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PHOSPHORUS USE AND MANAGEMENT BASED ON FERTILIZER PLACEMENT, RATE OF APPLICATION, AND SOIL BIOTA IN NO-TILL SITUATIONS

BY

BRENNAN ALEXANDER BINGHAM LEWIS

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Plant Science

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2022

THESIS ACCEPTANCE PAGE

Brennan Lewis

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I dedicate this thesis to the following:

My wonderful girlfriend, Allison Shorter, who throughout this journey has had nothing but belief, support, and encouragement for me, even when I did not feel that way about myself. My wonderful girlfriend, Allison Shorter, who has believed in me and has supported and encouraged me throughout this illuminating journey.

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ABSTRACT

PHOSPORUS USE AND MANAGEMENT BASED ON FERTILIZER PLACEMENT, RATE OF APPLICATION, AND SOIL BIOTA IN NO-TILL SITUATIONS

BRENNAN ALEXANDER BINGHAM LEWIS

2022

Phosphorus (P) pollution has become a concern among multiple scientific organizations as it leads to eutrophication, an algal bloom that depletes lacustrine and marine ecosystems of native species. Multiple strategies can be implemented to reduce phosphorus loss from agriculture fields, which is often implicated as a cause of eutrophication. Soil phosphorus chemistry results in phosphate fertilizers absorbing to clay minerals over time. Soil phosphorus is lost from agricultural fields primarily through wind and water erosion. No-till practices prevent soil erosion, which reduces the phosphorus from loading into waterways. Fertilizer placement affects phosphorus loss. Surface application of phosphorus fertilizers increases the risk of loss as rainfall can dissolve the fertilizer and move it into waterways. Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that infect plant roots and form a symbiotic relationship. AMF exchange water and plant nutrients, like phosphorus, with the plant for carbon. Field management practices that support the existence of healthy AMF populations in an agriculture field may allow for a reduction of phosphorus fertilizers. In turn, reduced phosphorus fertilizer rates may result in healthier stream, river, and lake ecosystems. Three different studies took place at Dakota Lakes Research Farm; (1) a phosphorus (P) fertilizer rate study; (2) phosphorus soil placement study; and (3) an AMF soil population study based on fertilizer rate. There were no significant differences between the P-rate treatments [0 lbs MAP (Check), 100 lbs MAP, and 200 lbs MAP, extra fertilizer applied in 2014, 2017, and 2019] and the impact on corn and soybean yield. The P-rate treatments did significantly change the phosphorus soil test levels within the field, with the 200 lbs of MAP having higher soil test levels than the 100 lbs of MAP, and both having higher soil test levels than the Check. Results from the placement study suggest that surface or band applied P did not impact plant tissue concentration, yield, and soil test phosphorus. The arbuscular mycorrhizal fungi populations were affected by the different phosphorus fertilizer rate treatments. The check treatment had significantly more fungi present than the treatments where extra fertilizer was added.

CHAPTER 1- LITERATURE REVIEW

Eutrophication and Phosphorus Fertilizers:

Fertilizer phosphorus has received intense scrutiny over the last 20 years as a cause of aquatic ecosystems degradation. One of the main factors causing the degradation is a process called eutrophication. Eutrophication is a process in which an excess amount of nutrient is present in a body of water, causing rapid growth of algae and plant life, which can deplete the water of oxygen. In South Dakota, eutrophication affects a sizable proportion of lakes in the Big Sioux River and the Missouri River basin areas (Roberts, 2022). Tools have been developed to help farmers understand the possible phosphorus loss risk that could occur in their fields, such as the Minnesota Phosphorus Index. South Dakota does not currently have its own Phosphorus Index; however, the Minnesota Phosphorus Index can be used. The Minnesota Phosphorus Index models the 3 major pathways of phosphorus loss: erosion, rainfall runoff, and snow runoff. The program also compares management practices of fields to find the optimal management that reduces phosphorus loss risk (Lewandowski et al., 2006).

There are two different categories of pollution: point source and nonpoint source pollution. Point source pollution can be traced back to a single point leading to where the pollutant is coming from, such as sewage treatment plants. Nonpoint source pollution is a pollutant that cannot be traced back to one single point (Inslee, 2021). Runoff of nutrients from agricultural fields falls into the nonpoint source pollution category. This requires that everyone involved in agriculture carefully examines all aspects of phosphorus use. Management tactics should (1) prevent environmental damage from phosphorus, such as eutrophication and (2) maintain high yields to feed a growing world population.

The two main contributions to nonpoint source phosphorus pollution are synthetic fertilizers and animal manures. In rangeland situations, animal manures do not cause an issue due to there being a low density of animals. Phosphorus pollution occurs most often from areas like feedlots where there is a high density of animals (Raton et al., 2001). This occurs from over application of the high amount of manure to fields or a direct loss from the manure piles at the feedlots. When looking at fertilizer source effect on movement to water ways, synthetic fertilizers contain more dissolved phosphorus. Dissolved phosphorus is a more bioavailable source of phosphorus in runoff water than manures (Kumaragamage et al., 2011).

Synthetic fertilizers and manures are transported in two forms, particulate P and dissolved P (Ruark et al., 2006; Kumaragamage et al., 2011; Kumaragamage and Akinremi, 2018; Zhang et al., 2018). Management practices affect the form in which phosphorus can be lost from the field. In cultivated fields, particulate P is lost. However, in no-till fields dissolved phosphorus is the primary form lost (Ruark et al., 2006; Potter et al., 2006). Particulate phosphorus loss occurs when phosphorus that has been adsorbed to soil particles is eroded from the field due to wind or water erosion. Particulate P is suspended in moving water or settles out into sediment when water recedes. Due to the phosphorus soil adsorption, varying amounts of P can be released from the soil under different circumstances. The mechanics for how this happens: 1) Sorbed particulate phosphorus in waterways is in chemical equilibrium with dissolved phosphorus, 2) The bioavailable phosphorus is used. 3) The sorbed phosphorus desorbs to maintain the equilibrium (Carpenter, 2005). This makes the particulate phosphorus a long-term source of phosphorus in waterways (Zhang et al., 2018). Dissolved phosphorus is phosphorus that has been dissolved in water. This form of phosphorus is readily available for uptake by biotic organism making it a short-term source of phosphorus (Ruark et al., 2006).

Particulate P and dissolved P are transported to waterways in two different transport methods, surface transport and subsurface transport. Surface transport occurs when phosphorus is lost from the soil surface and moves to waterways without going into the soil. If phosphorus soil concentration is very high, water will carry excess phosphorus that has not sorbed to the soil through tile drainage systems and be deposited into other bodies of water. Phosphorus also can be lost from the surface of the soil when phosphorus desorbs from the soil. Rainfall will desorb soil attached phosphorus and dissolve P into the water (Kaiser, 2018). The dissolved phosphorus is transported to larger bodies of water. Subsurface transport occurs when phosphorus leaches and moves by lateral flow and vertical flow out of the field (Raton et al., 2001; Rinderer et al., 2021). Most dissolved phosphorus (90%) moves vertically through the soil profile while the remaining 10% moves laterally in the soil (Rinderer et al., 2021).

Phosphorus movement to aquatic ecosystems is influenced by how phosphorus fertilizers are applied to the soil. Fertilizers containing phosphorus that are applied on the soil surface primarily lose the phosphorus nutrient via runoff. This happens because the fertilizer is directly in contact with water when rain or irrigation occurs. Movement of phosphorus in the soil is by diffusion which results in little movement in the soil matrix. The rate of diffusion is influenced by 3 factors. The first factor is the amount of phosphorus that is applied to the soil. The second is the soil water content and the bulk density. Finally, the third is the chemical reaction with phosphorus and the soil (Eghball et al., 1995). Banded P applications place the P close or in direct contact with the germinating seed. Eghball (1995) reported that when P fertilizer is banded at 123 lbs/acre, phosphorus moved only 1.6 inches in the soil from the center of the band. Banding P fertilizer may be a management tactic to reduce loss of P and prevent environmental problems like eutrophication.

Simard et al. (2000) documented total phosphorus and particulate phosphorus movement through a soil profile to field drainage ways. Three main conclusions came from this research: 1) Course and fine textured soils are prone to phosphorus losses by preferential flow through natural macropores and artificial drainage. 2) Enhancing a soil's water infiltration capability can reduce total phosphorus loss but may enhance particulate phosphorus losses when artificial drainage is used. 3) The management practices of a field have a great amount of influence on the amount of phosphorus lost and the form of phosphorus lost (Simard et al., 2000). Open inlets from artificial agriculture tile line drainage systems are one of the largest sources of phosphorus loss from a field. Open inlets are a part of the tile system that connects the field surface to the subsurface tile line. Open inlets are not covered in the field. Through an open inlet, phosphorus in soil solution can drain directly into the water from the field. Blind inlets are open inlets that have been capped and filled with gravel and soil to promote the filtration of water as it moves to the subsurface tile line (Kleinman et al., 2015). The use of a blind inlet can reduce phosphorus lost from a field by 50% or greater. When tile lines are used in cooperation with no-till practices, greater losses of dissolved phosphorus are observed due to more soil macropores leading directly to the tile lines (Kleinman et al.). The use of various combinations of management practices can have a detrimental effect on phosphorus losses when the practices are combined in the wrong ways.

Farmer interest in improving P management in fields can be initiated through discussions of the cost of lost P fertilizer. The USGS SPARROW models from 2002 predict that on an annual basis over 5.2 million pounds of phosphorus are lost due to exceeding the state's watershed assimilative capacity (Preston et al., 2011). The 2021 price for diammonium phosphate fertilizer was approximately $717/ton (Stockton and Burford, 2021), which calculates out to $1.28/lb phosphorus. That means that there is over $6.5 million lost annually from farmers due to phosphorus loss.

Farming is not the only industry that suffers from poor phosphorus fertilizer management. The recreational fishing industry is affected by degradation of waterways. Annually, fishing accounts for $683 million in revenue to the state of South Dakota (South Dakota Game Fish and Parks, 2022). Eutrophication of lakes harms game fish populations while also increasing the number of undesirable fish within these aquatic ecosystems. The common carp is considered an undesirable fish that is adapted for life in eutrophic conditions (Weber and Brown, 2009). The increased eutrophication decreases the population of game fish (bass, crappie, bluegill, and walleye) who rely on sight to hunt (Jackson et al., 2010). Eutrophication itself also harms game fish species by depleting oxygen from the water. The decrease in game fish due to eutrophication and the increase of nuisance fish has the potential to lead to decreased revenue for the state of South Dakota.

Other recreational water activities are impacted by eutrophication as well. Many states in the Midwest close lakes to swimming due to harmful algal blooms. These blooms are caused by cyanobacteria which produce chemicals that are harmful to humans and other animals (Watson et al., 2015). One of the most limiting factors in cyanobacteria growth is phosphorus, with approximately 67% of lakes effected by harmful algal blooms having cyanobacteria present (Wurtsbaugh et al., 2019). Phosphorus from agricultural contributes to this pollution. Reports indicate phosphorus contributed by agricultural practices contributes up to 75% of phosphorus lost from the field (Wurtsbaugh et al., 2019). This high amount of fertilizer loss is a major contributor to the production of the harmful cyanobacteria.

Phosphorus causes issues in waterways. Measures to reduce phosphorus concentrations in runoff that cause eutrophication must be implemented by landowners. The implementation of no-till management of agricultural lands has shown promise in reducing phosphorus losses to the environment. Utilizing no-till management reduces the amount of runoff that occurs from agricultural fields in three of the four preferential flow methods. When banded into the soil phosphorus only moves a couple of eighths of an inch from where the fertilizer granule was placed (Pagliari, 2017). No-till also promotes the soil microorganism that can help to release phosphorus that has been tied up in the soil. Reducing the loss of phosphorus from runoff and increasing the amount of soil biota that increases the release of phosphorus also allows for the reduction of inputs that are needed in a field. This allows for soil phosphorus levels to be maintained in lower solubility forms.

Soil Phosphorus Reactions and World Reserves of Phosphorus

Each year, farmers apply phosphate fertilizers to grow crops. Phosphorus soil chemistry is unique for two reasons. Phosphorus exists in the soil as an anion but does not leach. Phosphorus does not leach because of soil attachment. Phosphorus exists in three pools in the soils: (1) in soil solution, usually a couple lbs/a (2) active or labile, less than 100 lbs/a; and (3) fixed or non-labile, 100-1000 lbs/a. Phosphorus applied as inorganic fertilizers will remain in the soil for a few days to two weeks after application in the spring. Research at U of M has shown that fertilizer P will remain intact in the soil for 4-6 months when fall applied due to colder winter temperatures slowing P reactions between the solution and labile pools. Over time, fertilizer P will slowly migrate from the pool of solution P to non-labile. An agricultural management method that allows farmers to access the non-labile pool of P that has been amassed with yearly fertilizer additions would benefit the environment in at least two separate channels, preventing eutrophication and reducing the carbon footprint of phosphorus fertilizer mining.

Assessing the non-labile soil P pool is important for food security because the global supply of readily available rock phosphate is dwindling. Phosphorus fertilizers start with the mining of rock phosphate. Current estimates place depletion of rock phosphate from 50 (Boiarkina et al., 2018) to 300 years (Oloo and Asbon, 2020). Mining and transporting rock phosphate leaves a carbon footprint that has an environmental impact separate from eutrophication. The United States imported over 8 million short dry tons of phosphate fertilizers from Morocco and Russia between 2017-2019 (Kearns et al., 2020). The production of phosphorus fertilizers produces CO_2 in large quantities due to all the production steps. West and Marland (2002) found that phosphorus fertilizer production produces 165 kilograms of carbon for every metric ton of fertilizer produced from all the energy sources used for production and postproduction (West and Marland, 2002). The West and Marland study only considered US produced fertilizers and assumed the distance that the fertilizer traveled to be either 160 or 800 kilometers. With transportation from other countries, the amount of carbon produced will be higher than what was reported in the study.

To reduce the impact of phosphorus fertilizers in greenhouse gas production, there are a variety of methods that can be implemented to improve phosphorus use efficiency. Phosphorus binds with clay particles in the soil that can be eroded from a field by wind or water. Preventing loss of phosphorus through erosion will reduce the amount of fertilizer applied. Optimizing land use and maintaining soil quality on production lands is crucial (Schröder et al., 2011). This would allow for a longer period that soil phosphorus is accessible to plants.

Arbuscular mycorrhizal fungi (AMF) can be used as a tool to access the fixed pools of phosphorus. AMF greatly increases the surface area for plants to uptake nutrients when a relationship is formed between the plant roots and AMF (Marschner and Dell, 1994; Bagyaraj et al., 2015). AMF can release fixed phosphorus in the fixed phosphorus pools by producing organic acids that release phosphorus bound to iron ions (Andrino et al., 2021). AMF can release fixed phosphorus, however, the main contribution to plants from AMF is increasing the surface area the plants have for nutrient uptake.

Improving fertilizer recommendations will also increase phosphorus use efficiency. Current fertilizer recommendations are used to raise the soil test levels within a field. There are studies that have been conducted that show having soil phosphorus levels at an average to high level can be maintained at those levels for several years without the addition of fertilizer (Schröder et al., 2011). The reason the level can be maintained for several years is from the legacy and native phosphorus that is in the soil

(Withers et al., 2014). Using the principle of the legacy and native phosphorus soil test, levels can be maintained at lower levels. Applying enough phosphorus for the crop in the cropping season and utilizing native phosphorus can reduce the amount of phosphorus fertilizers that needs to be applied.

Soil Phosphorus Fixation, Soil Testing for Phosphorus, Philosophy to P Management in the Field, Phosphorus Fertilizer Placement and Arbuscular Mycorrhizal Fungi

South Dakota Soil test phosphorus levels (Olsen P) have been reported to vary from as low as 2.5 ppm up to 35 ppm. SDSU provides fertilizer recommendations for soil test levels ranging from 0 ppm (very low) to 21+ ppm (very high) (Murrell and Munson, 1999; Gerwing et al., 2020).

As previously mentioned, there are 3 different pools of phosphorus in the soil. If P is not used by the plant, it migrates from solution P to active P pool, to fixed P. There are a variety of different factors that affect the phosphorus fixation capacity of a soil. Soil physical, chemical, and biological factors affect the fixation capacity of a soil. Finer textured soils containing more clay have higher amounts of aluminum, iron, and calcium allowing for greater phosphorus fixation (“Important Factors Affecting Crop Response to Phosphorus,” 1999). In soils with lower temperatures, mineralization of organic phosphorus is decreases due to decreased microbial activity (Prasad and Chakraborty, 2019). The pH of the soil affects the phosphorus fixation capacity of a soil. Iron and aluminum are the primary ions that fix phosphorus in soils with pH below 6 and calcium in soils with pH above 7(Silva, 2012). The biological factor that affects phosphorus availability is the crop residue and soils which are laden with microorganisms.

Microorganisms also require P for metabolic activities. Uptake of P by soil biology is called immobilization(“Important Factors Affecting Crop Response to Phosphorus,” 1999).

Phosphorus fertilizer recommendations have been developed by land grant universities using a soil test phosphorus value and crop yield goal. The three most common soil test methods to measure phosphorus are Olsen, Bray-1, and the Mehlich-3 soil tests. The Olsen method is used for soils that are considered neutral or high-pH soil. The Bray-1 method is used for neutral or acidic soils. Mehlich-3 was developed for use in acidic, neutral, and basic soils (Sawyer and Mallarino, 1999). These soil tests are used to measure the most easily dissolved or desorbed forms of the phosphorus in the soil (Mckenzie and Bremer, 2003). The results from the soil tests are used to predict the probability of a crop’s response to phosphorus fertilization (Lee, 2021). Current recommended P fertilization rates from soil testing laboratories are based on an estimation of the P supplying ability of the soil and the projected need of the crop at the stated yield goal (Watson and Mullen, 2007).

Recommendations assume P fertilization is done by surface broadcast applications and that conventional tillage practices are used (Mallarino, 2009). There is substantial evidence that banding of P near or with the seed increases the efficiency of P crop uptake. Banding phosphorus fertilizers has the potential to allow for phosphorus fertilizer application rates to be reduced (Stecker et al.; Lu et al., 2019). There is also evidence that promoting healthy root systems and using fertilizer placement techniques can increase phosphorus (P) crop use efficiency (Campos et al., 2018). If all these steps are combined, it is probable that soil test P levels (P solubility) can be intentionally maintained at levels lower than those currently recommended without experiencing yield losses due to P deficiency. The net result should be a reduction in the potential for movement of phosphorus to aquatic ecosystems and more efficient use of P fertilizers.

The South Dakota State University (SDSU) fertilizer recommendation guide has five classifications for soil test phosphorus: very low, low, medium, high, and very high (Gerwing et al., 2020). For each of these categories there is a corresponding probability of response to fertilizer application. At the very low category, there is an >80% chance of response to fertilizer application. There is a 60-80%, 40-60%, 20-40% and <20% chance respectively for the low, medium, high, and very high categories.

SDSU has developed equations to determine the amount of P_2O_5 that needs to be applied per acre for crops commonly grown in SD. The equations are based on the Olsen soil phosphorus test. For each different crop and crop use, the structure of the formula has a crop nutrient requirement constant, and a second constant derived from a logarithmic regression. The other important components in the formula are the yield goal and the soil test phosphorus value (Gerwing et al., 2020). The SDSU formula for lbs-P2O5/A for corn is (0.7[crop nutrient requirement] -(0.044 [logarithmic regression constant] *soil test phosphorus) *yield goal) and the equation for soybean is (1.55- (0.14* soil test phosphorus) * yield goal).

There are different mindsets in managing phosphorus in the soil. One method is the sufficiency approach. The sufficiency approach applies enough phosphorus to the soil to sustain the crop planted for one growing season. Another method is the build and maintain approach. This method builds the soil test levels of phosphorus by applying an excess of phosphorus fertilizer to the soil. Once the desired soil test level is attained phosphorus fertilizer is applied at rates to maintain the test level as well as grow the season crop. (Macnack et al., 2017). The sufficiency approach applies the least amount of fertilizer to the soil while still providing enough nutrients to reach the intended yield goal of the crop (Shapiro et al., 2017). Applying nutrients to only feed the crop and not raise soil test levels will reduce the amount of phosphorus that can be lost from the field causing pollution of waterways. The build and maintain approach keeps phosphorus levels within the field, typically higher than the sufficiency approach. (Macnack et al., 2017). The soil test level in the build and maintain method does not recommend applying fertilizer if soil phosphorus levels are above the critical level.

It is generally accepted that conventionally tilled fields apply phosphorus fertilizers to the soil surface. These fertilizers are then tilled into the soil, incorporating the phosphorus with the soil. In no-till fields phosphorus fertilizers can be applied to either the soil surface or by banding the phosphorus fertilizer. Surface applying the fertilizer is less efficient in no-till fields as phosphorus moves by diffusion. Without incorporation of the fertilizer, the phosphorus will be trapped within the soil surface. Banding of the phosphorus fertilizers places the plant nutrients in the rooting zone of the plant (Farmaha et al., 2012; Alam et al., 2018).

Placement of fertilizers influences the use efficiency of phosphorus fertilizers. The use of surface applications of fertilizer (broadcast) allows for a greater chance for loss of soluble inorganic phosphorus through runoff from the field (Baker et al., 1982; Ruark et al., 2006). Applying phosphorus fertilizers in a band in the soil helps to reduce the loss of the soluble inorganic phosphorus (Kimmell et al., 2001). Smith et al. (2016) found when fertilizers were banded into the soil there was a reduction of 85 to 90% of soluble phosphorus loss when compared to the surface applied treatments (Smith et al., 2016).

Placing fertilizer in the soil reduces loss of soluble phosphorus from the field. However, phosphorus use efficiency can still be increased. Fertilizer should be placed in the right area in relation to the crop seed to increase use efficiency. Yield was optimized when phosphorus fertilizer was placed at 15 cm and 10 cm for corn and wheat (Smith et al., 2016). Broadcast and banded application methods are the two most common methods for applying fertilizers. Scientific knowledge increasingly indicates that under management systems where enhanced biological activity is promoted, these phosphorus fertilizer application methods behave differently as compared to traditional systems (Verzeaux et al., 2016).

Soil microorganisms also play a significant role in the release of phosphorus from the fixed P pool and phosphorus uptake by plants. The most well know organisms that aid plant phosphorus utilization are arbuscular mycorrhizal fungi. The large surface area of arbuscular mycorrhizal fungi hyphae and their ability to produce organic acids that solubilize insoluble mineral phosphate makes the fungi valuable symbiotes to plants. One of the problems with AM fungi in field situations is that AM fungi tend not to colonize plants when there is enough phosphorus available in the field (Gyaneshwar et al., 2002).

Mycorrhizal fungi are not the only soil microorganisms that can solubilize low solubility forms of phosphorus. There are P-solubilizing bacteria in the soil that constitute anywhere from 1-50% of the respective population of microorganisms in the soil. Arbuscular mycorrhizal fungi only constitute 0.1-0.5 %. The bacteria that solubilize phosphorus most effectively were found to be those located in the rhizosphere; but under sub-culturing, the bacteria lose their solubilizing ability. Unlike bacteria, fungi maintain their ability to solubilize phosphorus when sub-cultured, and in general have a greater capability to solubilize phosphorus. In no-till fields, higher activity of mycorrhizal fungi is reported. Tillage can destroy the fungal hyphae network. Maintenance of the hyphae network is important for AMF survival. No-till management of agriculture fields is reported to better maintain a population of AMF populations as compared to tilled fields.

Phosphorus solubilizing micro-organisms release protons or use organic acids to release phosphorus that isn’t readily available. When phosphorus is bound with calcium these organisms release exudates that decrease soil pH and cause releases of calcium bound P. When phosphorus is bound with iron or aluminum, microbes will release organic acids that can dissolve phosphate by chelating the ions and releasing the phosphorus. Another way of releasing phosphorus is by using phosphate-solubilizing micro-organisms that produce acid to directly dissolve mineral phosphate (Gyaneshwar et al., 2002). Once the phosphorus is released it can be taken up by the plants.

CHAPTER 2- SOIL TEST PHOSPHORUS LEVELS BASED ON RATE OF PHOSPHORUS FERTILIZER APPLICATION IN LONG TERM NO-TILL SITUATIONS

Introduction

Phosphorus (P) loading of waterways due to over-fertilization of P and erosion losses is a global problem. Loss of P from agriculture fields is considered a non-point source of pollution. The loads come from an increased use of phosphorus fertilizers and will continue to increase as global population growth escalates food demands. The increase in eutrophication will lead to economic loss: affecting fisheries, drinking water purification, as well as several other problems derived from eutrophication (Dodds et al., 2009).

The world's population growth escalates the need for food. Thus, agriculture needs to produce more crops on land already in use or bring more land into production. According to Mogollón et al. (2018) in their paper "Future Agricultural Phosphorus Demand According to the Shared Socioeconomic Pathways," it is likely that by 2050 phosphorus inputs could rise anywhere from 51-86% (Mogollón et al., 2018). More of the world's reserves will need to be mined because of the increased need for phosphorus fertilizers. Estimates place the world's phosphorus reserves, being used at the current and projected rates, could run out anywhere between the years 2170 and 2300 (Boiarkina et al., 2018; Daneshgar et al., 2018; Oloo and Asbon, 2020). Methods need to be developed to change current phosphorus fertilizer recommendations associated with increased food demands to create a more sustainable process.

The management of the field influences the rate that phosphorus can be lost from a field. Under management where tillage is frequently used, phosphorus losses from the field occurs at a higher rate than fields that are managed under no-till management practices (Buchanan and King, 1993). The losses of total phosphorus are greater in fields that have undergone tillage practices due to the destruction of soil structure. The tillage breaks up the soil structure, allowing for an increased rate of erosion to occur (Mikha et al., 2013; Jin et al., 2021). The eroded material carries phosphorus that has been bound to the soil material and enters streams, rivers, and lakes. While no-till management typically reduces soil erosion and eliminates some for the P losses due to wind and water erosion, P can still be lost from no-till fields. This loss occurs when phosphorus fertilizers are applied to the surface of the soil and rainfall events occur (Mcdowell et al.,).

Loss of phosphorus from fields is also affected by the rate that phosphorus is applied to the fields. The higher the rates of phosphorus applied, the more likely a loss will occur under the appropriate conditions (Tarkalson and Mikkelsen, 2004). During the first spring rainfall event the phosphorus load contained in the runoff increased based on the fertilizer rate. The higher phosphorus concentrations in the runoff come from the higher fertilizer application rates. (Shuman, 2002). The higher P load can be attributed to the sorption and desorption chemistry between phosphorus and soil. More P fertilizer is associated with increased amount of dissolved phosphorus (Kleinman et al., 2002). Fertilizer recommendation guides state that when soil tests phosphorus levels are very low, low, and medium, the addition of phosphorus fertilizers should have a correlating yield response (Slaton et al., 2005). This is not always the case though. In a study conducted by Rebecca Helget (2016), fields designated as “low” testing phosphorus only one of three fields had a positive response to the added phosphorus fertilizer (Helget, 2016). It has also been recognized that as the rate of phosphorus is increased the uptake efficiency of the plant is decreased. This occurs because there is more phosphorus available than what the plant requires to complete its lifecycle (Sun et al., 2015).

In SD, low soil test P and minor to zero yield response in corn and soybeans have been reported by numerous farmers who have incorporated the use of no-till for years. The objective of this experiment was to evaluate P fertilizer responses in a long term no-till management rotation that includes corn and soybean. The hypothesis is that soil test phosphorus can be intentionally maintained at low levels without seeing a significant loss in yield.

Materials and Methods

The field study is located at Dakota Lakes Research Farm, 18 miles Southeast of Pierre, SD. This study started in November of 2017. The fields used in this experiment have been farmed using no-till techniques since 1990. Phosphorus fertilizer applications had been used in a manner that reduced Olsen soil-test values to the 5-ppm range. Fertilizer applications were made based on how much phosphorus the crop would remove in the cropping season. The cropping sequence is a Corn-Corn-Soybean-Wheat-Soybean rotation. After wheat harvest, a cover crop is planted and terminated the following spring. Treatments for this were increasing rates of phosphorus fertilizers. Test parameters evaluated were soil test phosphorus levels, yield, phosphorus movement after application, and phosphorus accumulation in night crawler burrows.

Field Site:

The study takes place at Dakota Lakes Research Farm, established in 1990. The soil type is a Lowry silt loam. The field size is 1.8-hectacres. The center of the field has coordinates of 44.288243, -100.001752. Plot sizes were 103.6 meters in length by 6.1

meters wide. The length of the field is 161.5 meters, and the width of the field is 103.6 meters (Figure 2.1).

Shortly after winter wheat harvest a cover crop of oats (53 lbs/acre), barley (17.5 lbs/acre), and millet (29.5 lbs/acre) was planted on August 9, 2019. Volunteer winter what was allowed to grow as part of the cover-crop. The cover crop was planted using a John Deere 750 drill. After the cover crop was established a 1.2-meter-wide strip that ran perpendicular to each treatment was terminated before the first frost could occur. The rest of the cover crop was sprayed off on June 19, 2020, after the soybeans had reached the V3 growth stage. On May 14, 2020, soybeans (Pioneer P29A85L) were planted via a custom-built planter. Seeds were placed into the field in 50.8 centimeter spacing at 175,000 pure live seeds/acre. On May 11, 2021, the same planter was used to place corn seed (Pioneer P0220AM) at a rate of 36,000 pure live seeds/acre. During planting, fertilizer was side banded at the same depth as the seed (3.8 centimeters,) in a trench 7.6 centimeters to the side of the seed.

The bulk MAP fertilizer treatments were applied in the fall of 2017, 2019, and 2021 using a no-till drill with openers spaced 19 cm apart to place the fertilizer in bands at a depth of 3.8 centimeters. Soil test phosphorus levels had begun to be drawn down since the 2014 inception of this study at Dakota Lakes Research Farm. Each planting season phosphorus fertilizers were applied at a rate consistent with the removal rate of that cropping season’s crop. By applying fertilizer at a rate consistent with the crops removal rate, any excess phosphorus removed would lower the soil test levels.

Cropping System:

2020 Cropping Year:

Soil samples were collected on May 11, before planting, and November 4, post-harvest. Samples were taken at the 0-8 cm, 8-15 cm, 15-30 cm, and 30.-60 cm soil depths. Four cores at each depth were taken at four different points throughout each treatment strip (Figure 2.1). Each treatment had a total of 16 cores taken at each depth that were mixed into a composite sample. Due to there being no difference between any of the three treatments at the 30-60 cm depth the results of those soil samples will not be reported further.

Tissue analysis occurred four times in the 2020 growing season for soybean samples. Samples were taken at the V3 growth stage, the R1 growth stage, the R3 growth stage and the R6 growth stage. These samples were taken on May 15, June 23, July 9, and September 25, respectively. Samples were taken in 1.5-meter segments at four points throughout each treatment within the field for a total of 6 meters of samples taken. Four samples were taken by using a rice knife to cut the soybeans approximately 5 centimeters above the soil surface in each treatment. All four samples from an individual treatment are then combined to form a composite sample that was sent to Ward Laboratories for analysis. Ward Laboratories is located at 4007 Cherry Ave. in Kearney, NE.

A small trial was added in the 2020 growing season. The area was 1.5-meters wide and ran the width of the field across all treatments. In the fall of 2019, a 1.5-meter strip of the cover crop planted in the field was sprayed off using Round-up (glyphosate) herbicide. The purpose of this trial was to investigate if terminating a crop before a killing frost would result in (1) phosphorus remaining in plant cells or (2) phosphorus would be released by cells through rupture from frost. The residue samples, consisting of the cover crop that was killed off and residue from the last cropping season, were taken twice in the 2020 cropping season. The first sample was taken on May 15, a day after soybeans were planted in the termination study area, as well as outside of the termination study area. This was the only time residue samples were taken in the termination study area. The samples were tested to determine if early termination of the cover crop using an herbicide would stop loss of phosphorus from cellular rupture caused by frost. This was tested using inductively coupled argon plasma. Residue sampling occurred again on October 17 post-harvest. This residue sample was taken in the normal area of the field only and not in the termination study area. The October residue samples consisted of the soybean tissue that had gone through the combine and was tested using traditional tissue testing.

Residue samples were taken using a PVC plastic pipe square measuring 38cm x 38 cm. Four sections were sampled within each treatment over the total area. The square was placed in between planted rows and the residue on the surface was collected within the square. It is important to note that not all residue was able to be collected due to it being in heavy contact with the soil. The residue that had soil attached to it was not collected, as the soil could skew the laboratory analysis.

In May, the Cornell Water Infiltrometer was used to collect water samples in the soil phosphorus test level study field. The infiltrometer was also used in the termination area to assess phosphorus loss from the terminated cover crop area. The tests were run side by side with the infiltrometer being run in the termination study area first and secondly, in the P soil test study area. Some problems with this study occurred in both the rate and termination study areas. First, not all treatments produced run-off. In the high-water infiltration rate treatment, the Cornell Water Infiltrometer was not able to produce enough simulated rainfall to produce the runoff. The treatments that did produce runoff had their volumes measured. The first 250 mL of runoff was collected for analysis by Ward Laboratories. However, the results for the study are inconclusive and will not be discussed here. The results and discussion for the runoff and soil solution is found in appendix A.

The soybeans were harvested on October 8 using a 9410 John Deere combine mounted with a 6.1-meter-wide flex header equipped with a finger air reel. Grain samples from each treatment and replication were measured for moisture content. The moisture was collected first and then grain samples were dried until they did not lose any more weight from moisture loss. The weight was recorded, and the samples were sent to Ward laboratories for the routine tissue test analysis.

2021 Cropping Year:

Soil samples were taken one day post-plant on May 5. Samples were taken in each treatment in each rep. Samples were collected at four depths: 0-8 cm, 8-15 cm, 15-30 cm, and 30-60 cm, at four different locations throughout the treatment. The 16 cores were combined to make a composite sample. Soil samples were collected on October 29, after harvest, using the same method as described above. Due to there being no difference between the three different treatments at the 30-60 cm depth the results of those soil samples will not be reported further.

On August 3, soil samples were collected to measure the effect of earthworm burrows on phosphorus movement. Earthworm burrows were found by searching in between rows for the bottom corn leaf that had been dragged into the earthworm burrow. Once an earthworm burrow was found, a golf cup cutter was used to collect a soil core of 20 cm in depth directly over the burrow. The core is pressed out of the golf cup cutter leaving a cylinder of soil. The core is broken into disks that were 5 centimeters in height. Two types of samples were taken from these cores, a non-worm hole sample and a worm hole sample. The non-worm hole samples were taken from the edge of the disk and put into the non-worm hole sample container. The worm hole sample was taken by using a knife to cut around the worm hole, within 2 cm of the hole's location. This process is repeated with each disk that is broken off the core. It required 10 cores to make a composite sample with enough soil to be tested. The worm hole samples, and non-worm hole samples were then bagged and sent to Ward Laboratories for analysis.

Tissue samples were collected at four different growth stages throughout the 2021 cropping season. Samples were taken at the V3, V7, VT, and R6 growth stages. On June 10, a sample of 6 meters of row was gathered when the corn plants were at the V3 growth stage. Samples were collected in each treatment for each replication. Samples were gathered in 4 different 1.5-meter sections using a rice knife to cut the plant between 3 and 5 centimeters above the soil surface. After collecting the 4 different 1.5-meter sections, the sections were combined to form a composite sample. The samples were weighed and then oven dried for 10 days at a temperature of 80 degrees Celsius. After oven drying, the weight of the samples was recorded and packed to be shipped to Ward Laboratories for analysis.

The V7 tissue samples were taken on July 2. Samples were taken in 6-meter segments following the same collection pattern as with the V3 samples. Whole plants were not taken at the V7 growth stage, instead the uppermost collared leaf was removed from the plant and collected for the sample. The third set of tissue samples were collected on July 19 when the corn had reached the tassel growth stage or VT. The VT samples were collected in the same way that the V7 tissue samples except the leaf immediately below the ear leaf was selected. Both the V7 and the VT tissue samples were air dried for 10 days in a forced air dryer prior to being sent to Ward Laboratories for analysis.

On October 7, 2021, corn samples were hand harvested at the R6 growth stage. After collection, the corn plant was cut 15 centimeters above the soil surface, bundled together, and brought out of the field. The total number of plants was recorded. The ears were stripped from the plant while leaving the husks on the plant. The weight of the ears and plants were recorded. A subsample was taken where 10 plants were randomly selected. The subsample plants were cut into 61-centimeter sections where they were weighed, recorded, and bagged for drying. After the plants were harvested, 10 ears were randomly selected, weighed, and bagged for drying. The stalk samples were dried until easily broken by hand and weighed. The remaining stalks were shredded and weighed. The subsamples were sent to Ward Laboratories for analysis. Grain ears were oven dried for one week at 63 degrees Celsius. Once the drying was complete, the ears were hand shucked. The grain was weighed and sent to Ward Laboratories for analysis. The cobs were weighed to obtain a total weight of grain and cobs. After weighing, the cobs were discarded.

Runoff testing was conducted differently in 2021 as compared to 2020. The Cornell infiltrometer was not used. Instead, a rainfall simulator was constructed to perform the runoff testing. The runoff testing was conducted twice in 2021 cropping year. The first test (in-season) was on June 17 where the first replication of the study was completed. On June 21, 3 more replications were completed, and on June 22nd the final replication was completed. On October 23, runoff testing was conducted post-harvest. Due to being able to drive in the field the tests were able to be conducted in one day.

The rainfall simulator was constructed out of angle and flat iron measuring 50.8 cm x 76.2 cm. The dimensions were based on farmers' fields row width. The dimensions of the frame allow for the simulator to be used in different crop row widths. During the simulated rainfall event, 10 cm of water were applied in an average time of 12 to 15 minutes. A high application rate was required to produce runoff. The high-water infiltration capacity of the soils in this study is due to extended years in no-till management. Soil solution access tubes were inserted prior to the start of the simulated rainfall. A vacuum was drawn on the tubes and water was collected after a 24-hour period. Samples were sent to Ward Laboratories for analysis.

The results for the runoff and soil solution were inconclusive. Therefore, the results will not be discussed. However, the results and discussion for the runoff and soil solution can be found in appendix A.

Statistical Analysis:

Soil sample results were split by each depth and ran separately at each depth by the treatment and block. Analysis was conducted on plant tissue, grain, yield, runoff, and soil solution water by phosphorus fertilizer treatment and depth for soil samples. The worm burrow data was segregated by treatment, block, and location of the sample (inside or around the worm hole). ANOVA analysis was conducted using R programming version 4.0.2 (2020-06-22) (R Core Team, 2020). After ANOVA analysis, Least Significant Difference values were generated using the *lsd.test* function in R (Steel and Torrie, 1986). The *TukeyHSD* function was used for pairwise mean comparison (Yandell, 1997; Miller, 2012). For data visualization, the function *boxplot* was used as well as the *ggplot2* and *dplyr* package (Wickham et al., 2019).

Results

Soil Samples

The spring 2020 cropping season soil test phosphorus (STP) values measured by the Olsen phosphorus test were observed to differ by treatments based on $\alpha=0.01$ significance level. The differences were observed in the 0-8 cm depth. Treatment differences were not observed for the 8-15 cm and 15-30 cm depths. Fall soil samples did not show any difference between treatments over the three depths. Significant differences between treatments can be observed in table 2.1. Each individual ANOVA table and Tukey mean comparison table can be seen in appendix A.

In the spring of the 2021 cropping season, a significant difference was observed at the $\alpha=0.05$ significance level in the Olsen phosphorus test. A significant difference was observed in the 0-8 cm sampling depth. There was no difference between treatments at the other soil sample depths. Phosphorus fertilizer treatments did not affect soil test phosphorus for soil test depth: 0-8 cm, 8-15 cm, and 15-30 cm depths. Statistical results are reported in table 2.2. Individual ANOVA tables and Tukey mean comparison tables are in appendix A.

In both 2020 and 2021, the Mehlich, Bray and Total Phosphorus tests were run in conjunction with the Olsen soil test. In the Spring of 2020, all tests indicated a significant difference between treatments. Soil samples collected in fall 2020 and tested using the Olsen, Bray, and Mehlich showed no differences between MAP fertilizer treatments. However, the Total Phosphorus test results were different between fertilizer treatments (Table 2.3). In the Spring of 2021, soil P test results measured by the Olsen soil phosphorus were significantly different between MAP fertilizer treatments. Soil test P results between MAP fertilizer treatments were not different between any of the three test methods.

The worm burrow analysis did not show any significant differences between treatments. The analysis did not show any significant difference between the inside of the worm hole as compared to around the worm hole among MAP fertilizer treatments (Table 2.9).

Tissue Samples

At the V3 and full plant maturity growth stages, there were no significant differences between fertilizer treatments. At the R1 growth stage, there was a significant difference between treatments at the $\alpha=0.05$ significance level (Table 2.5). At harvest, there was no significant difference in the grain yield between fertilizer treatments. Grain analysis showed a significant difference of P_2O_5 removal by the grain between treatments at the $\alpha=0.1$ significance level (Table 2.6).

The V3, V7, and VT tissue samples of the 2021 corn plants did not show any significant difference between treatments. At full maturity, there was a significant difference between the fertilizer treatments at the α=0.05 significance level for plant P concentration (Table 2.7). There was no significant difference between treatments, in terms of yield. P removal and P_2O_5 had a significant value at the α=0.1 significance level (Table 2.7).

Discussion

The industry standard for soil sampling is at 0-15 cm soil depth. The study conducted at Dakota Lakes Research Farm sampled at 0-8 cm, then 8-15 cm sampling depth to accentuate the differences between treatments that were applied. Other studies have sub-divided the sampling depth for P (Slaton et al., 2005; Mikha et al., 2013; Pavinato et al., 2017). In the 2020 soybean growing season, there was a significant difference between the treatments, at the 8 cm sampling depth, where an extra 200 lbs of MAP fertilizer was applied per application and the check treatment. A significant difference was observed between the 200 lbs and 100 lbs/A of MAP treatment. Fertilizer applications of 100 and 200 lbs-MAP/acre were applied in 2014, 2017, and 2019, to create different P soil test levels. By the fall of 2020, post-harvest, soil test levels between treatments did not show any significant difference between the three treatments. This was due to the transition of the phosphorus to a less soluble form as well as the uptake of phosphorus by the soybeans up through harvest.

In the spring of 2021, results from the soil samples at the 0-38 cm depth were different between 200 lbs of MAP and the check treatment. The return to significance from the fall of 2020 to the spring of 2021 is due to the decomposition of the soybean litter that was left after harvest and the movement of non-labile phosphorus to the labile phosphorus pool. The release of the phosphorus after harvest is consistent with findings of Buchanan and King (1993) who found that within 40 weeks, 50% of phosphorus from crop residue was released in no-till fields (Buchanan and King, 1993). Throughout the 2021 growing season, phosphorus uptake of the corn and movement of labile phosphorus to non-labile pools led to no significant difference at any soil sampling depth in the fall, post-harvest.

In the 2020 growing season, there was no significant difference in phosphorus concentrations within plant tissue at the V3 growth stage. The study conducted by Helget (2016) found plant tissue P was widely variable at early soybean growth stages of soybean. (Helget, 2016). This would suggest that location or some other environmental factor would affect the uptake of phosphorus and not rate. The next plant tissue was analyzed during the R1 growth stage. Significant differences in plant tissue P were observed among fertilizer treatments at this growth stage. Treatments where higher rates of MAP fertilizer were applied had higher concentrations of phosphorus within the plant tissue. At the R1 growth stage, plants are hypothesized to increase nutrients for grain production; Helget’s research supports this (Helget, 2016). At harvest, there was no significant difference in tissue concentrations of phosphorus. At this point in the growing season, the vegetative portion of the plant does not need the phosphorus for the tissue and the plant has translocated phosphorus to the seed. The research done at Dakota Lakes Research Farm partly supports this. When a statistical analysis was run on the phosphorus concentration in the harvested grain a significant difference was observed between the check treatment and the MAP applied at 200 lbs per acre treatment. However, no difference between either treatment was shown when compared to the 100 lbs of MAP applied per acre treatment.

Phosphorus concentrations in corn tissue in the 2021 growing season did not show any significant difference through any of the vegetative stages. Samples were again taken prior to harvest when the plants had reached full maturity and were observed to be different between the MAP fertilizer treatments. The findings of this research agree with the findings of Barry and Miller (1989). They found the V3 growth stage, with varying levels of phosphorus applied in nutrient solutions, had no difference in uptake. Barry and Miller found that at the V6 and silking there was a significant difference between the different rates of phosphorus applied in plant tissue concentrations (Barry and Miller, 1989). The factor that accounts for the differences seen between Barry and Miller, 1989, and the research at Dakota Lakes Research Farm is that the study conducted by Barry and Miller (1989) phosphorus solutions were applied daily. The study conducted at Dakota Lakes Research Farm applied the treatment on an annual basis. As will be discussed in Chapter 4 Arbuscular mycorrhizal fungi have also influenced the results seen at Dakota Lakes Research Farm. At full maturity, the results from Dakota Lakes Research Farm are comparable to that of Barry and Miller (1989) where the plant phosphorus was higher in treatments when higher concentrations of phosphorus fertilizers were applied. This is further explained by Bąk et al. (2016) where there were significant differences in P concentrations based on fertilizer rate at maturity. Bąk et al. (2016) found that at lower rates of phosphorus, the efficiency of the plant uptake increased allowing better transfer of phosphorus to the grain (Bąk et al., 2016). Less efficient transfer in the plant causes the phosphorus to increase as it is not passing to the grain.

At harvest, there was no significant difference between the three treatments in terms of yield or P_2O_5 removal per acre. The findings conflict with the findings of Bąk et al., 2016 and Barry and Miller, 1989, where both studies indicate that there was a difference between the lowest and highest rate treatments (Barry and Miller, 1989; Bąk et al., 2016). Fertilizer rates are not the only factor affecting the yield and grain phosphorus concentrations. Tillage practices affect the availability of phosphorus. Bąk et al. (2016) and Barry and Miller's (1989) research did not document the type of tillage used in the experiments (Barry and Miller, 1989; Bąk et al., 2016). As will be explored further in Chapter 4 arbuscular mycorrhizal fungi plays a role to provide phosphorus that is not measured by traditional soil test methods. Arbuscular mycorrhizal fungi survive in no-till soils because the hyphae are not destroyed in tillage. The combination of diverse crop rotations and long term no-till changes the soil biology and has an impact on phosphorus soil availability.

The results from the earthworm burrow tests were not affected by MAP fertilizer treatments. Earthworm casts contain a more significant amount of phosphorus than normal soil (le Bayon and Binet, 2006). It was theorized that phosphorus dissolved in water would be deposited within worm burrows as the water infiltrated into the soil profile, but this was not observed at Dakota Lakes Research Farm. Another theory as to why there may be increased levels of phosphorus within earthworm burrows is the burrows become microbial hotspots that release phosphorus. This would explain why there were no significant results found at Dakota Lakes Research Farm as their management practices benefit the soil microbial pools. Hoang et al., 2016 confirmed that in the earthworm burrow there is a spike in the amount of microorganism's present

(Hoang et al., 2016) Therefore, soils that contain a high amount of microorganisms would not demonstrate a change.

Conclusion

It was found that varying the rate of phosphorus fertilizer will significantly increase the soil test levels in the surface soil, at the 0-8 m sample depth (Table 2.1-Table 2.4). In the fall of 2020 and 2021, there was no significant difference in the Olsen soil test levels. However, soil test P was impacted by MAP fertilizer treatments. (Table 2.3). The same was not observed for total phosphorus in 2021 fall soil samples. The addition of bulk applied phosphorus fertilizers will increase the soil test levels of a field. However, this does not mean increased soil test levels will increase yield. In the study there was no significant difference between the yield and the different phosphorus treatments. (Table 2.6 and Table 2.8) This indicates that there are other factors besides the amount of fertilizer applied to a field that contributes to yield. In Chapter 4 the contributions of arbuscular mycorrhizal fungi to plant uptake of phosphorus will be discussed.

Even though yield was not affected by the different phosphorus application rates, the concentrations of plant phosphorus were affected by the phosphorus fertilizer rates (Table 2.5- Table 2.8). In 2020, at the R1 growth stage, plant concentrations of phosphorus had significant variance based on the fertilizer rate treatment. As the season progressed, treatments with higher rates of MAP fertilizer were correlated with higher plant tissue P concentrations. At harvest, the soybean tissue phosphorus concentrations were no longer significantly different. However, the grain concentrations did show significant differences based on treatments (Table 2.6 & Table 2.7). As the soybean plants matured after R1, the plant diverts phosphorus to the grain for storage. Hence, at full maturity, the tissue did not show any significant difference.

In 2021, differences in corn tissue P were observed at full maturity. Significant differences in plant concentrations were not observed earlier in the season, like with soybean at the R1 growth stage, due to the soil test levels being much lower in the spring of 2021 than the spring of 2020. There was not as much phosphorus in the soil to be drawn from so plant concentrations were not as varied earlier in the season as in 2020.

The infiltration and runoff portion of the study are not presented but can be viewed in Appendix A. An important note from this study is that the percent runoff from the fields at Dakota Lakes Research Farm is very low. The average is less than 10% runoff with the Cornell water infiltrometer and less than 5% when using the rainfall simulator (Table 2.10). Runoff rates are low due to years of increasing the soil structure from no-till and promotion of biodiversity of organisms living in the soil. With the focus on the promotion of biodiversity in no-till soil, earthworms and their burrows were thought to influence the movement of phosphorus. However, our test found that earthworm burrows do not act as a confluence for phosphorus (Table 2.9).

Tables:

Table 2.1 Olsen Soil Test Phosphorus Levels in the 2020 Soybean Cropping Season in the P Rate Experiment Field

	Olsen Soil Test Levels (ppm) Spring			Olsen Soil Test Levels (ppm) Fall		
	0-3”	3-6”	6-12”	0-3”	3-6”	6-12”
Check	7.7 b	5.2 a	3.6 a	6.9 a	4.3 a	3.9 a
100 lbs MAP	11.2 b	5.2 a	3.7 a	7.0 a	4.3 a	3.9 a
200 lbs MAP	16.8 a	4.9 a	3.7 a	8.2 a	4.7 a	3.5 a
Significant	0.004	NS	NS	NS	NS	NS

Table 2.2 Olsen Soil Test Phosphorus Levels in the 2021 Corn Cropping Season in the Phosphorus Rate Experiment Field

	Olsen Soil Test Levels (ppm) Spring			Olsen Soil Test Levels (ppm) Fall		
	0-3”	3-6”	6-12”	0-3”	3-6”	6-12”
Check	6.0 b	6.3 a	5.4 a	7.2 a	6.9 a	6.9 a
100 lbs MAP	5.9 b	5.7 a	5.3 a	7.5 a	6.9 a	6.6 a
200 lbs MAP	7.6 a	7.2 a	5.4 a	7.3 a	7.0 a	6.8 a
Significant	0.04	NS	NS	NS	NS	NS

Table 2.3 Comparison of Four Different Soil Tests in the Spring and Fall of 2020 at the 0-3” Depth

	Spring 2020				Fall 2020			
	Olsen (ppm)	Total Phosphorus (ppm)	Mehlich (ppm)	Bray (ppm)	Olsen (ppm)	Total Phosphorus (ppm)	Mehlich (ppm)	Bray (ppm)
Check	8 b	661 ab	8 c	6 c	7 a	607 b	9 a	4 a
100 lbs MAP	11 b	643 b	13 b	10 b	7 a	617 ab	9 a	5 a
200 lbs MAP	17 a	699 a	22 a	17 a	8 a	644 a	11 a	6 a
Significant	0.004	0.07	< 0.005	< 0.005	NS	0.04	NS	NS

Table 2.4 Comparison of Four Different Soil Tests in the Spring and Fall of 2021 at the 0-3” Depth

	Spring 2021				Fall 2021			
	Olsen (ppm)	Total Phosphorus (ppm)	Mehlich (ppm)	Bray (ppm)	Olsen (ppm)	Total Phosphorus (ppm)	Mehlich (ppm)	Bray (ppm)
Check	6 b	589 a	5 a	3 a	7 a	612 a	7 a	3 a
100 lbs MAP	6 b	594 a	5 a	2 a	8 a	563 a	6 a	3 a
200 lbs MAP	8 a	637 a	8 a	4 a	7 a	572 a	8 a	3 a
Significant	0.04	NS	NS	NS	NS	NS	NS	NS

Table 2.5 Soybean Plant Tissue % Phosphorus Concentration throughout the 2020 cropping season in the Phosphorus Rate Experiment Field

	% P V3	% P R1	%P Harvest	%P in Grain
Check	0.394 a	0.340 b	0.110 a	0.429 b
100 lbs MAP	0.417 a	0.371 a	0.108 a	0.459 ab
200 lbs MAP	0.424 a	0.376 a	0.127 a	0.487 a
Signifcant	NS	0.01	NS	0.02

Table 2.6 Soybean Plant and Grain Phosphorus Concentrations, Pounds of P and P_2O_5 Removed, and Yield at Harvest in the 2020 Growing Season in the Phosphorus Rate Experiment Field

	Plant			Grain			Yield
	P (%)	P (lbs/a)	P_2O_5 (lbs/a)	P (%)	P (lbs/a)	P_2O_5 (lbs/a)	bushels/a
Check	0.110 a	34 a	77 a	0.429 b	16 b	36 b	61.1 a
100 lbs MAP	0.108 a	35 a	80 a	0.459 ab	17 ab	38 ab	60.6 a
200 lbs MAP	0.127 a	36 a	83 a	0.487 a	18 a	41 a	61.6 a
Significant	NS	NS	NS	0.02	0.06	0.06	NS

Table 2.7 Corn Plant Tissue % Phosphorus Concentration Throughout the 2021 Cropping Season in the Phosphorus Rate Experiment Field

	% P at V3	% P at V7	% P at VT	%P at Full Maturity
Check	0.503 a	0.333 a	0.282 a	0.073 b
100 lbs MAP	0.524 a	0.339 a	0.281 a	0.071 b
200 lbs MAP	0.562 a	0.371 a	0.311 a	0.113 a
Significant	NS	NS	NS	0.04

Table 2.8 Corn Plant and Grain Phosphorus Concentrations, Pounds of P and P_2O_5 Removed, and Yield at Harvest in the 2021 Growing Season in the Phosphorus Rate Experiment Field

	Plant			Grain			Yield
	P(%)	P (lbs/a)	P_2O_5 (lbs/a)	P (%)	P (lbs/a)	P_2O_5 (lbs/a)	bushels/a
Check	0.073 b	7.3 b	17 b	0.299 a	29.4 a	67.3 a	208 a
100 lbs MAP	0.071 b	7.4 b	17 b	0.32 a	31.9 a	73.1 a	209 a
200 lbs MAP	0.113 a	12.2 a	28 a	0.32 a	32.5 a	74.3 a	212 a
Significant	0.04	0.06	0.06	NS	NS	NS	NS

Table 2.9 Olsen Soil Phosphorus Analysis of Worm Burrows as Phosphorus Sinks in the 2021 Corn Growing Season

	Inside Worm Burrow (Olsen Soil Test ppm)	Around Worm Burrow (Olsen Soil Test ppm)
Check	7.1 a	7.3 a
100 lbs MAP	5.3 a	5.2 a
200 lbs MAP	5.3 a	8.3 a
Significance	NS	NS

Table 2.10 Average Amount of Water Applied, Average Runoff, and Average % Runoff by Treatment

	2020 Cornell Water Infiltrometer						2021 Rainfall Simulator		
	Sprayed			Not Sprayed			No Spray Treatment		
	Average Water Applied (mL)	Average Runoff Volume (mL)	Average % Runoff	Average Water Applied (mL)	Average Runoff Volume (mL)	Average % Runoff	Average Water Applied (mL)	Average Runoff Volume (mL)	Average Percent Runoff
Check	9855	350	4%	9585	613	6%	39329	1484	4%
100 lbs MAP	12132	424	3%	11475	920	8%	39329	1222	3%
200 lbs MAP	11133	1104	10%	10125	326	3%	39329	520	1%

Figures:

Figure 2.1: Arial image of the field where the different rates were applied to the soil. The central location of this field is 44.288243, -100.001752. The red box represents the location within the field where the study took place. The green boxes each represent 1 treatment pass in which samples were taken. The treatments are labeled with the pass number, the replication number, and the treatment in that pass. Each treatment was approximately 103.6 meters in length.

CHAPTER 3- PLACEMENT OF PHOSPHORUS FERTILIZERS IN NO-TILL SITUATIONS

Introduction

There are a variety of fertilizer forms as well as ways that fertilizers are applied to the soil. Some of the most common placement methods are broadcasting (surface application), banding, and placement of the fertilizers with the seed (Wiens et al., 2019). Phosphorus fertilizers come in two different forms that can be used, solid and liquid. Common solid inorganic fertilizers are MAP (mono-ammonium phosphate), DAP (di-ammonium phosphate), and TSP (triple super phosphate) (Darch et al., 2014; Jalali and Jalali, 2020). Liquid inorganic phosphorus fertilizer is most found as 10-34-0. Organic forms are most commonly various forms of manure from different livestock that can be in the form of solid or liquid (Jalali and Jalali, 2020).

How a phosphorus containing fertilizer is deposited on or in the soil can have an influence on the amount of the fertilizer that is lost due to runoff from the field. The use of liquid phosphorus is not as common as that of dry phosphorus fertilizers. Using liquid fertilizer does have an advantage over dry fertilizer in the case of runoff loss. In a study conducted by Sharpley and Syers (1983), the researchers found that surface application of liquid super phosphate fertilizer versus super phosphate in its dry form resulted in dissolved phosphorus and total phosphorus loss being reduced in the liquid fertilizer application (Sharpley and Syers, 1983). When fertilizers are applied into the soil, using either banding or injection techniques, the amount of dissolved phosphorus is reduced significantly compared to the same fertilizers applied on the surface (Williams et al., 2018). Both Williams and Sharpley and Syers agree that runoff loss is decreased with increased soil contact with the fertilizers (Sharpley and Syers, 1983; Williams et al., 2018).

The placement of phosphorus fertilizers can influence the uptake of phosphorus in plants. By placing the phosphorus fertilizer closer to the seed, the placement method allows for an increased uptake by the plants earlier in their growth cycle. (Rosa et al., 2020). Rosa et al. (2020) had these results with soybeans, while John MacLeod (1968) reached the same conclusion with corn (MacLeod, 1968). In soils with poor water holding capability, the use of deep banding is recommended. This allows for a more efficient use of phosphorus and water in the root system as it searches for water deeper in the soil profile (Kang et al., 2014). When soils have a sufficient amount water holding capabilities the use of regular banding is the most efficient application method for placing fertilizer (Freiling et al., 2022).

The macropores in the soil can also be affected by the placement of the fertilizers. The use of any planter or machinery that places fertilizer into the soil will end up disturbing or destroying the macropores that have been built in the soil. In the early spring when fertilizers are applied, the disruption of the macropores is a benefit because it helps prevent the loss of phosphorus through drain ways (Williams et al., 2018). The use of discs for tillage or for planting disrupts macropores thus blocking paths for phosphorus to exit the field. This is important for keeping the nutrients in the field until plants can utilize them and not lose the nutrients to either runoff or infiltration.

Management practices, such as tillage, affect how phosphorus fertilizers should be applied. While there is heated debate on what management practice is the correct method, fertilizer placement does respond differently based on the management practice used. When broadcasting, applying fertilizer to the soil surface, the most efficient use of the fertilizer requires the fertilizer to be incorporated into the soil (Borges and Mallarino, 2000). This will lead to distribution of phosphorus throughout the soil profile. When a no-till method is utilized, and a surface application is used, the stratification of phosphorus is most prevalent in the top 6 inches of the soil (Holanda et al., 2008). The use of banding in no-till and strip till practices reduces loss of phosphorus to runoff as well as provides localized nutrients at the placed depth (Fernández and Schaefer, 2012). When banding fertilizer, the depth that the fertilizer is applied also has an effect. Placing the fertilizer higher in the soil profile seems to have beneficial implications. The high band stimulates the root growth and uptake of the phosphorus nutrient allowing for more root biomass (Alam et al., 2018).

Materials and Methods:

The field study occurred at Dakota Lakes Research Farm located 18 miles Southeast of Pierre, SD. The sampling equipment used in this study was the same as the rate study. The cropping rotation is the same as described in Chapter 2. The study was established in November of 2017. The data being presented is from the 2020 and 2021 cropping seasons. The cropping sequence is a Corn-Corn-Soybean-Wheat-Soybean rotation. After wheat harvest, a cover crop is planted and terminated the following spring. The objective of this study was to find how placement methods of phosphorus fertilizers affected yield, grain and tissue phosphorus concentrations, and phosphorus movement after application.

Field Site:

Dakota Lakes Research Farm was established in 1990. The placement study field consists of three soil types: Canning loam, Dorna silt loam, and Lowry silt loam at 14.1%, 72%, and 13.9% total field area. The field size is 220 meters by 195 meters, with each treatment being 12.2 meters wide and running the length of the field. In 2020 soybeans, Pioneer P29A85L variety, were planted via a custom-built planter that placed the seeds into the field in 50 cm spacing at 175,000 pure live seeds/acre. In 2021, the same planter was used to place corn seed, Pioneer P0220AM variety, at a rate of 36,000 pure live seeds/acre.

Cropping System:

The field rotation is Corn-Corn-Soybean-Wheat-Soybean. The crop in 2020 was soybean and in 2021 corn was grown. The Dakota Lakes Research Farm is a strictly no-till farm and tillage has not been used since the inception of the farm in 1990.

2020 Cropping Year:

On August 7, 2019, a mix of 53 lbs of oats, 29.5 lbs of millet, and 17.5 lbs of barley were drilled into the field, using a John Deere 750 drill, as a cover crop. On July 1, the cover crop was terminated with herbicide. The soybeans were at the V4 growth stage. The cover crop was intended as another treatment in this study. However, due to an error when spraying the cover crop, this treatment was not included.

On May 12, 2020, Pioneer P29A85L variety soybeans were planted, and the two fertilizer treatments were applied at planting. The banded fertilizer consisted of a 90% monoammonium phosphate (MAP: 11-52-0) and 10% potash (KCl: 0-0-62) blend that was applied 7.6 centimeters to the side of the seed at a depth of 5 cm. The application rate was 38 lbs fertilizer blend per acre. The surface treatment was applied by constructing a broadcast bar for the planter. The hoses that fed the fertilizer for the banded treatment were then disconnected from the banding unit and attached to the broadcast bar allowing for the fertilizer to be broadcast onto the surface of the soil during the seeding process (Image 3.2 and 3.3). The fertilizer for the surface was the same as the banded treatment, a 90% MAP and 10% KCl blend applied at 38 lbs of the blend per acre. Five replications of each treatment were applied to the field. Each replication, for a single treatment, was 36 rows. The exception was the second banded replication, that treatment had a total of 60 rows. This was done so that there would be 36 rows in the plot and allowed for 24 rows of border.

Soil samples were taken on May 11 before the field was planted and sent to Ward Laboratories for analysis. During the 2020 cropping season, soil samples were only collected in the spring. The samples were taken at the 0-8 cm, 8-15 cm, 15-30 cm, and 30-60 cm depths. Four cores were taken in each span (Figure 1.1) for each treatment per replication for a total of 16 cores per depth. Each of the depths was mixed into a composite sample for each treatment in each replication. Due to there being no difference between the treatments at the 30-60 cm depth the results of those soil samples will not be reported further.

Tissue samples of the soybeans were taken three times throughout the growing season. The first sample was collected on June 23 at the V3 to V4 growth stage. The second samples were taken on July 13 at the R1 growth stage. Finally, the last set of samples were taken on September 26 prior to harvest. The samples were taken in 4-1.5-meter sections, for a total of 6 meter of row sampled. The plants were cut approximately two inches above the soil using a rice knife. One 1.5-meter section was taken in four different areas of each treatment. The 4 samples were combined into one composite sample for that treatment and sent for laboratory analysis. This was done for each treatment in each replication

In July, the Cornell Sprinkle Infiltrometer was used in combination with soil solution access tubes to measure: the amount of runoff occurring on the field, the amount of infiltration, also the phosphorus concentration of the infiltrated water. The infiltrometer was calibrated to apply 30 cm of water per hour. The infiltration ring that the infiltrometer sits on was pounded into the soil until the hose coming from the ring is flush and level with the ground inside the ring. A hole was then dug at the other end of the hose using a golf-cup cutter or a spade for the purpose of placing a beaker into the hole for runoff collection. A soil solution sampling probe was inserted near the outside of the ring at an angle that reached the center of the infiltration ring at 60 cm and a core was taken to 21 inches. The core was split into a 0-15 cm and a 15-30.5 cm section. The samples were weighed as moist soil, oven dried, and weighed after drying to determine moisture at the time of sampling. The cores were taken using a smaller probe the same size as the ceramic tip of the soil solution access tube. Then another three inches of soil was removed from the same hole, bringing the hole total to 60 cm.

A soil solution access tube was placed into the hole and snuggly pressed into place. Soil was packed around the top of the hole completing the seal allowing for a vacuum of 55-60 psi to be drawn into the tube. A second soil solution access tube was placed approximately three feet away where there wasn’t any infiltration test taking place. The purpose of the second tube was to see the difference in phosphorus movement when heavy amounts of water were applied as compared to where there was no water applied.

The Cornell infiltrometer was then filled, prepared, and placed on the infiltration ring. The air stop restricts water flow was removed at the same time as a timer was started. Once runoff had begun, the time was noted, and the runoff amount was measured after every 10 minutes for one hour. The first 250 mL of runoff was collected, bottled, and placed in a refrigerator until they were shipped to Ward Laboratories for analysis.

Water samples were collected each day for three days after the infiltration treatment was applied. The water was stored in a refrigerator in between each collection period. After the last collection, the water was measured for total volume collected. The samples were then sent to Ward Laboratories for analysis.

Because results were inconclusive, they will not be discussed. However, the results and discussion for the runoff and soil solution can be found in appendix B.

On October 8, 2020, the soybeans were harvested with a 9410 John Deere combine mounted with a 6-meter platform equipped with a finger air reel. Samples were taken from each replication of each treatment. Grain harvest moisture was collected at harvest. The grain was dried, and dry weight was collected.

2021 Cropping Year:

On May 11, 2021, Pioneer corn, variety P0220AM, was planted using the banded and surface application methods of applying fertilizer. The fertilizers were applied at a rate of 62.8 lbs of a 90% monoammonium phosphate (MAP: 11-52-0) and a 10% Potash (KCl: 0-0-62) blend per acre. The treatments followed the same structure as the 2020 cropping year. Liquid UAN and AMS (N and S fertilizes) at a nutrient rate of 45-0-0-4.5 was applied in the side band position on all treatments at seeding.

Soil samples were taken at two different periods during the 2021 cropping season, pre-plant, and post-harvest. The first set of samples were taken on May 11 where the surface applied portion of the study was sampled. On May 12, the banded portion of the study was sampled. Soil sample collection was the same between 2020 and 2021. Samples were collected at 0-8 cm, 8-15 cm, 15-30 cm, and 30-60 cm depths. In each treatment, 16 cores were taken at each depth. Four cores were taken at four different locations within a treatment to make a composite sample that was bagged and sent to Ward Laboratories for analysis. This process was repeated for each of the five replicates in the study. Soil samples were also collected on October 30 after the field was harvested using the same method as in May. The 30-60 cm samples were not collected. It was decided that these samples did not need to be taken due to the lack of variation amongst treatments.

In the 2021 cropping season, tissue samples were taken four times. The samples were taken at the V3, V7, VT, and R6 growth stages. The V3 tissue samples were taken on June 11. Four 1.5-meter sections of whole plants were cut at the soil level using a rice knife for a total of 6 meters sampled. The samples were weighed and then stored until they were placed in an oven for drying. On June 27’ the first half of the samples were placed in the oven to dry at a temperature of 80 degrees Celsius. After a 24-hour period, the weight was checked and marked on the bag. After another 6 hours, the weight of the same bag was checked again. The weight had not changed indicating the samples were dry. The bags were removed and weighed. The second half of the samples were then placed in the oven and the same process was repeated. The samples were then packaged and shipped to Ward Laboratories for analysis.

The second set of tissue samples was taken on July 6 at the V7 growth stage. The samples were taken using a similar method to the V3 plant samples. However, instead of taking the whole plant at the V7 growth stage, only the upper most collared leaf of the plant was taken. The same process was done with the third set of tissue samples taken on July 20, at the VT growth stage with the leaf immediately below the ear leaf was collected. The V7 leaf samples were air dried using forced air being blown through an air-drying setup. The samples were dried for 10 days before being packaged and shipped to Ward Laboratories

On October 8, 2021, corn samples were hand harvested. The corn was at the R6 growth stage at this time. Four 1.5-meter sections throughout each treatment in the field was collected for a total of 6-meters sampled per treatment. Each 1.5-meter section was to be packaged and sampled independently from the other samples of the same treatment and replication. The cobs were stripped from the stalk and weighed. The stalks were chopped into 60-centimeter sections for ease of handling and weighed with the husks of the corn ear. The stalk samples were air dried to remove moisture to mulch the samples.

When the stalks reached the point where they were easily broken by hand, they were fed through a garden mulching machine to shred the stalks. The total weight of the shredded stalks was recorded before taking a subsample of the shredded stalks that were sent for analysis. The ears were oven dried for the period of one week at a temperature of 63 degrees Celsius. After the ears were dried, the grain was hand shelled from the cob. The grain was weighed and bagged in a sealed bag. The de-kernelled cobs were also weighed and recorded. The grain samples were sent to Ward Laboratories for analysis.

Results for the runoff and soil solution were inconclusive. The tests were run too late in the season to obtain meaningful results. Multiple inches of irrigation water and rainfall had been applied to the field by the time the tests were run. By that point, the surface applied MAP fertilizer had been dissolved and moved into the soil or left the field via runoff. Therefore, the inconclusive results will not be discussed in this chapter. However, the results and discussion for the runoff and soil solution can be found in Appendix B.

Statistical Analysis:

Soil samples were split by depth. They were analyzed separately by depth, treatment, and block. The tests were ran using the value from the laboratory by the treatment for tissue, grain, yield, runoff, and soil solution water. ANOVA was administered using R programing R version 4.0.2 (2020-06-22) (R Core Team, 2020) where the *aov* function (Chambers et al., 1992) was used. After ANOVA was run, LSD numbers were applied using the *lsd.test* function in R (Steel and Torrie, 1986). The *Tukey HSD* function was used for pairwise mean comparison. The function *boxplot* was used as well as the *ggplot2* package for data visualization (Wickham, 2016).

Results

Soil Samples

In the 2020 cropping season, the soil test levels of phosphorus measured by the Olsen Phosphorus Test, in the spring, did not find any differences between the two treatments (Table 3.1). Fall soil samples were not taken in the 2020 cropping season.

In both the fall and spring of 2021, like in the 2020 cropping season, no differences were observed between treatments in the Olsen phosphorus test. (Table 3.2).

Tissue Samples

Soybean tissue samples throughout the 2020 growing season did not show any significant difference between phosphorus levels (Table 3.3). Grain analysis did not show any difference between phosphorus levels within the grain (Table 3.4).

The V3 tissue samples of the 2021 corn plants showed significant difference in tissue phosphorus concentration at the $\alpha=0.1$ significance level. However, an LSD test showed no different groupings between the treatments when administered at the same confidence intervals. The tissue samples at the V7, VT, and at full maturity did not show any significant difference between the two phosphorus placement methods (Table 3.5). There wasn’t any difference between treatments in yield, or the phosphorus content of the grain in the different treatments (Table 3.6).

Discussion

The industry standard for soil testing for nutrients ranges from a 0-15 cm to a 0-20 cm soil depth when sampling for surface level soil nutrients. In the study, the samples were split and taken at the 0-8 cm and 8-15 cm depths to better define the differences in the soil test levels between the treatments. The shift from the industry standard depth is not a new method as Alam et al. (2018), Borges and Mallarino (2000), Fernández and Schafer (2021), and Holanda et al. (1998) all used approximately the 0-3-inch (0-8 cm) soil sampling depth in experiments (Borges and Mallarino, 2000; Holanda et al., 2008; Fernández and Schaefer, 2012; Alam et al., 2018). The 2020 growing season did not produce any effect on the soil test levels of phosphorus due to the placement. Alam et al. (2018) and Borges and Mallarino (2000) observed similar results where the application method of the phosphorus fertilizer did not affect the soil test level phosphorus (Borges and Mallarino, 2000; Alam et al., 2018). The spring and fall of the 2021 cropping season revealed no differences between any of the treatments in terms of soil test levels. This observation is similar to results reported by Alam et al. (2018) (Alam et al., 2018). The results of the soil test did not reveal any differences between the different treatments.

Researchers have varying results on tissue analysis. Results from Rosa et al. (2020) showed significant differences between treatments at the V3 growth stage. Whereas the results from this experiment were more aligned with the results found by Borges and Mallarino (2000) (Borges and Mallarino, 2000; Rosa et al., 2020). The results of the tissue samples at the V3 growth stage did not show any significant difference between banding or surface application of the phosphorus fertilizer on the uptake of phosphorus in soybean plants. During later growth stages, particularly during the reproductive stages, the uptake of phosphorus is greatly increased in soybeans, as supported by Rose et al., 2020 (Rosa et al., 2020). However, the findings from the 2020 growing season at Dakota Lakes Research Farm did not support these findings. The field that the experiment took place in was an irrigated field, so the lack of rainfall in July when the R1 samples were taken, would not have influenced uptake. In the study conducted by Rosa et al. (2020), the lack of rainfall was suspected of influencing phosphorus uptake at the R1 growth stage (Rosa et al., 2020). A plant is a dynamic organism which translocates nutrients from plant tissues to the grain affecting tissue sampling. Plants also exist on circadian rhythms, an internal daily clock that governs metabolic activities. A plant tissue is a snapshot at only one time in the life cycle of the plant. Plant growth stage, environment, genetics, soil moisture, and soil biology have an impact on plant tissue sample results.

During early plant growth stages, phosphorus uptake is hypothesized to be more efficient when banded in the soil as compared to broadcast because banding places the fertilizer closer to the seed. (Freiling et al., 2022). The V3 growth stage revealed a significant difference at the α=0.1 significance level with banded treatments having a higher concentration of phosphorus within the plant tissue. There was one outlier, that if removed, would change the level of significance seen (Figure 2.17). This supports the findings of Freiling et al. (2022) who showed that plant uptake of phosphorus is greater when the fertilizer is placed in the soil within the vicinity of the seed (Freiling et al., 2022). Tissue samples from growing season through full maturity did not show any significant difference between the treatments. This result was expected as phosphorus levels do not vary much after the initial uptake of phosphorus needed by the plant (Al-Ansari, 1885).

The yield between the banded and surface application treatments in the 2020 growing season showed no significant difference between the two application methods.

However, there was a larger variation in yields within the surface application than that of the banded application. results from the soybean growing season are in line with the results found in Freiling et al., 2022, and Borges and Mallarino, 2000, where fertilizer placement did not affect the yield of soybeans. The 2021 corn growing season showed the yields between the banded and surface application methods in corn were not affected. The corn yield results were similar to the 2020 soybean results (Borges and Mallarino, 2000; Freiling et al., 2022). Freiling et al. (2022) found in 73% of the studies, banding phosphorus fertilizers resulted in a higher yield as compared to surface applied P fertilizer (Freiling et al., 2022).. (2018) (Alam et al., 2018). Alam et al. (2018) found that there was a difference in corn yields based on fertilizer placement (Alam et al., 2018). This is different than the results found at Dakota Lakes Research Farm where no difference in yield based on fertilizer placement was observed.

Freiling et al. (2022) report was a meta data analysis and did not include the tillage practices used (Freiling et al., 2022). The research summarized in this report is generated from no-till fields at Dakota Lakes Research Farm that have been in no-till for over 20 years. Fields that are managed with no-till practices may have responded differently to phosphorus fertilizer placement thus producing different yield results from other tillage practices. No-till practices and impact on soil biology may also explain the difference between the findings of this study and Freiling et al. (2022). Alam et al. (2018) investigated the effect of tillage practices with phosphorus fertilizer placement and had the same findings as Freiling et al. (2022). As suggested in Alam et al. (2018), the reasons that these differences may be observed between the studies is due to phosphorus stratification throughout the soil, differences in root growth, and the plant available water

(Alam et al., 2018). The study at Dakota Lakes did not look at root growth or the plant available water due to the field being irrigated. Adequate water, similar root densities and no difference between the soil test levels of phosphorus make the yields comparable.

The phosphorus removal in this study did not show any significant difference in the 2020 soybean growing season. The literature is divided on what method of phosphorus fertilizer application results in higher phosphorus accumulation in soybean grain. The results from Freiling et al. (2022) found that there was higher phosphorus uptake from banded applications, whereas Rosa et al. (2020) found that surface application of phosphorus fertilizers lead to higher phosphorus concentration in soybean grain (Rosa et al., 2020; Freiling et al., 2022). It could be assumed that differing environmental factors such as the amount of rain events that occurred, temperature, varieties, etc. may have had an effect in the differences between the studies of Freiling et al. (2022) and Rosa et al. (2020).

The 2021 corn growing season did not show a difference in the grain phosphorus concentrations between surface and banded treatments. This conflicts with the findings of Freiling et al. (2022) where banding fertilizer was correlated with increased phosphorus concentrations(Freiling et al., 2022). The difference between the two studies could be related to the differences in soil properties between the different sites.

Conclusion

According to the results from 2020 and 2021, there is no difference between applying phosphorus fertilizers in a band or by surface application (Table 3.1 & Table 3.2). Yield, (Table 3.4 & Table 3.6), tissue phosphorus concentration (Table 3.3- Table 3.6), and soil test phosphorus were similar between the two treatments. The literature is divided on fertilizer placement method impact on phosphorus plant tissue concentration. Results from the tests showed placement of phosphorus fertilizer had no effect on plant tissue phosphorus concentrations. The soil test levels of phosphorus were not affected by phosphorus fertilizer placement as expected.

Placement of phosphorus fertilizer is not only about providing nutrients to the plants. The environmental impact of fertilizer placement also needs to be addressed. The literature agrees that placing phosphorus fertilizers on the top of the soil, instead of in the soil, will lead to greater phosphorus loss from runoff. As stated before, no significant difference was observed in the phosphorus content of runoff water (Appendix B). The tests were run after multiple inches of rainfall and irrigation had been applied to the field, allowing for the surface applied phosphorus to be dissolved and move into the soil profile or to dissolve and runoff from the field.

Dakota Lakes Research Farm intentionally has drawn their soil test phosphorus levels to 5 ppm in all their fields. The intentional lowering of the soil test levels is to promote the levels of arbuscular mycorrhizal fungi. The fungi form a symbiotic relationship with plant roots providing phosphorus to the plant that would be unavailable to the plant without this relationship. The intentional lowering of the soil test levels was also done to reduce the amount of potential labile phosphorus that can move into aquatic ecosystems, while still maintaining yield at those decreased levels.

Tables:

Table 3.1 Olsen Soil Test Phosphorus Levels in the 2020 Soybean Cropping Season in the Phosphorus Placement Experiment

	Olsen Soil Test (ppm) Spring		
	0-3”	3-6”	6-12”
Surface Application	10.5 a	11.5 a	6.9 a
Banded	12.1 a	4.5 a	3.8 a
Significant	NS	NS	NS

Table 3.2 Olsen Soil Test Phosphorus Levels in the 2021 Corn Cropping Season in the Phosphorus Placement Experiment

	Olsen Soil Test (ppm) Spring			Olsen Soil Test (ppm) Fall		
	0-3”	3-6”	6-12”	0-3”	3-6”	6-12”
Surface Application	5.9 a	4.9 a	5.0 a	7.3 a	7.7 a	6.8 a
Banded	8.0 a	4.8 a	5.2 a	9.9 a	7.6 a	7.1 a
Significant	NS	NS	NS	NS	NS	NS

Table 3.3 Soybean Plant Tissue % Phosphorus Concentration Throughout the 2020 Cropping Season in the Phosphorus Placement Experiment Field

	% P at V3	% P at R1	%P in Grain
Surface Application	0.409 a	0.351 a	0.449 a
Banded	0.408 a	0.369 a	0.433 a
Significant	NS	NS	NS

Table 3.4 Soybean Plant and Grain Phosphorus Concentrations, Pounds of P and P_2O_5 Removed, and Yield at Harvest in the 2020 Growing Season in the Phosphorus Placement Experiment Field

	Grain			Yield
	P (%)	P (lbs/a)	P_2O_5 (lbs/a)	bushels/a
Surface Application	0.449 a	11.1 a	25.5 a	41.3 a
Banded	0.433 a	11.1 a	25.3 a	42.7 a
Significant	NS	NS	NS	NS

Table 3.5 Corn Plant Tissue % Phosphorus Concentration Throughout the 2021 Cropping Season in the Phosphorus Placement Experiment Field.

	% P V3	% P V7	% P VT	%P Full Maturity	% P in Grain
Surface Application	0.488 a	0.354 a	0.277 a	0.073 a	0.27 a
Banded	0.522 a	0.331 a	0.274 a	0.064 a	0.28 a
Significant	NS	NS	NS	NS	NS

Table 3.6 Corn Plant and Grain Phosphorus Concentrations, Pounds of P and P_2O_5 Removed, and Yield at Harvest in the 2021 Growing Season in the Phosphorus Placement Experiment Field

	Plant			Grain			Yield
	P(%)	P (lbs/a)	P_2O_5 (lbs/a)	P (%)	P (lbs/a)	P_2O_5 (lbs/a)	bushels/a
Surface Application	0.073 a	6.8 a	15.6 a	0.27 a	28 a	65 a	206 a
Banded	0.064 a	6.1 a	14 a	0.28 a	29 a	67 a	205 a
Significant	NS	NS	NS	NS	NS	NS	NS

Figure:

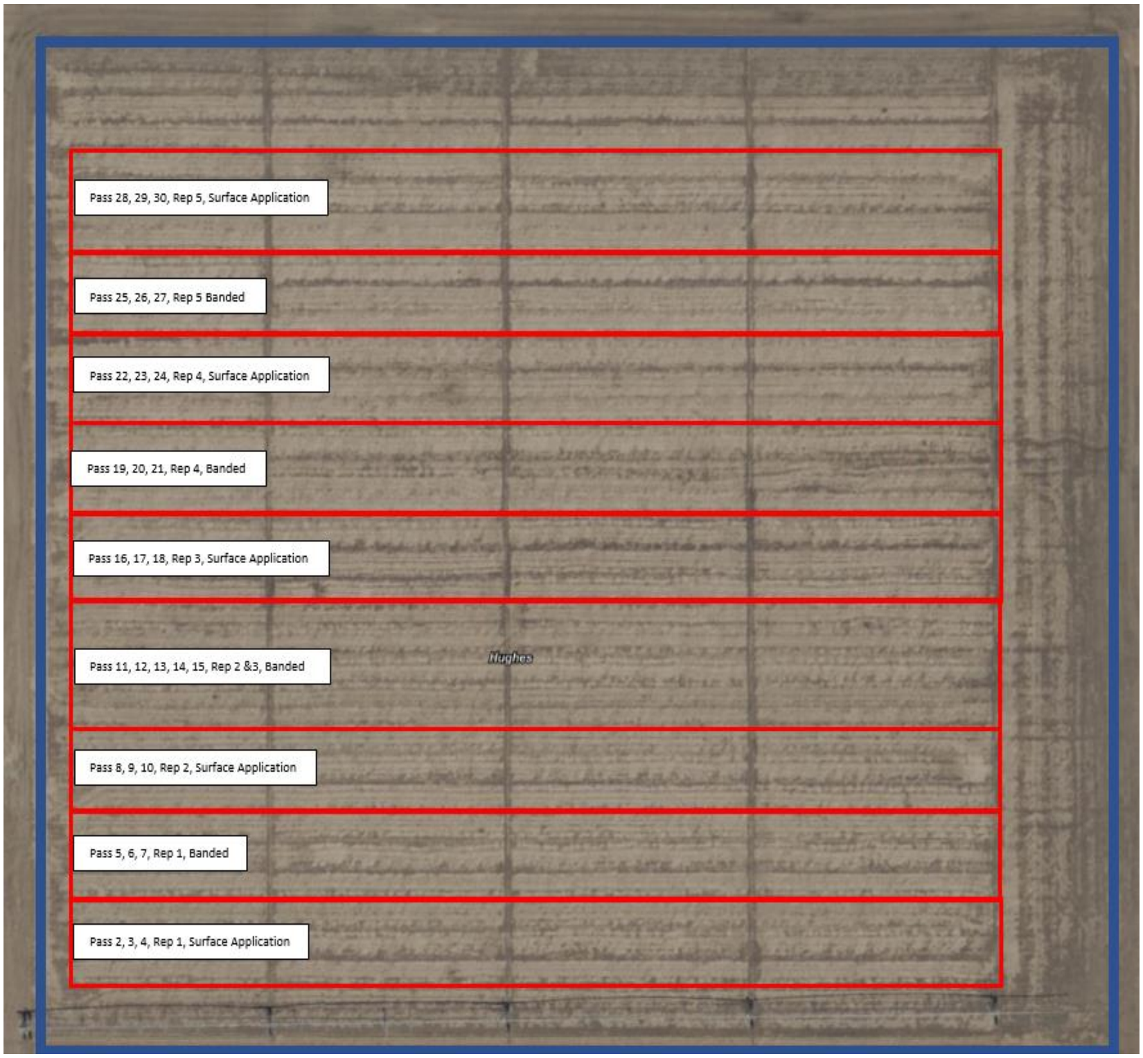

Figure 3.1: Arial Image of placement study field. The central location of this field is located at 44.290589, -99.998837 Each red box corresponds to a treatment replication with the passes, replication number, and treatment labeled within. The blue box is the border of the entire field where this study takes place. The length of the treatments is approximately 195 meters and treatments are 12.2 meters wide.

Figure 3.2: Image of Dakota Lakes Resarch Farm concept planter capable of side banding fertilizer as well as broadcasting. This distance image shows the fertilizer carry tubes hooked up to the surface application splash plates. Fertilizer is being applied in front of the planting unit that is planting soybeans in the 2020 growing season.

Figure 3.3: Up close image of the surface application portion of the planter. This image was captured mid application and fertilizer can be seen falling from the splash plates onto the soil

CHAPTER 4- ARBUSCULAR MYCORRHIZAL FUNGI’S RELATIONSHIP WITH ROW CROPS AND PHOSPHORUS UPTAKE BASED ON SOIL TEST PHOSPHORUS IN NO-TILL FIELDS

Introduction

Arbuscular mycorrhizal fungi (AMF) have a long beneficial history with higher plants, more specifically the plant roots, dating back hundreds of millions of years (Willis et al., 2012). A diversity of crops is thought to support arbuscular mycorrhizal fungi communities (Guzman et al., 2021). Without diversity, soil fungal communities’ activity is diminished. Field management techniques, such as diverse crop rotations, can restore AMF populations in soils(Guzman et al., 2021). Arbuscular mycorrhizal fungi are hypothesized to assist plant uptake of nutrients, such as phosphorus, nitrogen, potassium, zinc, and copper. Increased uptake occurs by the fungal hyphae extending the roots systems of the plant and increasing the surface absorption area of their host plant (Marschner and Dell, 1994). In addition to helping plants uptake nutrients, arbuscular mycorrhizal fungi also help with the uptake of water (de Moura et al., 2022).The plants provide carbon compounds to the fungi while the plants receive nutrients and water from the fungi (Barea et al., 2008). Arbuscular mycorrhizal fungi also help in the aggregation of soil, improving soil stability (Bearden and Petersen, 2000).

Arbuscular mycorrhizal fungi are affected by field management conditions. The use of tillage versus no-till management may influence fungal establishment in the soil. Disturbing the soil reduces the mass of the hyphae as well as the number of spores present in the soil (Kabir et al., 1998). In some cases, the first few years of transition to no-till is may reduce yield. However, if AMF fungi are present, the fungi may negate this effect, or at least reduce it (Wetzel et al., 2014). In no-till systems, chemical management for various detrimental pests are still needed. The use of pesticides has been shown to have a harmful effect on arbuscular mycorrhizal fungi, but the fungi have developed some mechanisms to reduce the effects of the chemicals to ensure its survival (Hage-Ahmed et al., 2019). The harmful effects of chemical application, such as herbicides fungicides, to mycorrhizal fungi are minimized in the no-till soils. This is due to increased mobility of water through the soil that moves chemicals in the water through the soil at a faster rate (Elias et al., 2018). Another important management consideration is mineral fertilizer application. Jiang et al. (2021) reported numerous studies that conclude addition of mineral fertilizers generally decreases diversity and amount of arbuscular mycorrhizal fungi (Jiang et al., 2021). The plants are lazy when mineral phosphorus is available and the plants do not need to form the symbiotic relationships with the fungi (Jiang et al., 2021)

There is significant proof that arbuscular mycorrhizal fungi improve phosphorus uptake in plants (Marschner and Dell, 1994). Measurement of AMF in the soil is accomplished by (1) most probable number (MPN) method or (2) phospholipid fatty acid analysis (PLFA) (Vestberg et al., 2012). The use of the PLFA method, used for finding the fraction of viable fungal hyphal biomass, involves extracting the 16:1ω5 signature fatty acid. The 16:1ω5 fatty acid is the primary marker in arbuscular mycorrhizal fungi that is key to identification (Ngosong et al., 2012). The most probable number method (MPN) is a dilution series that determines the presence or absence of the microorganisms based on the colonization of each dilution (Abinaya et al., 2018). There are drawbacks to each of the methods. The PLFA method has interference from other microorganisms due to other organisms' use of mycelia as a carbon source (Ngosong et al., 2012). The most probable number method is time consuming, with testing taking weeks to be completed. The MPN is not feasible for large scale testing (Roszell et al., 2021).

Materials and Methods

The field study occurred in an experiment field located at Dakota Lakes Research Farm located 18 miles Southeast of Pierre, SD, in conjunction with the experiments described in Chapters 2 and 3. The soil samples were taken using the same method as described in the previous chapters. The cropping rotation is the same as described in Chapter 2 and 3. The arbuscular mycorrhizal fungi study was started in November of 2017 at Dakota Lakes Research Farm. The laboratory portion of the study took place at the US Department of Agriculture-Agricultural Research Service in their on-site greenhouse and laboratory.

Field Site:

The field AMF study was in the same location as the soil phosphorus test study. The field size is 4.5-acre. The soil type is a Lowry silt loam. The treatments in the field run 103.6 meters in length and are 6.1 meters wide. The field is 161.5 meters by 103.6 meters (Figure 4.1). In 2020 soybeans, Pioneer P29A85L variety, were planted using a custom-built planter that drilled the seeds into the field in 50 cm spacing at 175,000 seeds/acre. In 2021, the same planter was used to drill corn seed, Pioneer P0220AM variety, at a rate of 35,000 seeds/acre. Soil samples for AMF analysis were collected from this field.

Experimental Design:

The experimental design for the arbuscular mycorrhizal fungi study is identical to the experimental design described in Chapter 2. Three different MAP fertilizer rates were evaluated on the impact of arbuscular mycorrhizal fungi population. Fertilizer treatments were check, where no extra fertilizer was applied besides the starter fertilizer, 100 lbs of mono-ammonium phosphate (MAP) applied per acre and 200 lbs of MAP applied per acre. The MAP treatments were applied in 6.1-meter-wide passes running the length of the field and applied in the Fall of 2014, 2017, and 2019. The fertilizer was placed 4 centimeters deep in 19-centimeter rows by a no till drill. The starter fertilizer was applied in a side band of 4 centimeters deep and 8 cm to the side of the seed at planting.

The USDA-ARS laboratory in Brookings, SD performed the MPN analysis. The most probable number of AMF propagules was ascertained using five levels of serial soil dilution performed in triplicate. This created a five by three most probable number matrix for each of the 15 soil samples collected at Dakota Lakes Research Farm.

Cropping System:

2020 Cropping year:

The soil samples for the laboratory studies and phospholipid fatty acid analysis were taken on July 10. One set of samples was taken from each of the 15 treatment areas, as described in chapter 2. The soil cores were taken from the 0–20-centimeter depth. Fifteen cores were taken from the three different areas within the sampling area. After each treatment was collected, the soil was placed into a labeled plastic bag and placed into a cooler with icepacks. The samples were brought back to the office building at Dakota Lakes Research Farm and the 15 samples were each weighed out to 500 grams of soil. The 500-gram samples were placed into new bags. They were then placed back into the cooler and kept on ice until they could be transported to the USDA-ARS lab. The rest of the soil from each sample was placed back into the bag and stored on ice in preparation to be sent to Ward Laboratories for phospholipid fatty acid analysis.

The 15 soil samples were put through a serial dilution with 5 levels at the USDA-ARS station. Three replicates were conducted for each sample. Bahai grass, a plant host for AMF, was planted and grown in each of the dilution series pots. The plants were harvested after 4 weeks of growth and the roots washed, cleared, and stained. The stained roots were then scored for the presence or absence of arbuscular mycorrhizal fungi structures. A more detailed version of the methods can be found in Lehman et al., 2012 and Lehman et al., 2019 (Lehman et al., 2012, 2019). Results were transformed by square root and addition of 1. This was done to make the variation more uniform as the data is dealing with counts.

The soybeans were harvested on October 8 using a John Deere 9410 John Deere combine with a 12- row head. Samples from each treatment in each replication were taken and measured for moisture. A total of 15 samples were collected for analysis. Grain samples were dried until the samples did not lose any more weight from moisture loss. The weight was recorded, and the samples were sent to Ward Laboratories for analysis.

2021 Cropping year:

The soil samples at the Dakota Lakes Research Farm field site for the 2021 cropping year were collected in much the same way as in the 2020 cropping year with the difference being the number of cores taken. In the 2021 cropping year, a total of 20 soil cores were taken. The samples were collected on June 15. The samples were transported to the Brookings, SD USDA-ARS laboratory on June 19 for MPN analysis. The remainder of the soil was packaged and sent to Ward Laboratories for phospholipid fatty acid analysis. The MPN method was the same as in 2020.

Corn samples were hand harvested at the R6 growth stage on October 7, 2021. The samples were taken in 1.5-meter sections from four points throughout each treatment. A total of 6 meters of row were harvested. The corn plant was cut approximately 15 centimeters above the soil surface, bundled together, and collected from the field. The total number of plants was recorded. Next, the ears were removed from the plant while leaving the husks on the plant. The weight of the ears and plants were recorded separately. A subsample of 10 randomly selected plants was set aside from the whole plant sample. The subsample plants were cut into 61-centimeter sections, weighed, and bagged for drying. After the plant's weights were measured and recorded, ten ears were randomly selected, weighed, and bagged for drying. The stalk samples were dried until easily broken by hand. Once the stalks had reached this point, stalks were weighed again and fed through a garden mulching machine to shred the stalks. The shredded stalk weight was recorded. A subsample was collected and sent to Ward Laboratories for analysis. The ears were oven dried for a period of one week at 63 degrees Celsius. Ears were hand shelled after drying. The grain was weighed and bagged in sealable bags and analyzed at Ward Laboratories. The cobs were also weighed to determine the total weight of grain and cobs. After weighing, the cobs were discarded.

Statistical Analysis:

ANOVA was run using R programing R version 4.0.2 (2020-06-22) (R Core Team, 2020) where the *aov* function (Chambers et al., 1992) was used. LSD numbers were generated using the *lsd.test* function in R (Steel and Torrie, 1986). The *TukeyHSD* function was used for pairwise mean comparison. The function *boxplot* and the *ggplot2* package was used for data visualization (Wickham, 2016). Phospholipid fatty acid data was left as the original data received from the laboratory. The most probable number assay data was transformed by taking the propagule number plus one and square root transformed.

Results:

The most probable number assay found that the AMF propagules in the soil were significantly different between the three fertilizer treatments in the 2020 soybean growing season. The check treatment which didn’t add any extra fertilizer was significantly different from the other two treatments. Where extra fertilizer was added in the fall of 2019, MAP added at 100 lbs/acre and MAP added at 200 lbs/acre, the two treatments were not significantly different from one another. However, the phospholipid fatty acid results showed there was no significant difference between the three treatments in terms of arbuscular mycorrhizal fungi biomass (Figure 4.1).

The 2021 corn cropping season results from the most probable number assay were much the same as the results from in 2020. Again, it was observed that the check treatment was significantly different from the MAP applied at 100 lbs/acre and the MAP applied at 200 lbs/acre treatment (Figure 4.1). An error in the data showed results indicating there were no mycorrhizal fungi in the sample which made it impossible to have a statistical analysis run on the phospholipid fatty acid analysis from 2021.

Yield samples from the 2020 and 2021 cropping seasons showed there was no significant difference between the three fertilizer treatments in terms of yield in either year. However, there was a significant difference in the P_2O_5 removal by soybean grain in 2020, at the α=0.1 significance level (Figure 4.1).

Discussion

Fertilizer is reported to reduce arbuscular mycorrhizal fungi (Jiang et al., 2021). The zero-phosphorus fertilizer treatment in 2020 and 2021 had significantly higher arbuscular mycorrhizal fungi as compared to treatments that received MAP fertilizer. When phosphorus fertilizers are applied at increased rates symbiotic infection by AMF are reported to decrease, but do not disappear entirely. Signaling that enough phosphorus is available is thought to occur between the plant and AMF organisms. The symbiotic relationship causes the plants to provide carbon to the fungi (Konvalinková et al., 2017 and Marschner and Dell, 1994).

It can even become detrimental to plants to have arbuscular mycorrhizal fungi relations when there is an abundance of phosphorus that is easy for plants to take up. The possible relationship then changes from mutualism to parasitism. The relationship requires carbon to be given by the plant to the fungi; however, the plant does not receive enough of a beneficial effect to make the relationship worth it. The plant will not look to have a symbiotic relationship with the arbuscular mycorrhizal fungi if there is enough phosphorus for the plant to uptake (Marschner and Dell, 1994).

Differences between monoammonium phosphate fertilizer (MAP) treatments were not observed in grain yields for both 2020 and 2021. Arbuscular mycorrhizal fungi may contribute to the lack of yield response to additional MAP fertilizer. No MAP fertilizer was used in the soil for the zero-fertilizer treatment. Lack of excess free phosphorus in the soil may have encouraged AMF root infection. Comparable results are reported by Wang et al. (2021) and Cely et al. (2016).

Arbuscular mycorrhizal fungi colonization significantly increased the yield of corn, Wang et al. (2021). Cely et al. (2016) reported that soybean yield increased when plants were inoculated with arbuscular mycorrhizal fungi under low soil test phosphorus conditions (Cely et al., 2016; Wang et al., 2021).

Methods to quantify soil AMF reported different results. Measurement of AMF using the most probable number method reported differences in populations between fertilizer treatments, whereas the PLFA method failed to report differences. According to “Neutral Lipid Fatty Acid Analysis Is a Sensitive Marker for Quantitative Estimation of Arbuscular Mycorrhizal Fungi in Agricultural Soil with Crops of Different Mycotrophy” by Vestberg et al. (2012), AMF colonization varied greatly, but PLFA did not support those findings (Vestberg et al., 2012). Instead, sources of high levels of the 16:1*w*5 fatty acid (a fatty acid often attributed to AMF) were hypothesized to come from other microbes or fungi.

Dakota Lakes Research Farm is a no-till farm. No-till promotes microbial diversity (Schmidt et al., 2018). With increased microbial diversity, background levels of 16:1w5 fatty acid affect differences between MPN and the PLFA. Ngosong et al. (2012) discussed using 16: 1ω5 signature fatty acid as a marker to measure PLFA. Ngosong et al. (2012) reported bacteria also had a high concentration of 16: 1ω5. The background 16: 1ω5 from bacteria may limit use of 16: 1ω5 fatty acid a reliable marker to measure AMF populations (Ngosong et al., 2012). Research of both Ngosong et al. (2012) and Vestberg et al. (2012) support the findings at Dakota Lakes Research Farm where no significant difference was observed between the three phosphorus fertilizer treatments when phospholipid fatty acid analysis was run. The MPN assay does not use fatty acid as a measurement, and this may explain observation of significant differences when this method is used to measure AMF soil populations

Most probable number assays directly count the number of potential infections of arbuscular mycorrhizal fungi in plant roots (Porter, 1979). Phospholipid fatty acid analysis measures the fatty acids of a cell's membrane, and in the case of arbuscular mycorrhizal fungi, the 16: 1ω5 fatty acid is used to estimate soil AMF populations (Olsson and Lekberg, 2022). As previously described, the 16: 1ω5 marker is not exclusive to arbuscular mycorrhizal fungi and is also found in bacteria. The PLFA method may overestimate AMF depending on the bacterial population of the soil. The most probable number assay is time intensive, taking multiple months to complete. Nevertheless, the fungal propagules numbers attained from the most probable number assay do represent the inoculum potential, which is an ecologically relevant pool (Lehman et al., 2019). The phospholipid fatty acid analysis is good for a quick result but is prone to overestimation of the fungal biomass in the soil.

Conclusion

The findings of the study support previous findings in the literature: test phosphorus levels increase; arbuscular mycorrhizal fungi decrease. The probable number assay is more accurate in long term no-till fields for finding the amount of arbuscular mycorrhizal fungi compared to the phospholipid fatty acid analysis. Phospholipid fatty acid analysis measures the 16: 1ω5 fatty acid marker to determine the amount of arbuscular mycorrhizal fungi, however the 16: 1ω5 fatty acid marker is also present in some bacteria. Dakota Lakes Research Farm's management practices promote biological activity in soil, including AMF and 16: 1ω5 fatty acid containing bacteria. Results from the phospholipid fatty acid analysis for arbuscular mycorrhizal fungi l were not significant due to there being a high-level background 16: 1ω5 fatty acid marker from bacteria. The background levels obscure the true arbuscular mycorrhizal fungi numbers produced from the phospholipid fatty acid analysis.

The increased presence of arbuscular mycorrhizal fungi in the zero-fertilizer treatment did not have as high of phosphorus concentrations in the plant, but yield did not significantly suffer. This result indicated that arbuscular mycorrhizal fungi supply enough phosphorus to the plant to attain yield that is not statistically different from yield where phosphorus fertilizers were added (Table 4.1). Other factors besides AMF play a role in why there is no difference between treatments in terms of yield, such as the variety planted or environmental factors. Regardless of the fertilizer treatment (0, 100, or 200 lbs of extra MAP), supplying some phosphorus fertilizer at plant will help sustain the crop through the growing season. Chapter 2 reported differences in soil test levels of phosphorus, however, over time the extra applied phosphorus becomes less soluble and less available to plants. the AMF values, as measured by MPN, decreased in the 100 and 200 lbs of MAP treatments. The solubilized phosphorus from the fertilizer resulted in grain yield that was not significantly different from the zero-fertilizer treatment.

Tables:

Table 4.1 Differences Between the Findings of the Most Probable Number Assay (MPN) and Phospholipid Fatty Acid Analysis (PLFA) as Compared to Yield and P_2O_5 Removal

	Most Probable Number		PLFA (%)	Yield (bu/a)		P_2O_5 Removal (lbs/a)	
	2020	2021	2020	2020	2021	2020	2021
Check	4.7 a	5.18 a	5.22 a	61.1 a	208 a	36 b	67 a
100 lbs MAP	0.7 b	0.96 b	5.42 a	60.6 a	209 a	38 ab	73 a
200 lbs MAP	0.7 b	0.98 b	4.92 a	61.6 a	212 a	41 a	74 a
Significant	0.002	0.003	NS	NS	NS	0.07	NS

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Figures:

Figure 4.1: Aerial image of the field in which arbuscular mycorrhizal fungi samples were taken. The central location of this field is 44.288243, -100.001752. This is the same field as in Chapter 2 and the treatments are labeled as they occur in the field. The red box represents the location within the field where the study took place. The green boxes each represent 1 treatment pass in which samples were taken. The treatments are labeled with the pass number, the replication number, and the treatment in that pass. Each treatment was approximately 103.6 meters in length.

APPENDIX A.

Construction and Operation of Rainfall Simulator

The construction of the rainfall simulator began by making the collection pans. A 7.6-centimeter-wide flat iron and 3.8-centimeter angle iron were cut to the length of 50.8 and 76.2 centimeter. A 50.8 x 76.2-centimeter square was made using flat iron. The angle iron was matched flush with one side of the flat iron square and welded so the angle protruded halfway down the flat iron. Before welding one of the 50.8-centimeter pieces of angle iron, a 3.8-centimeter hole was drilled in the center of the flat iron. The angle iron had a 5-centimeter piece cut from the center to allow for the hole in the flat iron to be unobstructed. A 10.1-centimeter by 4.1-centimeter diameter piece of tube stock was welded on over the hole for a hose to be connected to.

The Nutrient Research and Education Council (NREC) rainfall simulation machine was used as a template to apply water. An oscillating sprayer head would be used to simulate rainfall. The oscillating effect was achieved by using a John Deere wiper motor, part number RE151494, to swing the spray nozzle back and forth. The nozzle used was a TEEJET XR 8010 nozzle. A pressure regulator was attached to the hose leading to the spray nozzle to achieve the desired: pressure (16-18 psi), rate of water to the test area, application time (9-14 minutes). Water was pumped to the sprayer using a Remco Industries Proflo pump, capable of a 20 liter per minute flow rate. The frame was hammered into the ground until the angle iron was flush with the soil surface. The frame was orientated so the hose was following the slope of the field downhill. A hole was dug at the end of the hose for a beaker to be placed in it to collect the runoff. A ladder was placed over the frame that the rainfall simulator was attached to. Using the ladder, the rainfall simulator was at a height of 50.8 inches above the soil. A rain gauge was placed directly below the spray nozzle to measure the amount of water applied to the soil.

A sheet of plywood was placed over the rain gauge and frame to prevent water being applied to the soil before the desired pressure is reached. Once the pressure is set, the plywood was removed, and a timer was started simultaneously. The rainfall simulation was run until 10.1 cm of water was applied to the soil. When runoff started, the time was recorded. The first 250 mL of runoff were collected and stored in sample bottles. Water quality analysis was completed by Ward Laboratories.

A soil solution access tube was placed at the opposite end of the frame from where the hose is located. This was done after the frame was set and before the rainfall simulator was started. The soil solution access tube was placed at 61 cm below the rainfall simulator frame. A vacuum was drawn on the soil solution access tubes with a measured psi of 60 to draw in the soil water. After 2 days, the water from the soil solution access tube was collected and the pressure was checked. For an additional 2 days the soil solution water was collected. The collected soil solution water was measured, and the samples sent to Ward Laboratories for analysis.

Results for Runoff and Soil Solution Water Samples from the Soil Test Level Study

The Cornell water infiltrometer used in the 2020 soybean growing season revealed a significant difference, α=.01, in the P_2O_5 phosphorus concentrations found in the runoff between the area where herbicide was applied, and the non-herbicide effected area between the three fertilizer treatments. In the sprayed treatment, there was no difference between or within the treatments. The results for the soil solution access tube water collection did not show a difference between or within the treatments for total phosphorus in the herbicide effected and non-herbicide effected areas.

The rainfall simulator runoff results did not show significant differences between treatments. There was significant difference, at the α=0.01 significance level, between the replications. Soil solution water did not show any significant difference between the different treatments or within the replications.

Discussion for Runoff and Soil Solution Water Samples from the Soil Test Level Study

The Cornell water infiltrometer was used in 2020 to measure the infiltration and runoff in two different areas of the field, the 1.2 meter sprayed off swath and the rest of the field, which was not sprayed. A level of significant difference was observed between treatments in the regular portion of the field at the α=0.1 significance level. The check treatment sample had less phosphorus in the runoff water than the other treatments where extra phosphorus was applied. Comparable results were observed by Shuman (2002) and Tarkalson and Mikkelsen (2004), where concentrations of phosphorus in runoff water were elevated when higher rates of phosphorus fertilizers were applied (Shuman, 2002; Tarkalson and Mikkelsen, 2004). Phosphorus soil attachment happened slowly over time and is dependent on many factors such as pH, soil texture, etc. Phosphorus from fertilizer applied P remains in soil solution and is susceptible to loss through water run-off. There is a greater chance of more phosphorus in run-off water from soil when fertilizers are initially applied, and soil attachment has not occurred. The area where herbicide was applied by spraying did not show any difference between treatments. Nor was there a difference between the sprayed and non-sprayed areas interaction. These results are supported by the findings of Carver et al. (2022) where it was determined that cover crop termination methods did not significantly increase the risk of phosphorus loss (Carver, 2018). There was no significant difference in phosphorus concentrations of the soil solution water observed either. This is due to the phosphorus from the fertilizers already being sorbed to the soil and not in soil water solution pool. This idea is supported by the findings of Kleinman et al. (2002) where similar conclusions were reached.

The 2021 runoff results did not show any significant differences between treatments or between the soil solution water. This did occur two years after the application of the fertilizer treatments, so it would be reasonable to assume that with the increased time, the phosphorus has had more time to sorb to the soil. This can be seen in the study done by Shuman (2002) where after 2 years the amount of phosphorus in runoff was less than that of the previous year. Another reason no significant difference was not observed in the 2020 soil solution and the 2021 runoff and soil solution, was due to the fertilizer being applied in a band and not surface applied. Placing fertilizer into the soil has been proven in literature to reduce the amount of phosphorus lost from a field (Kleinman et al., 2002; Tarkalson and Mikkelsen, 2004).

Results for the Cover Crop Analysis in the Rate Study

The termination portion of the study revealed there was a significant difference between the PO_4 concentrations by each treatment, but no difference was observed between the sprayed and unsprayed treatments. Cover crop tissue sampling did show a significant difference in the concentration of phosphorus in the cover crop between treatments at the $\alpha=0.001$ significance level.

Discussion for the Cover Crop Analysis in the Rate Study

On April 15, 2020, the cover crop of oats, barley, and lentil were able to absorb the phosphorus that was left from the phosphorus applications done the previous fall. When the cover crop was sampled on May 15 there was a significant difference between the treatments, with the higher phosphorus rate treatments having a higher phosphorus content than that of the check treatment where no additional fertilizer was added. The higher application rate would allow for there to be a higher availability of phosphorus in those areas. This finding is supported by the findings of Pavinato et al. (2017) where the use of varying rates of phosphorus fertilizers affected the uptake rate of cover crops (Pavinato et al., 2017).

APPENDIX B

Construction and Use of Rainfall Simulator

The rainfall simulator construction was the same as described in Appendix A.

Results for Water Samples from Runoff and Soil Solution Water in the Placement Study

The Cornell water infiltrometer was used in the 2020 soybean growing season. There was no significant difference in the P_2O_5 phosphorus concentrations found in the runoff from the experiment. No difference was seen within the treatments either. The results for the soil solution access tube water collection did not show a difference between or within the treatments for total phosphorus.

Water samples collected using the rainfall simulator in the 2021 growing season showed no significant difference in the phosphorus concentrations in the collected runoff samples. There was no significant difference within treatments in the replications either. Soil solution access tubes collected water did not show any significant difference between treatments. There was a significant difference between the different replications within the treatments that was observed.

Discussion for Water Samples from Runoff and Soil Solution Water in the Placement Study

There was no difference between the phosphorus fertilizer placement treatments in terms of P_2O_5 loss from runoff during the 2020 soybean cropping season and the 2021 corn cropping season. The results from this study do not agree with the findings of Wiens et al. (2019). Wiens et al. (2019) found that broadcast application of phosphorus resulted in a significantly higher amount of dissolved phosphorus appearing in runoff (Wiens et al., 2019). The results that were seen at Dakota Lakes Research Farm were not similar because the experiment was conducted late in July. Over 12.7 cm of natural rainfall had occurred from May to July of 2020. The field also had multiple passes from irrigation which applied 5.1 centimeters of water per pass over the field. From May to June of 2021, the field received 10.1 centimeters of natural rainfall as well as irrigation. The significant amount of water applied to the field before the runoff experiments were conducted shows that from the time of application of phosphorus fertilizers there had been plenty of rainfall events to move the surface applied fertilizer. Collecting samples after the first rainfall event after the application of phosphorus fertilizer would contribute to the runoff data results.

Infiltrated water collected by soil solution access tubes did not show any significant differences between the different placement treatments, just as was seen in the runoff results. These results do not match what the literature has found. Williams et al.

(2018) found that broadcast phosphorus fertilizer applications significantly increase the amount of phosphorus that leaches in the soil profile. Like with the runoff, the experiment ran at Dakota Lakes Research Farm was conducted too late in the season to see any meaningful results. If the experiment was done directly after planting when the fertilizer was first applied, before the first rainfall event could affect the fertilizer, the results of the experiment may have shown a significant difference.

Conclusion for Water Samples from Runoff and Soil Solution Water in the Placement Study

The levels of phosphorus loss from runoff were similar for both years. In the runoff experiment, no significant result was observed due to the time of sampling. Samples were taken too late, after too many rainfall events, and irrigation to see any significant difference between the treatments. With a correction to the time of sampling, different results would be observed.

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