, in a randomized complete block design. A cover crop treatment consisted of cover crop (CC) or no cover crop (NC), was implemented with three P fertilizer management treatments; fall surface broadcast diammonium phosphate (FB), spring subsurface injected ammonium polyphosphate (SI), or no P fertilizer (NP). The first objective was accomplished by measuring the gross P pools such as total P (P_T), and total organic P (P_O), as well as bioavailable P pools such as water extractable P (P_W), and 2 mM citric acid extractable P (P_C), at the 0-5 cm depth (spring/fall 2018 and 2019), and 5-10/10-15 cm depths (fall 2018, and spring/fall 2019). Additionally, we used diffusive gradient thin films (P_{DGT}) to measured total soil-water available

P, and Mehlich-III (P_M) to measure the agronomically relevant P, at the 0-5 cm depth (spring/fall 2018 and 2019). The second objective was addressed by measuring MB-P, and P cycling enzyme activity (acid and alkaline phosphatase, and phosphodiesterase) at the 0-5 cm depth, in fall 2018 and spring/fall 2019. We documented P stratification of P_T in all treatments in fall 2018 and spring 2019, but reduced stratification in NP, and increased stratification in FB and SI by fall 2019. Total organic P was highest in the 5-10 cm depth in FB and SI in spring/fall 2019. While NP treatments almost always had less P than the fertilized treatments, it had either the same or more P_O than FB and SI. The labile pools of P, P_W and P_C, were stratified in FB*CC, FB*NC, SI*CC treatments but not in SI*NC, NP*NC, NP*CC in spring 2019 (P_W) and fall 2018 and spring 2019 (P_C). There were cover crop*P fertilizer interactions in the 0-5 cm depth where a SI*CC increased the amount of P compared to SI*NC in P_W (spring 2019), P_C (fall 2018 and spring 2019), and P_{DGT} (spring 2019). Cover crops did not affect the amount of P_W, P_C, P_{DGT}, or P_M in the 0-5 cm depth of NP or FB fertilizer management at any time. Cover crops reduced the amount of P_C at 5-10 cm (fall 2018 and spring 2019) and P_{DGT} at 10-15 cm (fall 2019). Almost identical P fertilizer * cover crop interactions from P_C and P_{DGT} was detected in MB-P in spring/fall 2019. Cover crops consistently increased P cycling enzyme activity compared to NC treatments. The MB-P was higher in fertilized plots compared to NP treatments in all seasons. Low MB-C:P in NP treatments suggest conditions for P immobilization by microorganisms, possibly contributing to organic P pools. These results suggest that cover crops could be translocating P in spring subsurface applied ammonium polyphosphate, that was then being stored in labile P pools, such as MB-P. At the same time, cover crops may be increasing the potential for organic P mineralization in all fertilizer management treatments. This body of research demonstrates that cover cropping and P fertilizer management in no-till corn-soybean

cropping systems interact, changing where and how P is stored and cycled. Further research will be necessary to develop more nuanced management recommendations to optimize soil fertility and reduce P loss to runoff.

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Dedication

This dissertation is dedicated to my daughters, Madeleine and Chloe Starr. You are what fuels me every day. Your love and support has made this work possible.

Chapter 1 - General Introduction

Agriculture and Water Quality

The agricultural community is responding to separate and often conflicting demands, to increase production to feed the growing global population and simultaneously reduce negative impacts on the environment. In the United States, the agriculture sector is crucial to the economy, making up 5.5% of the gross domestic product (GDP) and 10% of exports (USDA ERS, 2017). In the quest to boost production to provide plentiful and affordable crops for human consumption, pollution and degradation of natural resources has been an ever-persistent problem (Srivastava, Rishikesh, Sachchidanand, & Akhilesh, 2016). Agricultural production has been identified as a significant source of sediment, nitrogen (N) and phosphorous (P) pollution, leading to surface water quality issues (Sharpley & Tunney, 2000). The increase in P pollution to surface water is especially detrimental, although not exclusive, to freshwater bodies (Moody, 2011).

Nutrient and sediment loss have been the focus of much research and debate as they affect human and environmental health and harm fisheries, recreation, property values, and community wellbeing (Sharpley & Tunney, 2000). The effects of climate change are expected to exacerbate problems in agricultural production, such as drought, and impact environmental considerations like eutrophication (Zare, Nazari, Mohammady, Teimurian, & Brazrafshan, 2016; Carpenter, Booth, & Kucharik, 2017). Climate models predict more intense storms with longer periods of drought as global temperatures rise and weather patterns change (Reichenwaldt & Ghadouani, 2012; Paerl, Hall, Peierls, & Rossignol, 2014). Precipitation extremes have a log-linear relationship with P loading, where a handful of large rainfall events produce the majority

of the P load in runoff (Carpenter et al., 2017). Under conventional tillage, where the potential for erosion is higher, Fraser, Harrod, & Haygarth (1999) documented an increase of 100 kg sediment ha^{-1} h^{-1}, 303 g particulate P ha^{-1} h^{-1}, and 203 g dissolved reactive P ha^{-1} h^{-1} when rainfall intensity increased from 1 mm to 10 mm h^{-1}. Climate change predictions, in conjunction with the Revised Universal Soil Loss Equation (RUSLE) model, suggest that rainfall erosivity will increase by 10-35%, with corresponding predictions for soil loss (Zare et al., 2016). This is expected to impact the magnitude of erosion and nutrient pollution by impacting soil surface-freshwater interactions and water dynamics (Paerl & Paul, 2012; Paerl et al., 2014). More than 50% of U.S. croplands are on slopes > 2%, where 33% of the steepest land is expected to fall out of production due to erosion and loss of topsoil in the next 100 years (Montgomery, 2010).

Rainfall intensity and soil infiltration influence runoff characteristics (Kleinman, Srinivasan, Dell, & Schmidt, 2006). As the intensity of the rain or the slope of the field increases, the depth of surface soil that interacts with the rainfall increases. When rainfall increased from 50 to 160 mm h^{-1} on 2% slope, on conventionally tilled fields, the interaction depth between the surface soil and water increased 11.5 mm (Kleinman et al., 2006). On steeper slopes (>2%), this change in interaction depth occurred with less rain (Kleinman et al., 2006). The erosive power of rain events, the increased volume of water, and larger interaction depth is expected to increase the load and concentration of P in “pulse” discharges (Carpenter et al., 2017). This increased interaction depth between the soil surface and rain, and pulse discharge could affect the more than 50% of U.S. cropland with an elevated slope ratio.

Surface water quality is negatively impacted by crop production due to eroded sediment and nutrients (suspended and dissolved) in water runoff. These pollutants are transported to water bodies, either by surface or leaching, where they over-supply the aquatic ecosystem with

bioavailable nutrients and reduce water clarity (Chislock, Doster, Zitomer, & Wilson, 2013; Smith, Huang, & Haney, 2017). Some of these nutrients are then taken up by phytoplankton and result in excessive growth, known as algal blooms (Chislock et al., 2013; Carpenter et al., 2017). Algal blooms are the leading cause of anthropogenic eutrophication, where algal growth can limit light penetration, increase pH levels, and deplete dissolved inorganic carbon. When the phytoplankton die, bacteria decompose the biomass and consume the oxygen in the water, suffocating other organisms (Chislock et al., 2013). These blooms lead to fish kills, non-potable drinking water, and noxious smells. Eutrophication from P runoff is a dangerous and costly problem for many communities. Dodds et al. (2009) estimated the cost of freshwater eutrophication in the United States at $2.2 billion annually, with the majority of the direct costs borne by a handful of communities.

In addition to these devastating effects, some algal blooms produce harmful toxins further endangering community health, livestock, and wildlife (Boesch, Brinsfield, & Magnien, 2001; Paerl & Paul, 2012; Chislock et al., 2013). The toxic nature of the harmful algal blooms (HABs) is expected to be exacerbated under future climate conditions due to the toxinogenic cyanobacteria's ability to thrive in low light, warm, stagnant, nutrient rich conditions (Paerl & Paul, 2012; Reichwaldt & Ghadouani, 2012). In addition to increases in temperature, changes in rainfall patterns are also expected to increase the occurrence of HABs. As storm events are predicted to become more intense but increasingly sporadic, large inputs of nutrients from more erosive runoff are expected, followed by periods of drought and evaporation from water bodies, increasing nutrient concentrations and reducing water column mixing (Reichwaldt & Ghadouani, 2012).

Some research has shown that many forms of recalcitrant P in sediment such as apatite, previously thought unavailable to biota, are utilized by cyanobacteria (Bostrom, Persson, & Broberg, 1988; Okubo, Inoue, & Yokota, 2012). In addition to recalcitrant P, organic P often considered unavailable or resistant to degradation has been shown to contribute to algal growth (Turner, McKelvie, & Haygarth, 2002). This can occur via the release of orthophosphate from enzyme hydrolysis of organic molecules or direct uptake of organic P, such as the case of inositol-P, used by several algal species (Turner et al., 2002).

Despite the eutrophication of surface waters, some research suggests that in Europe, P fertilizers are still chronically over-applied (Jordan-Meille et al., 2012; Buczko et al. 2018). In the Unites States, research has demonstrated that P fertilizer applications elicit lower than expected crop responses (Dodd & Mallarino, 2005; Heckman et al., 2006). Some of these seemingly incongruent facts have been partially explained by a lack of updating nutrient needs for new varieties of crops, and inadequate soil P measurement methods and interpretation (Dodd & Mallarino, 2005; Jordan-Meille et al., 2012; Buczko et al. 2018). Despite increased research and mitigation techniques, pollution from agricultural production has remained a persistent and complex problem (Paerl et al., 2014; Dodd & Sharpley, 2016; Jarvie, et al., 2017).

Conservation Management, Soil Health, and Water Quality

To address the environmental concerns such as erosion, nutrient loss, and water pollution, the United States Department of Agriculture, Natural Resources Conservation Service (USDA-NRCS) promotes conservation practices that are designed to reduce erosion and pollution while improving soil health. This shift to emphasize soil health and conservation management techniques include changes in tillage practices and planting cover crops (NRCS, 2015; Dodd &

Sharpley, 2016). Conservation tillage and cover crops have been shown to improve soil health by increasing soil organic matter, reducing erosion and N loss, and improving aggregate stability. These practices have been shown to increase the microbial biomass pool by 50% (Simeone, Muller, Felgentreu, & Glaser, 2020) and reduce nitrate loss by 70-90% (Hanrahan et al., 2018).

No-till improves soil health by reducing the mechanical mixing of soil from conventional tillage, thereby preserving the aggregate structure and sequestering C in soil (Ogle, Swan, and Paustian, 2011). Good aggregation promotes an optimal balance of air and water in soil, promotes microbial activity and diversity, and reduces the potential for soil particles to succumb to erosive forces (Blanco-Canqui, Mikha, Presley, & Claassen, 2011). No-till has been shown to reduce sediment in runoff by up to 80%, in multiple ecoregions around the globe (Smith, Francesconi, Livingston, & Huang, 2015; TerAvest, Carpenter-Boggs, Thierfelder, & Reganold, 2015). Cover crops have been shown to further reduce erosion by increasing surface plant biomass, thereby reducing the erosive energy of wind and water while improving aggregate stability by increasing soil organic matter in the soil profile and increasing macropores from their root growth (Eichler-Lobermann, Kohne, Kowalski, & Schnug, 2008; Blanco-Canqui et al., 2011). Blanco-Canqui et al. (2011) demonstrated that cover crops enhanced the benefits of no-till management by increasing the soil organic carbon by 30-40%, mean weight diameter of the aggregates, and infiltration compared to no-till alone.

Cover cropping has long been recommended to improve soil physical properties but also as a means to make P more bioavailable (Eichler-Lobermann et al., 2008; Teboh & Frazen, 2011; Dodd & Sharpley, 2015). Cover crops, such as fava beans (*Vicia faba*), Phacelia or blue tansy (*Phacelia tanacetifolia*), buckwheat (*Fagopyrum esculenum*) and white lupine (*Lupinus albus*) can have high P uptake efficiency and access fractions not available to cash crops (Horst, Manh,

Jibrin, & Chude. 2001; Eichler-Lobermann et al., 2008; Teboh & Frazen, 2011). They can excrete mineral P solubilizing compounds and P_O hydrolyzing enzymes, and improve microorganism activity that can increase the volume of soil explored and compounds excreted that increase P availability; frequently in excess of their own needs (Schilling, Gransee, Deubel, Lezovic, & Ruppel, 1998; Richardson & Simpson, 2011; Eichler, 2004).

After cover crop termination, the decomposing organic matter can provide readily bioavailable P to microbes, which in turn becomes mineralized and available for crops (Dabney, 1998; Eichler-Lobermann et al., 2008). Eichler-Lobermann et al. (2008) determined that P mobilization from cover crop growth was the main mechanism of increased P availability in their study rather than the decomposing plant material. They found that the cover crop treatment increased the P content of the soil before the plant residue decomposed (Eichler-Lobermann et al., 2008). They demonstrated that planting blue tansy as a cover crop had a comparable effect to fertilizing soil with manure or mineral P fertilizers (Eichler-Lobermann et al., 2008). Although increased bioavailability of P from cover crops could be beneficial to crop yields and reduces dependence on inorganic fertilizer, it may have negative consequences if availability does not coincide with need (Eichler-Lobermann et al., 2008; Dodd & Sharpley, 2015).

Although these conservation practices have been credited with reducing erosion from fields and sediment in water bodies while also reducing nitrogen losses that negatively affect air and water quality (Daryanto, Wang, & Jacinthe, 2017), increasing soil organic matter (Hartl & Erhart, 2005), and improving microbial activity (Simeone et al., 2020), their impact on phosphorus loss is not clear (Dodd & Sharpley, 2016; Jarvie et al., 2017). In the Lake Erie Basin, a watershed that suffers from persistent HAB's, there was a period in the 1980's and 90's of improved water quality (Smith et al., 2015; Jarvie et al., 2017). Although it was speculated that

the implementation of conservation practices, such as cover cropping and no-till, would reduce phosphorus losses (Boesch et al., 2001; Sharpley, Foy, & Withers, 2000; Sharpley et al., 2015; Daryanto et al., 2017; Leinweber et al., 2018), the watershed has entered a period of re-eutrophication (Dodd & Sharpley, 2016; Jarvie et al., 2017; Smith et al., 2017). This reversal has been linked to increased dissolved bioavailable phosphorus, despite the wide adoption of the NRCS recommended conservation practices (Dodd & Sharpley, 2016; Jarvie et al., 2017).

Conservation Management and P loss

Phosphorus is cycled in the soil through a complex web of interactions. The form of the P compound, its storage in the soil, and the relative bioavailability are mediated by soil physical, chemical and biological properties. It is not clear what effect conservation management practices and the resulting changes to soil health parameters have on phosphorus cycling and loss. Although no-till and cover crop planting have been successful at reducing the amount of sediment carrying bound phosphorus particulate matter in runoff, some research has demonstrated that the amount of dissolved, bioavailable P has significantly increased (Smith et al., 2015; Daryanto et al., 2017; Smith et al., 2017). A growing body of evidence suggests that the benefits accrued by conservation practices are either ineffectual or negatively impacting phosphorus pollution (Boesch et al., 2000). A meta-analysis conducted by Daryanto et al. (2017) examined the link between particulate and dissolved phosphorus losses and no-till. They determined that there were reductions of total P and a 45% reduction of particulate P in dry years, on minimal slopes (<3%) but not different from conventional tillage in wet years, or on steeper slopes. Dissolved P was the same in no-till and conventional tillage in normal and dry years, while there was significant increase in concentration but not load in the wet years under

no-till (Daryanto et al., 2017). Tillage had no effect on P losses when planted with soybean or on steep slopes (4-9%). Benefits of reduced total P or particulate P under no-till were not evident after more than 10 yr of implementation (Daryanto et al., 2017). Other research has shown similar results where no-till increased dissolved reactive P (DRP) by 100% and decreased total P by 69% compared to rotational tillage (Smith et al., 2015).

Phosphorus fertilizer best management practices (BPM) have been developed to optimize fertilizer uptake by plants while decreasing loss to runoff and leachate. Research has documented an increase of DRP in runoff events as a function of water soluble P (WSP) in fertilizer sources such as triple superphosphate (79% WSP) and swine manure (70% WSP) (Kleinman, Srinivasan, Dell, & Schmidt, 2006; Shigaki, Sharpley, Prochnow, 2007; Shigaki & Sharpley, 2011). Kleinman et al. (2006) determined that the greatest total P losses came from sites with the higher transport potential/ lower P concentration (mid-slope) and lower total P but higher DRP was in lower transport potential (hill bottom) but high P concentration. They found that higher rainfall increased the volume of runoff and the total amount of P lost. Shigaki et al. (2007) demonstrated the importance of the degree of water solubility of the P source and rainfall intensity on P transportation. They found that all losses were significantly greater at 7.5 cm h^{-1} than 2.5 cm h^{-1} of rainfall and DRP loss increased as percent water solubility increased (Shigaki et al. 2007). Concentrations of DRP loss at 2.5 cm h^{-1} were 28.21 mg L^{-1} from triple superphosphate compared to 0.25 mg L^{-1} from rock phosphate (Shigaki et al. 2007). These results demonstrate the combined influence of precipitation event characteristics and nutrient solubility on P loss in the high rainfall scenarios predicted by climate models (Kleinman et al., 2006; Shigaki et al. 2007, Paerl et al., 2014). Question remains regarding the interaction between conservation management, such as no-till/cover cropping, and P fertilizer management.

There are several mechanisms that may be responsible for the unforeseen rise in P pollution due to no-till and cover crop implementation. Soil stratification and P accumulation at the soil surface have been identified as possible reasons that no-till has not been more effective (Smith et al., 2015; Smith et al., 2017; Dodd & Sharpley, 2016; Jarvie et al., 2017). In no-till management, P fertilizer is often broadcast on the soil surface, concentrating the nutrient in the top few mm of soil due to the lack of mechanical incorporation, which is where surface water has the most interaction (Sims, Edwards, Schoumans, & Simard, 2000). Smith et al. (2017) found that injecting liquid polyphosphate fertilizer had less soluble and total P loss than annual and biannual surface broadcasting in no-till fields. In addition to fertilizer, crop residues (from no-till) and cover crops are left to accumulate on the soil surface, creating a protective barrier but potentially concentrating bioavailable phosphorus and increasing stratification. Although Sharpley (2003) found that tilling manured soils can reduce P stratification, Smith et al. (2017) demonstrated that disking had the opposite effect, increasing the amount of stratification while Dodd, McDowell, and Condron (2014) measured only a month of reduced stratification from tilling no-till fields before no difference could be detected.

It has been suggested that the presence of plant residue at the surface and in root biomass (consisting of 7-86% P) is readily available after freeze-thaw cycles and may contribute to DRP in runoff and leachate (Liu, Khalaf, Ule'n, & Bergkvist, 2013). The dead plant cells can spill the available P onto soil surfaces after these cycles. Macropores that are left by the root growth and improved aggregation can create preferential pathways for the loss of the labile P (Liu, Ule'n, Bergkvist, & Aronsson, 2014). Liu et al. (2013) easily extracted nearly all P from cover crop biomass with water after a small number of freeze-thaw cycles in a simulated study. These plant

residue sources of P on the soil surface could increase soluble P surface runoff, while the increased infiltration from macropores could facilitate leaching (Jarvie et al, 2017).

In addition to P from plant residue, microbial biomass P (MB-P) (the main component of the active organic P pool) is readily available after not only freeze-thaw but dry-wet cycles that are common in most agriculturally productive areas. The accumulation of a large MB-P pool could potentially be detrimental to P losses in runoff, as the pool could be vulnerable to cell lysis from drought and storm patterns (Turner & Haygarth, 2001, Blackwell et al., 2010). The rapidly cycling, MB-P pool can make up 0.7-2.5% of total P in cropland and 2-7.5% for fertilized pasture (Oberson & Joner, 2005). He, Wu, O'Donnell, and Syers (1997) demonstrated a 200-500% enrichment of MB-P with applications of manure in pasture, while microbial biomass C (MB-C) only increased 15-20%. It is possible that fertilized, no-till, cover crop managed fields may share some characteristics with both cropland and pasture as the soil profile is left intact and cover crop residues remain in the system. It also may be comparable to manure applications generally, as labile C from crop residues and fertilizer application add labile nutrients to the system.

Organic P has been shown to provide similar amounts of P to crops as inorganic P in fertilized fields (Sharpley 1985). Organic P makes up 30-65% of the total P, and 5-52% of the total P are forms of organic P that are labile to moderately labile (Condron, Turner, & Cade-Menun, 2005). DeLuca et al. (2015) determined that over a large variety of soils in the United Kingdom, a decrease in $CaCl_2$ extractable, citrate extractable, and mineral occluded P was linked to a corresponding increase in phosphatase extractable P, suggesting that as inorganic P was diminished, organic P was available for mineralization. Studies have shown that dissolved and particulate organic P are a large portion of leachate, especially where soil organic matter has

increased (Dodd & Sharpley, 2015). In fact, organic P (other than phytate P) is more mobile than ortho-P, once it is in solution (Leytem, Mikkelsen, & Gilliam, 2002; Dodd & Sharpley, 2015). Toor, Condron, Di, Cameron, and Cade-Menun (2003) demonstrated that organic P accounted for more than 80% of the P in leachate from pasture regardless whether it was fertilized with manure or mineral P fertilizer. In addition to the loss of organic P in the study, they found that it was available for hydrolysis by enzymes in water during transport (Toor et al., 2003).

Higher mineralization rates have been documented in soils where soil health is improved (Oberson, Besson, Maire, & Sticher 1996; Oehl, Frossard, Fliessbach, Dubois, & Obersson, 2004; Requejo & Eichler-Lobermann, 2014). The P cycle is mediated by microbial activity and plants roots which are in turn influenced by soil health parameters such as organic matter, C quality, aggregation, and water dynamic which are sensitive to crop management. Extracellular enzymes excreted by roots and microorganisms enable the mineralization of organic P. The size of the microbial community and the quality of C have been shown to be key factors in the rate of P mineralization. Oehl et al., (2004) documented higher rates of basal organic P mineralization in organically managed compared to conventionally managed cropping systems. This increase in mineralization could account for increased bioavailable P loss in no-till/cover cropped systems that share similar soil health benefits as organic management. A carbon limitation in the soil systems have been shown to drive the mineralization of P rich organic matter (Spohn & Kuzyakov, 2013).

The fluctuations of soil C:N:P have been shown to influence microbial biomass C:N:P composition, induce microbial community structural changes, and change enzyme activity (Zhao et al. 2018). The nutrient demands of soil biota and soil stoichiometry illicit an enzyme response in order to make limiting nutrients available. Phosphorus demand and mineralization have been

linked to phosphatase production under differing P availability over a four year period (Olander & Vitousek, 2000), although mineralization of organic P can be driven by microbial C demand (Spohn & Kuzyakov, 2013). Marklein & Houlton (2012) and Olander & Vitousek (2000) demonstrated an increase in P availability suppressed phosphatase enzyme expression. However, higher N availability increased phosphatase expression. The quality of soil organic C and size of microbial biomass are important factors in rate of organic P turnover (Oberson & Joner, 2005), both of which are influenced by conservation management. Changes to soil stoichiometry such as labile C inputs from cover crop plant residues have been shown to cause short term N or P limitation and may promote the mineralization or mobilize less available forms of P into solution (Ehlers, Bakken, Frostegard, Frossard, & Bunemann, 2010; Kirkby et al 2014). Spatial variability in soil and high microbial activity in the rhizosphere could lead to a P limitation despite a high P status in the bulk soil leading to the mineralization of organic P. The application of manure has been shown to change microbial C:P ratios (He et al. 1997) and a quadratic relationship between P fertilizer and size of MB-P (Liu et al., 2008) illustrate the link between soil and microbial stoichiometry. Tillage, cover crops, and fertilizer management influence C:N:P ratios, as well as modifying different pools of each nutrient, changing nutrient demand, thus impacting biological and biogeochemical parameters (Gonzalez-Chavez et al., 2010). Changes to microbial parameters may change mineralization rates and how much and what forms of P are available for transportation by runoff or leachate water. It has been suggested that this chronic over-application of P fertilizer may in part be due to the soil test P measurements neglecting the role of organic P, rhizospheric processes, and soil buffering capacity that all contribute to the availability of P (Sims et al., 2000, DeLuca et al., 2015; Dodd & Sharpley, 2015).

Novel Phosphorus Testing Methods

The concentration of P in soil is often estimated by the ability of various extractants to extricate P from the soil. Frequently, these extractants are either acids or bases that solubilize some fraction of the mineral and organic P. Many agronomically relevant P extractants such as Colwell, Olsen, Bray-1, and Mehlich-3 were developed to estimate plant available P and were correlated to specific crop responses in particular regions/soil types (Tiessen & Moir, 2008). Traditional tests are reasonably well correlated with easily extractable inorganic P (Pote et al., 1999; Sims et al., 2000) but have less success at predicting crop responses to the applied fertilizer over multiple crops/locations, nor risk of potential P loss to surface water (Pautler & Sims, 2000; Menzies, Kusumo, & Moody, 2005; Moody, 2011; Jordan-Meille et al., 2012; Six, Smolders, & Merckx al., 2013; Dodd & Sharpley, 2015; Buczko et al., 2018). Six et al. (2013) found that common soil P tests, Colwell, Olsen, Bray-1, and Mehlich-3, could only explain <53% of the variation in yield response to increased P applications. The partial chemical extractions that characterize most traditional P measurement methods employ the separation of the solution/solid phases, which may interfere with the element partitioning and obscure their actual availability (Mason, Hamon, Nolan, Zhang, & Davidson, 2005). In addition to poor characterization of P, sampling techniques and P fertilizer recommendations have not changed with differences in tillage management and new crop cultivars (Dodd & Sharpley, 2015).

The characterization of P availability in the context of conservation management and soil health is a critical step in understanding how P (inorganic and organic) is contributing to both crop nutrition and water pollution. Organic acids are an important biotic uptake mechanism, where plants and microorganisms secrete acids to alter the rhizosphere to increase P availability (Jones, 1998; Richardson & Simpson, 2011; DeLuca et al., 2015; Menezes-Blackburn et al.,

2016). Organic acid extractants at concentrations similar to the soil ecosystem can range from 10-20 mM in the rhizosphere to 1-50 $\mu M^{-1}g$ in bulk soil. This approach may provide a more biologically focused way to measure P availability. Some research (Darch et al., 2016; DeLuca et al., 2015; Hayes, Richardson, & Simpson, 2000) proposed using citrate as alternative approach to measuring bioavailable P pools. Organic acids can complex metal cations such as aluminum (Al) and iron (Fe) to mobilize P (Jones, 1998). An extraction with 2 mM citric acid, at a 1:5 soil to extractant ratio would be comparable to soil conditions and provide a measure of citrate extractable, sorbed, and weakly bound P (Darch et al., 2016). Water extractable P is a biologically and environmentally relevant pool that estimates available P for immediate uptake from root exploration, or potentially lost from the soil surface in a rain event (Sharpley, Robinson, & Smith, 1995; DeLuca et al., 2015). Water extractable P has shown to predict yield response and dissolved P in runoff (Sharpley, Robinson, & Smith, 1995; Pote et al, 1999; Zehetner, Wuenscher, Peticzka, & Unterfrauner, 2018).

A relatively new method of P measurement, the diffusive gradient thin film (DGT), has been developed to measure the soil's ability to resupply P to the soil solution. It measures the concentration of P, assessing labile species, which indicates the species that contribute to the flux through processes of dissociation and desorption. The DGT method has several benefits to other P measurements; it is conducted with less physical disturbance, better predicts bioavailability and runoff risk, it is simple, and can be used in situ. It has some additional advantages over the anion exchange membrane (AEM), a resin method. It is conducted at closer to field conditions because it uses saturated soil instead of a slurry, there is no vigorous shaking, so particles are not abraded causing additional reactions, and is less subject to anionic interference due to the high affinity of the binding layer for P (Mason et al., 2005, Mason, McNeil, McLaughlin, & Zhang,

2010; Six et al., 2013). The diffusive layer of the DGT better represents the plant root conditions due to the limitation of flow to the binding layer, prevents contamination by particulates on the binding layer and allows more precise flux calculations than AEM (Mason et al., 2005, Mason et al., 2010).

The DGT mimics the uptake of phosphate by plant roots using a ferrihydrite based binding gel as an infinite sink (Menzies et al., 2005; Six, Pypers, Degryse, Smolders, & Merckx, 2012). The DGT binding layers accumulate phosphates when in contact with soil, measuring flux, not total concentrations (Zhang & Davidson, 2015). The concentration of the nutrient on the binding layers is a function of the amount and rate (using a time component in the equation) at which the soil can supply the nutrient from the pores and solid phase to solution (Six et al., 2012). Mason et al. (2010) determined that the DGT method accurately predicted yield increases of wheat in 18 of 20 field sites, while anion exchange membranes (AEM), a resin technique, predicted 14 of 20, while Colwell P modified with a P buffering index predicted 11 of 20. The Coldwell P test had no significant relationship to yield (Mason et al., 2010). Research has documented a greater relationship of the intensity based measure, DGT (r^2=0.84), than the quantity based measures of traditional soil P tests and AEM resin method (r^2=0.53). The efficacy of yield prediction of DGT devices on soybeans or any measurement of cover crops on available P using DGT methods have yet to be tested.

Diffusive gradient thin films not only predict yield response and agronomic utility of P applications more accurately than other soil testing methods but can also be used to predict P loss from agronomic fields to runoff (Menzies et al., 2005; Mason et al., 2005; Mason et al., 2010; Dougherty, Mason, Burkitt, & Milham, 2011; Christel et al., 2016). Dougherty et al. (2011) found a highly significant relationship between DGT measurement of soil P and runoff P

(r^2=0.84), offering an accurate method to predict dissolved P loss. The DGT method may have both economic and environmental benefits. A more accurate measure of plant available P supply (including contributions from organic fractions), which correlates better with crop yield, could benefit producers by providing more accurate fertilizer recommendations.

There is limited knowledge regarding the effect of cover crops, tillage, and P fertilizer management on organic P accumulation and resulting pool fluctuations. Despite the large percentage of P that is organic P in cultivated soil and runoff, the characterization and effect of this constituent is largely overlooked (Turner et al., 2002; Dodd & Sharpley, 2015). The relative bioavailability of organic P is an important factor when accounting for its contribution to crop nutrition or pollution risk. Organic P can be categorized by its availability to enzyme hydrolysis, the mechanism by which microorganisms cycle it through the soil system (Turner et al. 2002; Annaheim, Rufener, Frossard, & Bunemann, 2013). As conservation management improves soil health parameters, these parameters are simultaneously increasing microbial activity and the potential for mineralization while increasing or maintaining organic matter inputs, thus potentially increasing organic P.

Organic factions of P have a variety of chemical forms that dictate how available or resistant that molecule is to mineralization and its mobility in the soil profile (Turner et al., 2002; Condron et al., 2005). Inositol phosphates, a class of organic P once thought to be stable due to their binding affinity to soil particles, have been shown to be released into soil solution following dry/wet cycles (Turner & Haygarth, 2001). Organic P (estimated by unreactive P) has functional groups that can be measured based on the amount of organic P that can be hydrolyzed by adding different phosphatase enzymes to an extractant (Turner et al., 2002; Requejo & Eichler-Lobermann, 2014; Annaheim et al., 2013; DeLuca et al., 2015). It is possible to use phytase,

which has a low substrate specificity, to add to extractants for a gross measure of all readily hydrolysable organic P (Darch et al., 2016).

This measurement is important as it approximates the amount of potential P that could be mineralized by microbial activity in soil and aquatic environments. Enzyme hydrolysable P will be determined by adding phytase to sample aliquots from 2 mM citric acid extractions (Darch et al., 2016). Phytase is used to target all ester-P bonds and can be used to estimate the amount of bioavailable organic P is present in the soil (Hayes et al., 2000; Turner et al., 2002; He et al., 2007; Darch et al., 2016). The characterization of readily available organic P pools is a critical step in understanding how P (inorganic and organic) contributes to both crop nutrition and water pollution.

Research Rationale

It is important to understand how management (tillage, cover crop, etc.), along with fertilizer application and placement, may be contributing to dynamic soil health properties and the subsequent biogeochemical cycling, storage, and loss of P. As no-till is a commonly used conservation practice in many parts of the United States, this research seeks to understand the effects of cover crops and/or mineral fertilizer management on P pools and availability in no-till systems. Several knowledge gaps exist in respect to no-till, cover crops, and P fertilizer management in agricultural systems. How do cover crop and P fertilizer placement and timing impact microbial cycling in no-till systems? How do combinations of conservation management effect storage and stratification of P in soil? Will organic P accumulate and/or change under cover crop and/or fertilizer treatments? Will MB-P be correlated to soil test P or P_o? How do methods such as DGT compare to traditional soil test P methods when characterizing P

availability and potentially bioavailable P loss to runoff? We will determine the relationship between treatments, soil health parameters, and P pools in soil and runoff. We will use novel methodology such as the DGT device to measure the concentration of available P over a given time in the experimental units and relate these values to microbial activity and P_o. We will employ enzyme hydrolysable P methods to characterize the phytase hydrolysable P pools that are available for mineralization to connect P_o to mineralization potential and its role in P cycling, uptake, and loss.

Research Objectives

The goal of this research is to document linkages between management, soil health, and P cycling at the field scale. Ultimately, this knowledge will improve management recommendations and improve the sustainability of cropping systems in Kansas. The data collected from these sites will be used to address the following objectives:

Objective 1

We will quantify the effect of cover crops, and P fertilizer management effects on stratification and phosphorus availability in no-till corn-soybean cropping system

Hypotheses 1

i) Cover crops will increase the concentration of organic phosphorus

ii) Cover crops will interact with P fertilizer management and increase P bioavailability as measured by DGT, citrate extractable P, and water extractable P

iii) Fall broadcast diammonium phosphate and cover crop treatments will increase P stratification

Objective 2

We will document near surface effect of cover crops and P fertilizer management on microbial biomass P and phosphatase enzyme activity in a no-till corn-soybean rotation in northeastern Kansas

Hypotheses 2

i) Phosphorus fertilizer and cover crops will increase Mehlich-III extractable P in soil

ii) Phosphorus fertilizer applications will increase MB-P and decrease MB-C:P, cover crops will negate the decrease in MB-C:P

iii) Phosphorus fertilizer application will suppress phosphatase enzyme expression while cover crops will increase phosphatase enzyme activity

Objective 3

We will evaluate near surface effect of cover crops and P fertilizer management on bioavailable P_o and correlate the measure to total organic P and Mehlich-III P.

Hypotheses 3

i) Cover crops will increase the amount of phytase hydrolysable P

References

Annaheim, K. E., Rufener, C. B., Frossard, E., & Bunemann, E. K. 2013. Hydrolysis of organic phosphorus in soil water suspensions after addition of phosphatase enzymes. *Biology & Fertility of Soils*, 49, 1203-1213.

Blackwell, M. S. A., Brookes, P. C., de le Fuente-Martinez, N., Gordon, H., Murray, P. J., Snares, K. E., Williams, J. K., Bol, R., & Haygarth, P. M. 2010. Phosphorus solubilization and potential transfer to surface waters from the soil and microbial biomass following drying-rewetting and freeze-thawing. *Advanced Agronomy*, 106, 1-35.

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 & Water Management & Conservation*, 75(4), 1471-1482.

Boesch, D. F., Brinsfield, R. B., & Magnien, R. E. 2001. Chesapeake Bay eutrophication: Scientific understanding, ecosystem restoration, and challenges for agriculture. *Journal of Environmental Quality*, 30, 303-320.

Boström, B., Persson, G., & Broberg, B. 1988. Bioavailability of different phosphorus forms in freshwater systems. *Hydrobiologia*, 170, 133-155.

Buczko, U., van Laak, M., Eichler-Löbermann, B., Gans, W., Merbach, I., Panten, K., Peiter, E., Reitz, T., Spiegel, H., & von Tucher, S. 2018. Re-evaluation of the yield response to phosphorus fertilization based on meta-analyses of long-term field experiments. *Ambio,* 47(1), S50-S61.

Carpenter, S. R., Booth, E. G., & Kucharik, C. J. 2017. Extreme precipitation and phosphorus loads from two agricultural watersheds. *Limnology and Oceanography*,

Chislock, M. F., Doster, E., Zitomer, R. A., & Wilson, A. E. 2013. Eutrophication: Causes, consequences, and controls in aquatic ecosystems. *Nature Education Knowledge*, 4(4),10.

Christel, W., Lemming, C., Mundus, S., Bruun, S., Magrid, J., & Stroumann Jensen, L. 2016. Measuring phosphorus availability in recently fertilized soils with diffusive gradient in thin films (DGT) method – Challenges and opportunities. *Communications in Soil Science and Plant Analysis*, 47(5), 563-570.

Condron, L. M., Turner, B. L., & Cade-Menun, B. J. 2005. Chemistry and dynamics of soil organic phosphorus. In Sims, J. T., Sharpley, A. N. (Eds), *Phosphorus: agriculture and the environment* (pp 87-122). ASA, CSSA and SSSA, Madison, WI.

Dabney, S. M. 1998. Cover crop impacts on watershed hydrology. *Journal of Soil and Water Conservation*, 53(3), 207-215.

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.

Daryanto, S., Wang, L., & Jacinthe, P. A. 2017. Meta-analysis of phosphorus loss from no-till soils. *Journal of Environmental Quality*, 46, 1028-1037.

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

Dodd, R. J., & Mallarino, A. P. 2005. Soil-test phosphorus and crop grain yield responses to long-term phosphorus fertilization for corn-soybean rotations. *Soil Science Society of America*, 69(4), 1118-1128.

Dodd, R. J., McDowell, R. W., & Condron, L. M. 2014. Manipulation of fertilizer regimes in phosphorus enriched soils can reduce phosphorus loss to leachate through an increase in pasture and microbial biomass production. *Agriculture, Ecosystems and Environment*, 185, 65–76.

Dodd, R. J., & Sharpley, A. N. 2015. Recognizing the role of soil organic phosphorus in soil fertility and water quality. *Resources, Conservation and Recycling*, 105, 282-293.

Dodd, R. J., & Sharpley, A. N. 2016. Conservation practice effectiveness and adoption: unintended consequences and implications for sustainable phosphorus management. *Nutrient Cycling in Agroecosystems*, 104, 373-392.

Dodds, W. K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J., Schloesser, J. T., & Thornbrugh, D. J. 2009. Eutrophication of U.S. Freshwaters: Analysis of potential economic damages. *Environment Science & Technology*, 43(1), 12-19.

Dougherty, W. J., Mason, S. D., Burkitt, L. L., & Milham, P. J. 2011. Relationship between phosphorus concentration in surface runoff and a novel soil phosphorus test procedure (DGT) under simulated rainfall. *Soil Research*, 49, 523-528.

Ehlers, K., Bakken, L. R., Frostegard, A., Frossard, E., & Bunemann, E. K. 2010. Phosphorus limitation in a Ferralsol: Impact on microbial activity and cell internal P pools. *Soil Biology & Biochemistry*, 42, 558-566.

Eichler, B. 2004 *Opportunities to influence the P cycle in sustainable Agroecosystems*. Habilitation thesis [University of Rostock, Germany]. University of Rostock Research Database.

Eichler-Löbermann, B., Köhne, S., Kowalski, B., & Schnug, E. 2008. Effect of catch cropping on phosphorus bioavailability in comparison to organic and inorganic fertilization. *Journal of Plant Nutrition*, 31, 659-676.

Fraser, A. I., Harrod, T. R., & Haygarth, P. M. 1999 The effect of rainfall intensity on soil erosion and particulate phosphorous transfer from arable soils. *Water Science Technology*, 39(12), 41-45.

Gonzalez-Chavez, M. D. C. A., Aitkenhead-Peterson, J. A., Gentry, T. J., Zuberer, D., Hons, F., & Loeppert, R. 2010. Soil microbial community, C, N, and P responses to lonf-term tillage and crop rotation. *Soil & Tillage Research*, 106, 285-293.

Hanrahan, B. R., Tanka, J. L., Christophera, S. F., Mahla, U. H., Trentmana, M. T., & Royer, T. V. 2018. Winter cover crops reduce nitrate loss in an agricultural watershed in the central U.S. *Agriculture, Ecosystems and Environment*, 265, 513-523.

Hartl, W., & Erhart, E. 2005. Crop nitrogen recovery and soil nitrogen dynamics in a 10-year field experiment with biowaste compost. *Journal of Plant Nutrition and Soil Science*, 68, 781–788.

Hayes, J. E., Richardson, A. E., and Simpson, R. J. 2000. Components of organic phosphorus in soil extracts that are hydrolysed by phytase and acid phosphatase. *Biology and Fertility of Soils*, 32(4), 279-286.

He, Z., Cade-Menun, B. J., Toor, G. S., Fortuna, A., Honeycutt, C. W., & Sims, J. T. 2007. Comparison of phosphorus forms in wet and dried animal manures by solution phosphorus-31 nuclear magnetic resonance spectroscopy and enzymatic hydrolysis. *Journal of Environmental Quality*, 36, 1086-1095.

He, Z. L., Wu, J., O'Donnell, A. G., & Syers, J. K. 1997. Seasonal responses in microbial biomass carbon, phosphorus, and Sulphur in soils under pasture. *Biology and Fertility of Soils*, 24, 421-428.

Heckman, J. R., Jokela, W., Morris, T., Beegle, D. B., Sims, J. T., Coale, F. J., Herbert, S., Griffin, T., Hoskins, B., Jemison, J., Sullivan, W. M., Bhumbla, D., Estes, G., & Reid, W. S., 2006. Soil test calibration for predicting corn response to phosphorus in the Northeast USA. *Agronomy Journal*, 98, 280–288.

Horst, W. J., Manh, M., Jibrin, J. M., & Chude, V. O. 2001 Agronomic measures for increasing P availability to crops. *Plant and Soil*, 237, 211-223.

Jarvie, H. P., Johnson, L. T., Sharpley, A. N., Smith, D. R., Baker, D. B., Bruulsema, T. W., & Confesor, R. 2017. Increased soluble phosphorus loads to Lake Erie: Unintended consequences of conservation practices? *Journal of Environmental Quality*, 46, 123-132.

Jones, D. L. 1998. Organic acids in the rhizosphere: a critical review. *Plant and Soil*, 205, 25-44.

Jordan-Meille, L., Rubaek, G. H., Ehlert, P. A. I., Genot, V., Hofman, G., Goulding, K., Recknagel, J., Provolo, G., & Barraclough, P. 2012. An overview of fertilizer-P recommendations in Europe: soil testing, calibration and fertilizer recommendations. *Soil Use and Management*, 28, 419-435.

Kirkby, C. A., Richardson, A. E., Wade, L. J., Passioura, J. B., Batten, G. D., Blanchard, C., & Kirkegaard, J. A. 2014. Nutrient availability limits carbon sequestration in arable soils. *Soil Biology and Biochemistry*, 68, 402-409.

Kleinman, P. J., Srinivasan, M. S., Dell, C. J., & Schmidt, J. P. 2006. Role of rainfall intensity and hydrology in nutrient transport via surface runoff. *Journal of Environmental Quality*, 35(4), 1248-59.

Leytem, A. B., Mikkelsen, R. L., & Gilliam, J. W. 2002. Sorption of organic phosphorus compounds in Atlantic coastal plains soil. *Soil Science*, 167(10).

Leinweber, P., Bathmann, U., Buczko, U., Douhaire, C., Eichler-Löbermann, B., Frossard, E., Ekardt, F., Jarvie, H., Krämer, I., Kabbe, C., Lennartz, B., Mellander, P., Nausch, G., Ohtake, H., & Tränckner, J. 2018. Handling the phosphorus paradox in agriculture and natural ecosystems: Scarcity, necessity, and burden of P. *Ambio*, 47(1), S3-S19.

Liu, A., Hamel, C., Spedding, T., Zhang, T. Q., Mongeau, R., Lamarre, G. R., & Tremblay, G. 2008. Soil microbial carbon and phosphorus as influenced by phosphorus fertilization and tillage in a maize-soybean rotation in south-western Quebec. *Canadian Journal of Soil Science*, 88, 21-30.

Liu, J. R. Khalaf, R., Ule'n, B., & Bergkvist, G. 2013. Potential phosphorus release from catch crop shoots and roots after freeze-thawing. *Plant and Soil*, 371, 543-557.

Liu, J., Ulen, B., Bergkvist, G., & Aronsson, H. 2014. Freeze-thawing effects on phosphorus leaching from catch crops. *Nutrient Cycling in Agroecosystems*, 99, 17-30.

Marklein, A. R., & Houlton, B. Z. 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *The New Phytologist*, 193(3), 696-704.

Mason, S., Hamon, R., Nolan, A., Zhang, H., & Davison, W. 2005. Performance of a mixed binding layer for measuring anions and cations in a single assay using diffusive gradients in thin films technique. *Analytical Chemistry*, 77, 6339-6346.

Mason, S., McNeill, A., McLaughlin, M. J., & Zhang, H. 2010. Prediction of wheat response to an application of phosphorus under field conditions using diffusive gradients in thin-films (DGT) and extraction methods. *Plant Soil*, 337, 243-258.

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. *Environmental Science & Technology*, 50, 11521-11531.

Menzies, N. W., Kusumo, B., & Moody, P. W. 2005. Assessment of P availability in heavily fertilized soils using the diffusive gradient in thin films (DGT) technique. *Plant and Soil*, 269, 1-9.

Montgomery, D. R. 2010. Soil Erosion and Agricultural Sustainability. *Proceedings of the National Academy of Sciences of the United States of America*, 104(33), 13268-13272.

Moody, P. W. 2011. Environmental risk indicators for soil phosphorus status. *Soil Research*, 49, 247-252.

NRCS. 2015. *Soil health literature summary – Effects of conservation practices on soil properties in areas of crop land.* Natural Resources Conservation Service, National Soil Survey Center. United States Department of Agriculture.

Oberson, A., Besson, J. M., Maire, N., & Sticher, H. 1996. Microbial processes in soil organic phosphorus transformations in conventional and biological cropping systems. *Biology and Fertility of Soils*, 21(3).

Oberson, A., & Joner, E. J. 2005. Microbial turnover of phosphorus in soil. In Turner, B.J., Frossard, E., and Baldwin, D.S. (Eds.), *Organic Phosphorus in the Environment* (pp. 133-164). CAB International, Wallingford, U.K.

Oehl, F., Frossard, E., Fliessbach, A., Dubois, D., & Oberson, A. 2004. Basal organic phosphorus mineralization in soils under different farming systems. *Soil Biology & Biochemistry*, 36, 667-675.

Ogle, S. M., Swan, A., & Paustian, K. 2011. No-till management impacts on crop productivity, carbon input and soil carbon sequestration. *Agriculture, Ecosystems, & Environment*, 149, 37-49.

Okubo, Y., Inoue, T., & Yokota, K. 2012. Estimating bioavailability of soil particulate phosphorus to *Microcystis aeruginosa*. *Journal of Applied Phycology*, 24, 1503-1507.

Olander, L. P. & Vitousek, P. M. 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry*, 49(2), 175-190.

Paerl, H. W., & Paul, V. J. 2012. Climate change: Links to global expansion of harmful cyanobacteria. *Water Research*, 46, 1349-1363.

Paerl, H. W., Hall, N. S., Peierls, B. L., & Rossignol, K. L. 2014. Evolving paradigms and challenges in estuarine and coastal eutrophication dynamics in a culturally and climatically stressed world. *Estuaries and Coasts*, 37, 243-258.

Pautler, M. C., & Sims, J. T. 2000. Relationship between soil test phosphorus, soluble phosphorus, and phosphorus saturation in Delaware soils. *Soil Science Society of America Journal*, 64, 765-773.

Pote, D. H., Daniel, T. C., Nichols, D. J., Sharpley, A. N., Moore, P. A. Jr., Miller, D. M., & Edwards, D. R. 1999. Relationship between phosphorus levels in three Ultisols and phosphorus concentrations in runoff. *Journal of Environmental Quality*, 28, 170-175.

Requejo, M. I., & Eichler-Löbermann, B. 2014. Organic and inorganic phosphorus forms in soil as affected by long-term application of organic amendments. *Nutrient Cycling in Agroecosystems*, 100, 245-255.

Reichenwaldt, E. S., & Ghadouani, A. 2012. Effects of rainfall patterns on cyanobacterial blooms in a changing climate: Between simplistic scenarios and complex dynamics. *Water Research*, 40, 1372-1393.

Richardson, A. E., & Simpson, R. J. 2011 Soil microorganisms mediating phosphorus availability. *Plant Physiology,* 156(3), 989-996.

Schilling, G., Gransee, A., Deubel, G., Lezovic, S., & Ruppel, S. 1998. Phosphorus availability, root exudates, and microbial activity in the rhizosphere. *Z. Pflanzenern ahr. Bodenkd*, 161, 465-478.

Sharpley, A. N. 1985. Phosphorus cycling in unfertilized and fertilized agricultural soils. *Soil Science Society of America Journal*, 49, 905-911.

Sharpley, A. N. 2003. Soil mixing to decrease surface stratification of phosphorus in manured soils. *Journal of Environmental Quality*, 35, 1375-1384.

Sharpley, A. N., Bergstrom, L., Aronsson, H., Bechmann, M., Bolster, C. H., Borling, K., Diodjic, F., Jarvie, H. P., Schoumans, O. F., Stamm, C., Tonderski, K. S., Ule'n, B., Uusitalo, R., & Withers, P. J. A. 2015. Future agriculture with minimized phosphorus losses to waters: Research needs and direction. *Ambio*, 44(2), S163-S179.

Sharpley, A. N., & Smith, S. J. 1983. Distribution of phosphorus forms in virgin and cultivated soil and potential erosion losses. *Soil Science Society of America Journal*, 47, 581-586.

Sharpley, A. N., Robinson, J. S., & Smith, S. J. 1995. Bioavailable phosphorus dynamics in agricultural soils and effects on water quality. *Geoderma*, 67, 1-15.

Sharpley, A., & Tunney, H. 2000 Phosphorus research strategies to meet agricultural and environmental challenges of the 21st Century. *Journal of Environmental Quality*, 29, 176-181.

Shigaki, F., & Sharpley, A. 2011. Phosphorus sources and soil properties effects on phosphorus availability. *Soil Science*, 176(9).

Shigaki, F., Sharpley, A., & Prochnow, L. I. 2007. Rainfall intensity and phosphorus source effects on phosphorus transport in surface runoff from soil trays. *Science of the Total Environment*, 373, 334-343.

Simeone, G. D. R., Muller, M., Felgentreu, C., & Glaser, B. 2020. Soil microbial biomass and community composition as affected by cover crop diversity in a short-term field experiment on a podzolized Stagnosol Cambisol. *Journal of Plant Nutrition and Soil Science*, 183, 539-549.

Sims, J. T., Edwards, A. C., Schoumans, O. F., & Simard, R. R. 2000. Integrating soil phosphorus testing into environmentally based agriculture management practices. *Journal of Environmental Quality*, 29, 60-71.

Six, L., Pypers, P., Degryse, F., Smolders, E., & Merckx, R. 2012. The performance of DGT versus conventional soil phosphorus tests in tropical soils – An isotope dilution study. *Plant Soil*, 359, 267-279.

Six, L., Smolders, E., & Merckx, R. 2013. The performance of DGT versus conventional soil phosphorus tests in tropical soils – Maize and rice responses to P application. *Plant Soil*, 366, 49-66.

Smith, D. R., Huang, C., & Haney, R. L. 2017. Phosphorus fertilization, soil stratification, and potential water quality impacts. *Journal of Soil and Water Conservation*, 72(5), 417-424.

Smith, D. R., Francesconi, S. J., Livingston, S. J., & Huang, C. 2015. Phosphorus losses from monitored fields with conservation practices in the Lake Erie Basin, USA. *Ambio*, 44 (2), S319-S331.

Spohn, M., & Kuzyakov, Y. 2013. Phosphorus mineralization can be driven by microbial need for carbon. *Soil Biology & Biochemistry*, 61, 69-75.

Srivastava, P., Rishikesh, S., Sachchidanand, T., & Akhilesh, S. R. 2016. An urgent need for sustainable thinking in agriculture – an Indian scenario. *Ecological Indicators*, 67, 611-622.

Teboh, J. M., & Franzen, D. W. 2011. Buckwheat (*Fagopyrum esculentum* Moench) potential to contribute solubilized soil phosphorus to subsequent crops. *Communications in Soil Science and Plant Analysis*, 42(13), 1544-1550.

TerAvest, D., Carpenter-Boggs, L., Thierfelder, C., & Reganold, J.P. 2015. Crop production and soil water management in conservation agriculture, no-till, and conventional tillage systems in Malawi. *Agriculture, Ecosystems and Environment*, 212, 285–296.

Tiessen, H., & Moir, J. O. 2008. Characterization of available P by sequential extraction. In Carter, M.R., and Gregorich, E.G. (Eds), *Soil Sampling and Methods of Analysis* (2nd Edition). CRC Press, Boca Raton, FL.

Toor, G. S., Condron, L. M., Di, H. J., Cameron, K. C., & Cade-Menun, B. J. 2003. Characterization of organic phosphorus leachate from a grassland soil. *Soil Biology and Biochemistry*, 35, 1317-1323.

Turner, B. L., & Haygarth, P. M. 2001. Biogeochemistry: phosphorus solubilization in rewetted soils. *Nature*, 411, 258.

Turner, B. L., McKelvie, I. D., & Haygarth, P. M. 2002. Characterization of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biology and Biochemistry*, 34, 27-35.

Zare, M., Samani Nazari, A. A., Mohammady, M., Teimurian, T., & Brazrafshan, J. 2016. Simulations of soil erosion under the influence of climate change scenarios. *Environmental Earth Science*, 75, 1405.

Zehetner, F., Wuenscher, R., Peticzka, R., & Unterfrauner, H. 2018. Correlation of extractable phosphorus (P) with plant P uptake: 14 extraction methods applied to 50 agricultural soils from Central Europe. *Plant Soil Environment*, 64(4), 192-201.

Zhang, H., & Davison, W. 2015. Use of diffusive gradients in thin-films for studies of chemical speciation and bioavailability. *Environmental Chemistry*, 12, 85-101.

Zhao, F. Z., Ren, C. J., Han, X. H., Yang, G. H., Wang, J., & Doughty, R. 2018. Changes of soil microbial and enzyme activities are linked to soil C, N and P stoichiometry in afforested ecosystems. *Forest Ecology and Management*, 427, 289-295.

Chapter 2 - Cover Crops and Phosphorus Fertilizer Management Effects on Phosphorus Availability and Stratification

Abstract

No-till management increases stratification of P in soil. This enrichment of P at the soil surface from surface applied P fertilizer has been identified as a contributing factor to increased dissolved reactive P in runoff. Cover crops and phosphorus (P) fertilizer management have been suggested as ways to decrease P load in runoff. It is unknown how these management practices will affect the availability and distribution of P in no-till systems. The objective of this research was to document the effects of cover crops and P fertilizer management on the concentration and stratification of key P pools, and P supply to the soil solution in a no-till, corn-soybean cropping system. A no-till experiment was established in 2014, on 18 ~ 0.5 ha watersheds in northeastern Kansas. The experimental design was a randomized complete block designed with a 2 by 3 factorial treatment structure: two cover crop treatments [fall-sown cover crop (CC)/no cover crop (NC)] and three P fertilizer management treatments [27 kg P ha^{-1} fall broadcast (FB) diammonium phosphate, 27 kg P ha^{-1} spring injected (SI) ammonium polyphosphate, or no P fertilizer (NP)]. Samples were collected in spring/fall of 2018, and 2019. Samples were analyzed for total P (P_T), total organic P (P_O), Mehlich-III P (P_M), water extractable P (P_W), and citrate extractable P (P_C). In additionally, the P supply to soil solution was evaluated using diffusive gradient thin films (P_{DGT}). We detected P_T stratification in all fertilizer treatments in fall 2018 but observed a decreased concentration, from 344.8 to 320.7 ug P_T g^{-1} dry soil, at the 0-5 cm depth of NP treatments in fall 2019, resulting in reduced stratification. Organic P was higher at the 5-10 cm depth in FB and SI treatments, but higher at the 0-5 cm depth in NP. In the labile P pools

(P_W and P_C) FB*CC, FB*NC, and SI*CC were more stratified, than SI*NC, NP*NC, and NP*CC in fall 2018 and spring 2019. A P fertilizer*cover crop interaction in the 0-5 cm depth was detected in P_W spring 2019, P_C in fall 2018 and spring 2019 ($p<0.05$), P_{DGT} in spring 2019, and P_M in fall 2019 where SI had more labile P with a cover crop than without suggesting the cover crop was increasing P at the surface despite the subsurface placement. Additionally, a depth*cover crop interaction was documented in P_C where it was higher in NC compared to CC at the 10-15 cm depth in fall 2018 and spring 2019. The P_{DGT} corroborated the 0-5 cm P_C results in spring 2019 and additionally detected higher P_{DGT} in NC treatments compared to CC at the 10-15 cm depth in fall 2019. This research shows that cover crops can influence stratification in labile P pools in some P fertilizer management strategies. More research is needed to confirm the effect of increasing labile P concentrations at the surface in SI*CC treatments.

Introduction

Phosphorus fertilizers are applied to arable land to meet crop nutrient needs but have been identified as a potential source of water pollution (Jordan-Meille et al., 2012; Sharpley et al., 2015). Excess P nutrients from non-point sources such as agriculture can contribute to the eutrophication of surface water and potentially lead to harmful algal blooms (Sharpley et al., 2015; Jarvie et al 2017). To address the environmental concerns from agriculture, such as erosion, nutrient loss, and water pollution, State and Federal governments have promoted conservation practices that are designed to reduce erosion and nutrient pollution (Dodd & Sharpley, 2015; FAO, 2017). This shift emphasizes conservation management techniques include reducing or eliminating tillage practices and planting cover crops (Dodd & Sharpley, 2015; FAO, 2017; Duncan et al., 2019). Although no-till and cover crops have been shown to reduce some negative environmental impacts, their effect on phosphorus loss has not been clear (Smith, King, & Williams, 2015; Dodd & Sharpley, 2016; Blanco-Canqui, 2018; Duncan et al., 2019).

Although no-till and cover crop planting have been successful at reducing the amount of sediment bound phosphorus in runoff, some research has demonstrated that the amount of dissolved, bioavailable P has significantly increased (Smith et al., 2015; Daryanto, Wang, & Jacinthe, 2017; Smith, Huang, & Haney, 2017). A growing body of evidence suggests that the benefits accrued by conservation practices are either ineffectual or negatively impacting phosphorus pollution (Daryanto et al., 2017; Duncan et al., 2019). Some research has shown that no-till increased dissolved reactive P (DRP) by 100% and decreased total P by 69% compared to rotational tillage (Smith et al., 2015), and increased DRP when diammonium phosphate (DAP) was broadcast biannually to no-till, cover crop managed fields (Smith et al., 2017).

Soil stratification and P accumulation at the soil surface have been identified as possible reasons that no-till has not been more effective at reducing P losses (Smith et al., 2015; Dodd & Sharpley, 2016; Jarvie et al., 2017). In no-till management, P fertilizer is often broadcast on the soil surface, concentrating the nutrient in the top few cm of soil, due to the lack of mechanical incorporation, which is where surface water has the most interaction (Sims, Edwards, Schoumans, & Simard, 2000). Some research has recommended subsurface P fertilizer placement to reduce P loss to runoff (Schwab, Whitney, Kilgore, & Sweeney, 2006; Kleinman & Sharpley, 2011; Smith et al., 2017).

It is uncertain how cover crops in no-till cropping systems interact with P fertilizer management to impact stratification and how that affects P loss to runoff (Smith et al., 2017). While cover crops are a physical barrier that can reduce the amount of sediment bound P lost in runoff, it deposits plant residue on the soil surface after it is terminated (Liu, Khalaf, Ule'n, & Bergkvist, 2013; Varela et al., 2017). This decomposing plant residue can become a source of labile P (Liu et al., 2013; Varela et al., 2017). Moreover, the effect of cover crops on soil P concentration and distribution is variable by cover crop species (Eichler-Lobermann, Kohne, Kowalski, & Schnug, 2008; White & Weil, 2011). Eichley-Lobermann et al. (2008) determined that a purple tansy (*Phacelia tanacetifolia)* cover crop could mobilize recalcitrant P and increase P availability in the soil. White and Weil (2011) documented an increased soil P concentration at the tap root holes of forage radish (*Raphanus sativus*) cover crops but decreased P concentrations at 2.5-10 cm, while cereal rye decreased P concentrations over the 0-10 cm depth.

Common methods of soil P measurement can obfuscate some of the changes in P dynamics (Haney, Haney, Hossner, & Arnold, 2010; Dodd & Sharpley, 2015). Traditional, agronomic soil P tests such as Mehlich-III and Olsen extractions are well correlated with easily

extractable inorganic P (Pote et al., 1999; Sims et al., 2000) but are not a direct measure of soil P (Haney et al., 2010). Acidic or alkaline extractants (Mehlich-III pH 3 or Olsen pH 8.5) are used to solubilized mineral bound P and are agronomically correlated to a yield response from added P fertilizer, however they use extremely low or high pH in the extractant that does not reflect the actual soil pH that drives P solubility and availability in the field (Haney et al., 2010). Total P and total organic P assays can estimate the size of the gross P content of the soil but give little information on the relative bioavailability of soil P (O'Halloran & Cade-Menun, 2008).

Dilute organic acid extractants may provide a more biologically relevant method to measure P availability (Haney et al., 2010). Plants and microorganisms secrete organic acids to alter the rhizosphere to increase P bioavailability. Organic acids possess negative charges that allow them to chelate metal cations such as aluminum (Al) and iron (Fe), mobilize P held in humus-metal complexes, and displace orthophosphate anions from the soil matrix (Jones, 1998). Extractions with dilute organic acids at a similar pH to soil can mimic an important biotic uptake mechanism and provide a biologically relevant measure of available P (Jones, 1998; Li et al., 2007; Richardson et al., 2011; DeLuca et al., 2015; Menezes-Blackburn et al., 2016). Deluca et al (2015) and Hayes et al (2000) proposed using citrate as an alternative approach to measure citrate extractable P. Plant roots have an organic acid concentration of approximately 10-20 mM, while soil has a concentration of approximately1-50 μM^{-1}g soil (Jones, 1998). Soil extracted with 2 mM citric acid at a 5:1 solution to soil ratio would provide a measure of sorbed, and weakly bound P that would be available at biologically relevant concentrations (Darch et al., 2016).

In addition to citrate extractable P (P_C), the amount of water extractable P (P_W) is a biologically and environmentally relevant pool as it estimates the amount of soluble P that would be available for immediate uptake from root exploration, or potentially lost from soil in a rain

event (Paultner & Sims, 2000; Sharpley, Robinson, & Smith, 1995; DeLuca et al., 2015). Water extractable P is well established and correlated with dry matter yield response and dissolved orthophosphate in runoff (Sharpley et al., 1995; Pote et al, 1999; Zehetner, Wuenscher, Peticzka, & Unterfrauner, 2018).

A relatively new method of P measurement, the diffusive gradient thin films (DGT), has been developed to measures soil's ability to resupply P to the soil solution (Zhang, Davidson, Knight, & McGrath, 1998). The DGT attempts to mimic the uptake of phosphate by plant roots using a ferrihydrite based binding gel as an infinite sink (Zhang et al., 1998; Six, Pypers, Degryse, Smolders, & Merckx, 2012; Menezes-Blackburn et al., 2016). The DGT binding layers accumulate phosphates when in contact with soil, measuring flux, not total concentrations (Zhang & Davidson, 2015). The concentration of the nutrient on the binding layers is a function of the amount and rate at which the soil can supply the nutrient from the pores and solid phase to solution (Six et al., 2012). Research has documented a greater relationship of the supply based measure, DGT (r^2=0.84), than the quantity based measures of traditional soil P tests and anion exchange resin method (r^2=0.53) to crop yields (Mason, McNeil, McLaughlin, & Zhang, 2010), and P in runoff (r^2=0.84), (Dougherty, Mason, Burkitt, & Milham, 2011) offering an accurate method to predict P nutrient requirements of crops and dissolved P loss. A more accurate measure of plant available P supply, that includes contributions from organic fractions, may offer a more nuanced method of understanding conservation management implications.

In recent years there has been a wider adoption of cover crops and no-till management to reduce soil erosion and improve soil health (Smith et al., 2015; Jarvie et al., 2017), however there are few examples of experimental research that investigate the interaction of multiple conservation management techniques and P fertilizer management on stratification and

bioavailable P pools (Dodd & Sharpley 2015; Smith et al., 2017; Blanco-Canqui, 2018). In fact, in a recent review of cover crop research, Blanco-Canqui (2018) found only two research articles documenting the role of cover crops and P fertilizer management in conjunction with no-till (Kovar, Moorman, Singer, Cambardella & Tomer, 2011; Smith et al., 2017). The characterization of P availability in the context of conservation management is a critical step in understanding how P (inorganic and organic) is contributing to both crop nutrition and water pollution.

The objective of this research was to characterize the P pools at the 0-5, 5-10, and 10-15 cm depths using traditional and novel approaches to determine treatment effects on P concentration and bioavailability under multiple P fertilizer and conservation management scenarios. We hypothesized that i) P fertilizer application and cover crops would increase stratification ii) stratification would differ based on P pool measured iii) cover crops would increase the concentration of organic phosphorus iv) cover crops would interact with P fertilizer management and increase P bioavailability.

Materials and Methods

Site description

The experimental site is located at the Kansas Agricultural Watershed (KAW) Field Laboratory (39.134, -96.641) established in 2014. The site consists of agricultural fields along the Kansas River near Manhattan, KS. The site has a hot, humid continental climate, with a mean annual temperature of 12.7^{o}C, and 904 mm of precipitation annually (1981-2010 normal). Temperature and precipitation measurements were taken by the Ashland Bottoms Kansas Mesonet station, which is less than 1000 m from the experimental site.

Temperature from 2018 to June 2020 were within normal range of the 30 yr average for Riley county, KS (Fig. 2.1). Precipitation in 2017 was 17.5% lower than the 30 yr average (Fig. 2.2). Precipitation in 2018 was approximately 9% lower than the 30 year average but the period from January through July 2018 was 50% below average (Fig. 2.2). The precipitation in 2019 was approximately 16% above the 30 yr average (Fig.2.2).

The site is terraced into 18 watershed units that are approximately 0.5 ha each. The site has a slope of 6-8%. The soil is primarily eroded Smolan silty clay loam (fine, smectitic, mesic, Pachic Argiustoll) which is moderately well drained. Soil pH was 6.5-7.0, near optimum for soil P availability in all case (Havlin et al., 2013). The site has been in a continuous no-till, corn-soybean rotation since its establishment. Nitrogen fertilizer (28% urea ammonium nitrate) was injected below the surface at a uniform rate of 146 kg N^{-1} ha to all plots in corn years.

This experiment is organized in a randomized block, 2 by 3 factorial treatment design. The plots were blocked down the hill slope. The 18 watersheds were randomly assigned cover crop and fertilizer factors. The cover crop factor has two levels, cover crop (CC) or no cover crop (NC). Cover crops have been planted annually since 2015. The fertilizer treatment has three types of phosphorus fertilizer management: no P fertilizer (NP), spring injected ammonium polyphosphate (SI), and fall surface broadcast DAP (FB). Both fall broadcast and spring injected was applied at 27 kg P^{-1} ha annually prior to the cash crop (Table 2.1). The fall broadcast DAP [$(NH4)_2HPO_4$] was surface applied, annually, as a granule. The spring injected ammonium polyphosphate [$(NH_4PO_3)_n\,(OH)_2$] was applied annually, 4-8 cm below the soil surface with a corn planter.

Corn (*Zea mays* var DKC53-56) was planted in April and harvested in September 2017 (Table 2.1). A cover crop mix of triticale (*Triticosecale* var. TriCal 780) and rapeseed (*Brassica*

napus var Dwarf Essex) was sown following corn harvest (Table 2.1). A drought (Fig. 2) combined with a late planting date hindered establishment of the cover crop planted in 2018 (Table 2.1). In 2018, the cover crop was terminated in May, and soybeans (*Glycine max* var Liberty Link®) were sown (Table 2.1). A winter wheat and rapeseed mix was sown after soybean harvest in November 2018 (Table 2.1). The cover crop was terminated, and corn was planted April 26^{th,} 2019 (Table 2.1). In fall 2019, corn (hybrid DKC53-56) was harvested, and a cereal rye cover crop was planted in September (Table 2.1). Cover crops were terminated with glyphosate applied at 4.7 L ha^{-1} in the spring (Table 2.1). All plots were treated with 3.1 L ha^{-1} of Liberty® (BASF) and 1.1 L ha^{-1} clethodim for weed prevention post planting.

Composite soil samples consisting of 40 cores from randomly selected points across each plot were collected at a depth of 0-5 cm in spring 2018, and depths of 0-5, 5-10, and 10-15 cm in fall 2018, spring 2019, and fall 2019 (Table 2.1). The spring samples were always collected prior to cover crop termination and spring fertilizer application and fall samples were collected immediately after harvest but prior to fall fertilizer applications. Total P (P_T), and total organic P (P_O) were measured to estimate gross P pools. Water extractable P (P_W) and citrate (2 mM citric acid) extractable P (P_C) were used to mimic plant and microbial P acquisition mechanisms to estimate biologically dynamic pools. Mehlich-III (P_M) was used as an agronomically relevant P measure for the region. Diffusive gradient thin films (P_{DGT}) were selected to measure P that was made available to soil water, and not dependent on P extraction. All soil samples were sieved moist using a 2 mm sieve. Soil samples were subdivided and extracted in parallel for P_T, P_O, P_W, P_C, and P_{DGT}. Mehlich-III P was only measured at the 0-5 cm depth in all seasons. Diffusive gradient thin films were used to measure 0-5 cm in all seasons, and 5-10, and 10-15 cm only in fall 2019.

Methodology

Total P

Total P analysis was conducted at the Kansas State University Soils Lab. Briefly, P_T was determined by a salicylic sulfuric acid digestion, where 1 g of ground, air dried, soil was added to salicylic sulfuric and sodium thiosulfate and left overnight. The mixture was heated on a heating block at 200^{o}C for 1 hr and a further hour at 380^{o}C. The catalyst was added, and the samples were heated at 380^{o}C for an additional 3.5 hr. Samples were analyzed with analyzed by Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission (Varian Ltd, Mulgrave, Australia).

Total Organic P

Organic P was measured by the ignition method (O'Halloran and Cade-Menun, 2008; Walker and Adams, 1958). Two, 1 g subsamples of crushed, air dried soil from each sample were measured into 250 ml Erlenmeyer flasks. One was incinerated at 550^{o}C for 1 hr while the other was kept at room temperature. Both samples were shaken with 25 ml of 0.5 M H_2SO_4 for 16 hr. Samples were filtered using Whatman 42 filters for 30 min. Samples were analyzed using molybdate reactive P (MRP) (Murphy and Riley, 1962) with a Hitachi U-1100 spectrophotometer (Hitachi High-Tech Corporation, Tokyo, Japan). Organic P was determined by the difference between the incinerated and non-incinerated samples and corrected for blank P concentration (O'Halloran and Cade-Menun, 2008).

Water Extractable Phosphorus

A two gram subsample of air dried soil was weighed into 50 mL centrifuge tubes. Twenty milliliters of deionized (DI) water were added to the centrifuge tubes (Sharpley, Kleinmann, & Weld, 2008). The tubes were then shaken at low speed for 1 hr and then centrifuged at 10,000 rpm for 10 min. After centrifugation, supernatant was filtered through a 0.45 µm nylon syringe filter (Environmental Express, Charleston, SC) and analyzed colorimetrically for MRP using the Murphy and Riley molybdate blue method (1962) Sharpley, Kleinmann, & Weld, 2008).

Citrate Extractable P

Citrate extractable molybdate reactive P was assayed by weighing 8 g (dry soil equivalent) fresh, moist, sieved (2 mm sieve) soil samples into 250 ml Erlenmeyer flasks from each plot. Samples were extracted with 40 mL of 2 mM citric acid pH 5, equivalent to 10 µmol citric acid g^{-1} dry soil (Darch et al., 2016). Mixtures were then shaken for 30 min at 20^{o}C, centrifuged at 10,000 rpm for 10 min and filtered through Ahlstrom 74 filter paper into 50 mL Falcon tubes. The amount of P from each subsample was measured colorimetrically for MRP (Murphy and Riley, 1962; O'Halloran and Cade-Menun, 2008).

Soil Water P - Diffusive Gradient Thin Films

The ability of the soil to replenish P to solution, a measure of in-situ, bioavailable P, was measured using diffusive gradient thin films. A 40 g sub sample of air dried soil was placed in a 100 ml plastic cup for DGT analysis. DGT devices, LSLP-NP for deployment in soil, were purchased from DGT research (Lancaster, UK). Soil samples were moistened with 20 mL of DI water to approx. 50% water holding capacity 72 hr prior to the deployment of the device, then

adjusted to saturation 24 hr prior (Mason et al., 2005). Devices were deployed in the saturated samples for 48 h at 22°C, after which the devices were removed and rinsed with DI water (Mason et al., 2010, Dougherty et al., 2011; Zhang and Davison, 2015). The ferrihydrite gel layer was collected and eluted in 1 mL of 1 M HCl for 24 hr. The solution was analyzed colorimetrically for MRP (Murphy and Riley, 1962) on a Hitachi spectrophotometer U-1100 (Hitachi High-Tech Corporation, Tokyo, Japan).

The P concentration accumulated on the binding layer during the DGT device deployment was calculated (equations 1 and 2) (Zhang and Davison, 2015; Mason et al., 2005).

Mass of accumulated P on binding layer

$m = C_e (V_e + V_g) / P$

m = mass of P in binding layer

C_e = measured P concentration

V_e = volume of 1 M HCl

V_g = volume of the binding layer (given by DGT research)

P = elution efficiency in 1 M HCl (0.92) (Mason et al., 2005)

Equation 1. Mass of accumulated P on binding layer

Using the calculate mass of P collected by the iron oxide binding layer, the DGT time averaged concentration in solution (P_{DGT}) was calculated (Mason et al., 2005; Zhang et al., 1998):

Time averaged concentration in solution (P_{DGT}) = $m\Delta g/DAt$

Where –

m = Mass (μg) of accumulated P from the mixed binding layer

Δ g =Thickness of the diffusive layer and membrane filter (0.088 cm)

A = Area (cm^2) of exposed gel area (3.14 cm^2)

t = Time of deployment (s)

D = Diffusive coefficient (6.05 * $10^{-6}$$cm^2$/s, temperature corrected, given by https://www.dgtresearch.com/diffusion-coefficients/)

Equation 2. Calculation to determine PDGT after determining the mass of P on the iron oxide binding layer.

Mehlich-3 P

Mehlich-III P was analyzed at the Kansas State Soil Testing Lab. Briefly, 1 g crushed, air dried soil was extracted with the Mehlich-III extractant with a 1:10 soil weight to extractant volume and shaken for 5 min. The MRP was determined colorimetrically using the molybdate reactive P method (Murphy & Riley, 1962).

Statistical Analyses

Statistical analyses were conducted using a two way analysis of variance (ANOVA) to detect differences among cover crop and P fertilizer management treatments over all depths sampled. SAS version 9.4 software (Cary, NC, U.S.A.) was used with a PROC GLIMMIX, repeated measures of variance procedure. Depths were analyzed in the same model with slice partitioning to analyze each depth. Season/year was analyzed separately and not compared statistically due to high seasonal variability.

Results

Total P

0-15 cm profile

We did not document a depth*P fertilizer*cover crop interaction at any time (Table 2.2). In the seasons that were sampled at multiple depths (fall 2018 and spring/fall 2019), there was a consistent depth*fertilizer interaction (Table 2.3)., In fall 2018, all fertilizer treatments were stratified by depth, where 0-5 cm was greater than all other depths, but 5-10 cm was the same as 10-15 cm (Table 2.3). In spring 2019, FB and NP treatments had higher P_T in 0-5 cm than the other depths, but 5-10 cm was not different from 10-15 cm, however, SI had higher amounts of P_T at each depth (Table 2.3). In fall 2019, FB and SI were stratified with higher P_T in shallower compared to each deeper depth (i.e. 0-5 > 5-10 > 10-15 cm), while NP had greater P_T in 0-5 compared to 10-15 cm but 5-10 cm was the same as both, indicating a depth gradient where P_T concentration was reduced as depth increased (Table 2.3).

0-5 cm depth

In all seasons, there was a P fertilizer main effect where FB and SI had more P_T than NP, but FB and SI were not different ($p<0.001$) (Table 2.3).

5-10 and 10-15 cm depths

In all seasons, at the 5-10 and 10-15 cm depth, the interaction of cover crop and P fertilizer was not significant, and the main effect of cover crop was not significant. In fall 2018, all P fertilizer treatments were the same at 5-10 and 10-15 cm (Table 2.3). In spring 2019, at 5-10 cm, SI had higher P_T than NP, and FB was the same as both, however all P fertilizer treatments were the same at the 10-15 cm depth (Table 2.3).

Organic P

0-15 cm profile

Cover crops were not significant as a main effect, nor in an interaction, in any season (Table 2.2). No treatment effects were detected for P_O in fall 2018, the first season to be sampled at depth (Table 2.2). There was a depth*P fertilizer interaction ($p<0.05$) in spring 2019, where FB and SI had a higher concentration of P_O at the 5-10 cm depth, compared to 0-5 and 10-15 cm, while NP had a higher concentration at 0-5 and 5-10 cm depth than 10-15 cm (Fig. 2.3a). In fall 2019, there was a depth*P fertilizer interaction where there was no stratification in the NP treatment, while in FB and SI treatments, P_O was greater at the 5-10 cm compared to 0-5 and 10-15 cm depth ($p=0.02$) (Fig. 2.3b). Consistently, over all seasons, P_O was 40% of P_T in FB and SI treatments, and 60% in NP treatments at the 0-5 cm depth. At the 5-10 and 10-15 cm depth, P_O was 70% of P_T in all treatments.

0-5 cm depth

In spring and fall 2018, there was no treatment effect on P_O. In spring 2019, all P fertilizer treatments had the same P_O (Fig. 2.3a). In fall 2019, FB and NP had more P_O than SI (Fig. 2.3b).

5-10 and 10-15 cm depths

In fall 2018, we did not detect any treatment effects. In spring and fall 2019, all fertilizer treatments were the same at the 5-10 and 10-15 cm depths (i.e. at 5-10 cm FB=SI=NP, at 10-15 cm FB=SI=NP) (Fig. 2.3).

Water Extractable P

0-15 cm profile

In fall 2018, there was a P fertilizer main effect and a depth main effect (Table 2.2). Water extractable P was greater in SI compared to NP, and FB was equal to SI and NP ($p=0.03$). The P_W was stratified where 0-5 > 5-10 > 10-15 cm ($p<0.0001$). Cover crops were not significant.

In spring 2019, P_W had a cover crop*P fertilizer*depth interaction ($p=0.01$) where FB*NC, FB*CC, and SI*CC had a higher concentration of P_W at 0-5 cm than 5-10 and 10-15 cm (Table 2.4). SI*NC had a higher concentration of P_W at 0-5 cm than 5-10 cm but equal to the 10-15 cm depth (Table 2.4). The NP*CC and NP*NC treatments had the same amount of P_W at all depths (Table 2.4).

In fall 2019, there was a depth main effect (Table 2.2) where 0-5 cm had a higher concentration of P_W than 5-10 cm, and 5-10 cm was greater than 10-15 cm (Table 2.4). Phosphorus fertilizer management and cover crop treatments were not significant in fall 2019 (Table 2.2).

0-5 cm depth

In spring 2018, there was a P fertilizer * cover crop interaction where FB*CC and FB*NC had a greater concentration of P_W than SI*NC, NP*CC, and NP*NC (Table 2.5). The treatment SI*CC was greater than NP*CC and NP*NC but not statistically different from SI*NC despite having 4.04 compared to 2.32 ug P g^{-1} dry soil, or 74% higher P_W in SI*CC compared to SI*NC ($p=0.166$) (Table 2.5). In fall 2018, there was a fertilizer main effect where SI had greater P_W than NP, however FB was not different from either SI or NP ($p=0.05$) (Table 2.5). In spring 2019, there was a P fertilizer * cover crop interaction. The FB*CC, FB*NC, and SI*CC

treatments had more P_W than SI*NC, NP*CC, NP*NC, while SI*NC had higher P_W than NP*CC and NP*NC ($p<0.001$) (Table 2.5). There was no significant treatment effect at 0-5 cm depth in fall 2019 ($p=0.26$).

5-10 and 10-15 cm depth

In spring 2019, at the 5-10 cm depth, FB and SI had higher P than NP ($p=0.02$). Fall 2018 and 2019, 5-10 and 10-15 cm depth partitions were not significant.

Citrate Extractable P

0-15 cm profile

In fall 2018, all treatment main effects and interactions were significant (Table 2.2). A depth* P fertilizer * cover crop interaction was detected, and stratification was most severe in the SI*CC, followed by FB*NC, FB*CC ($p<0.04$) (Table 2.6). No stratification was detected in SI*NC, NP*CC and NP*NC treatments where 0-5 was equal to 5-10 and 10-15 cm (Table 2.6). Stratification was detected at every depth in the SI*CC treatments, where P_C was lower at each deeper depth (i.e. 0-5 > 5-10 > 10-15 cm). Fall broadcast treatments with and without cover crops had higher P_C at the 0-5 cm depth than other depths but 5-10 and 10-15 cm were the same (i.e. 0-5 > 5-10 = 10-15 cm) (Table 2.6).

In spring 2019, all treatment main effects and interactions were significant for P_C. A depth* P fertilizer * cover crop interaction was detected ($p<0.03$) (Table 2.6). The 0-5cm depth was greater than 5-10 and 10-15 cm depths, but 5-10 and 10-15 cm was the same in SI*CC, FB*CC, and FB*NC treatments. The SI*NC, NP*CC, and NP*NC were not stratified (i.e. 0-5 = 5-10 = 10-15 cm) (Table 2.6).

In fall 2019, we detected a depth * P fertilizer interaction where P_C was higher in the FB and SI treatments 0-5 cm depth than the 5-10 cm depth, but 5-10 compared to 10-15 cm, and 0-5 compared to 10-15 cm depths are the same (p=0.046) (Table 2.6). In the NP treatments, all depths were equal to one another (Table 2.6). There was no cover crop treatment effect (p=0.24).

We detected a depth * cover crop interaction in both fall 2018 and spring 2019 (p=0.03, 0.04 respectively) (Table 2.2). In fall 2018, at 0-5 cm depth, CC had more P_C than NC, while at the 10-15 cm depth, CC had less P_C than NC (Fig. 2.4a). In spring 2019, at 0-5 cm depth CC had the same amount of P_C as NC, however NC had more P_C than CC in depths 2 and 3 (Fig. 2.4b). The trend was numerically similar in fall 2019 at depth 3 where P_C was higher in NC than CC (p=0.12) (Fig. 2.4c).

0-5 cm depth

In spring 2018, a P fertilizer main effect was documented (Table 2.7). Fall broadcast P was greater than SI and NP, and SI was also greater than NP (p=0.0002). In fall 2018, SI*CC had more P_C than SI*NC, NP*CC, and NP*NC. The FB*CC and FB*NC treatments had more P_C than NP*CC and NP*NC (p=0.001) (Table 2.7). In spring 2019, SI*CC, FB*CC, and FB*NC had higher concentrations of P_C than SI*NC, NP*CC, and NP*NC (p<0.001) (Table 2.7). While the addition of a cover crop did not affect the result within the FB and NP treatments in spring 2019, cover crops increased P_C in SI P fertilizer management by 57%, from 2.42 to 5.55 ug P g^{-1} dry soil.

5-10 and 10-15 cm depth

Fall 2018, At 5-10 cm SI*CC was the only treatment to have more P_C than NP*CC and NP*NC (p=0.02) (Table 2.6). At 10-15 cm SI*NC had more and NP*CC had less P_C than all other treatments (Table 2.6). In spring 2019, at the 5-10 cm depth, SI*NC had more P_C than all

other treatments except NP*NC, while NP*NC was not different from all other treatments (p=0.01) (Table 2.6). At 10-15 cm, SI*NC and FB*NC had more P_C than NP*CC, FB*CC, and SI*CC (p=0.003) (Table 2.6). In fall 2019, there was no treatment effect at the 5-10 and 10-15 cm depth partitions.

Diffusive Gradient Thin films

Phosphorus fertilizer and cover crop main effects were detected in fall 2018. The FB and SI treatments had more P_{DGT} than NP (p=0.003) (Table 2.8). The CC treatment had 61 ug P_{DGT} ml^{-1} soil solution compared to the 36 ug P_{DGT} ml^{-1} soil solution in the NC treatment, a 41% increase. In spring 2019, we detected a fertilizer*cover crop treatment interaction. The FB*CC, FB*NC, and SI*CC P_{DGT} concentrations were greater than SI*NC, NP*CC, and NP*NC (Table 2.8). In fall 2019 all treatment combinations were statistically the same except SI*CC which had a greater P_{DGT} concentration than NP*CC and NP*NC (p=0.027) (Table 2.8). In fall 2019, P_{DGT} was measured for the 5-10 and 10-15 cm depths. The P_{DGT} was not significantly affected by treatments at the 5-10 cm depth. At the 10-15 cm depth NC had 8.29 ug P_{DGT} ml^{-1} soil solution compared to 7.64 ug P_{DGT} ml^{-1} soil solution in CC treatments, an 8.5% difference (data not shown).

Mehlich-III Extractable P

Mehlich-III P was measured at the 0-5 cm depth. The analysis documented a P fertilizer main effect spring and fall 2018, and spring 2019 where FB and SI had higher P_M than NP (Fig. 2.5). On average fertilized plots had 73.5 ug P_M g^{-1} dry soil in spring 2018 and 76.5 ug P_M g^{-1} dry soil in fall 2018 compared 18 and 16 ug P_M g^{-1} dry soil in NP respectively (Fig. 2.5). In spring

2019, the same trend was observed where FB and SI had 84.5 ug compared to 19 ug P_M g^{-1} dry soil in NP (Fig. 2.5). In fall 2019 there was a cover crop*P fertilizer interaction detected where SI*CC, SI*NC, FB*NC, and FB*CC were greater than NP*NC and NP*CC, and SI*CC was greater than FB*CC (p=0.017) (Fig.2.6). In all seasons, FB and SI had on average 300-500% greater P_M than NP treatments.

Discussion

Stratification

Nutrient stratification is a well-documented challenge in no-till management, where a lack of soil mixing leads to the concentration of nutrients at the surface (Schwab et al., 2006; Dodd & Sharpley, 2016; Smith et al., 2017). Deubel et al. (2011) and Smith et al. (2017) both detected stratification under conservation till/no-till respectively. Our results were consistent with other no-till results; differences between depths were detected in our no-till study by all extraction measures, in all seasons. There was consistent stratification and an interaction between depth and fertilizer in the gross P pools, P_T and P_O. Our P_T and P_O results ranged from 254-513 and 107-230 ug P g^{-1} dry soil, respectively, within ranges reported by Sharpley (1985) from fertilized and unfertilized sites in Oklahoma and Texas. The stratification results were similar to Smith et al. (2017) where stratification was detected in all P fertilizer and cover crop treatments, including the unfertilized control. However, our data showed that the P_T (Table 2.3) and P_O (Fig. 2.3) in NP treatments were stratified in 2018 but trended towards less stratification in spring/fall 2019. In fall 2018, the first season that we sampled at depth, all P fertilizer treatments at the 0-5 cm depth had higher P_T than 5-10, and 10-15 cm depths. But by fall 2019, however, the NP surface P_T had decreased so that 0-5 = 5-10 cm depth, while FB and SI had greater stratification

where 0-5 > 5-10 >10-15 cm. The move from stratification at the 0-5 cm depth in NP in fall 2018 to no stratification between the 0-5 and 5-10 cm depth, and declining values of the 0-5 cm depth suggests that due to a lack of P addition, the surface is being either depleted of P from crop removal and/or lost to erosion and runoff (Carver, 2018). To our knowledge this pattern has not been documented over time and depth in other research.

Interestingly, the P_O was primarily stratified by an increase of the 5-10 cm depth in the FB and SI treatments. By contrast, there was a trend in the NP treatment where the 0-5 cm depth was highest in P_O. Corn and soybean root length has been recorded at 70 to over 100 cm, and 60-95 cm respectively depending on year, tillage, and moisture, with the highest root density at the 0-20 cm depth (Dwyer, Stewart, & Balchin, 1988). The results in the FB and SI treatment with higher amounts of P_O at the 5-10 cm depth may be explained by an increase in P uptake in the roots of the crop, while higher P_O in NP 0-5 cm may reflect an increase in microbial immobilization of P as the soil system becomes P limited (Damon, Bowden, Rose, & Rengel, 2014). Phosphorus fertilizer applications have been shown to increase P uptake by crops (Carver, 2018). As crops decompose, the increase in P in the plant tissue may lead to a decrease in the C:P ratio. The change in C:P may influence the P mineralization or immobilization dynamics (Damon et al., 2014; Bünemann, 2015). As the NP plots are under cultivation but do not receive P fertilizer applications, it is possible that this result may reflect a net immobilization of P in the microbial biomass explains the increase in P_O as the soil C:P ratios widen (Liu et al., 2008; Damon et al., 201 4; Bünemann, 2016).

Results from P_T and P_O suggest some stratification of P is inherent in the top 0-15 cm of soil in no-till systems even with subsurface P fertilizer application or no P fertilizer. Depletion or loss of P from the cropping system at the 0-5 cm depth may explain the reduction of P_T

stratification in NP treatments. It is possible that the accumulation of P_O that we observed at the 5-10cm depth in both the fertilizer treatments may reflect high PO in the residual plant roots fertilized treatments and changing C:P ratios, that can influence whether organic P is mineralized or immobilized (Damon et al., 2014; Varela et al., 2017).

The labile pools of P, P_W and P_C, were stratified in FB*CC, FB*NC, SI*CC treatments but not in SI*NC, NP*NC, NP*CC in spring 2019 (P_W in Table 2.4) and fall 2018 and spring 2019 (P_C in Table 2.6). The P_C in NP treatments were not stratified in fall 2019, while FB and SI had higher P at the 0-5 cm depth. We detected a similar range of P_W, 0.8-9.4 ug g^{-1} dry soil, as the 1.82-6.02 ug g^{-1} dry soil reported in Smith et al. (2017). Smith et al. (2017) detected less P_W stratification in subsurface applied polyphosphate than other treatments which is consistent with our SI*NC results. Unfortunately, Smith et al. (2017) did not include a subsurface polyphosphate * cover crop treatment, so no comparison could be made. However, our results were in agreement with those of Smith et al. (2017) who documented no difference in stratification between DAP fertilizer applications managed with and without a cover crop.

Cover crop interactions

Cover crops had an inconsistent effect on the P pools measured. The gross pools we measured had no cover crop effect, while more labile pools (P_W and P_C) and the P in soil solution (P_{DGT}) revealed a cover crop interaction. A review by Bünemann (2016) found that organic P mineralization positively correlated to soil organic C but negatively correlated to inorganic P. Despite cover crops increasing carbon metrics in the treatments, from the same soil samples (Starr et al., 2019), we did not detect any cover crop treatment effect in P_O. We did see some evidence of a P fertilizer effect on the proportion of P_O where, in general, in P fertilized plots, P_O

was approximately 40% of P_T, while it was 60% of P_T in NP treatments. Microbial nutrient demand and mineralization/immobilization affects P_O concentrations (Bünemann, 2016). The increase in labile P from fertilizer may move microorganisms to mineralize organic matter and release inorganic P rather than re-incorporating it into organic forms (Spohn & Kuzyakov, 2013).

The biologically dynamic pools, as measured in this study with P_W, and P_C, revealed a more nuanced interaction between cover crops and P fertilizer management (Pote et al., 1999; Maguire & Sims, 2002; Haney et al., 2010). At the 0-5 cm depth in P_W in spring 2018 and 2019, P_C in fall 2018 and spring 2019, and P_{DGT} in spring/fall 2019 where the inclusion of a cover crop most frequently impacted spring injected polyphosphate fertilizer management, increasing the amount of bioavailable P compared to no cover crop present. The cover crop did not affect the NP of FB fertilizer treatments. Mehlich-III P results did not detect the P fertilizer * cover crop interactions until fall 2019 (Fig. 2.6), at which time it detected similar results to P_C, P_W, and P_{DGT} from earlier seasons. This suggests that it may not be as sensitive to early P changes but was able to detect interactions after enough time had passed.

In order to determine whether cover crops were translocating P or making more P available we examined the sectioned data to look for changes in cover crop effect within a depth. We detected a depth * cover crop interaction where P_C was higher in NC compared to CC treatments in fall 2018 (10-15 cm depth) and spring 2019 (5-10 and 10-15 cm depths). Additionally, the P_{DGT} fall 2019 10-15 cm results were consistent with P_C results, where NC had more soil solution P than CC treatments. These results are not consistent with Smith et al. (2017), who documented an increase in H3A (an organic acid extraction from the Haney Soil Test) extractable P at the 5-20 cm depth in plots that had cover crops and had been fertilized with surface broadcast DAP in a no-till system over two years. The increased P_C and P_{DGT} in NC

compared to CC at the 5-10 and 10-15 cm depths, combined with higher detected concentrations in SI*CC treatments at the 0-5 cm suggests that the cover crop is translocating P from lower depths to the surface. Kovar et al. (2011) detected higher stratification of bioavailable (exchange resin membrane) P in knife injected (20 cm deep) hog manure and no manure treatments compared to low disturbance injection (15 cm deep) hog manure. Better cover crop P uptake and biomass accumulation at the surface was attributed to less root disturbance from a low disturbance injection and showed a translocation of P from the application zone (Kovar et al., 2011). Although this study had different treatments to our own, it demonstrates a similar effect where a cover crop is moving P from a subsurface placement to the shallower depths.

While a cover crop effect was detected in SI treatments with and without cover crops, no such difference was detected in FB or NP fertilizer management regimes. It is possible that the lack of difference in FB reflects the high P availability in the top 0-5 cm from surface applied DAP. It is likely that the cover crop in FB treatments may access P at the surface and not need to translocate P from deeper depths. As the cover crop residue decomposes in the FB treatments, the P that was taken up by plant would be returned to the surface. Since the cover crop P is returned to the location where it had been applied, it may account for the lack of cover crop interaction with the P fertilizer management. The lack of cover crop effect in NP treatments may be explained by a P limitation in the soil, where the cover crops used in our study were not able to mobilize enough P from the soil to make a significant difference.

Potential Consequences for Water Quality

Conservation practices, such as no-till and cover crops, have been linked to increased dissolved P loss to surface water (Smith et al., 2015: Jarvie et al., 2017; Carver, 2018). In the

Lake Erie basin, increased conservation management implementation has coincided with a 65% higher delivery of dissolved reactive P to surface water (Jarvie et al., 2017). The interaction between spring injected ammonium polyphosphate and cover crops, where cover crops are increasing the labile P pools are the surface, could negatively impact P loss to runoff, as the 0-5 cm is the layer that interacts with precipitation (Kleiman et al., 2006). Numerically, the bioavailable P concentration in various pools (P_W, P_C) was 50-200% higher in SI*CC compared to SI*NC treatments in the 0-5 cm depth. While SI*CC ranged from 75% more to 20% less, SI*NC ranged from 43-65% less than FB*CC and FB*NC, highlighting the difference in surface dynamics of P fertilized treatments. Diffusive gradient thin films detected an average 125% higher P availability in SI*CC compared to SI*NC over the spring and fall 2019. This effect may partially explain why increased DRP concentrations have been detected in runoff from cover crop plots at the experimental site (Carver et al., 2018).

The interaction effects of P fertilizer management and a cover crop in a no-till system on soil P distribution have not previously been detected. To our knowledge, only one study has investigated the effects on P fertilizer management and multiple conservation management practices on P availability (Smith et al., 2017) but it did not include factorial combinations of the treatments. The lack of comparable studies makes it imperative that these results be confirmed in future research. The interpretation of the data in this study is limited by the short time frame and the lack of crop rotation replication. Further research that replicates crop cycles, different crop and cover crop species, and different locations will further illuminate the interaction between spring injected ammonium polyphosphate and cover crops. Documentation of this interactions will be important as both subsurface placement of P fertilizer and cover crops are frequently

recommended as best management practices to reduce P loss (Devlin et al., 2002; Kleinmann et al., 2015; Dodd & Sharpley, 2016) but may negate beneficial effects if implemented together.

Conclusion

Stratification of P_T and P_O was trending down over time in NP but increasing in FB and SI treatments. Total organic P was highest in NP treatments at the 0-5 cm depth but highest in FB and SI treatments 5-10 cm depth. Organic P was not changed by the presence of cover crops despite increased plant residue on the soil surface. Stratification of P_W and P_C was present in FB*NC, FB*CC, and SI*CC but not SI*NC, NP*CC, and NP*NC. Cover crops appeared to increase bioavailable P in SI at the 0-5 cm but a decrease in P_C at lower depths in two of the four seasons sampled. Labile P measures such as P_W, P_C, and P_{DGT} were sensitive and detected treatment interactions, while P_M detected interactions after several seasons. Gross P measures detected depth and P fertilizer main effects but not cover crop effects. The results of this research suggest that cover crops may be interacting with P fertilizer in different ways at different depths by changing where and how soil P stored, in particular the amount of labile P that is maintained in sorbed, and weakly bound state to cations, and organometal complexes. An important next step will be to confirm the effects of spring injected ammonium polyphosphate and cover crop combinations, and further investigate potential mechanisms for P fertilizer * cover crop interactions in no-till systems.

References

Blanco-Canqui, H. 2018. Cover crops and water quality. *Agronomy Journal*, 110(5):1633-1647.

Bunemann, E. K. 2015. Assessment of gross and net mineralization rates of soil organic phosphorus: A review. *Soil Biology & Biochemistry*, 89, 82-98.

Carver, R. E. 2018. *Cover crop and phosphorus fertilizer management effects on phosphorus loss and nutrient cycling* [Master's thesis, Kansas State University]. K-State Research Exchange.

Damon, P. M., Bowden, B., Rose, T., & Rengel, Z. 2014. Crop residue contributions to phosphorus pools in agricultural soils: A review. *Soil Biology & Biochemistry*, 74, 127-137.

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.

Daryanto, S., Wang, L., & Jacinthe, P. A. 2017. Meta-analysis of phosphorus loss from no-till soils. *Journal of Environmental Quality*, 46, 1028-1037.

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

Deubel, A., Hofmann, B., & Orzessek, D. 2011. Long-term effects of tillage on stratification and plant availability of phosphate and potassium in a loess chernozem. *Soil & Tillage Research*, 117, 85-92.

Devlin, D. L., McVay, K., Pierzynski, G. M., & Janssen, K. 2002. *Best management practices for phosphorus*. MF-2321. Kansas State University Agricultural Experiment Station and Cooperative Extension Service.

Dodd, R. J. & Sharpley, A. N. 2015. Recognizing the role of soil organic phosphorus in soil fertility and water quality. *Resources, Conservation and Recycling*, 105, 282-293.

Dodd, R. J. & Sharpley, A. N. 2016. Conservation practice effectiveness and adoption: unintended consequences and implications for sustainable phosphorus management. *Nutrient Cycling in Agroecosystems*, 104, 373-392.

Dougherty, W. J., Mason, S. D., Burkitt, L. L., & Milham, P. J. 2011. Relationship between phosphorus concentration in surface runoff and a novel soil phosphorus test procedure (DGT) under simulated rainfall. *Soil Research*, 49, 523-528.

Duncan, E. W., Osmond, D. L., Shrober, A. L., Starr, L., Tomlinson, P., Kovar, J. L., Moorman, T. B., Peterson, H. M., Fiorellino, N. M., & Reid, K. 2019. Phosphorus and soil health management practices. *Agricultural & Environmental Letters*, 4, 190014.

Dwyer, L. M., Stewart, D. W., & Balchin, D. 1988. Rooting characteristics of corn, soybeans and barley as a function of available water and soil physical characteristics. *Canadian Journal of Soil Science*, 68, 121-132.

Eichler-Löbermann, B., Köhne, S., Kowalski, B., & Schnug, E. 2008. Effect of catch cropping on phosphorus bioavailability in comparison to organic and inorganic fertilization. *Journal of Plant Nutrition*, 31, 659-676.

FAO, 2017. *Food and Agriculture Organization of the United Nations: Conservation Agriculture.* Available at: http://www.fao.org/ag/ca (Accessed July 22, 2020).

Haney, R. L., Haney, E. B., Hossner, L. R., & Arnold, J. G. 2010. Modifications to the new soil extractant H3A-1: A multinutrient extractant. *Communications in Soil Science and Plant Analysis*, 41, 1513-1523.

Hayes, J. E., Richardson, A. E., and Simpson, R. J. 2000. Components of organic phosphorus in soil extracts that are hydrolysed by phytase and acid phosphatase. *Biology and Fertility of Soils*, 32(4), 279-286.

Jarvie, H. P., Johnson, L. T., Sharpley, A. N., Smith, D. R., Baker, D. B., Bruulsema, T. W., & Confesor, R. 2017. Increased soluble phosphorus loads to Lake Erie: Unintended consequences of conservation practices? *Journal of Environmental Quality*, 46, 123-132.

Jones, D. L. 1998. Organic acids in the rhizosphere: a critical review. *Plant and Soil*, 205, 25-44.

Jordan-Meille, L., Rubaek, G. H., Ehlert, P. A. I., Genot, V., Hofman, G., Goulding, K., Recknagel, J., Provolo, G., & Barraclough, P. 2012. An overview of fertilizer-P recommendations in Europe: soil testing, calibration and fertilizer recommendations. *Soil Use and Management*, 28, 419-435.

Kleinmann, P. J. A., Sharpley, A., Withers, P. J. A., Bergstrom, L., Johnson, L. T., & Doody, D. G. 2015. Implementing agricultural phosphorus science and management to combat eutrophication. *Ambio*, 44(2), S297-S310.

Kleinman, P. J., Srinivasan, M. S., Dell, C. J., & Schmidt, J. P. 2006. Role of rainfall intensity and hydrology in nutrient transport via surface runoff. *Journal of Environmental Quality*, 35(4), 1248-59.

Kleinman, P. J., Sharpley, A. N., McDowell, R. W., Flaten, D. N., Buda, A. R., Tao, L., Bergstrom, L., & Zhu, Q. 2011. Managing agricultural phosphorus for water quality protection: Principles for progress. *Plant Soil*, 349, 169-182.

Kovar, J. L., Moorman, T. B., Singer, J. W., Cambardella, C. A., & Tomer, M. D. 2011. Swine manure injection with low-disturbance applicator and cover crops reduce phosphorus losses. *Journal of Environmental Quality*, 40, 329-336.

Li, L., Li, S. M., Sun, J. H., Zhou, L., Bao, X. G., Zhang, H. G., & Zhang, F. S. 2007. Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. *PNAS*, 104 (27), 11192-11196.

Liu, A., Hamel, C., Spedding, T., Zhang, T. Q., Mongeau, R., Lamarre, G. R., & Tremblay, G. 2008. Soil microbial carbon and phosphorus as influenced by phosphorus fertilization and tillage in a maize-soybean rotation in south-western Quebec. *Canadian Journal of Soil Science*, 88, 21-30.

Liu, J. R. Khalaf, R., Ule'n, B., & Bergkvist, G. 2013. Potential phosphorus release from catch crop shoots and roots after freeze-thawing. *Plant and Soil*, 371, 543-557.

Maguire, R. O., & Sims, J. T. 2002. Soil testing to predict phosphorus leaching. Journal of Environmental Quality, 31(5), 1601-1609.

Mason, S., Hamon, R., Nolan, A., Zhang, H., & Davison, W. 2005. Performance of a mixed binding layer for measuring anions and cations in a single assay using diffusive gradients in thin films technique. *Analytical Chemistry*, 77, 6339-6346.

Mason, S., McNeill, A., McLaughlin, M. J., & Zhang, H. 2010. Prediction of wheat response to an application of phosphorus under field conditions using diffusive gradients in thin-films (DGT) and extraction methods. *Plant Soil*, 337, 243-258.

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. *Environmental Science & Technology*, 50, 11521-11531.

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

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* (pp. 279-280). Canadian Society of Soil Science, Taylor & Francis Group.

Pautler, M. C., & Sims, J. T. 2000. Relationship between soil test phosphorus, soluble phosphorus, and phosphorus saturation in Delaware soils. *Soil Science Society of America Journal*, 64, 765-773.

Pote, D. H., Daniel, T. C., Nichols, D. J., Sharpley, A. N., Moore, P. A. Jr., Miller, D. M., & Edwards, D. R. 1999. Relationship between phosphorus levels in three Ultisols and phosphorus concentrations in runoff. *Journal of Environmental Quality*, 28, 170-175.

Richardson, A. E., Lynch, J. P., Ryan, P. R., Delhaize, E., Smith, F. A., Smith, S. E., Harvey, P. R, Ryan, M. H., Veneklaas, E. J., Lambers, H., Oberson, A., Culvenor, R. A., & Simpson, R. J. 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant Soil*, 349, 121-156.

Schwab, G. J., Whitney, D. A., Kilgore, G. L., & Sweeney, D. W. 2006. Tillage and phosphorus management effects on crop production in soils with phosphorus stratification. *Agronomy Journal*, 98(3), 430-435.

Sharpley, A. N. 1985. Phosphorus cycling in unfertilized and fertilized agricultural soils. Soil Science Society of America Journal, 49, 905-911.

Sharpley, A. N., Bergström, L., Aronsson, H., Bechmann, M., Bolster, C. F., Börling, K., Djodjic, F., Jarvie, H. P., Schoumans, O. F., Stamm, C., Tonderski, K. S., Ulen, B., Uusitalo, R., & Withers, P. J. A. 2015. Future agriculture with minimized phosphorus losses to waters: Research needs and direction. *Ambio*, 44(2), S163-S179.

Sharpley, A. N., Kleinman, P. J. A., & Weld, J. L. 2008. Environmental soil phosphorus indices. In Carter, M. R., and Gregorich, E. G. (Eds), *Soil Sampling and Methods of Analysis* (pp.143-145). Canadian Society of Soil Science, Taylor & Francis Group.

Sharpley, A. N., Robinson, J. S., & Smith, S. J. 1995. Bioavailable phosphorus dynamics in agricultural soils and effects on water quality. *Geoderma*, 67, 1-15.

Sims, J. T., Edwards, A. C., Schoumans, O. F., & Simard, R. R. 2000. Integrating soil phosphorus testing into environmentally based agriculture management practices. *Journal of Environmental Quality*, 29, 60-71.

Six, L., Pypers, P., Degryse, F., Smolders, E., & Merckx, R. 2012. The performance of DGT versus conventional soil phosphorus tests in tropical soils – An isotope dilution study. *Plant Soil*, 359, 267-279.

Smith, D. R., Francesconi, S. J., Livingston, S. J., & Huang, C. 2015. Phosphorus losses from monitored fields with conservation practices in the Lake Erie Basin, USA. *Ambio*, 44 (2), S319-S331.

Smith, D. R., Huang, C., & Haney, R. L. 2017. Phosphorus fertilization, soil stratification, and potential water quality impacts. *Journal of Soil and Water Conservation*, 72(5), 417-424.

Spohn, M., & Kuzyakov, Y. 2013. Phosphorus mineralization can be driven by microbial need for carbon. *Soil Biology & Biochemistry*, 61, 69-75.

Starr, L. M., Tomlinson, P. J., Nelson, N. O., Stewart, C. L., Roozeboom, K. L., Kluitenberg, G. J., and Presley, D. R. 2019. Effects of cover crops and phosphorus fertilizer management on soil health parameters in a no-till corn-soybean cropping system in Riley County, Kansas. *Kansas Agricultural Experiment Station Research Reports*, 5(6).

Varela, M. F., Barraco, M., Gili, A., Taboada, M. A., & Rubio, G. 2017. Biomass decomposition and phosphorus release from residues of cover crops under no-tillage. *Agronomy Journal*, 109(1), 317-326.

Walker, T. W., & Adams, A. F. R. 1958. Studies on soil organic matter. Influence of phosphorus content of parent materials on accumulation of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. *Soil Science*, 85, 307-318.

White, C. M., & Weil, R. R. 2011. Forage radish cover crops increase soil test phosphorus surrounding radish taproot holes. *Soil Science Society of America Journal*, 75(1), 121-130.

Wendling, M., Buchi, L., Amosse, C., Sinaj, S., Walter, A., & Charles, R. 2016. Influence of root and leaf traits on the uptake of nutrients in cover crops. *Plant Soil*, 409, 419-434.

Zehetner, F., Wuenscher, R., Peticzka, R., & Unterfrauner, H. 2018. Correlation of extractable phosphorus (P) with plant P uptake: 14 extraction methods applied to 50 agricultural soils from Central Europe. *Plant Soil Environment*, 64 (4), 192-201.

Zhang, H., & Davison, W. 2015. Use of diffusive gradients in thin-films for studies of chemical speciation and bioavailability. *Environmental Chemistry*, 12, 85-101.

Zhang, H., Davison, W., Knight, B., and McGrath, S. 1998. In situ measurements of solution concentrations and fluxes of trace metals in soils using DGT. *Environmental Science & Technology*, 32(5), 704-710.

Figures

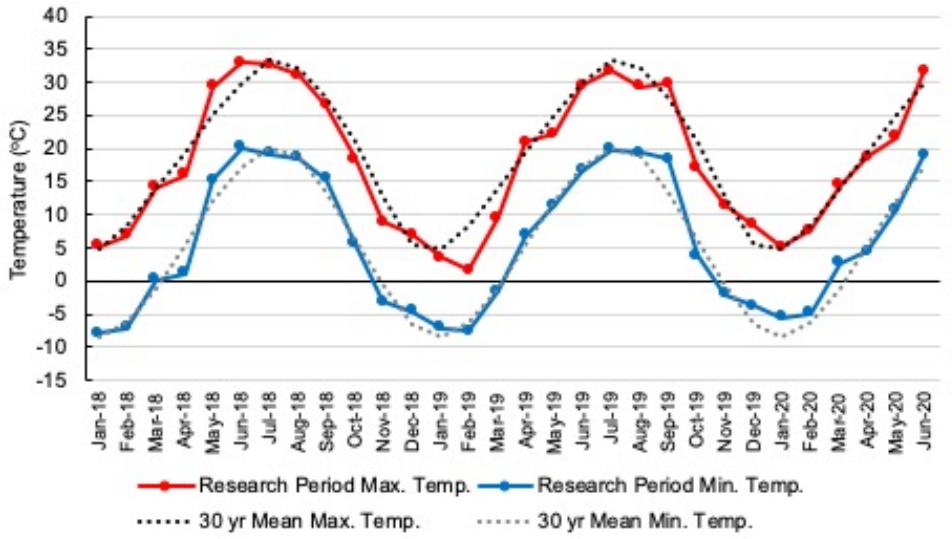


Figure 2.1 Mean monthly maximum and minimum air temperature from 01/2018 – 06/2020 at the Ashland Bottoms Mesonet Station (operated by Kansas State University), located <1000 m from the research site relative to the 30-yr (1980 – 2010) averages for the county (source: Kansas State Weather Data Library).

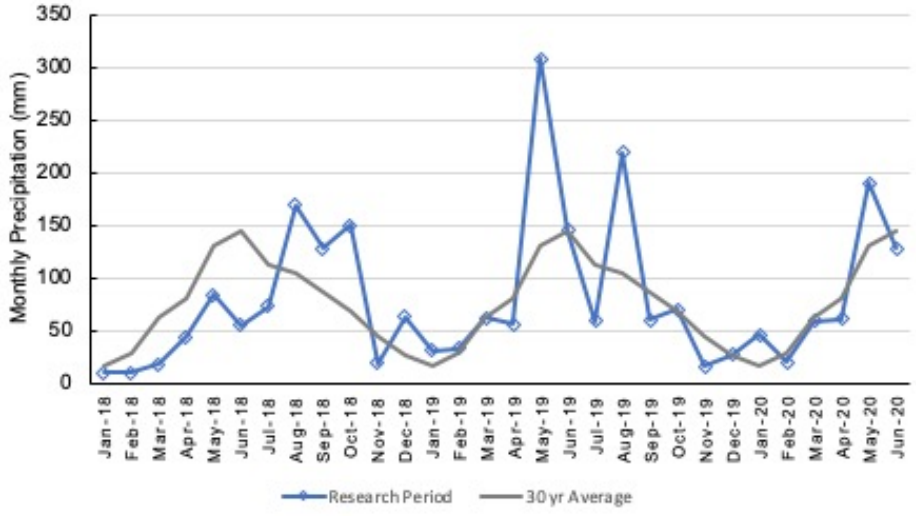


Figure 2.2 Monthly precipitation (mm) from 01/2018 – 06/2020 at the Ashland Bottoms Mesonet Station (operated by Kansas State University), located <1000 m from the research site.

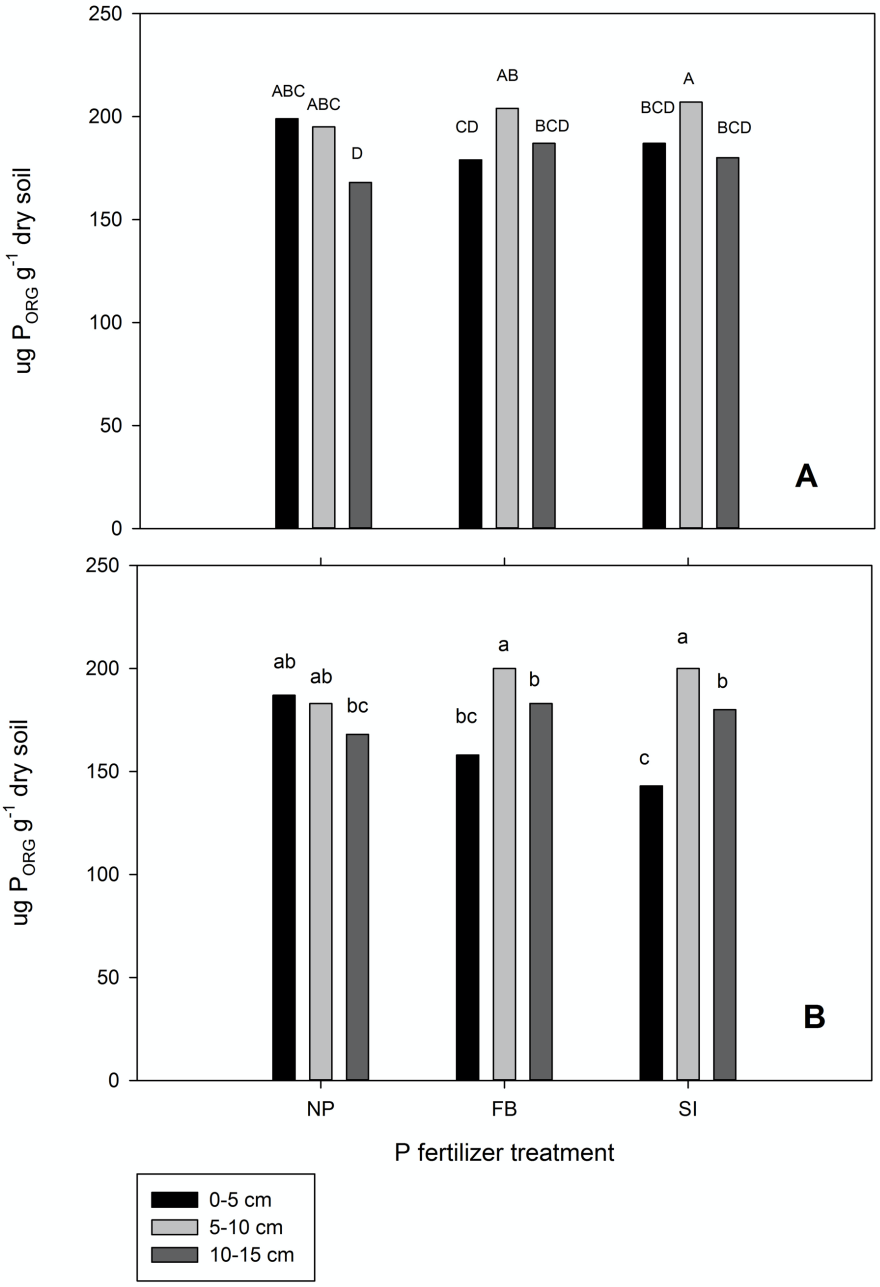


Figure 2.3. Total organic P concentration (ug P_O g^{-1} dry soil) treatment means of depth*P fertilizer interaction at 0-5, 5-10, and 10-15 cm A) Spring 2019 B) Fall 2019. Letters signify significant differences ($p<0.05$). Figure abbreviations: fall broadcast diammonium phosphate (FB), subsurface spring injected ammonium polyphosphate (SI), and no P fertilizer (NP).

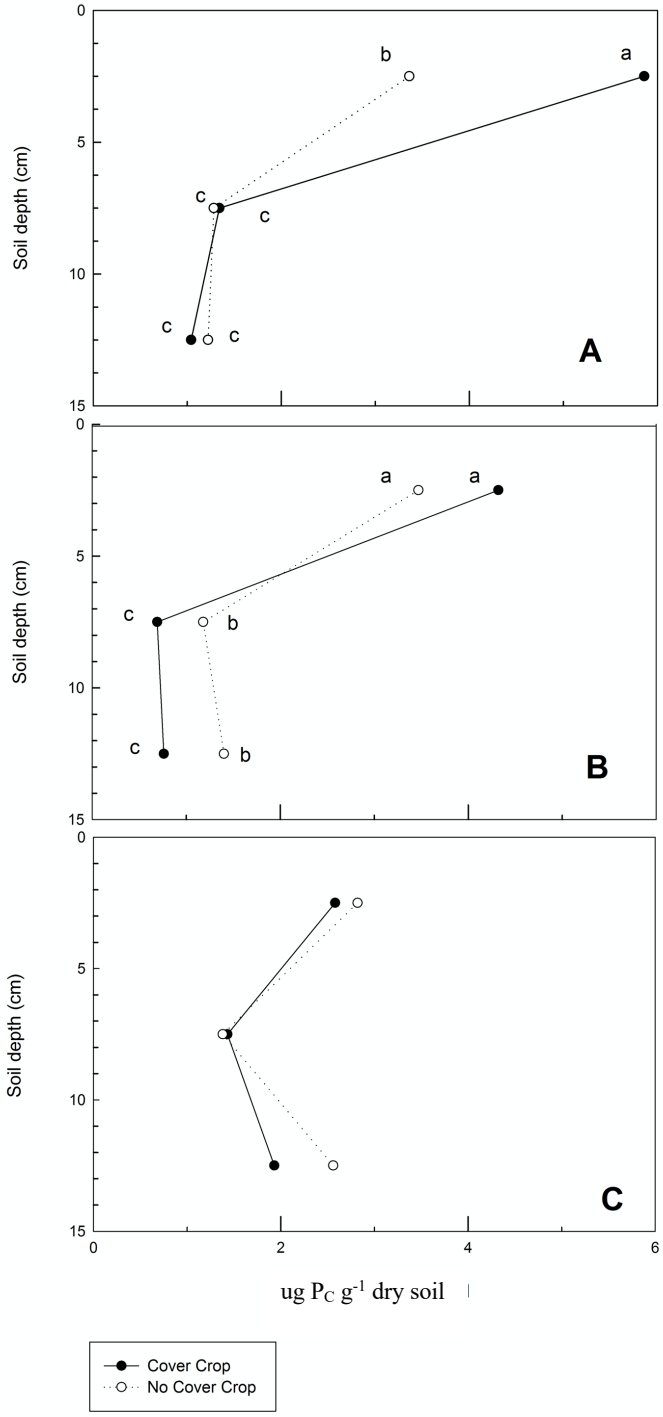


Figure 2.4. Citrate extractable P (ug P_C g^{-1} dry soil) depth * cover crop interaction A) Fall 2018 B) Spring 2019 C) Fall 2019. Letters signify significant differences ($p<0.05$).

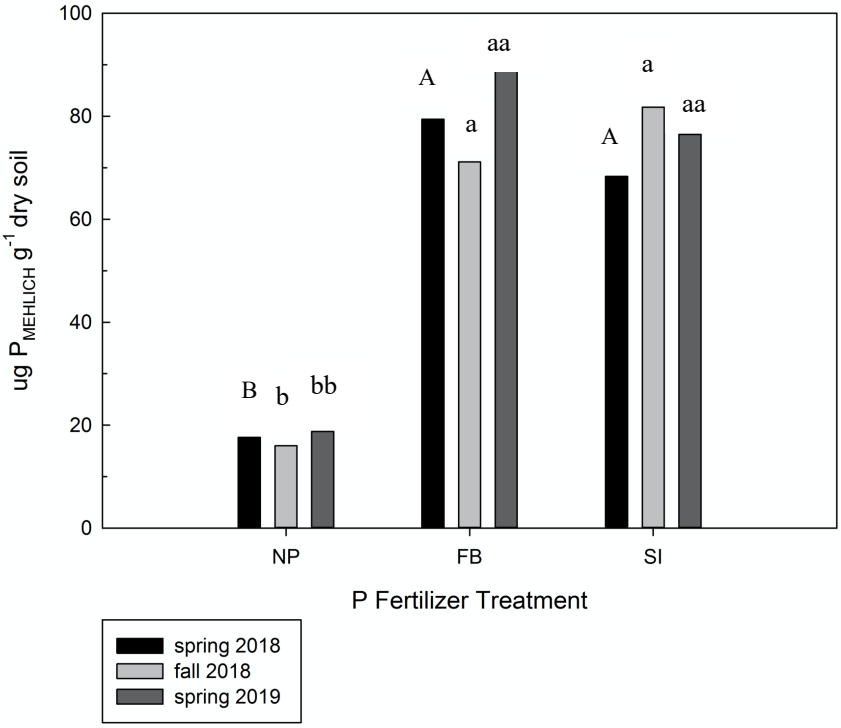


Figure 2.5. Mehlich-III extractable P (ug P_M g-1 dry soil) fertilizer main effect treatments means at 0-5 cm depth in spring 2018, fall 2018, and spring 2019. Letters indicate significant differences ($p<0.05$). Seasons not compared with one another. Figure abbreviations: fall broadcast diammonium phosphate (FB), spring injected ammonium polyphosphate (SI), no P fertilizer (NP).

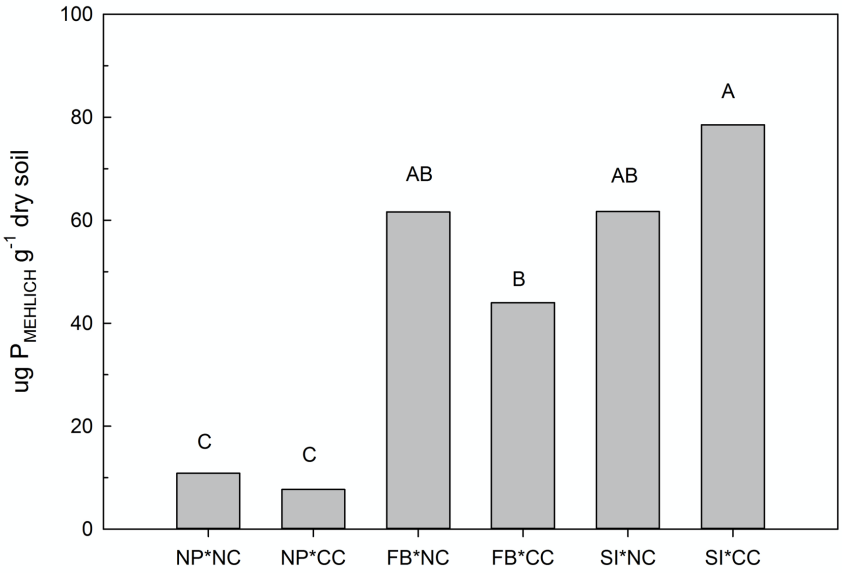


Figure 2.6. Mehlich-III extractable P (ug P_M g-1 dry soil) treatment means of a P fertilizer * cover crop interaction in fall 2019. Letters indicate significant differences ($p<0.05$). Figure abbreviations: fall broadcast diammonium phosphate (FB), spring injected ammonium polyphosphate (SI), no P fertilizer (NP), cover crop (CC), and no cover crop (NC).

Tables

Table 2.1. Summary of cropping system management, soil sample collection, and mean grain yield/ cover crop biomass production.

Date	Field operation	Details
2017		
24 April	Corn planted	64,000 seeds ha^{-1} *Zea mays* var. DKC53-56
26 April	N fertilizer applied	Urea ammonium nitrate injected at 146 kg N ha^{-1}
20 Sept.	Corn harvested	Mean grain yield 6,660 kg ha^{-1}
21Sept.	Cover crop planted	64 kg ha^{-1} triticale (*Triticosecale* var. TriCal 780) and 4.5 kg ha^{-1} rapeseed (*Brassica napus* var. Dwarf Essex)
28 Nov.	Fall-broadcast P applied	27 kg P ha^{-1} diammonium phosphate
2018		
04 May	Spring soil samples collected	0-5 cm depth
08 May	Spring injected P applied	27 kg ha^{-1} ammonium polyphosphate
09 May	Soybeans planted	320,000 seeds ha^{-1} *Glycine max* var. Liberty Link®
10 May	Cover crop terminated	Terminated with glyphosate 4.7 L ha^{-1} Mean biomass 2,391 kg ha^{-1}
01 Nov.	Soybeans harvested	Mean grain yield 1,809 kg ha^{-1}
02 Nov.	Cover crops planted	56 kg ha^{-1} winter wheat (*Triticum aestivum* var. 1863 Foundation Seed) and 6 kg ha^{-1} rapeseed (*Brassica napus* var. Dwarf Essex)
19 Nov.	Fall soil samples collected	0-5, 5-10, and 10-15 cm depths
21 Dec.	Fall-broadcast P applied	27 kg P ha^{-1} diammonium phosphate

2019		
22 April	Spring soil samples collected	0-5, 5-10, and 10-15 cm depths
24 April	N fertilizer applied	Urea ammonium nitrate injected at 146 kg N ha^{-1}
25 April	Corn planted	64,000 seeds ha^{-1} *Zea mays* var. DKC53-56
26 April	Cover crop terminated	Terminated with glyphosate 4.7 L ha^{-1} Mean biomass 312 kg ha^{-1}
18 Sept.	Corn harvested	Mean grain yield 9,720 kg ha^{-1}
26 Sept.	Fall soil samples collected	0-5, 5-10, and 10-15 cm depths

Table 2.2. ANOVA table showing *p-values* for soil total P, total organic P, water extractable P, citrate extractable P. In spring 2018, soil was sampled at the 0-5 cm depth only and did not include depth as an effect. Soil was sampled at 0-5, 5-10, and 10-15 cm depths in fall 2018, spring 2019, and fall 2019. Seasons are not compared to one another**.**

Effect	Spring 2018	Fall 2018	Spring 2019	Fall 2019
		----------Total Phosphorus----------		
P fertilizer management	0.001	0.002	0.0001	0.001
Cover crop	0.56	0.10	0.67	0.21
P fertilizer * Cover crop	0.35	0.34	0.81	0.52
Depth	n/a	< 0.0001	<0.0001	<0.0001
Depth * P fertilizer	n/a	0.001	0.001	0.001
Depth * Cover crop	n/a	0.09	0.71	0.37
Depth * P fertilizer * cover crop	n/a	0.85	0.74	0.49
		----------Total Organic Phosphorus----------		
P fertilizer management	0.06	0.50	0.86	0.81
Cover crop	0.66	0.94	0.77	0.52
P fertilizer * Cover crop	0.78	0.61	0.36	0.63
Depth	n/a	0.74	0.002	0.0001
Depth * P fertilizer	n/a	0.11	0.05	0.02
Depth * Cover crop	n/a	0.20	0.53	0.99
Depth * P fertilizer * cover crop	n/a	0.81	0.57	0.98
		----------Water Extractable Phosphorus----------		
P fertilizer management	<0.0001	0.03	<0.0001	0.17
Cover crop	0.52	0.28	0.86	0.83
P fertilizer * Cover crop	0.04	0.42	0.36	0.26
Depth	n/a	<0.0001	<0.0001	0.0001
Depth * P fertilizer	n/a	0.15	<0.0001	0.10
Depth * Cover crop	n/a	0.36	0.38	0.86
Depth * P fertilizer * cover crop	n/a	0.53	0.007	0.57
		----------Citrate Extractable Phosphorus----------		
P fertilizer management	0.0002	0.0003	<0.0001	0.03
Cover crop	0.89	0.04	0.66	0.43

P fertilizer * Cover crop	0.11	0.02	0.18	0.50
Depth	n/a	<0.0001	<0.0001	0.001
Depth * P fertilizer	n/a	0.003	<0.0001	0.046
Depth * Cover crop	n/a	0.03	0.04	0.39
Depth * P fertilizer * cover crop	n/a	0.02	0.03	0.51

Table 2.3. Soil total P (ug P_T g^{-1} dry soil) depth*P fertilizer interaction treatment means in fall 2018, spring 2019, and fall 2019. Spring 2018 was only sampled at 0-5 cm depth. Letters signify significant differences ($p<0.05$). Seasons are not compared to one another. Table abbreviations: fall broadcast diammonium phosphate (FB), subsurface spring injected ammonium polyphosphate (SI), and no P fertilizer (NP).

	2018		**2019**	
	Spring*	**Fall**	**Spring**	**Fall**
Crop growing prior to sampling	*Triticale and Rapeseed (if present)*	*Soybean*	*Winter Wheat and Rapeseed (if present)*	*Corn*
NP 0-5 cm	327.8 B	344.8 B	352.5 B	320.7 BC
NP 5-10 cm	n/a	274.3 C	279.2 DE	298.7 CD
NP 10-15 cm	n/a	274.5 C	281.3 E	290.8 D
FB 0-5 cm	411 A	429.0 A	465.8 A	395.3 A
FB 5-10 cm	n/a	281.3 C	292.8 CD	312.7 BC
FB 10-15 cm	n/a	280.2 C	281.3 DE	286.3 D
SI 0-5 cm	392.5 A	436.2 A	451.7 A	419.8 A
SI 5-10 cm	n/a	287.0 C	302.7 C	331.0 B
SI 10-15 cm	n/a	287.2 C	281.8 DE	296.8 CD
SE	17.26	*12.0*	*13.52*	*14.35*
p value	0.001	*< 0.001*	*< 0.001*	*< 0.001*

* Spring 2018 only had one depth and was analyzed with an ANOVA model without the depth component.

Table 2.4. Water extractable P (ug P_W g^{-1} dry soil) depth*P fertilizer*cover crop interaction treatment means in fall 2018, spring 2019, and fall 2019. Letters signify significant differences ($p<0.05$). Seasons are not compared to one another**.** Table abbreviations: fall broadcast diammonium phosphate (FB), subsurface spring injected ammonium polyphosphate (SI), and no P fertilizer (NP)**,** and cover crops are present (CC) or absent (NC).

		Fall 2018	**Spring 2019**	**Fall 2019**
Treatment	*Depth (cm)*	*Soybean*	*Winter Wheat and Rapeseed (if present))*	*Corn*
NP*NC	0-5	1.08	1.72 C	1.47
NP*NC	5-10	0.80	1.27 C	0.32
NP*NC	10-15	0.49	1.19 C	0.40
NP*CC	0-5	3.54	1.69 C	2.43
NP*CC	5-10	0.80	1.32 C	0.23
NP*CC	10-15	0.50	1.51 C	0.32
FB*NC	0-5	4.45	8.43 A	2.06
FB*NC	5-10	1.19	1.57 C	0.65
FB*NC	10-15	0.69	1.66 C	0.40
FB*CC	0-5	3.86	6.90 A	3.93
FB*CC	5-10	0.98	1.91 C	0.81
FB*CC	10-15	0.70	1.20 C	0.57
SI*NC	0-5	4.57	4.29 B	5.25
SI*NC	5-10	1.20	1.63 C	0.73
SI*NC	10-15	0.78	2.85 BC	0.39
SI*CC	0-5	6.75	6.92 A	3.37
SI*CC	5-10	1.09	1.65 C	0.49
SI*CC	10-15	0.5	1.12 C	0.23
*Depth*Fert*Cover*	*SE*	*1.20*	*0.51*	*1.12*
	p value	*0.53*	*0.007*	*0.57*

Table 2.5. Water extractable P (ug P_W g^{-1} dry soil), 0-5 cm treatments means, in spring and fall 2018, 2019. Letters signify significant differences ($p<0.05$). Seasons are not compared to one another. Table abbreviations: fall broadcast diammonium phosphate (FB), subsurface spring injected ammonium polyphosphate (SI), and no P fertilizer (NP)**,** and cover crops are present (CC) or absent (NC)

	2018		**2019**	
	Spring*	**Fall**	**Spring**	**Fall**
Crop growing prior to sampling	*Triticale and Rapeseed (if present)*	*Soybean*	*Winter Wheat and Rapeseed (if present))*	*Corn*
NP*NC	1.47 C	1.08	1.72 C	1.47
NP*CC	1.48 C	3.54	1.69 C	2.43
FB*NC	6.00 A	4.45	8.43 A	2.06
FB*CC	5.01 A	3.86	6.9 A	3.93
SI*NC	2.32 BC	4.57	4.29 B	5.25
SI*CC	4.04 AB	6.75	6.92 A	3.37
*SE fert*cover*	*0.48*	*1.18*	*0.51*	*1.08*
p value	*0.04*	*0.11*	*< 0.001*	*0.24*
NP	1.48 C	2.31 B	1.70 C	1.95
FB	5.50 A	4.16 B	7.67 A	2.99
SI	3.18 B	5.66 A	5.61 B	4.31
SE fertilizer	*0.36*	*0.86*	*0.36*	*0.76*
p value	*<0.0001*	*0.05*	*<0.0001*	*0.13*
No	3.26	3.54	4.82	2.93
CC	*3.51*	4.72	*5.17*	*3.24*
SE cover	*0.3*	*0.72*	*0.29*	*0.62*
p value	*0.52*	*0.2*	*0.41*	*0.73*

* Spring 2018 only had one depth and was analyzed with an ANOVA model without the depth component. Fall 2018, spring/fall 2019 results are from the depth partitioning of the full 0-15 cm results.

Table 2.6. Citrate extractable P (ug P_C g^{-1} dry soil) depth*P fertilizer*cover crop, and depth*P fertilizer interaction treatment means in fall 2018, spring 2019, and fall 2019. Letters signify significant differences ($p<0.05$). Seasons are not compared to one another. Table abbreviations: fall broadcast diammonium phosphate (FB), subsurface spring injected ammonium polyphosphate (SI), and no P fertilizer (NP), and cover crops are present (CC) or absent (NC).

		Fall 2018	**Spring 2019**	**Fall 2019**
Treatment	*Depth (cm)*	*Soybean*	*Winter Wheat and Rapeseed (if present))*	*Corn*
NP*NC	0-5	0.91 CD	0.5 C	0.91
NP*NC	5-10	1.00 CD	1.13 BC	1.41
NP*NC	10-15	1.18 C	1.18 BC	1.48
NP*CC	0-5	0.94 CD	0.59 C	0.86
NP*CC	5-10	0.86 CD	0.66 C	1.69
NP*CC	10-15	0.75 D	0.85 C	2.03
FB*NC	0-5	5.73 B	7.5 A	3.67
FB*NC	5-10	1.43 C	0.84 C	1.52
FB*NC	10-15	1.07 C	1.33 BC	2.91
FB*CC	0-5	6.03 B	6.83 A	3.14
FB*CC	5-10	1.4 C	0.62 C	0.94
FB*CC	10-15	1.29 C	0.68 C	1.89
SI*NC	0-5	3.43 BC	2.42 B	3.88
SI*NC	5-10	1.40 C	1.58 BC	1.22
SI*NC	10-15	1.40 C	1.69 B	3.28
SI*CC	0-5	10.62 A	5.55 A	3.75
SI*CC	5-10	1.77 C	0.80 C	1.66
SI*CC	10-15	1.07 C	0.76 C	1.87
*Depth*Fert*Cover*	*SE*	*1.24*	*0.65*	*1.06*
	p value	*0.02*	*0.03*	*0.51*
NP	0-5	0.92 CD	0.54 D	0.89 C
NP	5-10	0.93 D	0.90 CD	1.55 C
NP	10-15	0.96 D	1.02 CD	1.76 BC
FB	0-5	5.88 A	7.17 A	3.41 AB
FB	5-10	1.42 BC	0.73 D	1.23 C
FB	10-15	1.18 CD	1.01 CD	2.40 ABC
SI	0-5	7.02 A	3.99 B	3.82 A
SI	5-10	1.59 B	1.18 C	1.44 C
SI	10-15	1.24 C	1.22 C	2.58 ABC
*Depth*Fert*	*SE*	*0.88*	*0.36*	*0.75*
	p value	*0.003*	*<0.001*	*0.046*

Table 2.7. Citrate extractable P (ug P_C g^{-1} dry soil), 0-5 cm treatment means, in spring and fall 2018, 2019. Letters signify significant differences ($p<0.05$). Seasons are not compared to one another. Table abbreviations: fall broadcast diammonium phosphate (FB), subsurface spring injected ammonium polyphosphate (SI), and no P fertilizer (NP)**,** and cover crops are present (CC) or absent (NC).

	2018		**2019**	
	Spring*	**Fall**	**Spring**	**Fall**
Crop growing prior to sampling	*Triticale and Rapeseed (if present)*	*Soybean*	*Winter Wheat and Rapeseed (if present)*	*Corn*
NP*NC	1.44	0.91 B	0.50 B	0.91
NP*CC	1.21	0.94 B	0.59 B	0.86
FB*NC	13.94	5.73 AB	7.50 A	3.67
FB*CC	10.51	6.03 AB	6.83 A	3.14
SI*NC	4.03	3.43 B	2.42 B	3.88
SI*CC	8.24	10.62 A	5.55 A	3.75
*SE fert*cover*	*1.62*	*1.32*	*0.634*	*1.06*
p value	*0.11*	*0.02*	*0.02*	*0.97*
NP	1.33 C	0.93 B	0.54 C	0.89 B
FB	12.23 A	5.88 A	7.17 A	3.41 A
SI	6.13 B	7.02 A	3.99 B	3.82 A
SE fertilizer	*1.14*	*1.04*	*0.47*	*0.75*
p value	*0.0002*	*0.001*	*<0.0001*	*0.04*
No	6.47	3.36	3.47	2.82
CC	6.65	5.86	4.32	2.58
SE cover	*0.93*	*0.93*	*0.40*	*0.61*
p value	*0.89*	*0.02*	*0.12*	*0.79*

* Spring 2018 only had one depth and was analyzed with an ANOVA model without the depth component. Fall 2018, spring/fall 2019 results are from the depth partitioning of the full 0-15 cm results.

Table 2.8. P supplied to soil solution (ug P_{DGT} ml^{-1} soil solution) treatment means at the 0-5 cm depth, as measured by diffusive gradient thin films, in spring and fall 2018 and 2019. Letters signify significant differences ($p<0.05$). Seasons are not compared to one another. Table abbreviations: fall broadcast diammonium phosphate (FB), subsurface spring injected ammonium polyphosphate (SI), and no P fertilizer (NP), and cover crops are present (CC) or absent (NC).

	2018		**2019**	
	Spring*	**Fall**	**Spring**	**Fall**
Crop growing prior to sampling	*Triticale and Rapeseed (if present)*	*Soybean*	*Winter Wheat and Rapeseed (if present)*	*Corn*
NP*NC	10.22	9.95	7.81 B	25.94 B
NP*CC	8.89	23.79	7.81 B	8.95 B
FB*NC	101.24	52.27	90.75 A	42.93 AB
FB*CC	88.47	60.52	66.95 A	33.46 AB
SI*NC	42.16	45.35	36.74 B	34.76 AB
SI*CC	61.06	98.85	81.03 A	76.58 A
*SE fert*cover*	*13.15*	*13.31*	*8.77*	*9.83*
p value	*0.42*	*0.17*	*0.009*	*0.03*
NP	9.55 C	16.87 B	7.81 B	17.44 B
FB	94.86 A	56.40 A	78.85 A	38.19 B
SI	51.61 B	72.10 A	58.88 A	55.67 A
SE fertilizer	*10.21*	*10.33*	*6.26*	*6.95*
p value	*<0.0001*	*0.003*	*<0.0001*	*0.01*
No	51.21	35.86 B	45.1	34.54
CC	52.80	61.06 A	51.93	39.66
SE cover	*9.03*	*9.12*	*5.16*	*5.67*
p value	*0.87*	*0.03*	*0.36*	*0.54*

* Spring 2018 only had one depth and was analyzed with an ANOVA model without the depth component. Fall 2018, spring/fall 2019 results are from the depth partitioning of the full 0-15 cm results.

Chapter 3 - Near Surface Effects of Cover Crops and P Fertilizer Management on Microbial Biomass P and Phosphatase Enzyme Activity

Abstract

Cover crops phosphorus (P) fertilizer management are common approaches to reducing nutrient loss and improving crop nutrient use efficiency. Potential changes in nutrient availability and soil organic matter from these management decisions could affect microbial biomass (MB) stoichiometry, MB-P, and the expression of P cycling enzymes. The microbial biomass is important pool of labile P and could potentially be a source of P pollution to surface water. Increased P enzyme activity may indicate increased organic P hydrolysis, while MB-C:P can dictate whether P is immobilized back into MB. The objective of this research was to understand the effects of cover crops, and P fertilizer management on MB-P, MB-C:P, and the concentration of P cycling enzymes in soil. Our hypothesis is that the combination of fertilizer and cover crop will leading to a large bioavailable P pool in the 0-5 cm soil layer. A no-till, corn-soybean rotation was established in 2014, with 18, ~ 0.5 ha watersheds in northeastern Kansas. The experimental design was a randomized complete block designed with a 2*3 factorial treatment structure: two cover crop treatments [fall-sown cover crop (CC)/no cover crop (NC)] and three P management treatments [27 kg P^{-1} ha fall broadcast (FB), spring injected (SI), or no P application (NP)]. Samples were collected from 0-5 cm, in fall 2018, and spring/fall 2019. Samples were analyzed for Mehlich-III P (P_M), MB-P, and three P enzyme activity potentials (acid phosphatase, alkaline phosphatase, and phosphodiesterase). In all instances, enzyme activity for acid phosphatase greater in CC versus NC ($p<0.01$). Alkaline phosphatase, and

phosphodiesterase were greater in CC treatments in fall 2018 and fall 2019. Microbial biomass P was on average was five-fold higher in the P fertilized than unfertilized treatments ($p<0.001$), with the similar results in MB-C:P. We detected a MB-P cover crop * P fertilizer interaction in fall 2018 and spring 2019, where CC increased the amount of P in SI*CC treatments. No P fertilizer treatments had consistently less MB-P and higher MB-C:P, suggesting conditions for P immobilization. Our results suggest that the combination of P fertilizer and cover crops may increase the amount of MB-P that is in SI*CC treatments. This requires further investigation because both SI and CC are recommended management practices.

Introduction

The eutrophication of surface water from nutrient loss is a serious pollution problem (Dodd & Sharpley, 2015). It is estimated that it cost the United States $2.2 billion per year (Dodds et al., 2009). In many freshwater systems, the primary cause of eutrophication is the enrichment of phosphorus (P) which can be lost in runoff from agricultural fields either dissolved in the water or bound to eroded sediment (Smith, Huang, & Haney, 2017; Duncan et al, 2019). To address deleterious environmental effects from agriculture, the United States Department of Agriculture, Natural Resources Conservation Service (USDA-NRCS) promotes conservation practices that are designed to improve environmental outcomes and benefit soil health (NRCS, 2015).

Conservation management techniques include reduced/no tillage, planting cover crops, and managing fertilizer to minimize nutrient loss (NRCS, 2015; Dodd & Sharpley, 2016). Conservation tillage and cover crops have been shown to improve soil health by increasing soil organic matter, increase microbial biomass (MB) and diversity, reducing erosion and N loss (NRCS, 2015; Blanco-Canqui, 2018). For example, cover crop research has documented 50% increases in the microbial biomass pool (Simeone, Muller, Felgentreu, & Glaser, 2020) and 70-90% reductions of nitrate leaching (Hanrahan et al., 2018). The four R's of nutrient stewardship (right source, rate, time, and place) have been used to guide the development of best management practices (BMP) for specific regions and crops to reduce the amount of nutrient loss. Smith et al. (2017) documented a reduction in soluble and total P in runoff when subsurface applying liquid polyphosphate fertilizer in a no-till cropping system.

Cover cropping can increase P bioavailability in soil (Eichler-Lobermann, Köhne, Kowalski, & Schnug, 2008; Dodd & Sharpley, 2015). Cover crops can have high P uptake

efficiency and access fractions not available to cash crops by excreting P solubilizing compounds, changing soil pH and enhancing microbial activity that can mineralize organic P (P_O) (Schilling, Gransee, Deubel, Lezovic, & Ruppel, 1998; Kamh, Horst, Amer, Mostafa, & Maier, 1999; Horst, Manh, Jibrin, & Chude, 2001; Eichler-Lobermann et al., 2008). In addition to decomposing cover crop residue as a P source, Eichler-Lobermann et al. (2008) determined that P mobilization in soil from cover crop growth was the main mechanism of increased P availability. They found that the cover crop treatment increased the P content of the soil before the plant residue could decompose (Eichler-Lobermann et al., 2008).

Higher mineralization rates have been documented in soils where labile C is available, prompting microorganisms to excrete enzymes to meet nutrient demands (Oberson, Besson, Maire, & Sticher, 1996; Oehl, Frossard, Fliessbach, Dubois, & Oberson, 2004; Requejo & Eichler-Lobermann, 2014). Phosphatases, such as phosphomonoesterases (acid and alkaline phosphatase), and phosphodiesterase, play a significant role in the mineralization of P_O to orthophosphate (Tabatabai & Bremner, 1969; Browman & Tabatabai, 1978). Acid and alkaline phosphatase primarily hydrolyze phosphomonoesters by catalyzing the cleavage of phosphate bonds, while phosphodiesterase catalyzes reactions that degrade phosphodiesters such as nucleic acids and phospholipids. Olander and Vitousek (2000) demonstrated an inverse relationship between increased P fertilizer applications and phosphatase expression, suggesting that microorganisms would be less likely to excrete P cycling enzymes to hydrolyze P_O if the soil had high P availability, thus suppressing P_O mineralization. Although it is generally accepted that high nutrient availability can suppress enzyme activity, this assumption is not well understood in agricultural systems where nutrient availability and demand change rapidly. Tiecher, dos Santos,

and Calegari (2012) showed an increase in potential acid phosphatase activity under cover crops despite P fertilizer applications in a maize-soybean cropping system.

Although long term, global studies have shown the MB-C:N:P ratio to be well constrained at 60:7:1 in cropland (Cleveland & Liptzin, 2007; Xu, Thornton, & Post, 2013), some research suggests that MB stoichiometry is more plastic over the smaller scales, and can be modified by tillage, crops, and fertilizer (Liu et al., 2008; Crews & Brooks, 2014). The fluctuations in soil stoichiometry have been shown to influence microbial abundance, induce microbial community changes, influence enzyme activity, and MB-C:N:P (Zhao et al. 2018). He, Wu, O'Donnell, & Syers (1997) demonstrated a 200-500% enrichment of MB-P with applications of manure, while MB-C only increased 15-20%. Other research has demonstrated that combinations of P fertilizer applications, perennial crops, and reduced tillage can increase MBP (Liu et al., 2008; Teicher et al., 2012; Crews & Brookes, 2014). These results suggest that at some temporal scales, the combination of P inputs and increased organic matter (either from conservation management or manure) can enrich the P content of microbial cells and/or favor bacterial groups with higher affinity to immobilize.

The labile nutrients stored in the cells of microorganism can contribute to soil fertility but could also be lost in runoff (Liu et al., 2008; Dodd & Sharpley, 2015). The rapidly cycling, MB-P pool can make up 0.7-2.5% of total P in cropland and 2-7.5% of fertilized pasture (Oberson & Joner, 2005). Phosphorus in MB, the main component of the active P_O pool, is easily lysed after freeze-thaw and dry-wet cycles that are common in many agriculturally productive areas (Turner & Haygarth, 2001; Blackwell et al., 2010). Drought and rewetting can lyse up to 70% of microbial cells, which are concentrated in the soil surface, and most likely to interact with rainfall (Blackwell et al., 2010).

Increasing available P, either from mobilization, mineralization, or enrichment of MB- P, may have negative consequences if availability does not coincide with cash crop nutrient requirements (Eichler-Lobermann et al., 2008; Dodd & Sharpley, 2015). With the increased implementation of conservation management techniques and importance of minimizing agricultural pollution to water, it is critical to understand how changes in management (and their interaction) may be contributing to changes in biogeochemical cycling, and loss of P. The objective of this research was to document the impact of cover crops and two common P fertilizer management systems on a no-till corn-soybean cropping system in Kansas using biological parameters related to P cycling. We used the following hypotheses to guide our research: i) Phosphorus fertilizer applications with cover crops will increase MB-P, ii) No P fertilizer applications will decrease the MB-P and increase the MB-C:P, ii) Phosphorus fertilizer application will suppress phosphatase enzyme expression.

Materials and Methods

Site description

The experimental site is located at the Kansas Agricultural Watershed (KAW) Field Laboratory (39.134, -96.641), approximately 9 km southwest of Manhattan, Kansas. The site consists of upland agricultural fields along the Kansas River. The site has a hot, humid continental climate, with a mean annual temperature of 12.7^{o}C, and 904 mm of precipitation annually. It is terraced into 18 watershed units that are approximately 0.5 ha each. The site has a slope of 6-8% on primarily eroded Smolan silty clay loam. The site has been in a continuous no-till, corn-soybean rotation since its establishment in 2014.

This experiment is organized in a 2 by 3 factorial treatment design. The 18 watersheds were randomly assigned cover crop and fertilizer factors. The cover crop factor has two levels, cover crop (CC) or no cover crop (NC) and the fertilizer factor, has 3 types of phosphorus fertilizer management, no fertilizer (NP), spring injected (SI), and fall surface broadcast (FB). Cover crops have been planted annually since 2015 and have included: winter wheat before soybean in 2016, triticale and rapeseed before corn in 2017, and before soybean in 2018, and winter wheat and rapeseed before corn in 2019 (Table 3.1). The same amount of P was applied in both the spring injected and fall broadcast applications. The fall broadcast treatment was applied as diammonium phosphate (DAP) at 135 kg ha^{-1} (27 kg P ha^{-1}) and spring injected application was applied as ammonium polyphosphate at 131 L ha^{-1} (27 kg P ha^{-1}). Nitrogen fertilizer, 28% urea ammonium nitrate, was injected below the surface at a uniform rate of 146 kg N ha ^{1} for all plots in corn years.

Temperature and precipitation measurements were taken by the Ashland Bottoms Kansas Mesonet station (Kansas State University Research and Extension), which is less than 1000 m from the experimental site. Temperatures from 2018 to June 2020 were within normal range of the 30 year average for Riley county (Weather Data Library, Kansas Mesonet). Precipitation in 2018 was approximately 9% lower than the 30 year average but the period from January through July 2018 was 50% below average (Fig. 3.1). The precipitation in 2019 was approximately 16% above the 30 yr average (Fig.3.1). Mean soil temperatures at the 0-5 cm depth dipped below freezing in January 2018 and 2019 (Fig. 3.2). The mean monthly soil temperature during the soil sampling periods ranged from 3.6-28.7^{o}C (Table 3.1). Cover crops were planted immediately after cover crop planting but soil sampling during fall 2018 collection was delayed by three weeks due to weather and was notably cold and wet and (Table 3.1).

Methodology

Soil cores were collected at each sampling point at 0-5 cm depth. The samples were collected in spring, prior to cover crop termination and spring fertilizer application and in fall, immediately after harvest but prior to fall fertilizer applications. All soil samples were sieved moist using a 2 mm sieve. Soil samples were collected in spring and fall 2018 and 2019 (Table 3.1). Soil samples were analyzed for MB-P (fall 2018 – fall 2019), acid and alkaline phosphatase (spring 2018 - fall 2019), phosphodiesterase (spring 2018 - fall 2019), Mehlich-III extractable P (P_M), water extractable P (P_W) and total P (P_T) (spring 2018 - fall 2019).

Microbial Biomass P

Microbial biomass P was determined by the difference in P from fumigated and unfumigated soil samples. Two, 8 g (dry soil equivalent) fresh, sieved (2 mm sieve) soil subsamples were weighed into 250 ml Erlenmeyer flasks from each plot. One subsample was fumigated, and the other was incubated without fumigation. Fumigation procedures follow the chloroform ($CHCl_3$) fumigation method for MB-C (Brooks, Powlson, & Jenkinson, 1982; Vance, Brookes, & Jenkinson, 1987). After incubation, fumigated and unfumigated samples were extracted with 40 mL of 2 mM citric acid pH 5. Samples were then shaken for 30 min at 20^{o}C, centrifuged at 10,000 rpm for 10 min and filtered with Ahlstrom 74 filter paper into 50 mL Falcon tubes. The amount of molybdate reactive P (MRP) from each subsample was measured colorimetrically using the molybdate blue method (Murphy & Riley, 1962; O'Halloran & Cade-Menun, 2006). The value of the unfumigated sample was used as a measure of readily available orthophosphate while MB-P was calculated:

MB-P ($\mu g\ g^{-1}$ dry soil) = ($P_{fumigated}$ - $P_{unfumigated}$)

The MB-C to MB-P ratio (Xu et al., 2013) was calculated from the presented MB-P data, and Starr et al. (2018) MB-C data set, which were the same soil samples analyzed for MB-C. Microbial biomass C was estimated using chloroform ($CHCl_3$) fumigation method for MB-C (Vance, Brookes, & Jenkinson, 1987).

Enzyme activity

The procedure for assaying the acid and alkaline phosphatase, and phosphodiesterase was the same but used different buffers and substrates (Table 3.2; Tabatabai, 1994). In all enzyme assays, three 0.5 g subsamples of each moist soil sample were weighed into 20 ml scintillation vials labelled A, B, and C. To start the reaction, 2 mL of the start buffer was added to each vial and 0.5 mL of the substrate to vials A and B. Vials were incubated at 37ºC for an hour (Table 3.2). To stop the reaction, 0.5 mL of $CaCl_2$ and 2 mL of the stop buffer were added (Table 3.2). After the stop buffer, 0.5 mL of the substrate was added to the C vial. The mixture was filtered through 12.5 cm diameter cellulose filter paper w/2 μm pore size (Ahlstrom 642) for 30 min. Samples were analyzed with a spectrophotometer (U-1100, Hitachi High-Tech Corporation, Tokyo, Japan) at 400 nm to quantify p-nitrophenol (pNP). The enzyme activity was measured as mg pNP per kg soil per hour.

Mehlich-3 P

Mehlich-III P was analyzed at the Kansas State Soil Testing Lab. Briefly, 1 g crushed, air dried soil was extracted with the Mehlich-III extractant with a 1:10 soil weight to extractant

volume and shaken for 5 min. The MRP was determined colorimetrically using the molybdate reactive P method (Murphy & Riley, 1962).

Water Extractable Phosphorus

A two gram subsample of air dried soil was weighed into 50 mL centrifuge tubes. Twenty milliliters of deionized (DI) water were added to the centrifuge tubes. The tubes were then shaken at low speed for 1 hr and then centrifuged at 10,000 rpm for 10 min. After centrifugation, supernatant was filtered through a 0.45 μm nylon syringe filter (Environmental Express, Charleston, SC) and analyzed colorimetrically for MRP according Murphy and Riley molybdate blue method (1962).

Total P

Total P analysis was conducted at the Kansas State University Soils Lab. Briefly, P_T was determined by a salicylic sulfuric acid digestion, where 1 g of ground, air dried, soil was added to salicylic sulfuric and sodium thiosulfate and left overnight. The mixture was heated on a heating block at 200ºC for 1 hr and a further hour at 380ºC. The catalyst was added, and the samples were heated at 380ºC for an additional 3.5 hr. Samples were analyzed with analyzed by Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission (Varian Ltd, Mulgrave, Australia).

Statistical Analyses

Treatment effects were detected using a two-way analysis of variance (ANOVA), using SAS version 9.4 software (Cary, NC, U.S.A.) was used with a PROC GLIMMIX, repeated

measures of variance procedure. All data was normally distributed except for MB-C:P. Microbial biomass C:P data was transformed with a log transformation. General linear regressions were conducted using SAS code PROC REG. Season/years were analyzed separately and not compared statistically.

Results

Microbial biomass P

Microbial biomass P was not measured in spring 2018. We detected a main fertilizer P effect in fall 2018 (Table 3.3) where FB and SI had 220% more MB-P than the NP treatment ($p=0.009$). In spring 2019, a cover crop*P fertilizer interaction was detected ($p=0.017$) (Fig. 3.3a). The presence of a cover crop did not statistically change the amount of MB-P in each respective P fertilizer treatment (i.e., FB*NC=FB*CC, SI*NC=SI*CC, and NP*NC=NP*CC) but SI*CC was equivalent to FB*NC and FB*CC while SI*NC was significantly lower (Fig. 3.3a). All P fertilized treatments had higher MB-P than NP, with or without a cover crop (Fig 3.3a). A similar effect was detected in fall 2019, however SI*CC had 67% greater MB-P than SI*NC ($p=0.05$) (Fig. 3.3b). Over all seasons, MB-P was correlated to P_M ($p<0.001$) (Fig. 3.4), P_W ($r^2=0.55$) (Fig. 3.5), and P_T ($r^2=0.53$) ($p=0.01$) (Fig. 3.6).

Microbial biomass carbon to phosphorus ratio

The MB-C:P ratio was significant in all seasons for a P fertilizer management effect (Table 3.3) where FB and SI had a lower ratio than NP ($p<0.05$) (Fig. 3.7). In fall 2018, the MB-C:P was 47:1 for FB, 60:1 for SI, and 200:1 for NP. In spring 2019, FB and SI were 14 :1 and

21:1 respectively, and 245:1 in NP treatments. In fall 2019 FB and SI MB-C:P ratios were 61:1 and 78:1 respectively while NP was 1500:1.

Phosphatase enzyme activity

We did not detect an interaction or a P fertilizer main effect in acid phosphatase activity (Table 3.3). Acid phosphatase activity was higher in cover crop treatments compared to no cover crop in spring and fall 2018 and spring and fall 2019 (Table 3.4).

Alkaline phosphatase activity had a cover crop main effect (Table 3.3), where activity was higher in the cover crop treatments compared to no cover crop in spring 2018, fall 2018, and spring 2019 (Table 3.4). In fall 2018, there was a cover crop*P fertilizer management interaction where NP*CC had greater alkaline phosphatase activity than all other treatment combinations, SI*CC and FB*CC had higher activity than NP*NC and FB*NC, and SI*NC was not different than any treatment combination other than NP*CC ($p=0.004$) (Table 3.4). In addition to the interaction, fall 2018 had higher alkaline phosphatase activity in cover crop vs. no cover crop treatments ($p<0.0001$), and a fertilizer main effect where SI and NP were greater than FB ($p=0.03$) (Table 3.4). In spring 2019, there was higher alkaline phosphatase activity in cover crop compared to no cover crop treatments. There was no treatment effect detected in fall 2019.

We did not detect an interaction or a P fertilizer main effect in phosphodiesterase activity at any time (Table 3.3). Phosphodiesterase activity was higher in cover crop treatments in spring/fall 2018, and spring 2019 but not fall 2019 (Table 3.5).

Discussion

We determined that MB-P was most closely correlated with the Mehlich-III P status in the soil (Fig. 3.4) but was also correlated to water extractable P (Fig. 3.5), and total soil P (Fig. 3.6), highlighting the close relationship between the microbial community and soil P status. Microbial biomass P was higher in P fertilized plots in all seasons, reflecting the higher P status in the soil, and demonstrating a plasticity in microbial biomass stoichiometry over the given time scale. This result was consistent with Liu et al. (2008) that documented higher MB-P with P fertilizer applications of 40 kg P_2O_5 ha^{-1} in a maize-soybean cropping system. The increase of MB-P with P fertilizer applications can be attributed to either a larger general biomass or a change in relative abundance of fungi to bacteria. In general, bacteria have a lower C:P ratio than fungi. If P fertilizer applications favor bacteria over fungi (Liu, et al., 2008), it is possible that the MB-P could reflect the relative abundance. Additionally, microorganisms' MB-P is sensitive to moisture deficits due to a lack of P diffusion in the soil (Liu et al., 2008). It is likely that the MB-P results reflect differences of crop and weather, in conjunction with treatment effects.

An interesting P fertilizer * cover crop interaction was detected in spring 2019 where FB had more MB-P than SI treatments if no cover crop was present, however if a cover crop was added to the SI treatment, it had the same amount of MB-P compared to FB*NC and FB*CC. In spring 2019 SI*CC had 52% more MB-P than SI*NC (twelve months after SI fertilizer application). The effect was not only detected right after cover crop termination in spring 2019 but persisted after the harvest of a corn crop in fall 2019. In the fall sampling, the same pattern was detected but the difference was significant between SI*CC and SI*NC and had increased to 67% despite a P fertilizer application post cover crop termination and no growing cover crop for

6 months. We did not detect a difference between CC or NC within the FB or NP fertilizer management in any sampling.

In concurrent work at the experimental site, we detected patterns in soil P availability (Chapter 2), that were similar to patterns in MB-P where cover crop increased the P concentration in surface soil for SI treatment but had no effect on P concentration in surface soil for the FB treatment. Varela et al. (2017) found that a decaying cover crop released 2 to 16 kg P ha^{-1} during the growth of the cash crop, of which 53-100% was released as inorganic P. The increase in MB-P in SI*CC treatments could be due to differences in available P from decaying cover crop residues (Liu et al., 2014), the increased P favoring bacteria over fungi (Liu et al., 2008), increased mineralization of organic P (Olander & Vitousek, 2000; Teicher et al., 2012; Spohn & Kuzyakov, 2013), mineral P mobilization and/or translocation by the cover crop from a greater volume of soil (Eichler-Lobermann, et al., 2008), or likely a combination of the above. These results may suggest that the spring subsurface placement of P is interacting with the cover crop to enrich both plant available P (Chapter 2) and the MB-P in the top 5 cm of soil. It is possible that we did not detect this interaction in FB treatments due to the high concentration of available P that was applied to the surface. Due to the poor mobility of P in soil, the MB-P is likely reflecting the P availability within their immediate vicinity (Liu et al., 2008). The cover crop in the case of FB may not change the concentration of P at the surface because it is applied to the surface, which is taken up by the cover crop and redeposited at the surface during decomposition. In NP treatments, the cover crop may not have access to enough P to make a significant difference. It will be critical to further investigate and confirm this interaction between cover crop and P fertilizer management.

The MB-C:P ratio compares the amount of P held in microbial cells compared to the size of the microbial pool (Xu et al., 2013). This is important because it allows us to separate an increase in MB-P due to a larger microbial pool, from an increase due to a shift in microbial cell stoichiometry and/or shift in microbial community. A global average of C:P in soil microbial biomass is approximately 60:1 (Cleveland & Liptzin, 2007). Xu et al. (2013) further categorized global biomes, calculating a C:P in cropland of 60:1, and 169:1 in pasture. In grassland, Griffiths, Spilles, & Bonkowski (2012) documented a constrained MB-C:P at optimal soil nutrient levels, but an over saturation of MB-P at a P fertilization rate of 30 kg ha^{-1} $year^{-1}$. Our experimental results showed a consistent P fertilizer treatment effect in the given seasons, where MB-C:P was significantly lower in the FB (from 14-61) and SI (from 21-78) compared to NP (from 200-1500). The first MB-C:P measurement in fall 2018 was almost 200:1 in NP plots, growing to over 1500 in fall 2019. This most likely reflects an absence of P additions and the P removal by cash crops since 2014. The repeated cropping without P fertilizer additions showed a high (and increasing) MB-C:P suggesting that conditions were likely to favor microbial immobilization of any available P (Griffiths et al., 2012; Damon, Bowden, Rose, & Rengel, 2014).

Both P fertilizer treatment MB-C:P amounts were not different from each other despite the difference in timing, placement, and source of P. Interestingly, in spring 2019, FB and SI MB-C:P ratios were 14:1 and 21: respectively, well below the expected 60:1. These ratios are lower than expected despite six months since the last FB, and twelve months since the last SI fertilizer applications. Similarly, Griffiths et al. (2012) reported a MB-C:P of 29:1 in treatments that had 30 kg P ha^{-1} $year^{-1}$ in a grassland, P fertilizer study. The lower MB-C:P ratios in spring suggest that the microbial community would be mineralizing P and potentially increasing its

availability for plant uptake and/or loss as dissolved reactive P in runoff. Unfortunately, in Kansas, this documented period of low MB-C:P coincides with a seasonal period of high precipitation (Fig.3.2). In fall 2019, FB and SI MB-C:P ratios returned to 61 and 78, closer to the 47-61 range of fall 2018, and closer to the expected MB-C:P 60:1 ratio (Cleveland & Liptzin, 2007; Xu et a., 2013). Microbial diversity is sensitive to soil moisture and plant type (Liu et al., 2008). A different crop and/or weather could significantly change the community of microorganisms thus changing their C:P composition from inherent physiological differences. Multiple spring measures and replicated crop/cover crop combinations will be necessary to predict future MB-P, or MB-C:P trends.

All measured phosphatase enzymes showed greater potential activity with cover crop treatments in spring/fall 2018, and spring 2019. Contrary to the findings from a fertilized natural system by Olander & Vitousek (2000), the P fertilizer treatments in our study did not suppress P cycling enzyme activity. Ai et al. (2012) documented a reduction in enzyme expression in the rhizosphere but an increase in the bulk soil with inorganic fertilizer applications in a winter wheat-summer maize rotation, suggesting that the expression of phosphatase enzymes is more nuanced than a strict response to P availability. In general, cover crops increased potential phosphatase enzyme activity which was consistent with the findings of Tiecher et al. (2012) and Hai-Ming et al. (2014). Cover crops are known for increasing soil organic C and MB-C (Nevins et al., 2018; Kim, et al., 2020). It is likely that an increase in soil active C (Starr et al., 2019) from cover crops increased the demand for P, increasing the activity of phosphatase enzymes. Phosphodiesterase is expressed in order to hydrolyzed phosphodiesters, such as those in DNA and RNA (Browman & Tabatabai, 1978). The increase in phosphodiesterase we detected in cover crop treatments could be related to increased DNA and RNA from higher microbial and

cover crop biomass. In fall 2019, acid phosphatase was the only enzyme that demonstrated a cover crop response. Acid phosphatase is excreted by plants and microorganisms, while alkaline phosphatase is considered a product of only microorganisms (Luo, Meng, & Gu, 2017). As cover crops would be excreting acid phosphatase, and non-cover crop plots would not have that activity, may explain the treatment effect. The increase in potential phosphatase enzyme activity with cover crops suggests that the mineralization of organic P compounds could be enhanced under the treatment (Ai et al., 2012; Hallama et al., 2019), possible contributing to the differences we have detected in the SI*CC treatment combinations.

Fall 2018, alkaline phosphatase activity was the only instance of a cover crop*P fertilizer interaction. The NP*CC treatment had significantly greater activity than all other treatments. Alkaline phosphatase is excreted by microorganisms; therefore, the high activity may be the microbial community's effort to mobilize P in a low P environment. The cover crop influence in the NP*CC treatment is highlighted by at 73% increase of alkaline phosphate activity when compared to NP*NC. The results would be similar to Olander & Vitousek (2000), where phosphatase enzymes activity was enhanced in low P environments, especially where C and N availability was good. The fall 2018 sampling was proceeded by a soybean crop but sampling was delayed by 19 days after crop harvest due to adverse weather, with freezing temperatures at night. It is possible that the alkaline phosphatase activity was the result of higher microbial activity due to the breakdown of the high C:N soybean residues.

Conclusions

We detected P fertilizer main effects in MB-P, and MB-C:P where P fertilizer additions increased MB-P and decreased MB-C:P compared to no P fertilizer over the fall 2018-2019

period. An interaction of cover crops and P fertilizer applications was demonstrated in the MB-P, where an interaction was detected in spring and fall 2019. In both seasons, SI*NC was less than FB*NC and FB*CC but the addition of a cover crop to the SI treatment (SI*CC) made it equal to the FB*NC and FB*CC treatments. The concentration of MB-P in the spring injected ammonium polyphosphate treatment was increased by 52 and 67% in the presence of a cover crop compared to no cover crop over spring and fall 2019, despite a six month period without a cover crop growing at the time of fall 2019 sampling. We did not detect the same effect in the fall broadcast DAP or the no P fertilizer treatments. Microbial biomass P was significantly correlated with P_M (r^2=0.72), P_W (r^2=0.55), and P_T (r^2=0.53) aggregated over all seasons. Cover crops consistently increased the potential phosphatase enzyme activity, suggesting that cover cropping has the potential to enhance organic P hydrolysis. The wide MBC:P ratio in NP treatments suggests that any hydrolyzed organic P might be immobilized, while it would likely be mineralized in FB and SI treatments. We did not detect a suppression of phosphatase enzymes from P fertilizer treatments.

These results suggest that P fertilizer management can interact with cover crops in our no-till corn-soybean cropping systems which may have implications when considering water quality goals, as microbial cells are sensitive to lysis and loss wet/dry periods. It is important that these interactions are further investigated over longer time periods, with other crops and locations to confirm the outcome and predict the interaction between cover cropping and P fertilizer management.

References

Ai, C., Liang, G., Sun, J., Wang, X., & Zhou, W. 2012. Responses of extracellular enzyme activities and microbial community in both the rhizosphere and bulk soil to long-term fertilization practices in a fluvo-aquic soil. *Geoderma*, 173-174, 330-338.

Blackwell, M. S. A., Brookes, P. C., de le Fuente-Martinez, N., Gordon, H., Murray, P. J., Snares, K. E., Williams, J. K., Bol, R., & Haygarth, P. M. 2010. Phosphorus solubilization and potential transfer to surface waters from the soil and microbial biomass following drying-rewetting and freeze-thawing. *Advanced Agronomy*, 106, 1-35.

Blanco-Canqui, H. 2018. Cover crops and water quality. *Agronomy Journal*, 110(5), 1633-1647.

Brookes, P. C., Powlson, D. S., & Jenkinson, D.S. 1982. Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry*, 14, 319-329.

Browman, M. G. & Tabatabai, M. A. 1978. Phosphodiesterase activity in soils. *Soil Science Society American Journal,* 42, 284-290.

Cleveland, C. C., & Liptzin, D. 2007. C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry*, 85, 235-252.

Crews, T. E., & Brookes, P. C. 2014. Changes in soil phosphorus forms through time in perennial versus annual agroecosystems. *Agriculture, Ecosystems and Environment*, 184, 168-181.

Damon, P. M., Bowden, B., Rose, T., & Rengel, Z. 2014. Crop residue contributions to phosphorus pools in agricultural soils: A review. *Soil Biology & Biochemistry*, 74, 127-137.

Dodd, R. J. & Sharpley, A. N. 2015. Recognizing the role of soil organic phosphorus in soil fertility and water quality. *Resources, Conservation and Recycling*, 105, 282-293.

Dodd, R. J. & Sharpley, A. N. 2016. Conservation practice effectiveness and adoption: unintended consequences and implications for sustainable phosphorus management. *Nutrient Cycling in Agroecosystems*, 104, 373-392.

Dodds, W. K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J., Schloesser, J. T., & Thornbrugh, D. J. 2009. Eutrophication of U.S. Freshwaters: Analysis of potential economic damages. *Environment Science & Technology*, 43(1), 12-19.

Duncan, E. W., Osmond, D. L., Shrober, A. L., Starr, L., Tomlinson, P., Kovar, J. L., Moorman, T. B., Peterson, H. M., Fiorellino, N. M., & Reid, K. 2019. Phosphorus and soil health management practices. *Agricultural & Environmental Letters*, 4, 190014.

Eichler-Löbermann, B., Köhne, S., Kowalski, B., & Schnug, E. 2008. Effect of catch cropping on phosphorus bioavailability in comparison to organic and inorganic fertilization. *Journal of Plant Nutrition*, 31, 659-676.

Griffiths, B. S., Spilles, A., & Bonkowski, M. 2012. C:N:P stoichiometry and nutrient limitation of the soil microbial biomass in a grazed grassland site under experimental P limitation or excess. *Ecological Processess*, 1(6).

Hai-Ming, T., Xiao-Ping, X., Wen-Guang, T., Ye-Chun, L., Ke, W., & Guang-Li, Y. 2014. Effects of winter cover crops residue returning returning on soil enzyme activities and soil microbial community in double-cropping rice fields. *PLoS ONE*, 9(6), e100443.

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

Hanrahan, B. R., Tanka, J. L., Christophera, S. F., Mahla, U. H., Trentmana, M. T., & Royer, T. V. 2018. Winter cover crops reduce nitrate loss in an agricultural watershed in the central U.S. Agriculture. *Ecosystems and Environment*, 265, 513-523.

He, Z. L., Wu, J., O'Donnell, A. G., & Syers, J. K. 1997. Seasonal responses in microbial biomass carbon, phosphorus and sulphur in soil under pasture. *Biology & Fertility of Soil*, 24, 421-428.

Horst, W. J., Manh, M., Jibrin, J. M., & Chude, V. O. 2001 Agronomic measures for increasing P availability to crops. *Plant and Soil*, 237, 211-223.

Kamh, M., Horst, W. J., Amer, F., Mostafa, H., & Maier, P. 1999. Mobilization of soil and fertilizer phosphate by cover crops. *Plant and Soil*, 211, 19-27.

Kim, N., Zabaloy, M. C., Guan, K., Villamil, M. B. 2020. Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biology & Biochemistry*, 142, 107701.

Liu, A., Hamel, C., Spedding, T., Zhang, T. Q., Mongeau, R., Lamarre, G. R., & Tremblay, G. 2008. Soil microbial carbon and phosphorus as influenced by phosphorus fertilization and tillage in a maize-soybean rotation in south-western Quebec. *Canadian Journal of Soil Science*, 88, 21-30.

Luo, L., Meng, H., & Gu, J-D. 2017. Microbial extracellular enzymes in biogeochemical cycling of ecosystems. *Journal of Environmental Management*, 197, 539-549.

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

Natural Resources Conservation Service. 2015. *Soil Health Literature Summary – Effects of Conservation Practices on Soil Properties in Areas of Cropland.* National Resource

Conservation Service, National Soil Survey Center. United States Department of Agriculture.

Nevins, C. J., Lacey, C., & Armstrong, S. 2018. The synchrony of cover crop decomposition, enzyme activity, and nitrogen availability in a corn agroecosystem in the Midwest United States. *Soil & Tillage Research*, 197, 104518.

Oberson, A., Besson, J. M., Maire, N., & Sticher, H. 1996. Microbial processes in soil organic phosphorus transformations in conventional and biological cropping systems. *Biology and Fertility of Soils*, 21, 3.

Oberson, A., & Joner, E. J. 2005. Microbial turnover of phosphorus in soil. In Turner, B.J., Frossard, E., & Baldwin, D.S. (Eds.), *Organic Phosphorus in the Environment* (pp. 133-164). CAB International, Wallingford, U.K.

Oehl, F., Frossard, E., Fliessbach, A., Dubois, D., & Oberson, A. 2004. Basal organic phosphorus mineralization in soils under different farming systems. *Soil Biology & Biochemistry*, 36, 667-675.

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.

Olander, L. P. & Vitousek, P. M. 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry*, 49(2), 175-190.

Requejo, M. I. & Eichler-Löbermann, B. 2014. Organic and inorganic phosphorus forms in soil as affected by long-term application of organic amendments. *Nutrient Cycling in Agroecosystems*, 100, 245-255.

Schilling, G., Gransee, A., Deubel, G., Lezovic, S., & Ruppel, S. 1998. Phosphorus availability, root exudates, and microbial activity in the rhizosphere. *Z. Pflanzenern ahr. Bodenkd*, 161, 465-478.

Simeone, G. D. R., Muller, M., Felgentreu, C., & Glaser, B. 2020. Soil microbial biomass and community composition as affected by cover crop diversity in a short-term field experiment on a podzolized Stagnosol Cambisol. *Journal of Plant Nutrition and Soil Science*, 183, 539-549.

Smith, D. R., Huang, C., and Haney, R. L. 2017. Phosphorus fertilization, soil stratification, and potential water quality impacts. *Journal of Soil and Water Conservation*, 72(5), 417-424.

Spohn, M., & Kuzyakov, Y. 2013. Phosphorus mineralization can be driven by microbial need for carbon. *Soil Biology & Biochemistry*, 61, 69-75.

Starr, L. M., Tomlinson, P. J., Nelson, N. O., Stewart, C. L., Roozeboom, K. L., Kluitenberg, G. J., and Presley, D. R. 2019. Effects of cover crops and phosphorus fertilizer management on soil health parameters in a no-till corn-soybean cropping system in Riley County, Kansas. *Kansas Agricultural Experiment Station Research Reports*, 5(6).

Tabatabai, M. A. 1994 Soil enzymes. In R.W. Weaver, J.S. Angle, and P.S. Bottomley (Eds.) *Methods of soil analysis. Part 2. Microbiological and Biochemical Properties* (pp. 801-834). Soil Science Society of America, Madison, WI.

Tabatabai, M. A. & Bremner, J. M. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology & Biochemistry*, 1, 301-307.

Tiecher, T., dos Santos, D. R., & Calegari, A. 2012. Soil organic phosphorus forms under different soil management systems and winter crops, in a long term experiment. *Soil & Tillage Research*, 124, 57-67.

Turner, B. L., & Haygarth, P. M. 2001. Biogeochemistry: phosphorus solubilization in rewetted soils. *Nature*, 411, 258.

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry*, 19(6), 703-707.

Varela, M. F., Barraco, M., Gili, A., Taboada, M. A., & Rubio, G. 2017. Biomass decomposition and phosphorus release from residues of cover crops under no-tillage. *Agronomy Journal*, 109(1), 317-326.

Xu, X., Thornton, P. E., & Post, W. M. 2013. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Global Ecology and Biogeography*, 22, 737-749.

Zhao, F. Z., Ren, C. J., Han, X. H., Yang, G. H., Wang, J., & Doughty, R. 2018. Changes of soil microbial and enzyme activities are linked to soil C, N and P stoichiometry in afforested ecosystems. *Forest Ecology and Management*, 427, 289-295.

Figures

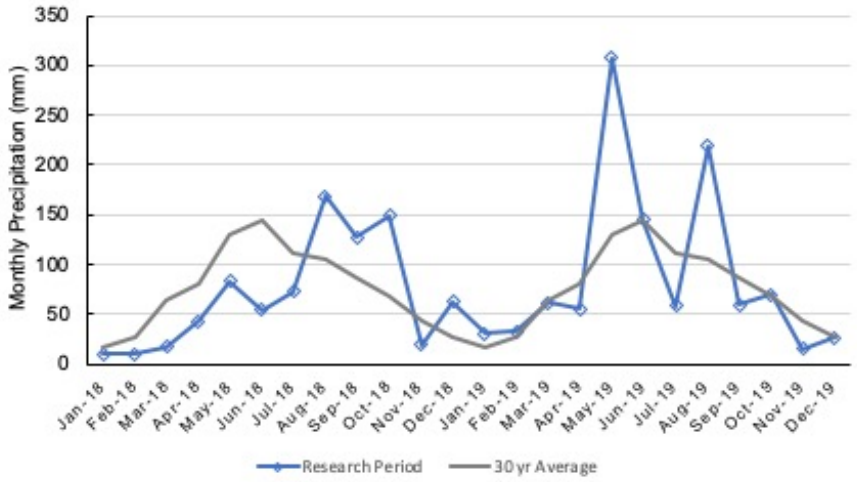


Figure 3.1 Monthly precipitation (mm) from 01/2018 – 12/2019 at the Ashland Bottoms Mesonet Station (Kansas State University) located <1000 m from the research site, relative to the 30-yr (1980 – 2010) averages for the county (source: Kansas State Weather Data Library).

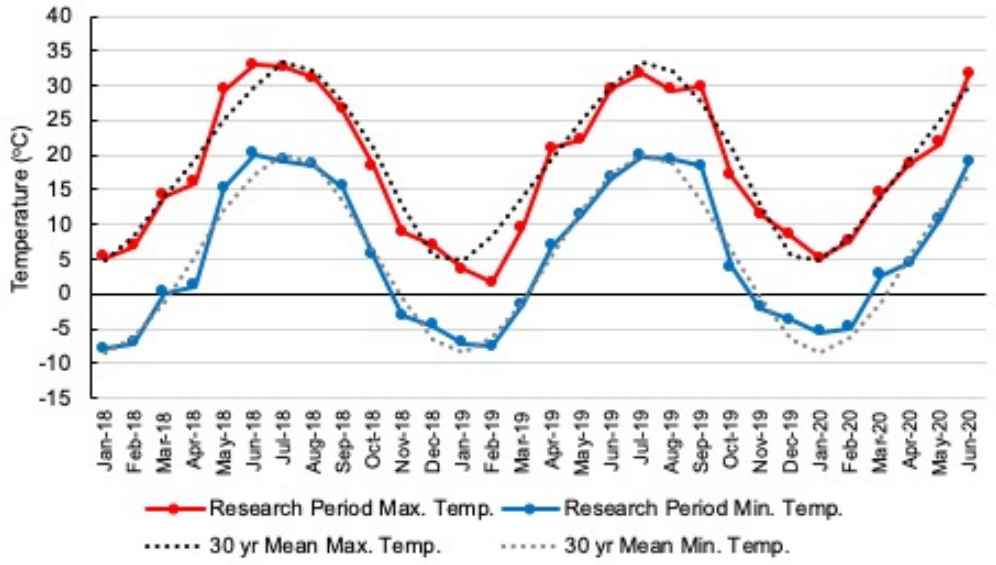


Figure 3.2 Maximum and minimum soil temperature in top 5 cm from 01/2018 – 06/2020 at the Ashland Bottoms Mesonet Station (Kansas State University) located <1000 m from the research site.

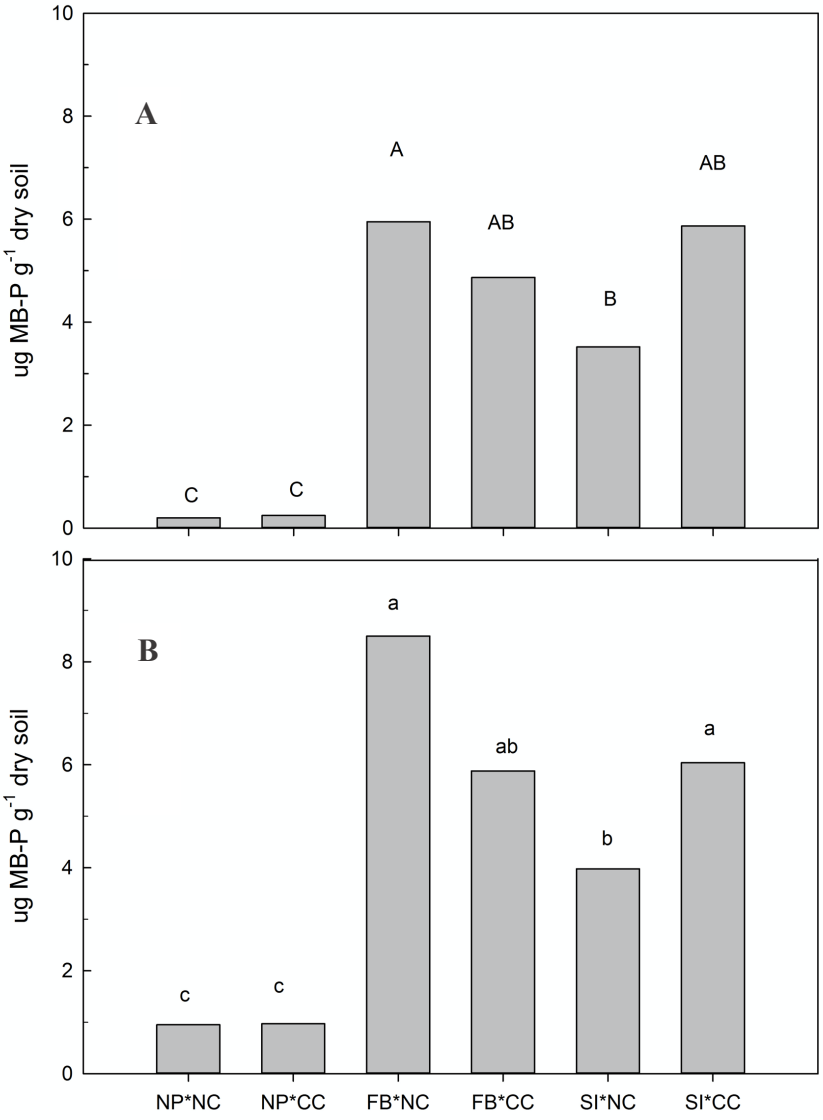


Figure 3.3 a. Spring 2019 b. Fall 2019 cover crop * P fertilizer management interaction effect on microbial biomass P concentrations (p=0.0017). Figure abbreviations: fall broadcast diammonium phosphate (FB), spring injected ammonium polyphosphate (SI), no P fertilizer (NP), cover crop (CC), and no cover crop (NC). Letters indicate significant differences ($p<0.05$).

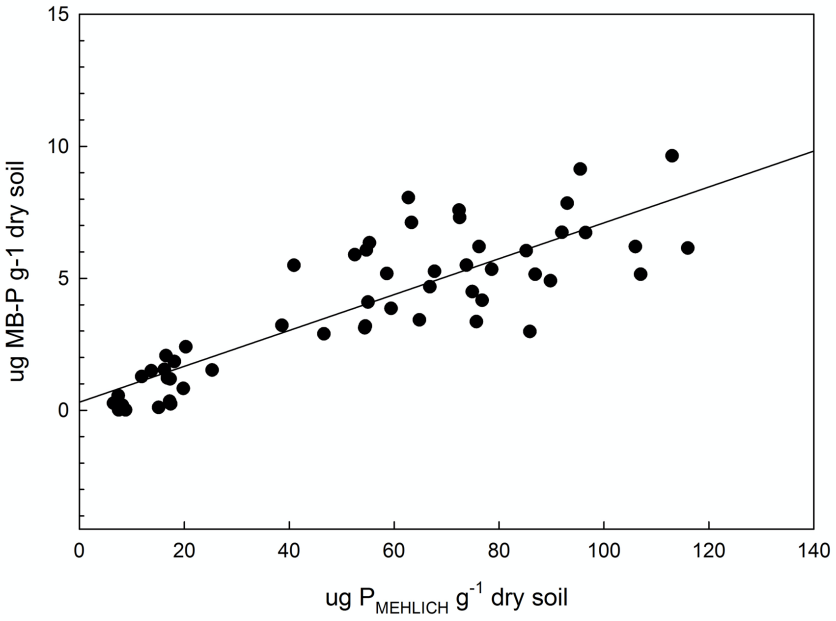


Figure 3.4 Microbial biomass P was correlated to Mehlich-III extractable P over fall 2018, and spring/fall 2019 (r^2=0.72) ($p<0.001$).

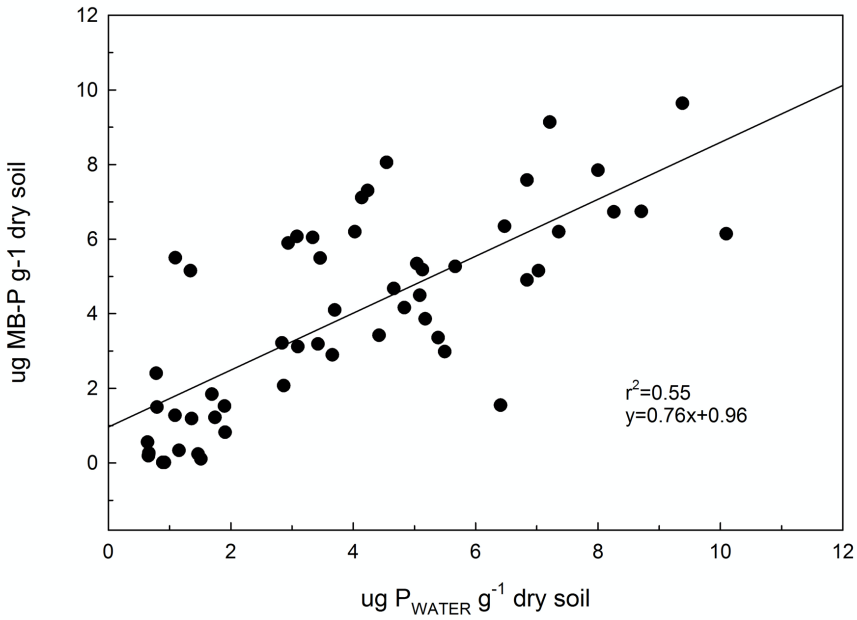


Figure 3.5 Microbial biomass P was correlated to water extractable P over fall 2018, and spring/fall 2019 (r^2=0.55) ($p<0.001$).

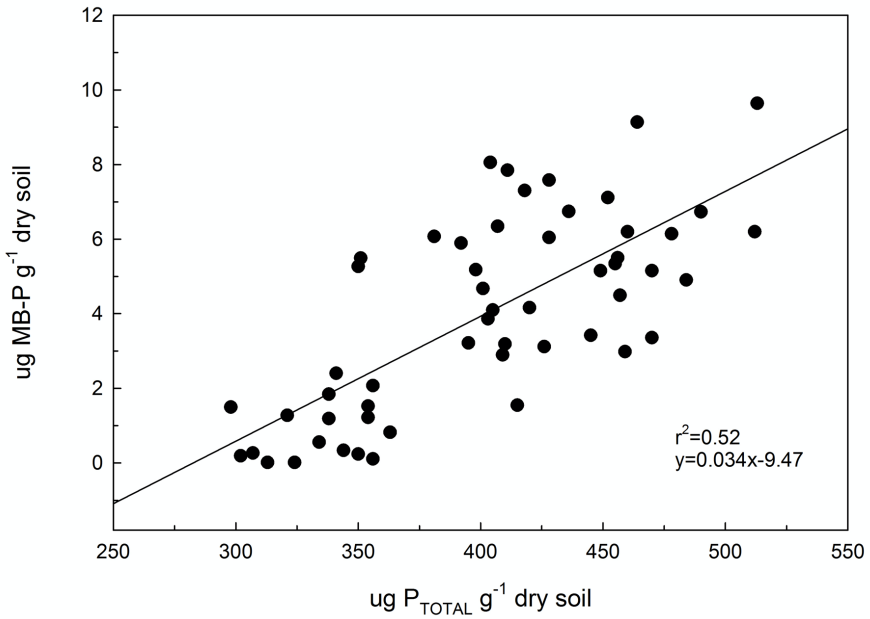


Figure 3.6 Microbial biomass P was correlated to soil total P over fall 2018, and spring/fall 2019 (r^2=0.53) (p=0.01).

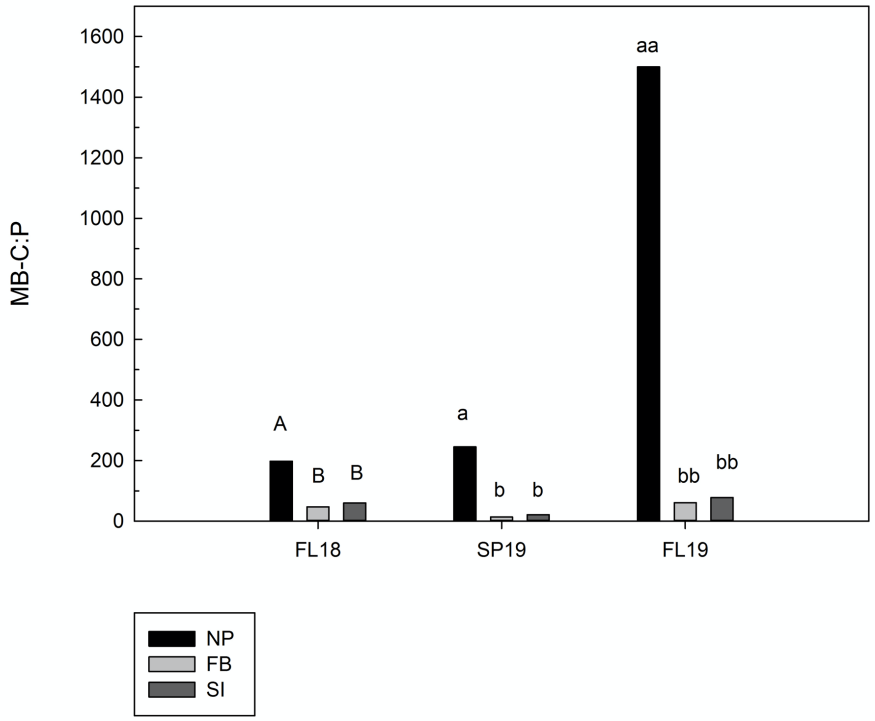


Figure 3.7 Microbial biomass C:P in fall 2018, and spring/fall 2019, at the 0-5 cm depth. In every case, FB and SI were greater than NP ($p<0.05$) (seasons not compared to each other). Figure abbreviations: fall broadcast diammonium phosphate (FB), spring injected ammonium polyphosphate (SI), no P fertilizer (NP). Letters indicate significant differences ($p<0.05$).

Tables

Table 3.1 Summary of cropping system, sampling dates, mean monthly 0-5 cm soil temperature and gravimetric soil moisture at sampling.

	Cropping Year 2017	**Cropping Year 2018**		**Cropping Year 2019**
Crop or Cover Crop	Triticale and Rapeseed	Soybeans	Winter Wheat and Rapeseed	Corn
Soil Sampling	Spring 2018 (05/07/18)	Fall 2018 (11/19/18)	Spring 2019 (04/22/19)	Fall 2019 (10/15/19)
Mean 0-5 cm Soil Max. and Min. Temp during sampling (°C)	28.7/19.3	6.6/3.6	18.1/10.6	15.1/11.9
Mean Gravimetric Soil Moisture (g water g^{-1} dry soil)	0.17	0.24	0.16	0.19

Table 3.2 Summary of reagents used for enzyme activity assays.

Enzyme	**Substrate**	**Start Buffer**	**Stop Buffer**
Alkaline phosphatase	0.05 M ρ-nitrophenyl phosphate	MUB pH 11	0.5 M NaOH
Acid phosphatase	0.05 M ρ-ntrophenyl phosphate	MUB pH 6.5	0.5 M NaOH
Phosphodiesterase	0.05 M bis-ρ-nitrophenyl phosphate	0.05 M THAM pH 8.0	0.1 M THAM pH 12

Table 3.3. ANOVA table showing *p-values* for soil microbial biomass P, microbial biomass C:P, acid phosphatase activity, alkaline phosphatase activity, phosphodiesterase activity. Microbial biomass P and C:P was not measured in spring 2018. Seasons are not compared to one another.

Effect	Spring 2018	Fall 2018	Spring 2019	Fall 2019
		----------Microbial Biomass Phosphorus----------		
P fertilizer management	n/a	0.001	<0.0001	0.001
Cover crop	n/a	0.19	0.74	0.40
P fertilizer * Cover crop	n/a	0.55	0.02	0.046
		---------- Microbial Biomass Carbon:Phosphorus----------		
P fertilizer management	n/a	0.01	0.0003	0.0002
Cover crop	n/a	0.83	0.72	0.88
P fertilizer * Cover crop	n/a	0.90	0.57	0.86
		----------Acid Phosphatase----------		
P fertilizer management	0.45	0.12	0.31	0.29
Cover crop	0.0001	0.002	0.002	0.03
P fertilizer * Cover crop	0.11	0.23	0.79	0.48
		----------Alkaline Phosphatase----------		
P fertilizer management	0.59	0.03	0.23	0.34
Cover crop	0.008	<0.0001	0.009	0.54
P fertilizer * Cover crop	0.96	0.004	0.79	0.29
		----------Phosphodiesterase----------		
P fertilizer management	0.96	0.10	0.40	0.14
Cover crop	0.001	0.008	0.002	0.45
P fertilizer * Cover crop	0.45	0.12	0.23	0.40

Table 3.4 Acid and alkaline phosphatase enzyme activity from spring 2018 – fall 2019 in the 0-5 cm depth of soil. Seasons were not compared. Letters indicate significant differences ($p<0.05$). Table abbreviations: fall broadcast diammonium phosphate (FB), spring injected ammonium polyphosphate (SI), and no P fertilizer applied (NP) and cover crop treatments - cover crops (CC) and no cover crop (NC).

	Acid Phosphatase				**Alkaline Phosphatase**			
	SP18	**FL18**	**SP19**	**FL19**	**SP18**	**FL18**	**SP19**	**FL19**
NP*NC	103.22	27.39	187.55	188.15	20.83	33.11 C	46.87	62.80
NP*CC	120.4	34.97	217.71	195.01	33.60	57.26 A	58.86	74.79
FB*NC	97.51	26.25	198.91	184.01	15.44	32.22 C	34.34	54.00
FB*CC	141.04	33.34	240.59	215.86	29.48	43.19 B	52.58	62.52
SI*NC	101.07	32.31	194.38	196.02	17.95	42 BC	44.57	70.19
SI*CC	127.61	34.65	239.13	221.93	29.32	45.06 B	56.34	60.47
*SE fert*cover*	*8.75*	*1.74*	*24.5*	*10.41*	*4.71*	*2.37*	*6.51*	*6.92*
p-value	*0.12*	*0.25*	*0.79*	*0.48*	*0.96*	*0.004*	*0.79*	*0.29*
NP	111.81	31.18	202.63	191.58	27.22	45.19 A	52.86	68.80
FB	119.27	29.80	219.75	199.94	22.46	37.71 B	43.46	58.26
SI	114.34	33.48	216.75	208.97	23.63	43.53 A	50.46	65.33
SE fertilizer	*7.75*	*1.31*	*23.19*	*7.36*	*3.34*	*1.68*	*5.32*	*4.89*
p-value	*0.45*	*0.12*	*0.31*	*0.29*	*0.59*	*0.03*	*0.23*	*0.34*
NC	100.6 B	28.65 B	193.61 B	189.39 B	18.07 B	35.78 B	41.92 B	62.33
CC	129.69 A	34.32 A	232.48 A	210.93 A	30.80 A	48.50 A	55.93 A	65.93
SE cover	*7.39*	*1.13*	*22.74*	*6.01*	*2.72*	*1.37*	*4.86*	*4*
p-value	*0.0001*	*0.002*	*0.002*	*0.03*	*0.01*	*<0.0001*	*0.01*	*0.54*

Table 3.5 Phosphodiesterase enzyme activity from spring 2018 – fall 2019 in the 0-5 cm depth of soil. Seasons were not compared. Letters indicate significant differences ($p<0.05$). Table abbreviations: fall broadcast diammonium phosphate (FB), spring injected ammonium polyphosphate (SI), and no P fertilizer applied (NP) and cover crop treatments - cover crops (CC) and no cover crop (NC).

	Phosphodiesterase			
	SP18	**FL18**	**SP19**	**FL19**
NP*NC	19.73	30.51	46.62	57.34
NP*CC	36.90	50.43	69.56	73.84
FB*NC	20.44	26.46	41.31	45.59
FB*CC	34.79	35.91	61.45	51.38
SI*NC	22.80	35.81	51.70	64.87
SI*CC	32.58	37.87	58.28	58.22
FB*NC	20.44	26.46	41.31	45.59
FB*CC	34.79	35.91	61.45	51.38
*SE fert*cover*	*2.82*	*3.96*	*5.05*	*8.16*
p-value	*0.45*	*0.12*	*0.23*	*0.40*
NP	28.31	40.47	58.09	65.59
FB	27.62	31.19	51.38	48.49
SI	27.69	36.84	54.99	61.55
SE fertilizer	*1.99*	*2.85*	*3.78*	*5.77*
p-value	*0.96*	*0.10*	*0.40*	*0.14*
NC	20.99 B	30.93 B	46.55 B	55.94
CC	34.76 A	41.40 A	63.09 A	61.15
SE cover	*1.63*	*2.37*	*3.24*	*4.71*
p-value	*0.0001*	*0.01*	*0.002*	*0.45*

Chapter 4 - Enzyme Hydrolysis

Abstract

Labile forms of organic P (P_o) can be an important source of P for crop nutrition but may also be lost in runoff. Organic P in runoff can be mineralized during transport thus making it a potential source of nutrient pollution. Despite its impact on crop nutrition and receiving waters, labile Po is rarely considered or measured in traditional soil test P assays. Enzyme hydrolysable Po is a way to measure the labile fraction of Po by adding different P cycling enzymes to soil extracts and measuring the change in molybdate reactive P. Darch et al. (2016) suggested using phytase as a simple method to measure the whole enzyme labile P pool, as phytase has a low substrate specificity and targets all ester-P bonds. The method proposed adding phytase to a 2 mM citric acid soil extraction to mimic organic acids exuded in the rhizosphere as a P acquisition strategy. The objective of this work was to adapt the phytase hydrolysable P method to measure the labile pool of Po at an experimental no-till, corn-soybean cropping system in NE Kansas. The experimental design was a randomized complete block designed with a 2*3 factorial treatment structure: two cover crop treatments [fall-sown cover crop (CC)/no cover crop (NC)] and three P fertilizer management treatments [27 kg P ha^{-1} fall broadcast (FB) diammonium phosphate, 27 kg P ha^{-1} spring injected (SI) ammonium polyphosphate, or no P fertilizer (NP)]. Samples were collected at 0-5 cm in fall of 2018, and spring/fall 2019. Samples were extracted with 2 mM citric acid buffer (pH 5.0) and analyzed for phytase hydrolysable P ($P_{PHYTASE}$). A regression was conducted with the $P_{PHYTASE}$ resultsP_O, and Mehlich-III extractable P (P_M). Phytase hydrolysable P was higher in FB and SI compared to NP treatments in fall 2018 ($p=0.003$) but not different in

spring/fall 2019. The $P_{PHYTASE}$ results were significantly correlated to P_M in fall 2018 (r^2=0.34) and P_O in spring (r^2=0.3) but was not correlated to either measure in fall 2019.

Introduction

Organic P (P_o) has been shown to provide similar amounts of P to crops as inorganic P in fertilized fields (Sharpley 1985). Organic P makes up 30-65% of the total P (P_T), and 5-52% of the P_T are forms of P_o that are labile to moderately labile (Condron, Turner, & Cade-Menun, 2005). DeLuca et al. (2015) determined that over a large variety of soils in the United Kingdom, a decrease in $CaCl_2$ extractable, citrate extractable, and mineral occluded P was linked to a corresponding increase in phosphatase extractable P, suggesting that as inorganic P was diminished, organic P was available for mineralization. Studies have shown that dissolved and particulate organic P are a large portion of leachate, especially where soil organic matter has increased (Dodd & Sharpley, 2015). In fact, P_o (other than phytate P) is more mobile than orthophosphate, once it is in solution (Leytem, Mikkelsen, & Gilliam, 2002; Dodd & Sharpley, 2015). Toor, Condron, Di, Cameron, & Cade-Menun (2003) demonstrated that P_o accounted for more than 80% of the P in leachate regardless whether the pasture was fertilized with manure or mineral P fertilizer. In addition to the loss of P_o in the study, they found that P_o was available for hydrolysis by enzymes in water during transport (Toor et al 2003).

Recently there has been a call to re-evaluate the role of P_o, both as a supply of P and a contributing factor to P water pollution (Dodd & Sharpley, 2015). There is limited knowledge regarding the effect of cover crops and tillage on P_o accumulation and resulting P_o pool fluctuations. Despite the large percentage of P that is P_o in cultivated soil and runoff, the characterization and effect of this constituent is largely overlooked (Turner, McKelvie, & Haygarth, 2002; Dodd & Sharpley, 2015). The relative bioavailability of P_o is an important factor when accounting for its contribution to crop nutrition or pollution risk. Organic P can be categorized by its availability for enzyme hydrolysis, the mechanism by which microorganisms

cycle it through the soil system (Turner et al. 2002; Annaheim et al., 2013). As conservation management improves soil health parameters, these parameters are simultaneously increasing microbial activity and the potential for mineralization while increasing or maintaining organic matter inputs, thus potentially increasing the potential for P_o loss. Organic factions of P have a variety of chemical forms that dictate how available or resistant that molecule is to mineralization and its mobility in the soil profile (Turner et al., 2002; Condron et al., 2005). Inositol phosphates, a class of P_o once thought to be stable due to their binding affinity to soil particles, have been shown to be released into soil solution following dry/wet cycles (Turner & Haygarth, 2001). Organic P (estimated by unreactive P) has functional groups that can be measured based on the amount of P_o that can be hydrolyzed by adding different phosphatase enzymes to an extractant (Turner et al., 2002; Requejo & Eichler-Lobermann, 2014; Annaheim et al., 2013; DeLuca et al., 2015).

Phytase has a low substrate specificity, hydrolyzing a wide range of organic P forms, targeting all ester-P bonds. Due to this characteristic, phytase can be used to characterize the size of P_o pool that is available from hydrolysis and readily bioavailable (He, Cade-Menun, Toor, Fortuna, Honeycutt, & Sims, 2007; Johnson & Hill, 2010; Darch et al., 2016). Darch et al. (2016) suggested using phytase as a rapid measure of bioavailable organic P. A common P acquisition strategy for plants is to excrete organic acids to solubilize P and enzymes to hydrolyze organic P forms. To mimic these acquisition strategies, citric acid has been used to extract P for enzyme hydrolysis assays (Hayes, Richardson, & Simpson, 2000; Darch et al., 2016). Hayes et al. (2000) showed that 50 mM citric acid was an effective extractant of organic P, allowing up to 79% of organic P to be extracted and readily hydrolyzed by commercial phytase. Darch et al. (2016)

suggested using a 2 mM citric acid extraction followed by the enzyme hydrolysis, as it mimicked more realistic organic acid concentrations in the rhizosphere.

The characterization of P_o pools is a critical step in understanding how P (inorganic and organic) contributes to both crop nutrition and water pollution. The relative availability of the organic compound and size of their pools may improve our understanding of the impact of conservation management. The objective of this research was to document the effects of cover crops and P fertilizer management a no-till, corn-soybean cropping system on P_o. We used the following hypotheses to guide our investigation i) cover crops would increase the amount of phytase hydrolysable Po ($P_{PHYTASE}$) found in the 0-5 cm layer of soil ii) the amount of $P_{PHYTASE}$ will be correlated to the amount of P_O.

Materials and Methods

Site description

The experiment was established in 18 terraced watersheds, located at the Kansas Agricultural Watershed (KAW) Field Laboratory (39.134, -96.641), near the Kansas State University, Manhattan, Kansas. The site a mean annual temperature of 12.7^o C, and 904 mm of precipitation annually. The site has a slope of 6-8% on primarily eroded Smolan silty clay loam. Each watershed is approximately 0.5 ha and has been a continuous no-till, corn-soybean rotation since 2014.

This experiment is organized in a 2 by 3 factorial treatment design. The cover crop factor has two levels, cover crop (CC) or no cover crop (NC) and a fertilizer factor, has three types of phosphorus fertilizer application: no fertilizer (NP), spring injected ammonium polyphosphate (SI), and fall surface broadcast diammonium phosphate (FB). The 18 watersheds were randomly

assigned cover crop and fertilizer factors. Cover crops have been planted annually since 2015 and have included: winter wheat in 2016, triticale and rapeseed in 2017 and 2018, and winter wheat and rapeseed in 2019. Soybeans were harvested in 2018 and corn was harvested in 2019. The 27 kg P ha^{-1} was applied in both the spring injected and fall broadcast applications. Nitrogen fertilizer, 28% urea ammonium nitrate, was injected below the surface at a uniform rate of 146 kg N ha^{1} for all plots in corn years.

Forty soil cores were collected and combined for a composite sample from each watershed. Soil samples were collected in fall 2018, and spring/fall 2019 from the 0-5 cm depth. Spring samples were collected prior to cover crop termination and SI application, and fall samples were collected prior to crop harvest and FB application.

Soil Preparation and Extraction

The fresh soil was sieved through a 2 mm sieve and refrigerated. Eight grams of fresh soil was weighed into an Erlenmeyer flask and shaken for 30 min with 40 mL of 2 mM citric acid (pH 5.0). The supernatant was transferred to a 50 mL falcon tube and centrifuged for 10 min at 10,000 g. After centrifugation, the supernatant was filtered with Whatman 42 filter paper into a clean falcon tube and refrigerated.

Buffer and Phytase Preparation

A 0.5 M sodium acetate buffer was used to prepare the phytase. It was made by dissolving 47.63 g of sodium acetate, and 13.51 g of acetic acid in 500 mL of DI water. After reagents were dissolved, 0.041 g of $MgCl_2$ is added as an enzyme activator and DI water was added to bring the buffer to 1 L volume. This was made in 13 L batches as additional buffer was

used in the dialysis step, used to purify the phytase. A 100 mM sodium azide reagent was prepared by dissolving 0.652 g NaN_3 into 100 mL DI water.

Phytase from wheat (Sigma-Aldrich EC. 3.1.3.26 P1259) was used as a phytase source. A final concentration of 0.1 units per mL was used to ensure full hydrolysis of all ester-P bonds (Darch et al., 2016). One enzyme unit is equal to the liberation of 1 μmol of product per minute at a specified temperature and pH (www.sigmaaldrich.com). The certificate of analysis had the specification of phytase from wheat as ≥ 0.01 units per mg, with a test of activity of 0.07 units per mg. Previous research has documented orthophosphate contamination in phytase derived from wheat and has required purification through dialysis (He, Griffin, & Honeycutt, 2004; Zhu et al., 2013; Darch et al., 2016). One hundred mg of phytase was added 0.5 M sodium acetate buffer to a final volume of 10 mL and loaded into Spectra/Por Float-A-Lyzer (MWCO: 3500-5000 Spectrum Laboratories Inc.). The dialysis tube was suspended in 2 L of 0.5 M sodium acetate buffer on a stir plate. The buffer was replaced 6 times in a 24 hour period. The purified enzyme has then aliquoted into five – 2 mL microcentrifuge tubes and centrifuged for 10 min at 10,000 g. The purified phytase was used within 24 hr of dialysis.

Enzyme hydrolysis

For each soil sample, a 1.8 ml aliquot of the soil supernatant was added to three vials, labelled A, B, and C. A 0.2 mL aliquot of the prepared phytase was added to the A and B vials, while a 0.2 mL aliquot of the 0.5 M sodium acetate buffer was added to the C vial (Darch et al., 2016). A 0.3 mL aliquot of 100 mM sodium azide was added to all three vials to prevent microbial activity. The vials were incubated for 16 hr at 37°C. The vials were analyzed colorimetrically for molybdate reactive P (MRP) using the Murphy Riley method immediately

following incubation (Murphy & Riley, 1962) with a Hitachi U-1100 spectrophotometer (Hitachi High-Tech Corporation, Tokyo, Japan). Phytase hydrolysable P was calculated as the MRP with phytase addition – MRP without phytase.

Total Organic P

Organic P was measured by the ignition method (O'Halloran & Cade-Menun, 2006). Two, 1 g subsamples of crushed, air dried soil from each sample were measured into 250 ml Erlenmeyer flasks. One was incinerated at 550ºC for 1 hr while the other was kept at room temperature. Both samples were shaken with 25 ml of 0.5 M H_2SO_4 for 16 hr. Samples were filtered using Whatman 42 filters for 30 min. Samples were analyzed using molybdate reactive P (Murphy & Riley, 1962) with a Hitachi spectrophotometer (Hitachi High-Tech Corporation, Tokyo, Japan). Organic P was determined by the difference between the incinerated and non-incinerated samples and corrected for blank P concentration (O'Halloran & Cade-Menun, 2006).

Mehlich-III P

Mehlich-III P (P_M) was analyzed at the Kansas State Soil Testing Lab. Briefly, 1 g crushed, air dried soil was extracted with the Mehlich-III extractant with a 1:10 soil weight to extractant volume and shaken for 5 min. The MRP is determined colorimetrically using the molybdate reactive P method (Murphy & Riley, 1962).

Statistical Analyses

Statistical analyses were conducted using a two way analysis of variance (ANOVA) to detect differences of $P_{PHYTASE}$ among cover crop and P fertilizer management treatments using

PROC GLIMMIX, repeated measures of variance procedure, SAS version 9.4 software (Cary, NC, U.S.A.). The concentration of $P_{PHYTASE}$ was regressed against the total organic P using a linear regression, PROC REG. Season/year was analyzed separately and not compared statistically.

Results

There was a significant P fertilizer effect in fall 2018, where SI and FB had a higher concentration of $P_{PHYTASE}$ than NP treatments ($p=0.003$) (Fig. 4.1). This result was not detected in spring or fall 2019 (Fig. 4.2). In general, $P_{PHYTASE}$ was approximately 1-3% of P_O in fall 2018, 4-5% in spring 2019, and 4-10% in fall 2019 (Fig. 4.3)

In fall 2018, $P_{PHYTASE}$ was correlated with P_M ($r^2=0.34$) ($p<0.001$) (Fig. 4.4) but not in other seasons or when aggregated across seasons ($p=0.44$). The concentration of $P_{PHYTASE}$ was correlated with total organic P in spring 2019 ($r^2=0.3$, $p=0.02$) (Fig. 4.5). If two outliers were removed from the spring 2019 data set, the correlation improved to $r^2=0.69$. This relationship was not observed in other seasons nor when all seasons were combined ($r^2=0.03$). Neither total P_O nor P_M was correlated with fall 2019. Other P measures such as water extractable P or 2 mM citric acid extractable P were not correlated to $P_{PHYTASE}$.

Discussion

Cover crops had no effect on $P_{PHYTASE}$ concentrations, in any season. We had expected cover crops to potentially access P that may not be available to the main crop and deposit P enriched residue on the soil surface, thus increasing the concentration of labile organic P at the surface (Eichler-Lobermann, Kohne, Kowalski, & Schnug, 2008, Liu, Khalaf, Ule'n, &

Bergkvist, 2013). We observed a P fertilizer main effect in $P_{PHYTASE}$, in fall 2018 suggesting that P fertilizer could be an important factor in the accumulation of bioavailable organic P, although this was not documented in other seasons, making it difficult to evaluate this effect. We extracted and hydrolyzed higher amounts of $P_{PHYTASE,}$ 1.57-15.32 ug $P_{PHYTASE}$ g^{-1} dry soil compared to the 3.35-6.15 ug $P_{PHYTASE}$ g^{-1} dry soil range of citric acid extracted Hayes et al. (2000), and 0.05-2.19 ug $P_{PHYTASE}$ g^{-1} dry soil range of Darch et al. (2016), but lower amounts than He et al. (1997) which had a range of 42.3-83.3 ug $P_{PHYTASE}$ g^{-1} dry soil when the soil was extracted with $NaHCO_3$.

At a landscape scale, DeLuca et al. (2015) documented an increase in enzyme hydrolysable P, at the same time that soluble P was declining. While our results were, in most cases, not significant, the patterns we documented were at odds with the results from DeLuca et al. (2015). We did not detect any inverse relationships with soluble orthophosphate and $P_{PHYTASE}$. In fact, in the same fall 2018 samples that we detected higher $P_{PHYTASE}$ in FB and SI compared to NP treatments, there was also significantly higher P_M. DeLuca et al. (2015) tested P pools at a landscape scale. It is possible that the relationship they detected were not relevant at the scale we were testing or that cropping systems may alter the inorganic/organic P relationships. Liu et al. (2014) documented an increase in P release from subsequent freeze-thaw cycles. It is possible that we detected a $P_{PHYTASE}$ response only in fall 2018 because soil sampling was delayed by 20 days after soybean harvest due to inclement weather. Several freeze-thaw cycles were recorded in the delay time possibly allowing for the lysing of plant cells and increased decomposition rate of soybean residue due to a high C:N ratio in the plant biomass. The time delay with potential for decomposition may have increased the amount of labile organic P that was leaked on the soil surface.

Phytase hydrolysable P was correlated with P_M in fall 2018, which would be consistent with the P fertilizer main effect detected in the same season. Phytase hydrolysable P was well correlated with P_O in the spring 2019 but did not appear to be related to any metric in fall 2019. It is possible that after the crop residues have decomposed over the fall making the amount of $P_{PHYTASE}$ well correlated with the agronomically relevant P_M, while the correlation of P_O in the spring suggests that the larger P_O pool may be adding labile compounds that are hydrolysable by phytase. This difference in correlation among seasons could indicate that different P pools may influence $P_{PHYTASE}$ over different seasons or crops but it will be important to confirm this relationship in other samplings. The lack of consistent results makes it difficult to draw any conclusions about the role of $P_{PHYTASE}$ or its relationship to other P measures in the no-till corn-soybean cropping system. We did see in fall 2018 and spring 2019, that the proportion of $P_{PHYTASE}$ to P_O varied over a small range, from 1-3% and 4-5% respectively. In fall 2019, however, we detected a much larger range of values, from 4-10%. Interestingly, within that season both FB and NP treatments only varied by 1% with or without the cover crops, while within the SI treatment P_{HYTASE} made up 10% of the P_O with a cover crop but only 4% without a cover crop.

There were some challenges to the phytase hydrolysable method and further method development is necessary. We had consistent difficulties purifying phytase from wheat and removing orthophosphate contamination. It may be advisable to test other sources of phytase. A phytase source with less orthophosphate contamination would decrease the time needed for the experiment and reduce background interference. In addition to purification challenges, other soil extractants should be examined. It is possible that the 2 mM citric acid extraction suggested by Darch et al. (2016) did not extract enough organic P from the soil in our given study. Several

other studies have used either 50 mM citric acid or 0.5 M $NaHCO_3$ to extract soils for enzyme hydrolysis (Hayes et al., 2000; He et al., 2004) which may increase the amount of P we can extract and improve the sensitivity of the analysis.

A valuable next step would be to conduct phytase hydrolysable P on runoff collected from the treatments to determine if the $P_{PHYTASE}$ status has a relationship with the P in runoff. In addition to applying this assay to runoff, it would be useful to further characterize the organic P forms detected in the soil using ^{31}P nuclear magnetic resonance spectroscopy.

Conclusion

We did not detect any evidence to support our hypothesis that cover crops would increase the amount of $P_{PHYTASE}$ found in the 0-5 cm layer of soil. We detected a fertilizer main effect in fall 2018, where there was significantly more $P_{PHYTASE}$ in FB and SI than NP treatments, but this effect was only detected in one season. The amount of $P_{PHYTASE}$ was correlated to P_O in spring 2019. Correlations between $P_{PHYTASE}$, P_O, and P_M suggests that $P_{PHYTASE}$ may be related to various P pools in different seasons. Further method development should be conducted to refine this metric for this experiment and location.

References

Annaheim, K. E., Rufener, C. B., Frossard, E., & Bunemann, E. K. 2013. Hydrolysis of organic phosphorus in soil water suspensions after addition of phosphatase enzymes. *Biology & Fertility of Soils*, 49, 1203-1213.

Condron, L. M., Turner, B. L., & Cade-Menun, B. J. 2005. Chemistry and dynamics of soil organic phosphorus. In Sims, J.T., Sharpley, A.N. (Eds), *Phosphorus: agriculture and the environment* (pp. 87-122). ASA, CSSA and SSSA, Madison, WI.

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 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. A., de Sosa, L. L., Cerda-Moreno, C., & Jones, D. L. 2015. A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. *Soil Biology and Biochemistry*, 88, 110-119.

Dodd, R. J., & Sharpley, A. N. 2015. Recognizing the role of soil organic phosphorus in soil fertility and water quality. *Resources, Conservation and Recycling*, 105, 282-293.

Eichler-Löbermann, B., Köhne, S., Kowalski, B., & Schnug, E. 2008. Effect of catch cropping on phosphorus bioavailability in comparison to organic and inorganic fertilization. *Journal of Plant Nutrition*, 31, 659-676.

Johnson, N. R., & Hill, J. E. 2010. Phosphorus species composition of poultry manure-amended soil using high-throughput enzymatic hydrolysis. *Soil Science Society of America Journal*, 74, 1786-1791.

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. *Biology and Fertility of Soils*, 32, 279-286.

He, Z., Cade-Menun, B. J., Toor, G. S., Fortuna, A., Honeycutt, C. W., & Sims, J.T. 2007. Comparison of phosphorus forms in wet and dried animal manures by solution phosphorus-31 nuclear magnetic resonance spectroscopy and enzymatic hydrolysis. *Journal of Environmental Quality*, 36, 1086-1095.

He, Z., Griffin, T.S., & Honeycutt, C.W. 2004. Enzymatic hydrolysis of organic phosphorus in swine manure and soil. *Journal of Environmental Quality*, 33, 367-372.

He, Z. L., Wu, J., O'Donnell, A. G., & Syers, J. K. 1997. Seasonal responses in microbial biomass carbon, phosphorus, and Sulphur in soils under pasture. *Biology and Fertility of Soils*, 24, 421-428.

Leytem, A. B., Mikkelsen, R. L., & Gilliam, J. W. 2002. Sorption of organic phosphorus compounds in Atlantic coastal plains soil. *Soil Science*, 167, 10.

Liu, J. R. Khalaf, R., Ule'n, B., & Bergkvist, G. 2013. Potential phosphorus release from catch crop shoots and roots after freeze-thawing. *Plant and Soil*, 371, 543-557.

Liu, J., Ulen, B., Bergkvist, G., & Aronsson, H. 2014. Freeze-thawing effects on phosphorus leaching from catch crops. *Nutrient Cycling in Agroecosystems*, 99, 17-30.

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

O'Halloran, I. P., & Cade-Menun, B. J. 2006. Chapter 24 Total and organic phosphorus. In Carter, M.R., and Gregorich, E.G. (Eds), *Soil Sampling and Methods of Analysis* (2nd edition). Canadian Society of Soil Science.

Requejo, M. I. & Eichler-Löbermann, B. 2014. Organic and inorganic phosphorus forms in soil as affected by long-term application of organic amendments. *Nutrient Cycling in Agroecosystems*, 100, 245-255.

Toor, G. S., Condron, L. M., Di, H. J., Cameron, K. C., & Cade-Menun, B. J. 2003. Characterization of organic phosphorus leachate from a grassland soil. *Soil Biology and Biochemistry*, 35, 1317-1323.

Turner, B. L., & Haygarth, P. M. 2001. Biogeochemistry: phosphorus solubilization in rewetted soils. *Nature*, 411, 258.

Turner, B. L., McKelvie, I. D., & Haygarth, P. M. 2002. Characterization of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biology and Biochemistry*, 34, 27-35.

Sharpley, A. N. 1985. Phosphorus cycling in unfertilized and fertilized agricultural soils. *Soil Science Society of America Journal*, 49, 905-911.

Zhu, Y., Wu, F., He, Z., Guo, J., Qu, X., Xie, F., Giesy, J.P., Liao, H., & Guo, F. 2013. Characterization of organic phosphorus in lake sediments by sequential fractionation and enzymatic hydrolysis. *Environmental Science & Technology*, 47, 7679-7687.

Figures

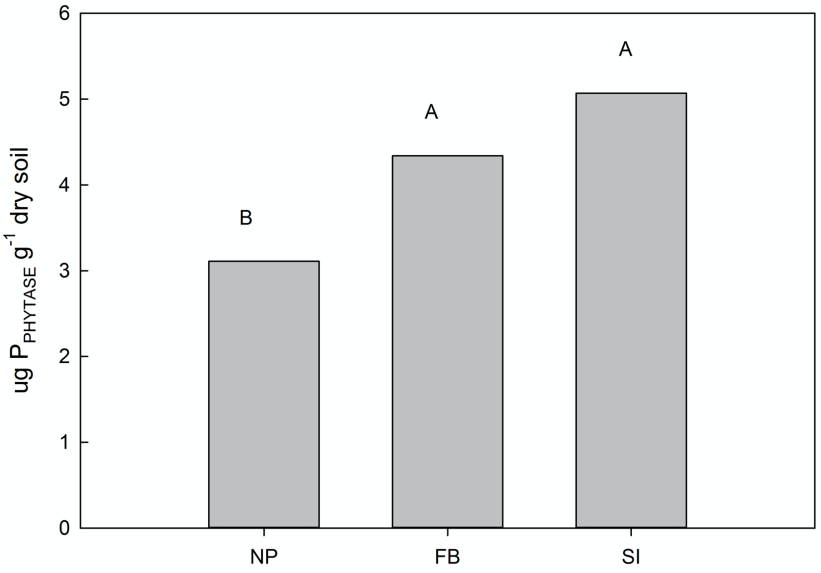


Figure 4.1. Fall 2018 phytase hydrolysable P (ug $P_{PHYTASE}$ g^{-1} dry soil) in P fertilizer treatments (p=0.003). Figure abbreviations: fall broadcast (FB), and spring injected (SI), no P fertilizer (NP).

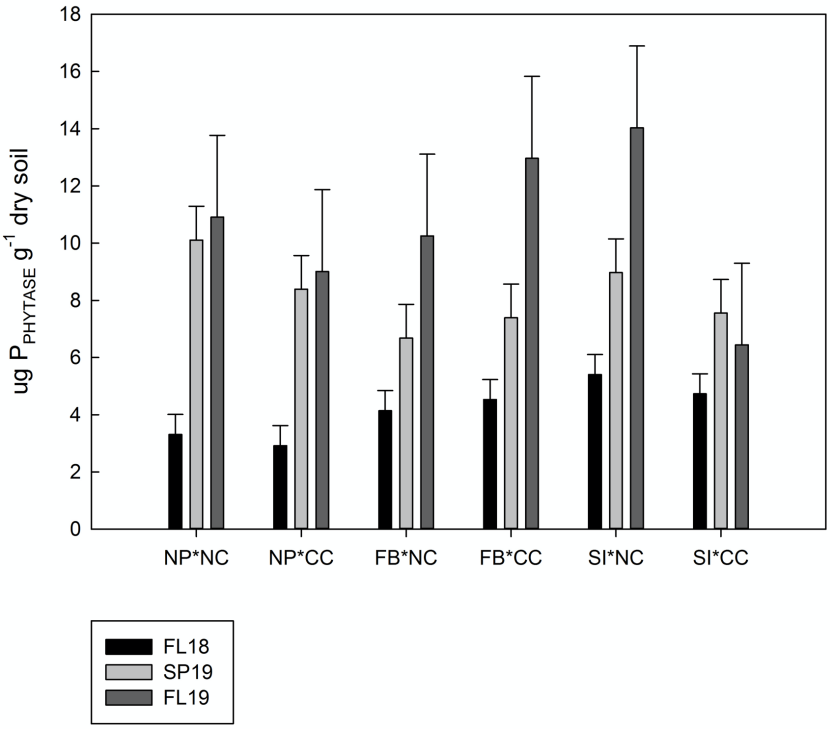


Figure 4.2. Phytase hydrolysable P (ug $P_{PHYTASE}$ g^{-1} dry soil) in all treatment combinations in fall 2018, and spring/fall 2019. Figure abbreviations: fall broadcast diammonium phosphate (FB), spring injected ammonium polyphosphate (SI), no P fertilizer (NP), cover crop (CC), and no cover crop (NC).

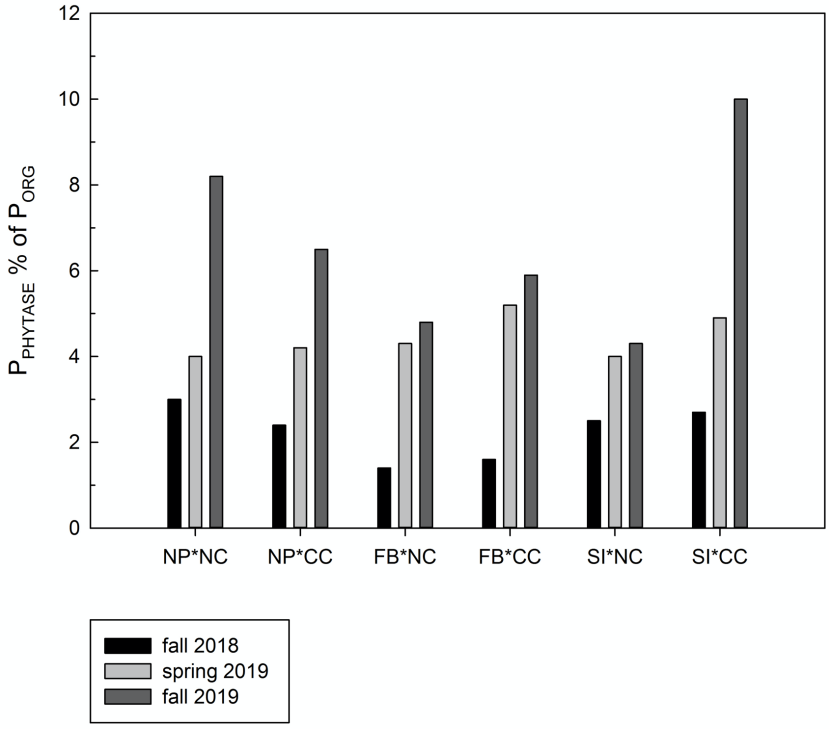


Figure 4.3. Percent of total organic P that is phytase hydrolyzable in fall 2018, and spring/fall 2019. Figure abbreviations: fall broadcast diammonium phosphate (FB), spring injected ammonium polyphosphate (SI), no P fertilizer (NP), cover crop (CC), and no cover crop (NC).

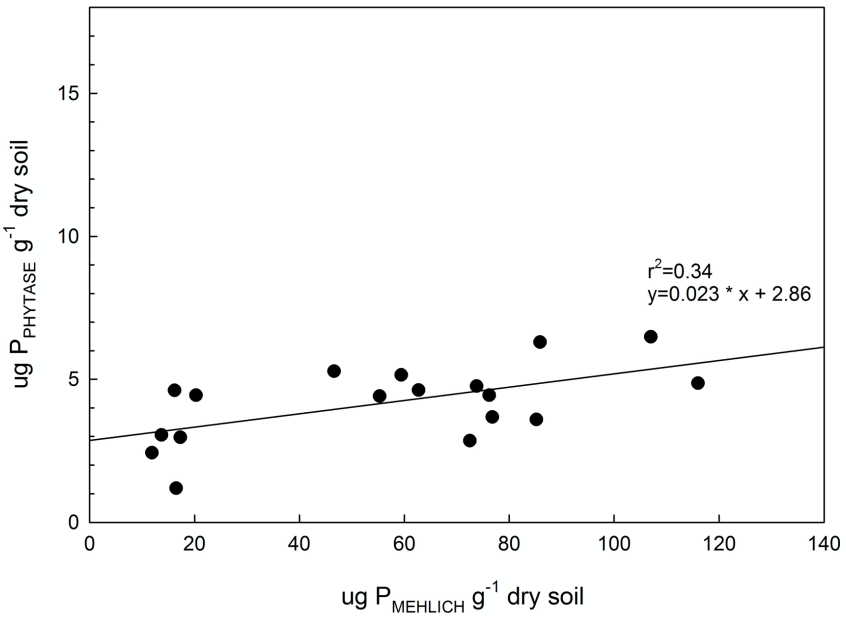


Figure 4.4. Correlation between phytase hydrolysable P and Mehlich-III extractable P in fall 2018 (r^2=0.34, p<0.001).

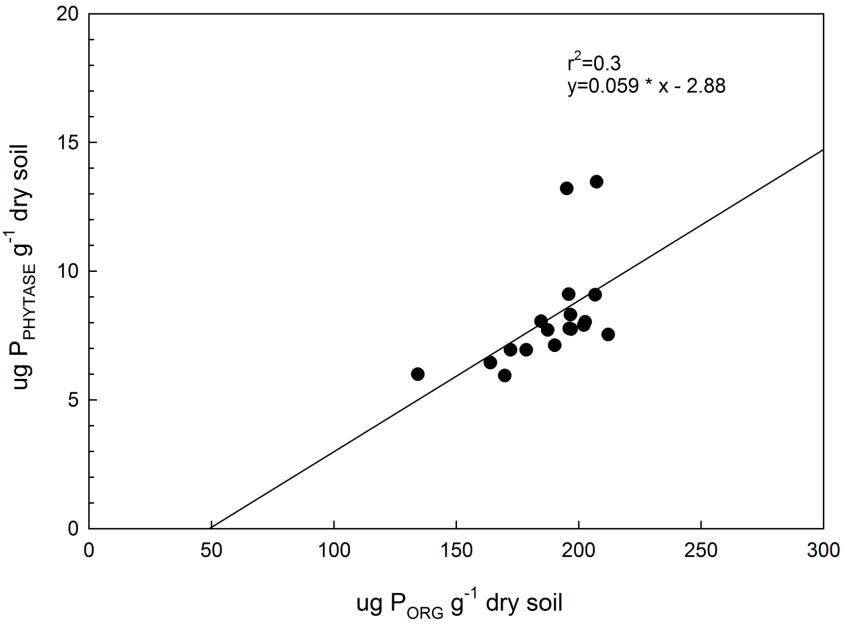


Figure 4.5. Correlation between phytase hydrolysable P and total organic P in spring 2019 (r^2=0.3, p=0.02).

Chapter 5 - Summary

Phosphorus loss from agricultural fields contributes to reduced water quality and eutrophication. Conservation management has been shown to decrease erosion and improve soil health, but little research has been conducted on how these practices interact with P fertilizer management. Although no-till and cover crops can reduce sediment loss and sediment bound P, a growing body of evidence has documented increased dissolved reactive P loss from these practices. Phosphorus stratification, increased cycling of organic P, and changes to bioavailable P pools have been suggested as causes of increased dissolved reactive P loss in runoff. Increased stratification has been documented in no-till cropping systems, while cover crops can increase microbial activity. The purpose of this research was to document how cover crops would impact stratification with differing P fertilizer management, and how P fertilizer interacted with cover crops to change biological cycling and P storage in a no-till cropping system. In addition to common soil P tests such as Mehlich-III (P_M), and total P (P_T), we investigated P pools that were biologically relevant such as total organic P (P_O), citrate extractable P (P_C), water extractable P (P_W), microbial biomass P (MB-P) and the P supply to the soil solution as measured by diffusive gradient thin films (P_{DGT}). We also measured the potential activity of P cycling enzymes, acid/alkaline phosphatase, and phosphodiesterase.

In chapter two, we documented increased stratification initially in all treatments, but stratification decreased in no P fertilizer (NP) treatments and increased in fall broadcast diammonium phosphate (FB). Stratification was greater in spring injected ammonium polyphosphate (SI) with a cover crop (CC) but not without one (NC). We chose to use biologically relevant measures of P pools such as, P_C, and P_W. These were critical in

understanding P storage under different management scenarios, as the 2 mM citric acid and water extractions mimicked plant P acquisition strategies. These measures allowed us to detect differences that were not documented with traditional soil test P methods. Our results demonstrated a repeated increase in bioavailable P in SI*CC treatments at the 0-5 cm, while SI*NC was frequently not different from the control. At the 5-10 and 10-15 cm depth we detected lower amount of P in CC treatments compared to NC. These results suggest that cover crops maybe moving P to the surface, but this effect is particularly prevalent where P fertilizer is injected below the surface.

In chapter 3, we measured the treatment effect of P fertilizer management and cover crops on MB-P, MB-C:P, and P cycling enzymes. We documented an increase in MB-P in FB and SI treatments compared to NP. Interestingly, we documented increased MB-P in SI*CC treatments, similar to the results of P_C, P_W, and P_{DGT} from chapter 2. Microbial biomass P was well correlated to P_M, suggesting that P_M may be reasonable estimate of treatment effects on MB-P. Microbial biomass C:P ratios were greater in NP treatments compared to SI and FB. The high MB-C:P in NP treatments suggest that P will be quickly immobilized by microorganisms. We consistently detected higher phosphatase enzyme activity in response to cover crops. We did not detect a suppression of phosphatase enzyme activity in response to P fertilizer despite high P availability. Increases in carbon availability in cover crop treatments could account for the lack of P fertilizer effect.

In chapter 4, we measured the treatment effects on the bioavailable organic P pool. We estimated the bioavailable fraction by adding phytase to soil extracts and measuring the amount of organic P that is hydrolyzed from organic P to orthophosphate ($P_{PHYTASE}$). We detected higher amounts of $P_{PHYTASE}$ in FB and SI treatments compared to NP in fall 2018.

Together these measurements indicate that cover crops interact with P fertilizer management in no-till systems in unforeseen ways. Most notably, they modified P stratification dynamics with SI management in labile P pools, increasing the concentration of P at the surface compared to the same fertilizer management with no cover crops. This is an important development as both subsurface P application and cover crops have been recommended as best management practices. In addition to stratification dynamics, they generally increased the activity of phosphatase enzymes at the 0-5 cm soil depth, which may potentially lead to increased organic P mineralization or immobilization by microorganisms. Phosphorus fertilizer applications had consistent impact on MB-P which is labile P pool that is sensitive to wet and dry periods. This increase in MB-P may contribute to increases in dissolved reactive P loss to runoff. The seasonal change in $P_{PHYTASE}$ correlation from P_O to P_M could suggest that the labile fractions of organic P may be related to different P pools based on decomposition and seasonal P storage.

Appendix A – Microbial Biomass Phosphorus Method Development

Introduction

Microbial biomass can be 2-3% of the organic matter in soil and 0.7-7.5% of TP, making it a large pool of labile nutrients (Brookes et al., 1982; Oehl et al., 2004). Microorganisms are sensitive to environmental changes, such as wet/dry or freeze/thaw cycles which can lyse cells and spill labile nutrients into the soil solution (Blackwell et al., 2010). Microbial biomass P (MB-P) is typically measured using a chloroform fumigation and 0.5 M $NaHCO_3$ extractant, originally developed by Hedley and Stuart (1982) and further modified to be conducted on fresh soil, with a 24 h fumigation period by Brookes et al. (1982) (Table 1). This method is widely accepted but has had less reproducibility and reduced sensitivity on soils with low pH (Potter et al., 1991; Wu et al., 2000) or high soil test P (Zhou et al., 2008) (Table 1). Microbial biomass P is a valuable and important metric as it represents a labile source of phosphorous that may be sensitive to management changes and may contribute to P loss in runoff from agricultural fields. Challenges inherent in the method may prevent wider adoption of the measurement in agronomic settings where high soil test P may be prevalent.

Rationale

Initial attempts to measure MB-P in a P experiment that was established in a no-till corn-soybean cropping system in NE Kansas highlighted challenges in using traditional MB-P methodology (Hedley and Stuart, 1982; Brookes et al., 1982). A lack of sensitivity using 0.5 M $NaHCO_3$ extractant in preliminary tests may be attributable to high background P in the soil. Hedley and Stuart (1982) and Zhou et al. (2008) identified high soil P as interference to

measuring MB-P (Table 1). Both sources recommended an orthophosphate extraction step prior to fumigation in soil with high P. Additionally, we found that, despite pre-acidification and adherence to Lachat methods, analyzing $NaHCO_3$ extracts on a Lachat QuickChem 8500 Series II Automated Ion Analyzer was not reliable. We propose modifying the Brookes et al. (1982) method with an alternative extractant to avoid the additional orthophosphate extraction which would improve efficiency and minimize interference of soil and soil microorganisms and improve automated analysis.

Several research articles have highlighted the value of extracting P using an extractants that are closer to soil conditions rather than some of the strong acids and bases commonly used (Hayes et al., 2000; Haney et al., 2010; Darch et al., 2016). Hayes et al. (2000) and Darch et al. (2016) proposed using a dilute citric acid extraction as an extractant for enzyme hydrolysis analyses, a method that uses the addition of enzymes to characterize labile nutrient pools. Darch et al. (2016) proposed a 2 mM citric acid at pH 5.0 because it was a concentration and pH that was similar to those found in the rhizosphere, thus mimicking conditions found in soil. By comparison, the common 0.5 M $NaHCO_3$ pH 8.5 extraction creates conditions that are likely to solubilize many mineral P containing compounds (Haney et al., 2010). Research has shown that citric acid was an efficient extractant for enzyme hydrolysis (Hayes et al., 2000; Darch et al., 2016); while Hayes et al. (2000) found that 0.5 M $NaHCO_3$ extracted smaller amounts of labile organic P and solubilized larger amounts of inorganic P. While MB-P is a different method to enzyme hydrolysis, we hypothesized that 2 mM citric acid may be a suitable option to extract relevant fractions of P for MB-P analysis, while minimizing the solubilization of mineral P fractions that may interfere with analysis.

Methods

Soil Description

The soil was sampled from an experimental site in NE Kansas, that is organized in a 2 by 3 factorial with cover crops and P fertilizer management treatments. Eighteen watersheds were arranged in a randomly complete block design. The cover crop factor has two levels, cover crop (CC) or no cover crop (NC) and there were three types of phosphorus fertilizer application, no fertilizer (NP), spring injected (SI) ammonium polyphosphate applied at 131 L ha^{-1} (27 kg P ha^{-1}) and fall surface broadcast (FB) diammonium phosphate (DAP) applied at 135 kg ha^{-1} (27 kg P ha^{-1}). Nitrogen fertilizer, 28% urea ammonium nitrate, was injected below the surface at a uniform rate of 146 kg N ha ^{1} for all plots. Forty soil cores were collected from the 0-5 cm depth from each watershed in May 2019. The soil pH was 6.5-7.0. Total P was significantly higher in FB and SI treatments compared to NP (data presented in Chapter 2).

Microbial biomass P

Soils were immediately refrigerated and sieved moist (2 mm sieve). Roots and other organic materials were removed during the sieving process. Eight grams of fresh soil from each sample was weighed into two 250 mL Erlenmeyer flasks. One flask was placed in a desiccator with 25 mL of $CHCl_3$ and three wet paper towels. The desiccator was evacuated with a vacuum pump for 5 min, sealed, and left for 24 h (Brookes et al., 1982). The other flask was incubated without fumigation.

After the 24 hr period, 40 mL of 0.5 M $NaHCO_3$ pH 8.5 or 2 mM citric acid pH 5.0 added and was shaken for 30 min. Once shaken, the sample was centrifuged at 10,000 rpm for 10 min and filtered with a Whatman 42 filter. The extracts were analyzed on a Lachat auto-nutrient analyzer using the Murphy Riley method (Murphy and Riley, 1962). Microbial biomass P was

determined by subtracting molybdate reactive P (MRP) of the unfumigated samples from the fumigated samples (Equation 1).

$$\text{MB-P } (\mu g\ g^{-1} \text{ dry soil}) = (P_{fumigated} - P_{unfumigated})$$

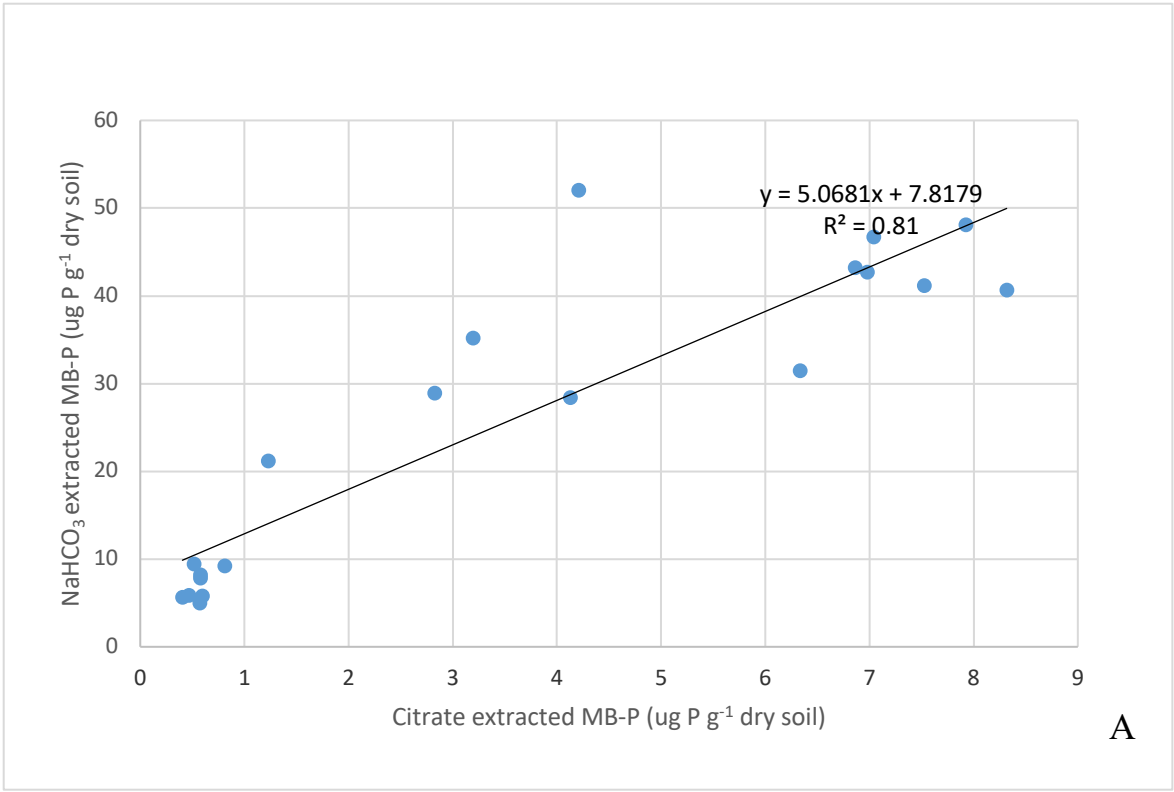
Equation 1. Calculation to determine microbial biomass P.

Results and Discussion

We were not able to detect any treatment effect on MB-P in samples extracted with 0.5 M $NaHCO_3$ pH 8.5, despite higher total P in FB and SI treatments. Out of the 21 samples tested, 5 of the results were negative after MB-P was calculated from the 0.5 M $NaHCO_3$ pH 8.5 extraction. From the 2 mM citric acid extraction, however, a cover crop*P fertilizer interaction was detected in MB-P ($p=0.017$). The interaction was detected in the SI treatments where SI*CC was equivalent to the highest treatment, FB*NC, while the SI*NC was not. Additionally, FB*NC, FB*CC, SI*CC, and SI*CC had higher MB-P than NP*NC and NP*CC.

The unfumigated extractions were well correlated to one another ($r^2=0.81$) (Fig. 1a), however the MB-P calculated from the respective extractions were not ($r^2=0.1$) (Fig.1b). The 0.5 M $NaHCO_3$ pH 8.5 extracted 4.95 – 52.07 ug MRP from the unfumigated soil compared to 0.4 – 8.31 ug from the 2 mM citric acid extraction.

Our preliminary tests suggest that the 2 mM citric acid extraction is a sensitive alternative to the 0.5 M $NaHCO_3$ pH 8.5 extractant. These results suggest further testes should be conducted to confirm the efficacy of the citric acid extractant on different soil types.


y = 5.0681x + 7.8179
R² = 0.81
NaHCO3 extracted MB-P (ug P g-1 dry soil)
Citrate extracted MB-P (ug P g-1 dry soil)
0
10
20
30
40
50
60
0
1
2
3
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8
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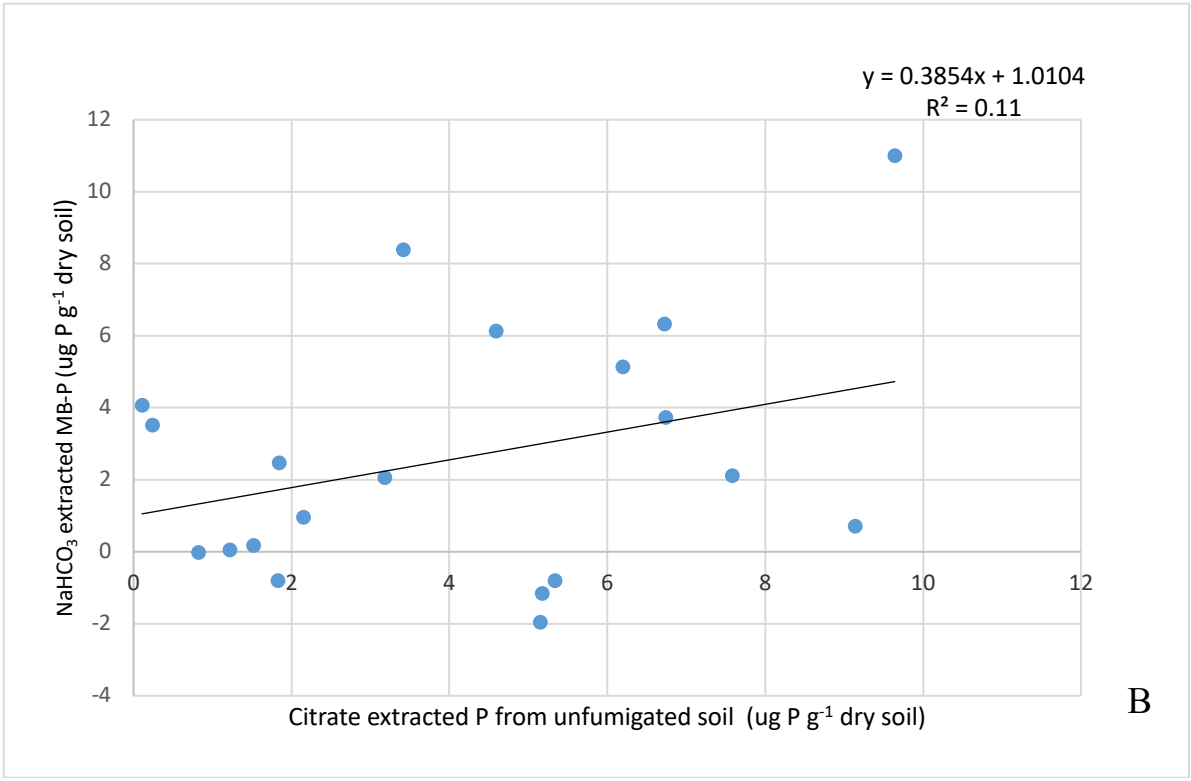

Figure 1. A) Correlation between *unfumigated* soil extracted with 0.5 M NaHCO3 pH 8.5 and 2 mM citric acid pH 5.0 (r^2=0.81) B) Correlation between MB-P calculated from 0.5 M NaHCO3 pH 8.5 and 2 mM citric acid pH 5.0 extractions (r^2=0.11). The MB-P measurements from both extractants in Fig.1a were well correlated with each other, while they are not correlated when used on unfumigated soil.

Table 1. Summary of microbial biomass P methods

Reference	Extractant	Soil to Extractant Ratio	Extraction Time	Soil pH	Notes
Hedley and Stewart, 1982	0.5 M $NaHCO_3$ pH 8.5	1:60 (0.5g:30 mL)	16 hr	7.0-7.4	**Original method** – soils dried, ground, brought to 60% field capacity, incubated for 21 days –then fumigated The soil is extracted with HCO_3^- prior to incubation to remove excess background P to reduce interference
Brookes, Powlson and Jenkins, 1982	0.5 M $NaHCO_3$ pH 8.5	1:20 (10g:200mL)	30 min	5.6-7.6	**Adapted method to use moist, unground (2 mm sieved) soil** – good discussion on soil sample weight and sieving
McLaughlin and Alston, 1986	Multiple – documents superior results from 0.5 M $NaHCO_3$ pH 8.5	1:20	Multiple	6.0-8.4	**Tested various extractants and biocides** (0.1 M and 0.5 M $NaHCO_3$ pH 8.5, 50 mM NaOH, 10mM $CaCl_2$, 50 mM H_2SO_4, 30mM NH_4F + 0.1 M HCl) and chloroform and hexanol biocides Used dried, ground, and remoistened soil.
Potter et al., 1991	0.5 M $NaHCO_3$ pH 8.5	Ratio not specified	16 hr	Not specified	**Identifies problems with original extractant in acid, weathered soils**. Air drying reduces resin extractable P, while grinding soil releases MB-P Tested on weathered soils from Georgia and Brazil Used dried, ground, and remoistened soil.

Kouno et al. 1995	Distilled water and anion exchange membrane	Multiple	1-24 hr	4.1 and 4.3	**AME strip were used to absorb released P** Chloroform liquid was added to water instead of incubation with chloroform vapor AME strips eluted with 0.5 M HCl
Wu et al., 2000	30mM NH_4F + 25mM HCl	1:4	30 min	3.6-5.9	**Proposes using Bray-1 as an extractant** (30mM NH_4F + 25mM HCl) for more reproducible results for extracting MB-P in acidic soil
Zhao et al., 2008	0.5 M $NaHCO_3$ pH 8.5	1:20	30 min	7.5 and 8.6	**Documented high background P interference** in calcareous soil Found that MBP could not be determined if Olsen P was >60 mg/kg. Suggested removal of inorganic P by resin or 0.5 M $NaHCO_3$ pH 8.5 prior to fumigation Used sieved, moist soil.
Blackwell et al., 2009	0.5 M $NaHCO_3$ pH 8.5	1:20	Not specified	Not specified	**Discusses the importance of including roots when measuring MB-P** Used sieved, moist soil. References *Snars et al., 2006 for modified MBP method but gives few details.
Crews and Brooks, 2014	Ion exchange resin strips	Not specified	Not specified	Not specified	Many details not specified but cites Kouno et al. (1995) methods using resin strip to measure P release Incubated remoistened soil prior to fumigation following Hedley and Stewart (1982)

References

Blackwell, M. S. A., Brookes, P. C., de le Fuente-Martinez, N., Gordon, H., Murray, P. J., Snares, K. E., Williams, J. K., Bol, R., & Haygarth, P. M. 2010. Phosphorus solubilization and potential transfer to surface waters from the soil and microbial biomass following drying-rewetting and freeze-thawing. *Advanced Agronomy*, 106, 1-35.

Blackwell, M. S. A., Williams, J. K., Snares, K. E., Brookes, P. C., de le Fuente-Martinez, N., Michallon, L., Murray, P. J., & Haygarth, P. M. 2009. Significance of root-attached soil and soil preparation for microbial biomass phosphorus measurement. *Soil Science Society of America Journal*, 73, 1861-1863.

Brookes, P. C., Powlson, D. S., & Jenkinson, D.S. 1982. Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry*, 14, 319-329.

Crews, T. E., & Brookes, P. C. 2014. Changes in soil phosphorus forms through time in perennial versus annual agroecosystems. *Agriculture, Ecosystems and Environment*, 184, 168-181.

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 soils by organic acid extraction and phosphatase hydrolysis. *Geoderma*, 284, 93-102.

Haney, R. L., Haney, E. B., Hossner, L. R., &Arnold, J. G. 2010. Modifications to the new soil extractant H3A-1: A multinutrient extractant. *Communications in Soil Science and Plant Analysis*, 41, 1513-1523.

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. *Biology and Fertility of Soils*, 32, 279-286.

Hedley, M. J., &Stuart, J. W. B. 1982. Method to measure microbial phosphate in soil. *Soil Biology and Biochemistry*, 14, 377-385.

Kouno, K., Tuchiya, Y., & Ando, T. 1995. Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. *Soil Biology and Biochemistry*, 27(10), 1353-1357.

McLaughlin, M. J.,& Alston, A. M. 1986. Measurement of phosphorus in the soil microbial biomass: A modified procedure for field soils. *Soil Biology and Biochemistry*, 18(4), 437-443.

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

Oehl, F., Frossard, E., Fliessbach, A., Dubois, D., & Oberson, A. 2004. Basal organic phosphorus mineralization in soils under different farming systems. *Soil Biology & Biochemistry*, 36, 667-675.

Potter, R. L., Jordan, C. F., Guedes, R. M., Batmanian, G. J., &Han, X. G. 1991. Assessment of a phosphorus fractionation method for soil: Problems for further investigation. *Agriculture, Ecosystems and Environment*, 34, 453-463.

Wu, J., He, Z. L., Wei, W. X., O'Donnell, A. G., & Syers, J. K. 2000. Quantifying microbial biomass phosphorus in acid soils. *Biology and Fertility of Soils*, 32, 500-507.

Zhao, X., Li, G., & Lin, Q. 2008. Interference of soil-extractable phosphorus in measuring soil microbial biomass phosphorus. *Communications in Soil Science and Plant Analysis*, 39, 9-10.

Appendix B – SAS Code

*Analysis of Variance – Mixed models wit*h random effects

```
TITLE "WEP";

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

PROC Print DATA=WEP PLOTS=diagnostics;

PROC glimmix data = WEP;
class block depth fert cover;
model response = fert|cover|depth/ddfm = satterth;
random block;
random _residual_/subject=block*cover*fert type = CSH;
lsmeans fert|cover|depth/lines cl;

slice fert*depth / sliceby=depth lines;
slice cover*depth / sliceby=depth lines;
slice fert*cover*depth/ sliceby=depth lines;
ods output Tests3=ANOVA2 lsmeans=Means2 LSMlines=lines2 SliceTests=sliceout2
slicelines=slines2;

Run;
```

General Linear Regression

```
data MBP_Mehlich;
input MBP Mehlich 3;
datalines:
;
PROC reg data=MBP_Mehlich;
Model MBP = Mehlich 3 / influence r;
Run;
```

Appendix C – Phosphatase Enzymes at 5-10 cm

Acid Phosphatase

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

Alkaline Phosphatase

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

Phosphodiesterase

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

Appendix D – Phosphatase Enzymes at 10-15 cm

Acid Phosphatase

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

Alkaline Phosphatase

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

Phosphodiesterase

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