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The Relationship Between Soil Test Phosphorus and Soil Biology - A Natural Management of Soil Phosphorus Through Conservation Practices

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THE RELATIONSHIP BETWEEN SOIL BIOLOGY AND SOIL TEST PHOSPHORUS-A NATURAL MANAGEMENT OF SOIL PHOSPHORUS THROUGH CONSERVATION PRACTICES

BY

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THESIS ACCEPTANCE PAGE

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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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The aforementioned thesis is dedicated to all researchers that have come before me, and those that will follow me. The pursuit of scientific understanding is an important part of the human spirit, and I applaud you for your efforts, no matter the field of study.

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CONTENTS

ABBREVIATIONS

°C	degrees Celsius
µm	micrometer
Al	Aluminum
AMF	Arbuscular Mycorrhizal Fungi
ATP	adenosine triphosphate
BMP	best management practices
C	carbon
Ca	Calcium
cm	centimeter
CO_2	carbon dioxide
DAP	diammonium phosphate
DNA	deoxyribose nucleic acid
ERGO	ergothioneine
Fe	Iron
g	grams
$H_2PO_4^-$	orthophosphate
ha	hectare
HPO_4^{2-}	orthophosphate
i.d.	inside diameter
kg	kilogram
L	liter
m	meters
m^2	meters squared
MAP	monoammonium phosphate
mg	milligram
mm	millimeter
MPN	most-probable-number
N	Nitrogen
ng	nanograms
NLFA	neutral lipid fatty acid
OM	organic matter
P	Phosphorus
PLFA	phospholipid fatty acid
ppm	parts per million
PR	Phosphate Rock
PSB	phosphate solubilizing bacteria
PSM	phosphate solubilizing microbes
S	sulfur
SDSU	South Dakota State University

STP	Soil Test Phosphorus
w/w	weight per weight
α	alpha

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ABSTRACT

THE RELATIONSHIP BETWEEN SOIL TEST PHOSPHORUS AND SOIL BIOLOGY-A NATURAL MANAGEMENT OF SOIL PHOSPHORUS THROUGH CONSERVATION PRACTICES

CLARENCE FREDERICK WINTER

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Phosphorus (P) management is an ongoing challenge in modern agriculture. Due to the importance of P in agriculture, P fertilizer additions are made throughout the U.S. to avoid nutrient-depleted soil. However, the application of P is problematic, as an excess of P in an agricultural system elevates the risk of environmental degradation, and current synthetic P sources are a fleeting resource. Additionally, a majority of P found in agricultural soils are insoluble, in the form of secondary and primary P minerals, making this large pool of P unavailable to plants. Fortunately, soil fungi organisms, such as arbuscular mycorrhizal fungi (AMF) have been studied extensively for their P sustainability potential, as these organisms are capable of dissolving insoluble complexes and providing P to host plants. The implementation of conservation practices like no-till have been found to affect soil test phosphorus (STP) level, because of the increase of AMF in these systems. The effect of varying STP levels on the formation of AMF in no-till managed soil has not been studied. Therefore, this study was conducted to 1) determine in a long-term no-till field the effect of different STP levels on crop yield and soil biological activity as measured by the phospholipid fatty acid (PLFA) and most-probable-number (MPN) assay and 2) determine if the more cost-effective PLFA test instead of the MPN test can be used to effectively identify any differences in AMF due to

STP level. At the Dakota Lakes Research Farm in Pierre, SD soil test levels were drawn down to 5 ppm Olsen P in 2014. A five-year crop rotation was implemented in this field prior to the study in 1994 (soybean-wheat/cover crop-soybean-corn-corn). To create field areas with low, medium, and very high soil test P categories within the field, P fertilizer rates of 0, 58, and 116 kg P_2O_5 were applied in randomized strips across the field in 2014. These rates were again applied to the same treatment areas in 2017, 2019, and 2021 to maintain three distinct soil test levels. After five years, there was not an economic advantage to maintaining STP level in the very high category (no P fertilization recommended) vs the low and medium STP categories (P fertilization recommended). One potential reason for this lack of yield difference between STP categories may be due to the greater amount of AMF fungi in the low (5 propagules gram^{-1}) compared to the medium (2 propagules gram^{-1}) and very high STP (1 propagule gram^{-1}) soil, as tested by the AMF most-probable-number (MPN) assay. Conversely, results from the PLFA assay were inconclusive, as soil fungi and bacteria only showed microbial differences in one year out of the five year study, as a result of stressful weather conditions. These results indicate that P fertilizer recommendations may need to be revisited in long-term no-till systems, as these systems appear to require less P to maintain crop yields than conventional tillage systems as a result of AMF accumulations with no-till, allowing for lower amounts of P fertilization.

CHAPTER 1 REVIEW OF LITERATURE

1.1 PHOSPHORUS

1.1.1 Phosphorus Importance

Crop fertility is a critical objective for producers during the growing season. Phosphorus (P) is considered a macronutrient that determines plant growth and productivity and is one of the most common yield-limiting factors in crop production (Q. Wu et al., 2022). Furthermore, P is a major component of nucleotides like adenosine triphosphate (ATP) and deoxyribonucleic acid (DNA) (Weil & Brady, 2016) that play a significant role in plant physiological responses. This highlights the molecular importance of P as life cannot exist without this element. In addition to its molecular functions, P is also a critical component for multiple metabolic processes in plants, including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis, and plant respiration (Weeks & Hettiarachchi, 2019). Although P exists in large quantities in most agricultural soils, a large percentage of the mass of P is only present in mineral and organic forms that are not available for plant uptake (Sawyer et al., 2000). This means that every year that these annual crops are used, there is a large portion of P that will need to be subsequently replaced using a number of fertilizer methods. From 2010 to 2021, the United States consumed an average of 4 million metric tons per year of P fertilizer to accommodate P-intensive annual crops (International Fertilizer Industry Association, 2023). This is done to avoid depleted and infertile soil that can disrupt the crop production enterprise and the food production system (A. E. Johnston et al., 2005). There is no replacement for P in an ecosystem, which has led to P fertilizers having a pivotal role in agricultural production.

Traditionally, P additions are carried out in an attempt to increase the growth of the root structure in plants. The abundance of P in the soil affects root morphological and physiological characteristics that are important for P uptake (Hajabbasi & Schumacher, 1994). Multiple experiments have been conducted to study the influx of P in several crop types and to determine the importance of P in root development. In many cases, split root techniques show that P uptake via roots have good correlation with root growth irrespective of root density or plant age (Newman & Andrews, 1973). This means that P uptake via the root system increases the surface area of the root system, which allows the plant to uptake more P, resulting in a continued cyclative process. Furthermore, research suggests that P nutrition is of utmost importance in the early parts of a growing season. As mentioned earlier, P is integral to energy reactions in the plant. If deficiencies are present in the soil solution in the early parts of the growing season, the plant may respond with certain adaptations that can ultimately reduce the potential yield of that plant. These adaptations include the diversion of resources to root production and increased root proliferation (Grant et al., 2001). Providing this valuable P nutrition to crops, especially at an earlier growth stage, is vital to the growth and yield of the crop in question. Because of this, P additions will continue to be important in the future of crop production.

1.1.2 Phosphorus Cycle

The supply of the biocritical component, P, is dependent on the cyclative properties of multiple chemical forms of phosphorus (Figure 1.1). The P cycle is a biogeochemical cycle that involves the cycle of P through the lithosphere, hydrosphere, and biosphere, and ultimately excludes the atmosphere, as P does not enter a gaseous phase readily (Schlesinger & Bernhardt, 2020). This is significant, as other biogeochemical processes, such as nitrogen (N), have an atmospheric phase, which provides a supply of this nutrient that can be manufactured through processes like the Haber-Bosch process (Rouwenhorst et al., 2021). This means that an understanding of the natural processes of these varying forms of phosphorus found in soil, and how these chemical forms interact with the larger environment is critical if responsible management of P is to be obtained.

Natural sources of P come from the weathering of various phosphate compounds, such as apatite and other sedimentary rocks (Adediran et al., 2020). Additionally, there are some parent materials of soil, such as phosphatic soils, that contain an additional source of natural P that is released slowly to the environment and is taken up by plants (Weeks & Hettiarachchi, 2019). However, natural sources of P in the soil have a very low concentration, especially when compared to other critical nutrients such as N, calcium (Ca), and sulfur (S). The chemical concentration of soil water P ranges from 0.001 mg L^{-1} to 1 mg L^{-1} (Weil & Brady, 2016).

Traditionally, plant roots absorb this P as inorganic phosphate ions HPO_4^{2-} and $H_2PO_4^-$. However, there is some research that suggests that plants can also uptake small amounts of organic forms of P in the soil (University of Hawai'i at Manoa, 2023). Under normal circumstances, organic forms of P, which include organic matter from plants and animal wastes, are converted to inorganic P sources by soil microbes through mineralization (Hyland, Ketterings, Dewing, et al., 2005). The form of inorganic P that is taken up by the plants is determined by the relative abundance of hydrogen ions, also known as the level of soil acidity or alkalinity. Once these forms of inorganic P are in the plant via root influx, a portion of the P is translocated to plant tissue. The fate of this P is determined by the fate of the plant in question; the plant can shed the leaves, the roots of the plant can die, or the plant tissue can be eaten by animals. In any case, P returns to the soil in the form of plant residues, leaf litter, and animal waste. Soil microorganisms that predominantly decomposes the residue that is left behind temporarily ties up a portion of this P in their cells (Weil & Brady, 2016). Eventually, the P is released into the soil solution via soil mineralization as previously mentioned.

There are multiple pathways in which P is lost in the soil ecosystem. One of these pathways is through plant removal, which in many cases is not returned via deposition of plant tissue like many cropping systems operate. However, there are other pathways of P loss in the P cycle that are much more devastating, which include wind and water erosion. Wind erosion is the loss of soil particles through wind erosivity forces, that have P attached to the soil particle surface. Water erosion also influences the loss of P in these systems, including surface runoff water, carrying dissolved forms of P attached to soil particles, and P leaching into groundwater. These two combined pathways contribute to over 50% of total P losses in agroecosystems (Alewell et al., 2020). In the case of soil erosion and surface runoff, environmental degradation is a risk due to the increase of eutrophication events from non-point P sources. This P enrichment in aquatic systems has a P concentration threshold that can be as low as 0.03 ppm of dissolved P (Hyland et al., 2005). In an effort to avoid P depleted soils, farmers apply organic forms of P such as manure and compost, or inorganic forms of synthetic P fertilizers. This P addition is essentially combatting the pathways losses described earlier, including crop removal, wind erosion, and water erosion through surface runoff.

1.1.3 Phosphorus Uptake by Agronomic Crops

The distribution of P in soils can be classified into four groups, which include primary P minerals (inorganic), secondary P minerals (inorganic), organic forms of P, and soil solution P. Approximately 65% of the total P found in the soil is in organic forms, while the remaining 35% of P is in inorganic forms. Examples of primary P minerals include variscite, apatite, and strengite, while secondary P minerals include Ca, iron (Fe), and aluminum (Al) phosphates (Prasad & Chakraborty, 2019). Phosphorus exists in the soil in large pools, almost exclusively as primary P minerals. These primary P minerals are not available for plant uptake and are not easily mineralized. However, through natural weathering events, soluble P is released in the soil that is available for plant uptake. This soluble portion of P is either taken up by plants or becomes attached to existing cations in the soil to become secondary P minerals (Ca, Fe, and Al). These secondary P minerals, although not available for plant uptake, can be mineralized by microorganisms that exist in the soil, and through this process are then available for plant uptake. This P returns to the soil in either plant (organic matter) or animal wastes, in the form of organic P. This pool is also not available for plant uptake but can go through the same mineralization process by microbes, to become available for plant uptake. Lastly, the smallest portion of P that exists in soils is the soil solution P, which exists mostly of inorganic P that is readily available for plant uptake (Prasad & Chakraborty, 2019).

Phosphorus is predominantly taken up by agronomic crops in the orthophosphate form ($H_2PO_4^-$ and HPO_4^{2-}). In acidic soil conditions (pH < 7), $H_2PO_4^-$ is the common orthophosphate that is available for plant uptake. These orthophosphate ions originate from primary and secondary minerals, as well as organic sources. Phosphorus ions in the soil are traditionally in low supply when looking at plant available forms of P. Other plant availability issues are due to the reactivity P has with soil surfaces making it relatively immobile in soil. Therefore, the method of P uptake through the system of mass flow is very minimal. This method of P movement is determined by the flow of water through a soil profile. Overall plant P uptake is driven by root interception and diffusion processes. However, phosphate ions are slow to move toward the root through diffusion. Diffusion has implications for the root zone, as the surrounding areas of the root are depleted during this process (Weil & Brady, 2016). An active and large root system is imperative for the supply of P to agronomic crops. In order for plants to access soil P through diffusion, they have to compensate by continually extending into new zones that have not been depleted. There are other factors that contribute to this depletion zone surrounding the root, including the soil physical factors, soil moisture, compaction, and soil temperatures. With this in mind, a large factor that determines the supply of P to plants is through root interception (Sawyer et al., 2000). Having an abundance of P in the soil solution and in proximity to plant roots is important for avoiding P deficiencies in agronomic crops. Furthermore, forming symbiotic relationships with mycorrhizal fungi in the soil increase the root surface area, which aids agronomic crops in the provision of P in the soil.

Another source of plant available P are organic P fractions, available after mineralization process by soil microbes. This mineralization occurs through a specific phosphatase-catalyzed biodegradation process from inorganic P, where solubility equals bioavailability. There are two common classes of organic P, which include monoesters and diesters, and monoesters compromise 50-70% of total organic P in soil (Cade-Menun et al., 2015). The organic P fraction of the soil is an important source of P fertility for plants, as a majority of the total P observed in ag soils is categorized as such. A majority of total soil P is categorized as organic, and is mainly derived from humus, phospholipids, and nucleic acids (Mylavarapu et al., 2021). To summarize, there is a small portion of P that is readily available for plant uptake when considering the whole mass of P that is found in soil. However, the portion of total P, which includes minerals and organic components of P, is in large supply, and over time become available for plant uptake. Once these forms become available, agronomic crops take up this P through diffusion or root interception, making the management of this P different than other nutrients that move through other forces, such as nitrogen (N) through mass flow.

1.1.4 Problems Associated with Phosphorus

There are several problems that exist with P management. Synthetic P fertilizers are created by blending phosphate rock (PR), a detrital sedimentary rock that has high amounts of phosphate minerals, and sulfuric acid to separate P and is the most common source of additions in cropping systems (FAO Land and Water Development Division, 2004). This PR is mined in centralized locations of the world (Khasawneh & Doll, 1979). Phosphate rock is formed by oceanic sedimentary deposits, and form over millions of years of P deposition (Filippelli, 2011). Contrary to existing mineral P in agricultural soils, PR is an example of a fleeting nonrenewable resource, creating a sustainability problem. The supply of this geologically derived P ranges from 50-500 years (Beardsley, 2011; Cordell, 2010). Coupled with the general supply of PR, there has been a trend of rising P fertilizer prices. Notable price spikes of PR were witnessed in 1975 and 2008 that were driven by global economic factors (Mew, 2016). The limited availability of this resource in the future, and the rising prices of agricultural inputs in general have the potential to create significant problems in food scarcity and agricultural production in the future.

Another problem associated with P in agricultural systems exists in the chemical nature of soil P. Ultimately, large pools of P exist in a large portion of the globe's agricultural soils, especially in regions where the soil is geologically new, and has not been subject to extensive weathering (Pagliari, 2018). Unfortunately, the negative charges of the orthophosphate anion ($H_2PO_4^-$ and HPO_4^{2-}) binds to other cations in the soil, creating insoluble complexes which are unavailable for P uptake by plants (Zee et al. 1988). Despite having anywhere from 112-3360 kg ha^{-1} of total P, a fraction of this is classified as soluble or labile in its availability to plants (Ohio State University, 2012). In the soil, phosphorus content is as much as 0.05% (w/w), however only 0.01% is available for plant uptake. This has to do with the complexes P forms in the soil (Alori et al., 2017). The chemical properties of soil determines the rate and types of these complexes; in acidic soil, P forms complexes with Fe^{3+} and Al^{3+} ions, and in basic soils, P forms complexes with $Ca^{2+.}$ These complexes arise from the general negative charge of orthophosphate, which attracts abundant ions in the soil (Strawn et al., 2015). This means that the longer plant-available forms of P reside in the soil solution, there is an elevated risk of this P being tied up and not being available for plant-uptake.

Another problem associated with soil P and P fertilization is the elevated risk of environmental degradation due to surface runoff. Rainfall events can force surface runoff of soils, that can enter aquatic ecosystems. In the event that soluble P rates are high, there is an increase of non-point P pollution that enters aquatic systems, resulting in eutrophication. Eutrophication is the enrichment of aquatic ecosystems with silt, nutrients, or pollutants that lead to algae blooms in these ecosystems, which cause a rise of carbon dioxide (CO_2) and a lack of oxygen as the algae are decomposed. This enrichment of water systems ultimately leads to the death of aquatic organisms (Walter, 1972). A common cause for elevated eutrophication is due to the continued overapplication of P fertilizers in an attempt to increase the amount of inorganic P in soil solution that contributes to crop growth (Bindraban et al., 2020). These problems are substantially elevated when an agricultural field is in close proximity to lakes and rivers, which is common in the US Midwest, such as the Great Lakes and the Missouri and Mississippi Rivers (Daniel et al., 1998). These persistent problems in P soil fertility create the need for producers to be more efficient in the use of P for agricultural production.

1.2 PHOSPHORUS MANAGEMENT

1.2.1 Phosphorus Application Methods

Phosphorus is a critical element used by plants and has a major role in a number of metabolic processes including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis, and plant respiration. Since its discovery in 1669 by alchemist Henning Brandt, P use has been widespread in fertilizer forms (Weeks & Hettiarachchi, 2019). In agricultural settings, the use of P fertilizer has led to tremendous improvements in crop yield across the globe, leading to major population increases and the improvement of nutrition for millions of people (Sharpley et al., 2018). There are multiple strategies that exist for producers to supply crops with this critical element.

Similarly, there are also multiple chemical forms of fertilizer that can be used for supplementation in crop production, in both organic and inorganic forms alike.

Organic P typically is in the form of compost or livestock manure. For farm operations that have a livestock enterprise, applying manure is a common practice to raise the P fertility in agricultural fields, and dispose of animal wastes responsibly. However, organic P must be mineralized by soil microbes, and at the time of application, is not readily available for plant uptake. Therefore, synthetic P application is common to supplement crops with immediately plant-available P forms, which can be in both solid and liquid form. Some examples of solid forms of P fertilizer include monoammonium phosphate (MAP; 11-52-0) or diammonium phosphate (DAP; 18-46-0). In SD, 67% of producers use the MAP form of fertilizer, followed by DAP at 30% (J. D. Clark et al., 2023). The most common form of liquid P fertilizer is ammonium polyphosphate (10-34-0). Traditionally, solid forms of P fertilizers are broadcast evenly across the field. From there, the delivery of this applied P is dependent on either precipitation to move this P into the soil, or to be incorporated using tillage methods. The latter is more commonly used, as P movement via mass flow is very slow, and cannot be delivered to the root zone in a timely manner for the subsequent crop to use. If no incorporation is used, this is known as the “top-dress” method, and can also be used in liquid forms, where the fertilizer is typically run through spraying equipment known as a stream bar. The top dress method is only recommended for forage crops due to the impracticality already mentioned (McKenzie, 2013).

A standard method of delivery for liquid P fertilizers involves using mechanical delivery systems on planting equipment. One example of mechanical delivery systems include seed-placed P systems, where fertilizer is placed in direct contact with the seed at planting. This method is sometimes referred to as “pop-up.” Another example of delivery systems for liquid P fertilizers is the “banding” method, where liquid fertilizer is placed in proximity to the seed at planting, and at the seed depth. The advantage of using either the banding or pop-up method is that it takes advantage of the fundamental properties of P movement in the soil; placing the P in proximity to the root zone allows the plant to expend less energy looking for this synthetic reserve and accelerates root growth. When looking at SD crop production, banding and pop-up methods of P fertilizer additions are more common in the western and central portions of the state which utilize no-till management more frequently. These farmers are unable to incorporate P fertilizers into the soil using tillage methods. However, when looking at SD as a whole, broadcast P applications, followed by tillage incorporation, is the most common method of P management, at 47% (J. D. Clark et al., 2023).

Another aspect to P management is the rate of P application and the timing of the fertilizer application. Typically, P application rates are determined by the crop grown, the yield goal of the farmer, and in some cases, the agricultural management practice that is used. In a SD survey in 2023, P rates for corn production ranged from 69 to 81 kg P ha^{-1}, with an average of 76 kg P ha^{-1} (J. D. Clark et al., 2023). Along with the rate of P fertilizer, application timing is an integral decision for crop producers in SD. Approximately 33% of P fertilizer applications for corn production in SD occur in the fall, and 50% of these additions occur in the spring. This varies from N application strategies, which have a much higher application frequency as a split application occurring in the middle of the season. This is likely due to the slow moving properties of

P in the soil, where high spring precipitation events will not lead to considerable losses of P. Additionally, early season P additions may aid the P to move to plant roots during the growing season. A large portion of SD farmers also only apply their P fertilizers for one growing season, while 13% of farmers apply P fertilizer to account for two or more growing seasons (J. D. Clark et al., 2023). Applying P fertilizers for one growing season at a time reduces the risk of P becoming unavailable to plants by binding to minerals in the soil, and also reduces the risk of eutrophication via surface runoff.

Stewardship of P application is another aspect of P management that has gained more focus in agriculture in recent years. Since the development of various synthetic P fertilizers, there has been a rising concern related to the potential of P fertilizers becoming an environmental hazard. This hazardous risk was spurred by the abundance and previous history of cheap access to these synthetic components. Meanwhile, in other parts of the globe, P fertilizers are not feasible due to a more recent rise in synthetic P prices and the inherent chemical properties of soil, leading to poor reactivity and efficacy (Blessing et al., 2017). With this in mind, it becomes clear that producers around the globe need a new approach to managing their P applications to better suit their economic needs and consider the potential environmental damage. To combat this, government officials imposed regulations on the fertilizer industry to create a guide for fertilizer use known as Best Management Practices (BMP) for numerous regions of the world. From this BMP approach, the 4R Nutrient Stewardship concept was developed, which implies applying the right source of nutrients, using the right rate, at the right time of the growing season, and in the right placement (A. M. Johnston & Bruulsema, 2014). This stewardship concept ultimately proposes an alternative strategy of fertilizer application that had not been considered in the past. Producers become more mindful in their approach of nutrient provision to their crops, and in many cases, reduces the number of synthetic products needed during a growing season. A reduction of inputs reduces the investment needed each year and reduces the risk of eutrophication to surrounding water sources. The 4R nutrient stewardship model has also adjusted the fertilizer recommendation model provided by land grant universities across the U.S. and is the basis of nutrient application research in the last several years. Rate, placement, timing, and fertilizer sources studies are ubiquitous in modern research grants, increasing regional fertility understanding.

1.2.2 Conventional P Management

Agricultural management practices affect P management in a major way. Examples of agricultural management practices that influence P dynamics include soil tillage operations, residue management, crop rotations, and many other choices that may affect the underlying principles of nutrient movement and the microbial activity that influences them. An example of a management system that is commonly used is conventional farming management. In conventional farming systems, mechanical equipment and agrochemicals are intensively used. One form of mechanical equipment that is commonly used in conventional systems is the use of many forms of tillage to disturb the soil. For millenniums, tillage equipment has been used on arable soil to prep the seed bed for planting, reduce weed population density, and mineralize residue for nutrient boosts. This broad management practice is still employed on a large portion of global agricultural land today. In SD, nearly 20% of farm ground uses conventional management (USDA NRCS, 2014). In regard to P management, conventional systems depend on the physical movement of mineral P to desired locations in the root zone. As mentioned previously, broadcasting, or top dressing P fertilizer is only suggested if the nutrients are incorporated into the soil. Conventional farming practices operate under the use of these agrochemicals, as well as combining the use of mechanized equipment. Additionally, reducing the residue on the surface of the soil is a catalyst for microbes in the soil to mineralize residue, leading to short bursts of nutrients from this decomposing material.

The biggest drawback to using conventional style agriculture is the effect of the environment. The disturbance of the soil impedes the ability of the soil to infiltrate water when compared to other management strategies (Bergtold & Sailus, 2020). This means that the agrochemicals that are applied are at risk of being lost due to rainfall events that cause surface runoff. More importantly, the tillage operations that conventional agriculture requires disrupt the microbial biome in the soil. Disrupting the soil severs fungal hyphae networks that are found in the soil and change the natural cyclative system that these organisms provide. This means that tillage operations disrupt the natural living cycle of mycorrhizae fungi (Kabir, 2005). Additionally, soil disturbance in the form of tillage affect the soil structure and leads to increased wind and water erosion (Seitz et al., 2019). If the soil cannot infiltrate water at effective rates, and the microbial organisms that reside in the soil cannot cycle the nutrients like they have evolved to, that means these conventional systems are dependent on the application of more agrochemicals to continue to produce the high yields that are demanded in modern agriculture.

1.2.3 No-till P Management

The ubiquitous use of tillage equipment in agriculture resulted in many environmental problems that led researchers to explore alternative methods of crop production. Additionally, the agricultural economic crisis in the later part of the 20th century catalyzed farmers and researchers to develop alternative farming methods that require less labor and are more profitable. Because of this, no-till farming has gained popularity in modern agriculture due to the efforts of these researchers pursuing a more environmentally sustainable system that is profitable for farmers. Phosphorus management in no-till systems is dependent on maintaining and feeding the microbial communities that reside there. Not disturbing the soil and maintaining the crop residue on the surface allows for various microbial organisms, like mycorrhizal fungi, to grow and create symbiosis with the crops that are grown there. In SD, the western and central regions of the state predominantly employ no-till methods due to the drier climates present there. From 2004 to 2014, SD saw an increase in no-till acreage in the state, from 37% to 45%, making this the most popular form of soil management (USDA NRCS, 2014). Reducing tillage events allow valuable precipitation events to enter the soil and reduce the effects of drought as a yield limiting factor. This soil management method reduces erosion events as well, making it an ideal farming management system.

Soil disturbance accelerates soil erosion and weathering processes, leading to irreversible soil losses and depletion known as soil degradation.. These inadvertent soil losses reduce the productive capacity of an ecosystem and results in alterations in water and energy balances, disrupting the cycles of carbon (C), N, S, P, and other elements (Lal & Stewart, 2012). Furthermore, government subsidization has transformed the most productive acres in the country into monoculture cropping systems that exacerbate these problems. Over time, observations of these destructive events and their effect on farming systems were noted and alternative farming systems were studied. These studies resulted in the advent of more sustainable farming practices like no-till and diverse cropping systems. Conservation practices such as no-till can increase soil microbial activities, soil moisture, organic matter, aggregate stability, cation exchange capacity, and crop yield, all while reducing the risk of soil degradation (Cannell & Hawes, 1994).

Utilizing no-till farming management creates an inability of incorporating solid forms of P fertilizers using tillage equipment. Because of this, top dressing, or seed placed P fertilizer is the common method of P application in no-till systems. In the event of seed placed or banded P fertilizer, precision agriculture techniques can be used to reduce the P inputs that a producer uses, limiting the dependency of agrochemicals including P fertilizers. In SD, on average, no-till producers apply 11.2 kilograms less P_2O_5 per hectare than conventional producers (J. D. Clark et al., 2023). Over long-term no-till management, it has been theorized that the margin of difference between P additions can be greater, as long-term no-till farmers have continued to decrease P additions in their agroecosystem. Other studies have also shown that no-till farming management decreases P applications in comparison with conventional management. In a study done in Milan, Tennessee, a comparison between these two management practices yielded that optimum P fertilizer application in a conventional system was 19 kg P ha^{-1} more than a no-till system (Howard et al., 2002). Furthermore, no-till studies have also seen that no-till management can increase P uptake efficiency of certain crops like corn (Xomphoutheb et al., 2020). In a case study done in a long-term no-till field in central

SD, corn yield increases were seen while simultaneously reducing P fertilizer additions by 30% (Anderson, 2016). These study results showcase the potential of no-till management in supplying P in sufficient amounts to crops, while simultaneously reducing the required P to optimize yield.

A sustainable provision of P in an agricultural system originates from the improvement of microbial communities in the soil, which are influenced by the utilized management practices of that system. Utilizing practices such as no-till, diverse cropping systems, cover crop implementation, and a reduction in synthetic inputs lead to greater C, N, and P cycling enzymes (Mbuthia et al., 2015). A large reason for this occurrence is the buildup of microbial organisms such as mycorrhizal and saprophytic fungi that do not exist in conventional systems, due to the severing of their mycorrhizal hyphae during a tillage operation (Jansa et al., 2003). Additionally, a key component of these microbial communities is the reduction of synthetic inputs as these additions have adverse effects on the functionality of the soil that it is applied to. Synthetic fertilizer additions over the long term inhibit the biodiversity in that soil by suppressing the role of cyclative organisms and enhancing the role of everything that feeds on the added nutrient (Tripathi et al., 2020). A reduction in tillage events and synthetic inputs ultimately increase the amount of organic P concentrations, rather than relying on inorganic forms. These management changes, combined with an application of manures can improve organic P and microbial pools in the soil (Dodd & Sharpley, 2015). This improvement of microbial efficiency and P sustainability is the foundation of the study being presented.

1.2.4 Soil Test Phosphorus

Collection of soil samples is critical to assess plant-available P, also known as the labile or soluble pool of P in the soil. These soluble P measurements are typically completed to construct accurate P fertilizer recommendations. The principles of P movement and supply to plants indicate that P does not readily move through the soil profile. Because of this, soil P testing is traditionally done in the shallow depths of soil. To obtain soil P results that result in coefficients for crop yield, 0-15cm, 5-15cm, or 5-10cm are commonly used. In no-till systems, P stratification can affect soil test analysis levels, and is an important aspect to consider (Reed et al., 2022). The proposed depths are dependent on the P application method, crop type, and farming management practices. For example, a 0-5cm soil sampling depth can be used to capture a banded portion of soluble P if collected as a cross-section volume.

Multiple soluble P measurements exist to determine available P including Olsen P, Bray P-1, Mehlich-I, Mehlich-III, Lancaster P, Morgan P, and Modified Morgan P tests, each using a different extractant, and methodology to collect soluble P (Bray & Kurtz L. T., 1945; Cox, 2001; Lyons et al., 2023; McIntosh, 1969; Mehlich, 1953, 1984; Morgan, 1941; Sterling Olsen, 1954). Each test has been developed to account for different biological, chemical, and physical factors in the soil. Each one of these tests follows a laboratory procedure that is outlined for the north central region of the U.S. (Frank et al., 2012). The Olsen P test is traditionally better suited for soils with higher pH values, typically >7. The Bray P-1 test has better performance in acidic soils with pH values <7 and has been shown to underestimate available P in neutral and basic environments. The Mehlich-III uses a more technical extraction protocol, using multiple acidic extractants. This test arose from the need for universal extractants for soil testing and follows similar characteristics as the Olsen test (A. Mallarino, 1995). Each soil test has an established critical value, where concentrations are deemed too low or detrimental for crop growth. However, some research suggests that yield response and phosphorus agronomic efficiency (PAE) is more significant in low soil test P levels (Ros et al., 2020). Some other factors that affect yield response and PAE in agroecosystems are climate, grass/legume mixtures, pH, and soil test levels/P application rates (L. J. R. da Silva et al., 2024).

1.2.5 Developing P Fertilizer Recommendations

Accurate fertilizer recommendations are a focus of producers and researchers for proper nutrient management and sound agronomic practices. In SD, fertilizer recommendation guides are created by South Dakota State University (SDSU) Extension personnel and are based on field research in SD and neighboring states. South Dakota State University fertilizer recommendation guide is provided in tables that were developed as part of continuing cooperation between surrounding states in an attempt to standardize recommendations. The recommendations that are present in these tables are generated by equations, with factors like yield goals and soil test levels included as variables that ultimately affect P fertilizer recommendations. The generation of these equations are based off of economic optimum fertilizer curves that are created through rate studies across the state, in varying climatic, and chemical conditions, and represent the probability of yield response to P fertilizer additions (J. Clark et al., 2023). Variations are observed in P recommendations as these yield goals and soil test values change. Furthermore, P fertilization recommendations change when considering the crop type that is grown for a specific growing season. The reasoning for this resides in the fact that crops vary in their P uptake intensity and demands for any given growing season. An example of these varying P fertilizer recommendations exists when comparing P applications rate in corn and soybeans. In soybean, P fertilization recommendations range from 0-120 kg ha^{-1} depending on soil test level and desired yield goal (Table 1.1). In corn, P fertilization recommendations range from 0-142 kg ha^{-1} depending on soil test level and desired yield goal (Table 1.2).

Other states in the U.S. Midwest also develop fertilizer recommendations based on soil test phosphorus (STP) categories, crop type, and yield goals. However, there are additional factors that are considered when looking at these other U.S. Midwest states, including region and irrigation practices, P application methods (banding vs broadcast), and maintenance P level strategies (Culman et al., 2020; Franzen, 2018; Kaiser et al., 2023). Between all of these states, a classified low STP testing soil would have a suggested P_2O_5 application that ranges from 62-92 kg ha^{-1}.

One factor that is not included in these P fertilizer recommendations among the U.S. Midwest is the use of no-till farming practices, and other management strategies that are growing in popularity for this region. In most cases, no-till is either not considered in these P fertility trials to determine recommendations, or the results from no-till are combined with the results from conventional farming practices. This is troublesome as the use of no-till has been found to influence yield response to P fertilization, as discussed in section 1.2.4. These discussed results suggest that the P dynamics in soil are significantly affected by the management practice that producers use and provide evidence that current P fertilizer recommendations may need to be reevaluated. Including no-till in the fertilizer recommendation guide or separating the no-till and conventional sites may provide more accurate P fertilizer estimates to producers that utilize these management practices. Factors such as these influence the influx of P into agronomic crops and should be considered when pursuing P fertility objectives.

1.3 BIOLOGICAL INFLUENCE ON PHOSPHORUS

1.3.1 Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi (*Glomeromycota*: AMF) are one of eight currently recognized divisions of fungi, with an approximated 230 species (Hibbett et al., 2007; Redecker & Raab, 2006). This division of fungi are organisms that form biological associations between themselves and terrestrial plants that are symbiotic in nature. Most of the microbial evidence that exists on AMF suggest that these organisms are dependent on terrestrial plants for C and their energy supply, but there has been some suggestion that that they can survive independently (Hempel et al., 2007). Arbuscular mycorrhizal fungi reproduce asexually through development of the hyphal tip to produce fungal spores, typically with a diameter of 80-500 µm (Schüβler et al., 2001). These spores play a role in colonization of terrestrial plant roots as a source of inoculum. Other inoculum sources include existing fungal hyphae and infected root fragments; however, spore presence and density have shown to have the most significant impact in root colonization (Zangaro et al., 2013). Upon germination, AMF hyphae penetrate the root cortical cells and expand intercellularly for their point of entry. After the AMF have entered the cortical cell walls, the AMF form tree-like branched structures called arbuscules, that then serve as the site of metabolic exchange between the fungus and the terrestrial host plant (Rodrigues & Rodrigues, 2019). Arbuscular mycorrhizal fungi are widely distributed among the world's soils and can form relationships with 80% of plant species, making AMF one of the most abundant microbial organisms in the rhizosphere (Smith & Read, 2008).

It has been theorized that AMF first created relationships with plants around 450 million years ago when plants first evolved from marine environments to terrestrial ones (Redecker et al., 2000). The AMF extended the root surface area of the underdeveloped plants and allowed the plants to thrive in terrestrial ecosystems. This mutually beneficial relationship has continually adapted over millions of years, allowing plants to grow in hostile environments and continue to be resilient in a host of environments. In this relationship, plants provide the fungal phylum *Glomeromycota* with photosynthetic C in exchange for ecological functions critical to plant growth (Willis et al., 2012). Some of these ecological functions include production of inorganic fertilizers (symbionts), upregulating tolerance mechanisms, and preventing down-regulation of key metabolic pathways. Arbuscular mycorrhizal fungi are also key in plants having tolerances to abiotic stresses like heat, drought, and salinity (Kula et al., 2005). The important ecosystem function that AMF provide that will be explored in this study include the acquisition of insoluble P and organic P and providing these forms of P to the plant in a usable form. Network formation by AMF with plant roots increase soluble P acquisition by producing organic acids and phosphatases, an enzyme that removes phosphate groups from a protein (Lee et al., 2014). This acquisition of P for plants has major implications on sustainable P usage, and could be a solution to overapplication of P, and environmental risks that were discussed in earlier chapters.

There are several agricultural management strategies that have been known to disrupt or limit the functionality of mycorrhizal fungi in the soil ecosystem. The first and most prominent is tilling the ground. Any soil disturbance in the ground damages and severs the mycorrhizal network in the soil, which kills the fungal organisms, thereby reducing the benefits to crop and soils that originate from AMF (Kabir, 2005). It has also been observed that the use of fertilizers, biocides, and monocultures also leads to a decline in mycorrhizal activity and functionality (Gosling et al., 2006). In this study, it is being theorized that AMF and its associations with plants are dissolving the insoluble P complexes in the soil, tapping into the reserves of the total P in the soil profile. This may allow for the lower STP soils to produce crops with similar yields when compared to the higher STP levels. Furthermore, it has also been theorized that intentionally keeping STP levels low allows for more mycorrhizal relationships, and greater fungal density. In low STP environments, the plant has to actively look for relationships to provide the soluble P, whereas the soils with higher STP have readily available P in the root zone. In this study, no-till management is used throughout the entire experimental area and is classified as a regenerative farm. This creates an ideal environment to study treatment effects of AMF in response to varying STP levels.

1.3.2 Other Organisms i.e. Bacteria, Actinomycetes, etc.

The microbial pool found in agricultural soils is of immense population density, whose full scope of the activity and functionality are not fully understood. In a single gram of agricultural soil, there can be billions of bacteria, with as many as 60,000 different bacterial species, most of which have not even been named, with varying capabilities (Reid & Wong, 2005). It is estimated that 10^4 to 10^8 actinomycete organisms exist in a single gram of agricultural soil (Barka et al., 2016). Along with soil fungi that has already been discussed, these organisms are in major supply in the soil, and their functionality is dynamic. Soil fungi have been heavily studied for their P solubilization potential and are the focus when discussing sustainable P management. However, other organisms exist that have exhibited properties that also solubilize P, creating a source of plant-available P, known as phosphate solubilizing microorganisms (PSM). Phosphate solubilizing microorganisms also include soil fungi in their classification. The PSM class of organisms create pools of plant-available P in one of two ways. First, they enhance the dissolution of insoluble P complexes attached to minerals through a combination of soil acidification and release of metal complexing agents. Second, PSM breakdown organic P found in soils through enzymatic processes, which is characteristic of P cycling in natural environments (Jones & Oburger, 2011). Typically, PSM exist in the top layer of the soil, with the highest concentration of organic matter. This layer provides an energy source for these microbes to carry out these cyclative processes.

In addition to other organisms contributing to the provision of P in agricultural soils, these PSMs also outnumber fungi in the microbial pool of soil. Phosphate solubilizing bacteria (PSB; the bacteria portion of PSMs) make up 1-50% of the total microbial population in soil, which is a much higher portion than fungi, which are responsible for this same solubilization process (Pandey et al., 2019). This solubilization process includes mineralizing organic P pools in the soil to create inorganic P ready for plant uptake, as well as solubilizing insoluble P complexes, similar to soil fungi. Gram-negative bacteria species make up a large portion of these PSB organisms, with a small addition of gram-positive bacteria species, as evidenced by a study done in Pakistan that aimed to classify this important pool or microorganisms (Tariq et al., 2022). These studies show the importance of analyzing the presence of bacterial communities when discussing the availability of P in conservation systems such as no-till.

1.3.3 Testing for Soil Biology

Soil biological activity is a key indicator for agricultural productivity and environmental quality. Furthermore, a large portion of soil health classification is determined by the measurement and functionality of biological activity in the soil. The distribution of functional groups in the soil, and how to differentiate between them provides valuable insights to the below-ground ecosystem, and how this is functioning in cooperation with the crops in a growing season. However, testing for microbial activity and functionality is relatively new, and methods are constantly being developed to help researchers and producers alike. This development will help these individuals better understand the functionality of soil, and what can be done to improve this activity.

Microbial soil testing can be accomplished in several ways. One of the more common tests that are employed by researchers and producers is the measurement of CO_2 bursts in a soil sample. Soil microbes respire CO_2 through the decomposition of organic matter (OM) and plant litter. Therefore, the relative measurement of CO_2 can determine the microbial activity that is present in that soil. However, differentiating between microbial organisms that are responsible for this CO_2 respiration is difficult to do. Another option for soil microbial testing includes the use of biomarker tests such as phospholipid fatty acid (PLFA) or neutral lipid fatty acid (NLFA) testing. These tests reveal the content of differing extractable phosphorylated-lipids known to be the constituents of cell walls of microbes. These lipids are easily extractable from the soil with the use of organic solvents. To interpret these tests, specific lipids are assigned as biomarkers or signatures for certain classifications of organisms (Frostegård et al., 2011). Unfortunately, some PLFA compounds are not specific to microorganisms, and are shared across microbial communities. Because of this, there is still some issues related to the differentiation between organisms that CO_2 burst test have. A more comprehensive microbial test that can differentiate these organisms has evolved to DNA sequencing and eco computing. These tests, offered by numerous companies, can identify the types of microbes that are present in a soil sample and offers a better measurement of potential activities in the soil microbiome. Having this information allows researchers and producers to make more targeted decisions for soil management and allows these individuals to consider some of the complex relationships that exist between plants, soil nutrients, and soil microbes.

Other methods exist to determine microbial life in soils that have been adapted and designed to identify specific microbial organisms. An example of one of these methods includes the most-probable-number (MPN) assay to determine AMF organisms. In this method, identification of AMF propagules is done on soil samples from a specific location, which includes spores, infected root pieces, and vegetative hyphal fragments, all of which represent total inoculum potential. This identification is done by using serially diluted soil in a sterile potting mix, with the addition of a host plant, such as Bahai grass (*Paspalum notatum* Flugge). After utilizing greenhouse techniques to grow this host plant, roots are removed, washed, and stained with trypan blue to identify these AMF structures under a microscope. The results of this assay yield the number of fungal propagules per gram of soil. This method is traditionally used by many soil microbiologists and has been previously documented in many studies (Douds et al., 2011; Lehman et al., 2012, 2019). The advantage of this assay is that the results are consistent and reliable, as it accounts for all of the total AMF inoculum potential present in each soil sample. However, the MPN analysis takes considerably longer than traditional microbial soil tests, and only gives results related to AMF organisms, and does not yield results for other important microbial groups like bacteria and actinomycete values. In order to get an accurate measurement of soil microbial life, researchers should be mindful of the advantages and disadvantages of the current microbial tests that exist, and prioritize what results are needed for their respective studies. Additionally, if reporting broad results of the microbial activity in soils, researchers should maybe consider analyzing soil microbial life utilizing multiple microbial methods.

1.3.4 Ergothioneine

Ergothioneine (ERGO) is an antioxidant and anti-inflammatory amino acid that naturally occurs in soil environments. It is commonly produced by non-yeast fungi, cyanobacteria, and mycobacteria. There is accumulated evidence that ERGO can be considered a longevity vitamin that can mitigate chronic disease of aging and thereby increase life expectancy (R. Beelman, 2024). ERGO is not produced by plants, rather it is found in plant products such as grain. This is linked to detrital or symbiotic soil fungi passing on ERGO to plant through their roots, specifically mycorrhizal fungi, which is a cornerstone of this project. Soil and crop management have been shown to affect ERGO concentrations, most notably tillage intensity (R. Beelman, 2024). This has to do with tillage reducing fungal populations, as mechanical implements permanently damage and sever the hyphae of mycorrhizal fungi. In a study done comparing tillage intensity, ERGO concentrations and also yield were dramatically influenced by the intensity of tillage where a 30% reduction of ERGO concentrations in harvested seed was found between moldboard plow treatments compared to no-till treatments (Beelman et al., 2021). This suggests that growing crops with conservative management practices, or practices that influence the population of fungi in a beneficial way can lead to higher concentrations of ERGO in crop tissue and can lead to better diets of humans (Carrara et al., 2023).

Ergothioneine is a sulfur-containing derivative of the amino acid histidine, which is obtained exclusively through human diet. Despite it being discovered over a century ago in rye ergot, its physiological function has not been clearly defined (B. D. Paul & Snyder, 2010). There are several unknowns related to ERGO, but the accumulation, tissue distribution, and scavenging properties signify the potential for ERGO to function as physiological antioxidant. Although ERGO is primarily produced by fungal organisms and transmitted through human diet, humans and mammalian species have shown accumulation of ERGO in various cells and tissues at high concentrations (100µM to 2mM) (Cheah & Halliwell, 2012). This is primarily attributed to the transmission of ERGO through soil-borne fungi or bacteria to plant tissue, which enters the food chain. This key idea reflects the notion that ERGO signifies a definitive connection between soil health and human health (R. Beelman et al., 2022). Although agricultural commodities like corn and soybeans are not traditionally used in human diets, if ERGO can be classified as a soil health category, it can be readily used to approximate fungi populations in the soil and can signify relative soil health.

1.4 CONCLUSIONS

Agricultural production in the United States is a critical component to the food production and economic system and is dependent on the supply and management of macronutrients such as P. Understanding the cyclative properties of P, as well as the physiological uptake of P is important in providing this nutrient to crops when they need it. Being capable of growing healthy, non-nutrient deficient crops will continue to be an important factor for a developing civilization. As crop yields continue to improve in our crop production systems, P fertilizer and its application will continue to be essential to producers across the U.S. However, when considering the supply of P for crops, producers need to be mindful of the problems associated with non-point P pollution to the environment, and the limited availability of synthetic P fertilizers in the future. When considering these factors, stewardship regarding P fertilizer applications needs to be achieved if agricultural production is to continue in the U.S.

Currently, there are multiple strategies that exist for managing this biocritical element in agricultural systems. Conventional P management involves physically moving P nutrients to the root zone by using soil tillage. This management style has been studied extensively as it is considered the standard management method by producers in the U.S. Conversely, no-till management typically utilizes seed placed or banded methods of P application to supply crops with this nutrient. Some studies have suggested that P dynamics in no-till systems are significantly changed, requiring less P to achieve optimum yield. The effect of no-till management on optimum P rates needs to be further studied in the U.S., and this study aims to provide evidence that optimum STP rates in no-till systems may need to be reevaluated and adjusted.

One potential reason that no-till research studies have found lower optimum P rates in comparison with conventional studies is the improvement of the soil microbial life, specifically soil fungi species like AMF. Arbuscular mycorrhizal fungi have been studied for their P sustainability potential in the past, as these organisms are capable of dissolving insoluble P complexes that account for the total P found in agricultural soils. Using farming management practices like no-till create more suitable environments for AMF organisms and allow for cropping systems to access the total P reserve and require less additions from synthetic P sources. Other microbial organisms, including soil bacteria may also be improved using these management systems. Measuring these microbial organisms in the soil has been a challenge in the past, however, as multiple assays exist to approximate these organisms, but have either been inconclusive (PLFA) or are time and labor intensive (MPN). Therefore, the objectives of this study were to 1) determine in a long-term no-till field the effect of different STP levels on crop yield and soil biological activity as measured by the PLFA and MPN assay and 2) determine if the more cost-effective PLFA test instead of the MPN test can be used to effectively identify any differences in AMF due to STP level.

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1.6 TABLES

Table 1.1. South Dakota State University P fertilization guide for soybean production in SD.

		Soil Test Phosphorus (ppm)				
		VL	L	M	H	VH
Yield	Bray-P1	0-5	6-10	11-15	16-20	21+
	Olsen P	0-3	4-7	12-15	12-15	16+
kg ha^{-1}				P_2O_5 kg ha^{-1}		
2016		45	26	11	0	0
2688		61	35	11	0	0
3360		75	44	12	0	0
4032		90	53	15	0	0
4704		105	62	17	0	0
5376		120	69	20	0	0

Table 1.2 South Dakota State University P fertilization guide for corn production in SD.

		Soil Test Phosphorus (ppm)				
		VL	L	M	H	VH
Yield	Bray-P1	0-5	6-10	11-15	16-20	21+
	Olsen P	0-3	4-7	12-15	12-15	16+
kg ha^{-1}				P_2O_5 kg ha^{-1}		
6272		71	52	31	12	0
7526		85	62	72	15	0
8781		100	72	44	17	0
10035		113	82	50	19	0
11290		128	92	57	21	0
12544		142	103	63	24	0

1.7 FIGURES

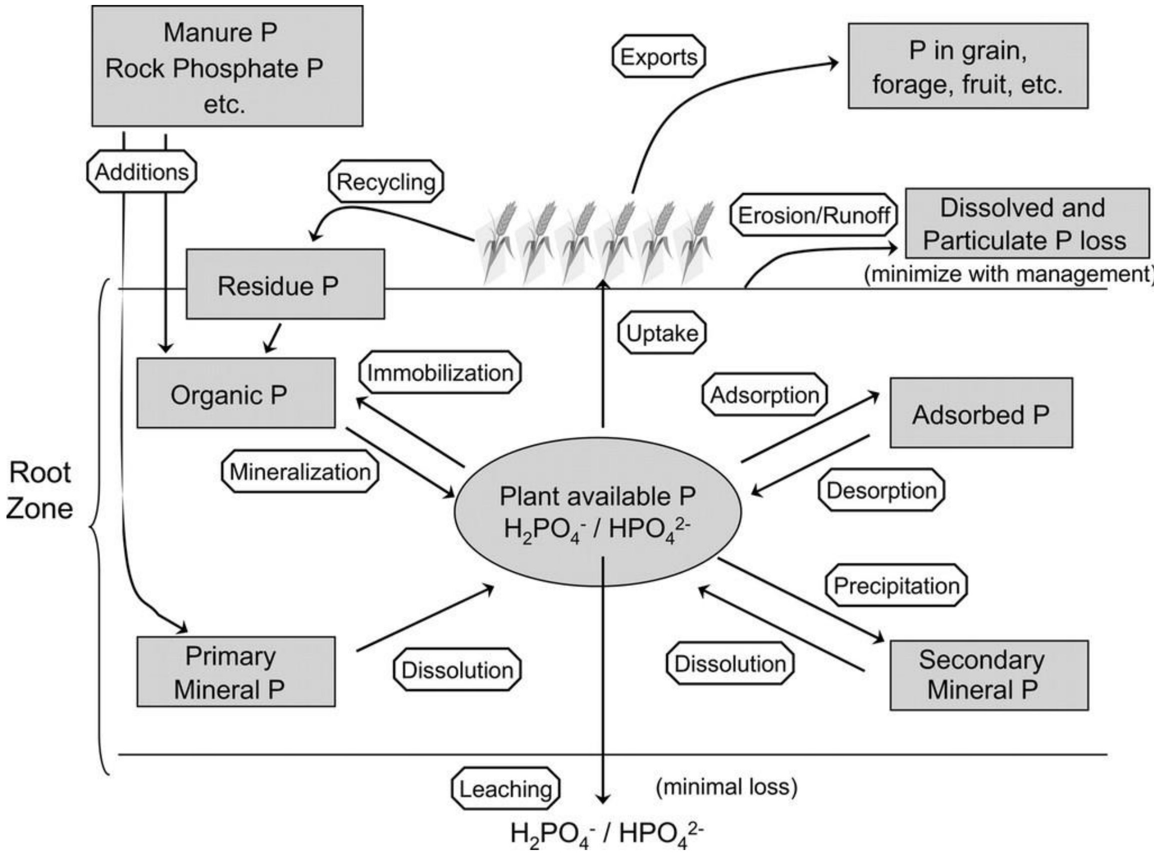

Figure 1.1. The P cycle in agricultural production systems, including P additions, exports, cycling, and transformations within the soil (Nelson & Janke, 2007).

CHAPTER 2 SOIL TEST PHOSPHORUS LEVEL AFFECTS MICROBIAL PARAMETERS

2.1 ABSTRACT

Using no-till may be able to lower the soil test phosphorus (STP) level needed to optimize crop yield due to the increase of arbuscular mycorrhizal fungi (AMF) in these systems. However, the effect of varying STP levels on the formation of AMF in no-till managed soil has not been studied. This study was conducted to 1) determine in a long-term no-till field the effect of different STP levels on crop yield and soil biological activity as measured by the phospholipid fatty acid (PLFA) and most-probable-number (MPN) assay and 2) determine if the more cost-effective PLFA test instead of the MPN test can be used to effectively identify any differences in AMF due to STP level. On a long-term no-till field in Pierre, SD, the effect of three STP level categories (low, medium, and very high) on AMF abundance, measured using the PLFA and MPN assays, and yield was investigated. From 2018 to 2022, there was no economic yield increase among the three STP categories, which may be explained by the greater amount of AMF propagules in the low (5 propagules g^{-1} soil) and medium (2 propagules g^{-1} soil) compared to the very high (1 propagules g^{-1} soil) STP category. Results from the PLFA assay were inconclusive as soil fungi and bacteria showed microbial differences in only one out of five years, as a result of stressful weather conditions. Therefore, in long-term no-till systems producers can reduce the STP level of their fields without experiencing an economically significant yield decline potentially due to greater AMF accumulations.

2.2 INTRODUCTION

Phosphorus (P) is an essential macronutrient that helps determine plant growth and productivity and is one of the most common yield-limiting factors in crop production (Q. Wu et al., 2022). Phosphorus is a major physiological component of molecular nucleotides, including adenosine triphosphate (ATP) and deoxyribonucleic acid (DNA), the energy currency and the genetic code for plants, respectively (Weil & Brady, 2016). In addition to molecular building-blocks, the availability of P for plants has implications on key metabolic processes, including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis, and plant respiration (Weeks & Hettiarachchi, 2019). Natural sources of P for plants come from the weathering of phosphate compounds found in sedimentary rocks as a part of the P cycle (Adediran et al., 2020). Additionally, organic matter (OM) in the form of plant residues also provide a natural source of P in the soil (Prasad & Chakraborty, 2019). When these natural sources of P in soil become low enough to reduce crop yield, producers typically apply organic forms of fertilizer (manure and compost) or inorganic forms of P fertilizer. Application of these fertilizers is done to supply crops with enough of this essential element to optimize yield, and avoid nutrient depleted and infertile soil. In commercial crop production, application of synthetic fertilizer is the most common practice and in the U.S. from 2010 to 2021, farmers applied an average of 4 million metric tons of P fertilizer per year (International Fertilizer Industry Association, 2023). It is important to note that there is no nutrient that can replace P in an agroecosystem, and because of this, application of P fertilizers has become an integral part of agricultural production.

To avoid P deficient soils and to help determine when producers will see a yield response to applying fertilizer, researchers developed fertilizer recommendations through years of fertility trials that take place in multiple locations. These recommendations are primarily based on the extraction of nutrients from soil such as P (Culman et al., 2023; Lyons et al., 2023; Sterling Olsen, 1954). Quantification of soil test P (STP) is commonly done with the use of an extraction solution like the Bray P-1, Olsen P, Mehlich-I, Mehlich-III, Lancaster P, Morgan P, or Modified Morgan P solutions (Bray & Kurtz, 1945; Cox, 2001; Lyons et al., 2023; McIntosh, 1969; Mehlich, 1953, 1984; Morgan, 1941; Sterling Olsen, 1954). These tests represent readily available P for plant uptake, but also represent the portion of P in the soil solution that is at most risk for fixation (Lehman & Taheri, 2017). The use of STP in P fertilizer recommendations is common across all states in the U.S., but other factors that vary by state may also be used including yield goal, regional location, irrigation methods, and P application methods (banding vs broadcast) (Clark et al., 2023; Culman et al., 2020; Franzen, 2018; Kaiser et al., 2023; Mallarino et al., 2013). One factor that is not included in current P recommendations is the use of no-till farming practices. This is problematic, as the use of no-till management can affect yield response to P. For example, a comparison of optimum P rates between conventional tillage and no till systems was done in Milan, Tennessee, which showed that conventional tillage systems had optimum P rates that were 19 kg P ha^{-1} higher than no-till systems (Howard et al., 2002). Further, in a study done in northeast China, continuous no-till management improved the uptake of P in corn (Xomphoutheb et al., 2020). However, the effect of no-till on the amount of STP needed to optimize yield has not been determined in the northern climate of SD. Determining the effect of no-till on P needs in SD is important as the number of no-till hectares has increased by 29% from 2004 to 2014, with 577,286 ha of farmland using no-till (USDA NRCS, 2014). Additionally, utilizing no-till, which may be able to subsequently lower P fertilization needs is advantageous as the mining of phosphate rock in centralized locations of the world is the main source of synthetic P, and the supply of this geological derived P ranges from only 50-500 years (Cordell, 2010; Khasawneh & Doll, 1979).

Given that synthetic P fertilizer is a finite commodity, alternative sources of plant available P are needed for the supply of this essential element. One such alternative may come from chemically-bound P sources already in the soil. Large pools of P exist in most agricultural soils, especially in regions where the soil is geologically new, and has not been subject to extensive weathering (Pagliari, 2018). Unfortunately, the negative charge of this P source binds to cations in the soil, creating insoluble complexes which are unavailable for P uptake by plants (van der Zee & van Riemsdijk, 1988). For plants to access this source of P in agricultural soils, these insoluble P complexes need to be dissolved and transported to plant roots.

One benefit of no-till compared to conventional tillage farming management is the increase in fungal organisms (Kabir, 2005). One of these soil fungi organisms, arbuscular mycorrhizal fungi (AMF) can dissolve the insoluble complexes of P found in the soil and transport this now available form to plants on their mycorrhizal hyphae (Willis et al., 2012). These AMF organisms function this way due to their evolution with plants over the last 460 million years and can form a symbiosis with 80% of terrestrial plant species (Redecker et al., 2000; Smith & Read, 2008). The advent of conservation practices like no-till and multi-species cropping systems create more suitable environments for these AMF organisms to accumulate and contribute to sustainable P management (Manoharan et al., 2017). Along with no-till, P fertilization also influences soil processes involving soil microorganisms, and increasing the amount of P has been found to reduce the colonization rate of AMF organisms with plant species (Beauregard et al., 2010; Menge et al., 1978). These results show no-till and P fertilization can affect AMF organisms and other microorganisms that are major contributors to P cycling in soils, which current P recommendations do not consider. Additionally, the effect of varying STP levels in a long-term no-till field on AMF is unknown.

To effectively improve P fertilizer recommendations for no-till management systems, it would be beneficial to accurately measure AMF and bacteria that are involved in solubilizing P. A common method to determine soil AMF inoculum potential is the AMF most-probable-number (MPN) assay (Erkmen, 2022), which consists of a serial dilution method of collected soils, paired with greenhouse growth techniques (Douds et al., 2011). The MPN assay is a reliable and consistent test to determine AMF organisms but takes extensive time and labor and does not yield results for other microbial organisms (Wilson & Trinick, 1983). An alternative method to determine soil microbial levels that is faster and more affordable consists of extracting and separating phospholipids from soil samples. This method determines AMF organisms in addition to varying soil microorganisms through the identification of biomarkers, which is the separation of taxonomic groups of soil microbes. This test is known as the phospholipid fatty acid (PLFA) assay (Buyer & Sasser, 2012). Despite the advantages of the PLFA assay, results from this assay have been inconsistent in the past, as some biomarkers are shared between microbial taxa (Frostegård et al., 2011). Therefore, the objectives of this study were to 1) determine in a long-term no-till field the effect of different STP levels on crop yield and soil biological activity as measured by the PLFA and MPN assay and 2) determine if the more cost-effective PLFA test instead of the MPN test can be used to effectively identify any differences in AMF due to STP level.

2.3 MATERIALS AND METHODS

2.3.1 Experimental Design

The experimental site was located at the Dakota Lakes Research Farm, east of Pierre, SD (44.288194, -100.001750). The soil type is classified as a Lowry silt loam (Course-silty, mixed, super active, mesic Typic Haplustolls). The soil pH of the site was 7.7, and the organic matter was 40 g kg^{-1}. The research area was arranged as a randomized complete block design, utilizing five replications. The total research area was 1.8 hectares in size, separated into 15 experimental units which were 6.1 m wide by 103.6 m in length for a total area of 632 m^2 (Figure 2.1). Prior to 2014, soluble P concentrations were drawn down to five mg kg^{-1} Olsen P through continuous crop production without P fertilizer additions, in order to establish a uniform low STP level. In the fall of 2014, low (4-7 mg kg^{-1}), medium (8-11 mg kg^{-1}), and very high (16+ mg kg^{-1}) soil test P category treatments for SD crops (Clark et al., 2023) were created by applying three rates of P (0, 112, or 224.2 kg P_2O_5 ha^{-1}) using monoammonium phosphate (52% P_2O_5), placed in the soil using a no-till drill at a depth of five cm and a spacing of 19 cm. To maintain the low, medium, and very high STP levels, the above P rates were again applied in 2017, 2019, and 2021. Each year P fertilizer was applied slightly less than removal rates to help maintain STP in each STP category. Phosphorus was applied using monoammonium phosphate at planting at 5cm from the row and 5cm deep. Phosphorus was applied at 38 kg ha^{-1} for corn, 20 kg ha^{-1} for soybeans, and 24 kg ha^{-1} for wheat.

In 1994, a five-year crop rotation [soybean (*Glycine max*)-wheat (*Triticum aestivum L*.)/cover crop-soybean-corn (*Zea mays*)-corn] was initiated on this no till, irrigated field. This rotation was implemented to increase crop diversity and reduce the risk of pest damage. The cover crop grown after wheat in the 2019 growing season was planted at 112 kg ha^{-1}. The mix of this cover crop consisted of 53% oats (*Avena sativa*), 29.5% millet (*Pennisetum glaucum*), and 17.5% barley (*Hordeum vulgare*). Nutrient management for this study consisted of applying nitrogen (N), potassium (K), and sulfur (S) following university recommended guidelines for each crop (Clark et al., 2023). For corn production, N was applied at rates that varied from 131 kg to 144.5 kg ha^{-1}, K was applied at a rate of 4.5 kg ha^{-1}, and S was applied at rates that varied from 4.5 to 6.7 kg ha^{-1}. For soybean production, N was applied at 4.3 kg ha^{-1}, and K was applied at 2.5 kg ha^{-1}. For wheat production, N was applied at 103 kg ha^{-1} and S was applied at 10.6 kg ha^{-1}. Irrigation practices were utilized during the growing season of each year starting in June using a lateral-move irrigation system. Irrigation timing and amount was based on tensiometer readings (Ministry of Agriculture, British Columbia, 2015). The reading from the tensiometer gauge represents the measure of soil water tension using a water tube, mechanical gauge, and ceramic tip (Cahn & Johnson, 2017).

2.3.2 Soil and Plant Sample Collection and Analysis

Soil samples for phospholipid fatty acid and AMF MPN were collected after the crop was established, ranging from 10-20 June of each growing season. Four cores were collected for these biological soil samples at a 0-to-15-cm depth in each treatment area utilizing a 3.2 cm i.d. soil probe to create a composite sample for each experimental unit. After collecting the biological soil samples, the samples were placed in coolers with ice packs. Soil used for PLFA analysis were sent to Ward Laboratories (Kearney, NE) and the soil for AMF MPN were transported to the USDA-ARS facility in Brookings, SD. Phospholipid fatty acid assays were completed following Buyer & Sasser (2012). Four main steps of the PLFA assay are used to identify these important biomarkers: drying and extraction, lipid separation, transesterification, and gas chromatography. Phospholipid fatty acid soil testing is a biological soil test that gives a representation of microbial biomass and activity. It is specifically an identification of microbial functional groups of interest through lipid biomarkers. This analysis yielded results for numerous parameters including total fungi biomass, total fungi percentage, AMF biomass, AMF percentage, total microbial biomass, diversity index, bacteria percentage, total bacteria biomass, actinomycete percentage, actinomycete biomass, gram-negative percentage, gram-negative biomass, gram-positive percentage, gram-positive biomass, and the ratio between fungi to bacteria. The full PLFA assay yields other microbial parameters, however, from 2018 to 2022, the listed parameters were consistently measured, and will be the focus of the discussion in this manuscript. The AMF MPN assay was completed as previously described by Douds et al. (2011) and Lehman et al. (2012, 2019) This assay involved the identification of AMF propagules including spores, infected root pieces, and vegetative hyphal fragments, that together represent AMF soil inoculum. Propagule numbers were measured using a most-probable-number (MPN) assay involving replications of serially diluted field soils in a sterile potting mix, and a plant host, Bahai grass (*Paspalum notatum* Flugge), grown in a greenhouse. Sterile potting soil alone was used as a negative control. The AMF MPN analysis yielded a value that represented the number of AMF propagules per gram of soil.

Soil nutrient testing was conducted on samples collected in the spring of each growing season to ensure STP categories were still in their respective range. From 2018-2019, four cores at a depth of 0-to-15 cm using a 2cm i.d. soil probe were collected from each experimental unit. Starting in 2020 to better sample the soil from within and between fertilizer bands a depth of 0-to-7.6 cm and 7.6-to-15 cm was collected. For the 0-to-7.6 cm soil samples, four samples were collected as a cross-section volume measurement. The cross-section volume collection was 38.1 cm wide, 7.6 cm deep, and 15 cm thick from the center of the crop row in each experimental unit. In this measurement, the 38.1 cm width captured two banded layers of P from pervious fertilization history. The remaining soil sampling depth, starting in 2020, included a 7.6-to-15-cm section that was separated from a 0-to-15 cm core, which was collected using a standard soil sampler 2 cm i.d., to get a soil representation below the banded layer of fertilizer. Soils collected from each depth at all sampling events were analyzed for soil test P concentration using the Olsen P extractant (Sterling Olsen, 1954), and followed the soil test procedure outlined for the north central region of the U.S. (Frank et al., 2012). For the 2020-2022 samples, the STP value from each depth was averaged together to calculate an STP value for the 0-to-15 cm depth.

Crops were harvested using a commercial grade combine, equipped with a grain scale, to determine grain yield from each experimental unit. The center 6.1 m was harvested within each experimental unit, using three separate 6.1 m wide combine headers used for the harvest of corn, soybeans, and wheat. The average from three moisture samples collected after harvesting each experimental unit was used to correct the harvest yield to 12.5% (wheat), 13% (soybeans), and 15.5% (corn) moisture.

2.3.2 Statistical Method

Soil microbial parameters and yield as affected by STP category were evaluated using the mixed analysis option in JMP Pro 17 (SAS Institute Inc., Cary, NC). In seven out of the 17 parameters (yield, diversity index, AMF percentage, bacteria percentage, gram-negative percentage, gram-positive percentage, and fungi to bacteria ratio), no data transformations were needed as residual plots showed normality and constant variance assumptions were met. Logarithmic transformations were used on nine out of the 16 soil parameters (total fungi biomass and percentage, AMF biomass, total biomass, bacterial biomass, actinomycete biomass and percentage, gram-negative biomass, and gram-positive biomass) before analysis to meet normality and constant variance assumptions. For total fungi biomass, four out of the five years used a logarithmic data transformation before analysis to meet normality and constant variance assumptions. However, in 2021, a logarithmic plus one data transformation was used to account for the zeros that were present in the data set for this sampling year. For the AMF MPN assay, a square root plus one transformation was used to meet normality and constant variance assumptions. Year, soil test phosphorus (STP) category, and their interaction were considered fixed effects. Block nested within year was considered a random effect. Analysis of variance (ANOVA) was used to evaluate differences between microbial parameters and the three STP categories employed in this study. When yield or a microbial parameter was significantly affected ($P \leq .05$) by STP or the STP x Year interaction, the LS means procedure with the Student's T method to adjust for multiple comparisons was used to determine differences among treatments. When the STP x year interaction was significant, years were evaluated individually.

2.4 RESULTS AND DISCUSSION

2.4.1 Weather

Soil sampling for biological testing was completed between 10-20 June of each year. This sampling date was selected so that the soil biological activity was high and provided a representative estimate of the measured microbial communities. The average temperature for the month of June ranged from 20.0 to 24.4 °C (Table 2.1). The monthly average temperature departure from normal varied among sampling years, but most of the sampling years were within 2 °C of the normal. The exception for this occurred in June 2021 where the average monthly temperature was 3.8 °C higher than the historical average. Monthly precipitation for June ranged from 15 to 104.6 mm. In three out of the five years, precipitation values were within 20 mm of the historical average (2018, 2020, and 2022). In two out of the five years, precipitation values were well below the historical averages. In 2019 and 2021, precipitation was 77.2 mm and 77.5 mm below the historical average, respectively.

2.4.2 Crop Yield

Soybean yield values ranged from 3539 kg ha^{-1} to 4534 kg ha^{-1}, wheat yields ranged from 3820 kg ha^{-1} to 3835 kg ha^{-1}, and corn yields ranged from 11512 kg ha^{-1} to 12295 kg ha^{-1} (Table 2.2). There were no observed differences in crop yield due to STP category in four out of the five years. There was a small corn yield difference in 2022, where compared to the medium and very high STP category, the low STP category yielded 403 to 485 kg ha^{-1} less. To avoid a yield reduction in low and medium soil test categories, university guidelines recommend applying 92 kg P_2O_5 ha^{-1} and 57 kg P_2O_5 ha^{-1}, respectively, which if using monoammonium phosphate (52% P_2O_5) would have cost \$191.05 ha^{-1} for the low STP category and cost \$118.68 ha^{-1} for the medium STP category (Quinn, 2024). Using the fall 2022 corn price, the profit difference between the very high STP category and the low and medium STP categories were \$112.21 ha^{-1} and \$16.03 ha^{-1}, respectively (USDA-NASS, 2024). In both instances, it was not economically advantageous to apply fertilizer as economic return (increase in corn price – P fertilizer cost) was -US\$78.84 ha^{-1} and -US\$102.65 ha^{-1} for the low and medium STP categories, respectively. Therefore, there was not an economic advantage to apply P fertilizer in the low and medium STP categories across all five years. A reason for the potential lack of difference in yield among the STP categories may be due to an increase in soil fungi in the low and medium STP categories as will be discussed in the upcoming sections. Soil fungi can improve access to P by the solubilization of low-reactivity P-sources and P-desorption from soil minerals (Vassileva et al., 2022).

The four years of no yield differences among STP categories were contrary to the current SD fertilizer guidelines where the low and medium category would normally be yield limiting without fertilization (Clark et al., 2023). Other U.S. Midwest states also recommend adding fertilizer to optimize corn yields in low and medium STP soils (STP < 12 mg kg^{-1} Olsen-P or < 16 mg kg^{-1} Bray P-1) (Culman et al., 2020; Franzen, 2018; Kaiser et al., 2023; Mallarino et al., 2013). The lack of economical yield differences seen among the STP categories of this study and fertilizer typically being recommended for these soils throughout the U.S. Midwest may be attributed to not including no-till sites or combining no-till and conventional till sites to calculate fertilizer recommendations. The factors other U.S. Midwest states use in their P recommendations vary by state. It is typical in the U.S. to use a soil test that measures P availability in their fertilizer recommendations (Lyons et al., 2023). In the U.S. Midwest, other management considerations are used in their P recommendations, such as region and irrigation practices (North Dakota), and P application methods (banding vs. broadcasting; Minnesota) (Franzen, 2018; Kaiser et al., 2023). However, studies show switching from tillage to no-till influences P fertilization needs. For example, a comparison of optimum P rates between conventional tillage and no till systems was done in Milan, Tennessee, which showed that conventional tillage systems had optimum P rates that were 19 kg P ha^{-1} higher than no-till systems (Howard et al., 2002). In a case study done on a farm with 20 years of no till management in central SD, corn yield increases were seen while simultaneously reducing P fertilizer applications by 30% of what was previously required under conventional tillage management (Anderson, 2016). Further, in a study done in northeast China, continuous no-till management improved the uptake of P in corn (Xomphoutheb et al., 2020). These combined results indicate that when using no-till compared to conventional tillage management, soluble P levels can be left at lower levels than would currently be considered sufficient with minimal impact on yield. Further, these results indicate that current P fertilizer recommendations may need to be updated for long-term no till systems.

2.4.3 Fungi Measurements

The STP categories alone or their interaction with year had a significant effect (α=0.05) on AMF most-probable-number (MPN), and PLFA parameters including total fungi biomass, fungal percentage, and AMF biomass (Table 2.3). Year had a significant effect (α=0.05) on the PLFA parameter AMF %. The STP categories regardless of year influenced the accumulation of AMF when measured with the MPN assay method (Table 2.4). The low STP category had the highest average accumulation of AMF propagules (five propagules $gram^{-1}$ soil), followed by the medium (two propagules $gram^{-1}$ soil) and lastly the very high STP category (one propagule $gram^{-1}$ soil). These results indicate that the abundance of inorganic P levels in the very high and medium STP categories reduced the amount of mycorrhizal fungal organisms present in the soil. Similar results were reported in a study completed in British Columbia, Canada where fungal biomass was reduced after multiple additions of manure and P fertilizers (Bittman et al., 2005). However, in a study done in east China in a silty clay loam paddy soil, inorganic P fertilizer additions did not affect AMF hyphae biomass when grown in a wheat-rice agroecosystem (Qin et al., 2015). An explanation for the lack of significant treatment effects in this wheat-rice system may be because waterlogged soil hinders AMF activity (Vallino et al., 2014).

The higher number of AMF organisms as STP category decreased (Table 2.4) may explain the lack of yield differences among the STP categories (Table 2.2) because AMF can dissolve insoluble P complexes and provide them to plants (Etesami et al., 2021). Therefore, the greater amount of AMF structures in the low STP category may be dissolving enough insoluble P complexes to make up for having a STP level lower than what is normally needed to optimize crop yield. These AMF organisms establish a symbiotic relationship with plants roots when the availability of P is low (Liang et al., 2022). This symbiosis increases the root surface area of the host plant, where in exchange for photosynthetic C from the plant, the plants receive a provision of P from previously insoluble sources (Willis et al., 2012). Another benefit of this symbiosis is AMF enhance stress resistance and photosynthetic processes of host crops, which can help maintain yield in low STP soils (S. Wu et al., 2022). The ecosystem benefit of AMF is evident in this study, where the low STP category had no economical yield difference compared to the medium and very high STP categories, as previously discussed in the crop yield section. This finding reinforces the idea of the potential ability of reducing the soluble P levels in no-till agricultural fields to supplement the development of mycorrhizal fungi that can help supply sufficient P to crops without significant yield reductions. Furthermore, the reduction of soluble P levels in agricultural fields lowers the risk of environmental degradation due to non-point P sources entering aquatic systems through agricultural run-off (Ghane, 2023).

Another method to measure AMF is through the PLFA assay. From 2018 to 2022, AMF biomass ranged from 13 ng g^{-1} (very high STP category 2021) to 407 ng g^{-1} (low STP category 2020) (Table 2.5). Contrary to our AMF from the MPN assay, in four out of the five years AMF biomass was similar among the STP categories. The only difference occurred in 2021 where the medium STP category had the highest AMF biomass, followed by the low and then the very high STP category. This result indicates that in 2021, similar to the AMF MPN results, as STP increased, the AMF biomass accumulations generally decreased. One reason for only finding differences in AMF with PLFA in 2021 may be due to higher than normal temperatures and lower than normal precipitation that occurred in the 2021 growing season (Table 2.1). This is significant, as soil temperature and moisture availability are critical environmental factors that influence fungal activity in soils (Paul & Clark F.E., 1996; PietikÃ¥inen et al., 2005). The lower moisture availability and high temperatures in 2021 reduced the measured AMF biomass across STP categories (Table 2.5). This overall reduction in AMF due to stressful conditions (low rainfall and high temperatures) may have made the identification of AMF differences between STP categories easier in 2021 because of the stress resistance properties of AMF (S. Wu et al., 2022). Therefore, these results also indicate that differences in AMF through PLFA may be more apparent under stressful environmental conditions like low rainfall and high temperatures opposed to when both rainfall and temperature are optimized for plant and microbial growth.

Overall, only one of five years of the PLFA assay AMF results were shown to be affected by STP category. Conversely, the use of the MPN assay showed differences in AMF among STP categories regardless of year. This result provides evidence that when approximating AMF species abundance, the AMF MPN assay compared to the PLFA assay results were more sensitive to the effect of the different STP categories. An explanation for this occurrence may be that the methodology used by the MPN method yields a higher number of microorganisms than other microbial measurements such as the PLFA method (Erkmen, 2022). Additionally, the interpretation of the PLFA assay can be troublesome, due to the shared biomarkers of microbiological taxa, such as AMF and soil bacteria (Frostegård et al., 2011). These results with the MPN and the PLFA assays suggest that researchers should be mindful of what AMF measurement they use when studying STP effects as the MPN method proved to be more sensitive and better able to find treatment differences.

Similar to AMF from the PLFA assay, the total fungi biomass and total fungi percentage were affected by the STP category x year interaction (Table 2.3) and both measurements were similar among the STP categories except in 2021 (Table 2.5). For total fungi percentage, the medium STP category had the highest measured values, and the low and very high STP categories were similar. For total fungi biomass, the medium category had the highest measured values, followed by the low STP category and then the very high STP category. Although differences were only significant in one of five years, there was a general trend observed each year where the low STP category, on average, yielded a higher amount of total fungi percentage and total fungi biomass compared to the medium and very high STP category. Therefore, similar to the AMF from PLFA assay results discussed earlier, these results also indicate that higher levels of inorganic P can reduce fungal amounts in the soil.

Contrary to the other fungal measurements, AMF percentage was not influenced by STP category or the interaction between STP and year (Table 2.3). However, AMF percentage was significantly affected by year. From 2018-2020, there were no differences in average AMF percentage, as values ranged from 4.9% to 5.8% (Table 2.6). In 2021, there was a significant decrease in AMF percentage to a value of 1.1%, followed by an increase of AMF percentage in 2022 to 3.2%. The effect of year on AMF percentage was likely due to yearly differences in temperature and precipitation. Evidence for this occurred as 2021 was the year with the lowest AMF percentage and it also had the highest average temperature of (24.4°C, +3.8°C from average) and the lowest amount of precipitation (15mm, -77.5mm from average) (Table 2.1). The remaining sampling years generally had higher and similar AMF percentages along with temperature or precipitation values that were closer to the historical averages.

2.4.4 Bacterial Measurements

The STP categories by year interaction had a significant effect on four out of the eight soil bacteria parameters tested (α=0.05) including bacteria percentage, gram negative percentage, gram-positive percentage, and actinomycete percentage (Table 2.3). The remaining four soil bacteria parameters tested (total bacteria biomass, gram-negative biomass, gram-positive biomass, and actinomycete biomass) were significantly affected by only year. For the bacterial measurements affected by the STP by year interaction the effect was generally minimal as differences only occurred 35% of the time and the specific effects varied by year and parameter. Bacteria percentage was affected by STP category the most often (in three years), followed by gram-positive bacteria (in two years), and lastly the actinomycete and gram-negative percentage (in one year). For bacteria percentage when differences occurred in 2020 and 2021, it was lowest in the low STP category, followed by the medium and very high STP category which were similar (Table 2.5). However, in 2022, the medium STP category had the lowest bacteria percentage, followed by the low and very high STP category which were similar. Gram-positive percentage showed differences in 2020 and 2021; in both years, the low STP category had the lowest percentage of gram-positive bacteria while the medium and very high STP category switched each year as to which one had the highest value. For gram-negative bacteria percentage, there were differences in 2021, where the medium STP category had the highest percentage, followed by the low and very high STP category which were similar. Actinomycete percentage showed differences in 2018, where the low STP category had the highest percentage, followed by the medium, and then the very high STP category.

When differences occurred for bacterial measurements, nearly half of the differences observed between STP categories occurred in 2021, which were most likely due to that year's above normal temperatures and below normal precipitation as discussed in the weather and fungi measurement sections. Evidence for the effect of temperature and moisture on bacterial measurements was also seen in a study in California where heating soil samples resulted in an exponential decline of soil bacteria as a function of soil moisture and temperature (Dunn et al., 1985). Therefore, similar to the PLFA fungi measurements, these results also suggest that with near normal temperature and precipitation generally soil bacteria measurements did not change due to varying STP levels, but under stressful climatic conditions, differences in bacterial measurements due to STP level were more common. The effect of STP level on bacterial measurements was inconsistent in this study but a study in Jiangxi, China found a change in bacterial functional groups with the addition of N or P fertilizers (Niu et al., 2020). Additionally, in a study in Brazil on a dark red latosol soil, additions of superphosphate increased the accumulation of gram-positive organisms in the soil (P. da Silva & Nahas, 2002). The results of these two studies may be contradictory to this study because of different cultivation settings (forested soil, China), climate (tropical, Brazil), or the use of conventional-till management as compared to no-till management utilized in this study.

The remaining bacterial parameters collected (total bacterial biomass, gram-negative biomass, gram-positive biomass, actinomycete biomass) were not influenced by STP category or the interaction between STP and Year (Table 2.3). Similar to the bacterial percentage-based measurements, these results indicate that the bacterial biomass measurements were not affected by varying STP levels. However, each of these parameters were affected by year. There was a pattern observed for biomass values for total bacteria, gram-negative, gram-positive, and actinomycetes, where the lowest measured value was observed in 2021 (229, 53, 179, 58 ng g^{-1}, respectively) and the highest measured value occurred in 2020 (3657, 1494, 2155, 797 ng g^{-1}, respectively) (Table 2.5). On average, all bacterial biomass values increased from 2018 to 2020, which had close to average precipitation and temperature for those growing seasons. During the lower-than-normal precipitation (-77.5mm from average) and higher-than-normal temperature (+3.8°C from average) year of 2021, bacterial biomass values decreased by an average of 160%, followed by biomass values increasing by an average of 105% in 2022. These results suggest that the decrease in precipitation and increase in temperature affected the biomass values in 2021. An alternative explanation for the difference in biomass values may be the addition of soybeans in the cropping system in 2020, which form a symbiosis with rhizobia bacteria to fix atmospheric N. Other studies have shown that rhizobia bacteria interact with other bacterial organisms in the soil and are involved in a diversity of interactions that affect the observed plant-microbe mechanisms (Granada Agudelo et al., 2023). These results suggest that year-to-year temperature and precipitation differences were likely the cause of the varying biomass measurements across years, as well as the possibility of bacteria dependent crops (soybeans) planted in this area influencing the accumulation of soil bacteria.

2.4.5 Soil Diversity Measurements

The STP categories alone or their interaction with year had a significant effect on two out of the three soil diversity parameters tested (α=0.05) including diversity index and fungi to bacteria ratio (Table 2.3). Year alone had a significant effect on the remaining soil diversity parameter of total biomass. Diversity index values were calculated using the Shannon Diversity Index and was calculated based on the relative abundance of different PLFA markers. The higher value of this index indicates greater diversity of all microbial parameters, while lower values indicate lower diversity. The measured diversity index was influenced by the interaction between STP and year (Table 2.3). The diversity index ranged from 1.00 (low and very high STP category 2021) to 1.68 (low STP category 2018). There were no differences in diversity index among STP categories in 4 out of the 5 years of the study. However, in 2021 the medium STP category had a higher diversity index number than the low and very high STP categories (Table 2.5). Although differences were only significant in one of five years, there was a general trend observed where the low STP category, on average, yielded a higher diversity index compared to the medium and very high STP category. These results suggest that as STP decreases, the general diversity of soil microbes in the soil increases. Having a higher biodiversity of soil microbes in the soil has several advantages, including better cycling of nutrients, suppression of pests, parasites, and disease, and aids the soil in the sequestration of carbon (C) (NSW Government, 2023). Similar results were found in a study completed in Ireland, where P fertilizer applications reduced the Shannon diversity index in the soil (Chhabra et al., 2013).

The measured fungi to bacteria ratio was influenced by STP categories (Table 2.4). Across years, the low STP category had the highest ratio of fungi to bacteria with an average of 0.23, followed by the medium STP category with a value of 0.21, and then the very high STP category with a value of 0.19. The fungi to bacteria ratio was also the only microbial parameter from the phospholipid fatty acid (PLFA) assay that was consistently influenced by STP category and did not vary by year. The low STP category had a greater ratio of fungi to bacteria when compared to the medium and very high STP category, which may have contributed to the ecosystem functions mentioned earlier, including limited crop yield decline as STP level decreased (Table 2.2). Similar results were found in a meta-analysis of peer-reviewed publications that discuss PLFA assays, where P fertilizer additions significantly reduced the fungi: bacteria ratio, especially in forest and grassland systems (W. Wu et al., 2022).

Contrary to the other diversity measurements, total biomass was not influenced by STP category or the interaction between STP and year (Table 2.3). However, total biomass was significantly affected by year. From 2018-2020, there was no difference in average total biomass, as values ranged from 2722 ng g^{-1} to 7219 ng g^{-1} (Table 2.6). In 2021, total biomass decreased to a value of 684 ng g^{-1}, followed by an increase of total biomass in 2022 to 1683 ng g^{-1}. The effect of year on total biomass was likely due to differences in temperature and precipitation that occurred in 2021. Evidence for a weather effect occurred as 2021 was the year with the lowest total biomass and it also had the highest average temperature of (24.4°C, +3.8°C from average) and the lowest amount of precipitation (15mm, -77.5mm from average) (Table 2.1). The remaining sampling years generally had higher and similar total biomass values along with temperature or precipitation values that were closer to historical averages. Another explanation could be the crop-type conversion that occurred in 2021, in which corn was planted after a three-year gap in this sequence. Other research has shown that fungi communities are more responsive to crop-type conversions and could account for this decrease in total biomass (Ai et al., 2018). However, the effect of crop-type conversion is not definitive in this study, as only one full crop rotation was analyzed. Therefore, these results suggest that the effect of environment was likely the main source of variation in total biomass.

2.5 CONCLUSIONS

The results from this study show that long-term no-till producers can reduce the STP level of their fields without experiencing an economically significant yield decline. Evidence for this is that in all five years of this study, there was not an economic advantage to maintaining STP level in the very high category (no P fertilization recommended) vs the low and medium STP categories (P fertilization recommended). These results also suggest that optimum STP levels in long-term no-till systems in SD need to be revisited and adjusted. One potential reason for this lack of yield difference between STP categories may be due to the greater amount of AMF fungi in the low compared to the medium and very high STP soil. Results from the AMF MPN assay showed that as inorganic P level increased, AMF fungi propagules decreased. These AMF organisms may be dissolving insoluble P complexes and providing it to the crops via root-AMF symbiosis in sufficient quantities that allowed the crops in the low and medium STP categories to yield similarly to crops in the very high STP category.

The effect of the three STP levels on the PLFA measurements evaluated in this study were inconsistent as differences in soil fungal and bacterial measurements were only seen 20% and 35% of the time, respectively. It is interesting to note that in the stressful weather conditions of 2021, 88% of the soil parameters tested in the PLFA assay showed differences among STP categories. These results suggest that in higher than normal temperature and lower than normal precipitation conditions, differences in soil fungi and bacteria due to STP level are more prevalent and in near normal weather conditions differences were minimal. The greater effect of STP on PLFA measurements in stressful weather conditions may be due to these measured organisms being more active in stressful compared to more normal weather conditions. Among the soil diversity measurements used in this study only the fungi to bacteria ratio was consistently affected by STP level, where the low STP category had a higher ratio, followed by the medium, and then the very high STP level. The remaining soil diversity measurements had a similar response to STP level as soil bacteria and fungi, where only in stressful weather conditions were there differences.

The more cost-effective PLFA assay was not as effective as the MPN assay in identifying differences in AMF in the soil as affected by varying STP levels. Results from the MPN assay were more consistent across all years, whereas the PLFA assay only showed differences in AMF populations in 2021. Although the PLFA assay has an advantage in providing results for multiple microbial taxa, the soil bacteria results from this assay were inconclusive. Furthermore, the AMF results from the PLFA assay were less responsive to varying STP levels compared to soil fungi when measured with the MPN assay. The results found in this study suggest that researchers should be mindful of what microbial measurement they use when studying treatment effects as in this study MPN proved to be more sensitive than the PLFA assay.

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2.7 TABLES

Table 2.1. Monthly air temperature, precipitation, and irrigation amounts for March through June at the Dakota Lakes Research Farm in Pierre, SD from 2018-2022, with the difference from the long term average in parentheses.

	March	April	May	June
2018				
Air Temp (°C)	0 (-1.7)	3.3 (-4.5)	17.8 (+3.9)	21.7 (+1.1)
Rain fall (mm)	14.2 (-7.6)	19.1 (-34.0)	56.6 (-23.9)	75.4 (-17)
Irrigation (mm)	0.0	0.0	25.4	19.1
2019				
Air Temp (°C)	-3.9 (-5.6)	7.2 (-0.6)	11.7 (-2.7)	20.0 (-0.6)
Rain fall (mm)	6.9 (-15)	20.6 (-32.5)	81.8 (+1.3)	15.2 (-77.2)
Irrigation (mm)	0.0	0.0	0.0	92.7
2020				
Air Temp (°C)	3.3 (+1.6)	6.7 (-1.1)	13.3 (-1.1)	22.2 (+1.6)
Rain fall (mm)	17.3 (-4.6)	7.6 (-45.5)	59.4 (-21.1)	104.6 (+12.2)
Irrigation (mm)	0.0	0.0	0.0	31.8
2021				
Air Temp (°C)	5.0 (+3.3)	7.2 (-0.6)	13.9 (-0.5)	24.4 (+3.8)
Rain fall (mm)	24.9 (+3.0)	28.7 (-24.4)	27.4 (-53.1)	15.0 (-77.5)
Irrigation (mm)	0.0	0.0	0.0	165.1
2022				
Air Temp (°C)	1.1 (-0.6)	5.6 (-2.2)	14.4 (+0.0)	21.1 (+0.5)
Rain fall (mm)	1.5 (-20.3)	67.8 (+14.7)	40.4 (-40.1)	91.7 (-0.8)
Irrigation (mm)	0.0	0.0	0.0	127.0

Source: South Dakota State University Dakota Lakes Research Farm weather station 2018-2022.

Note: Air Temp (°C) values first includes the average temperature for each month, followed by the difference between the average temperature and the historical average temperature for that region.

Note: Rain fall (mm) values first includes the total rainfall for each month, followed by the difference between the total rainfall amount and the historical rainfall average for that region.

Table 2.2. Crop yield as influenced by soil test phosphorus (STP) category for each year from 2018-2022.

STP Category	Crop	Yield (kg/ha)
2018		
Low	Soybean	3539a[a]
Medium	Soybean	3687a
Very High	Soybean	3683a
2019		
Low	Wheat/CC	3820a
Medium	Wheat/CC	3835a
Very High	Wheat/CC	3828a
2020		
Low	Soybean	4391a
Medium	Soybean	4405a
Very High	Soybean	4534a
2021		
Low	Corn	12030a
Medium	Corn	12114a
Very High	Corn	12295a
2022		
Low	Corn	11512b
Medium	Corn	11915a
Very High	Corn	11997a

[a]Means with different letters in the same column are statistically different ($P \leq .05$).

Table 2.3. Degrees of freedom (df), F-Ratio, and significance level for the effects of year, soil test phosphorus (STP) category, and their interaction on phospholipid fatty acid (PLFA) measurements, arbuscular mycorrhizal fungi (AMF), and most probable number (MPN) assay parameters in Pierre, SD from 2018-2022.

	F-ratio and significance level		
Soil Parameters	Year	Soil Test Phosphorus (STP) Category	STP x Year
Total Fungi Biomass, ng/g	17.53**	2.21	3.98**
Total Fungi, %	10.76**	2.81*	3.25**
AMF Biomass, ng/g	40.00**	2.53*	3.69**
AMF %	22.56**	1.22	1.02
AMF MPN	9.51**	37.17**	1.08
Total Biomass, ng/g	22.85**	0.68	2.04*
Diversity Index, ng/g	7.17**	2.56*	3.75**
Bacteria, %	26.13**	4.54**	5.30**
Total Bacteria Biomass, ng/g	31.44**	1.44	2.09*
Actinomycetes, %	1.18	1.1	2.27**
Actinomycetes Biomass, ng/g	42.66**	1.26	1.77
Gram (-), %	22.91**	0.26	3.34**
Gram (-) Biomass, ng/g	28.66**	0.53	1.47
Gram (+), %	2.18	3.29**	2.83**
Gram (+) Biomass, ng/g	34.26**	1.62	1.39
Fungi:Bacteria	9.13**	3.25**	1.11
Degrees of freedom (df)	4	2	8

** Significant at $\alpha = 0.05$

* Significant at $\alpha = 0.10$

Table 2.4. Microbial soil parameters as influenced by soil test phosphorus (STP) category averaged across all years.

	STP Category		
Soil Parameters	Low	Medium	Very High
AMF MPN	5a[a]	2b	1b
Fungi:Bacteria	0.23a	0.21ab	0.19b

[a]Means with different letters in the same row are statistically different ($P \leq .05$)

Table 2.5. Microbial soil parameters as influenced by the interaction between soil test phosphorus (STP) category and year from 2018-2022.

Soil Parameters	2018 STP Category			2019 STP Category			2020 STP Category			2021 STP Category			2022 STP Category		
	L	M	VH	L	M	VH	L	M	VH	L	M	VH	L	M	VH
Total Fungi Biomass, ng/g	270a[a]	331a	334a	373a	276a	336a	1159a	832a	888a	16b	62a	6c	148a	131a	178a
Total Fungi, %	13a	10a	11a	14a	11a	11a	14a	13a	12a	1b	3a	1b	9a	8a	10a
AMF Biomass, ng/g	104a	145a	145a	175a	157a	140a	407a	350a	347a	4b	10a	2c	52a	45a	65a
Diversity Index	1.68a	1.56a	1.54a	1.53a	1.47a	1.47a	1.55a	1.50a	1.48a	1.0b	1.3a	1.0b	1.46a	1.45a	1.48a
Bacteria, %	50a	44a	47a	54a	53a	52a	46b	54a	53a	26b	40a	39a	42a	40b	43a
Actinomycetes, %	11a	9ab	9b	11a	10a	10a	10a	12a	12a	6a	9a	11a	10a	11a	11a
Gram (-), %	19a	17a	18a	21a	19a	20a	20a	21a	22a	5b	12a	6b	15a	13a	14a
Gram (+), %	29a	26a	29a	33a	34a	32a	27b	33a	31ab	22b	28ab	34a	28a	28a	28a

[a]Means with different letters in the same row and within the same year are statistically different ($P \leq .05$).

L – Low STP Category

M – Medium STP Category

VH – Very High STP Category

Table 2.6. The effect of year on microbial soil parameters.

	Year				
Soil Parameters	2018	2019	2020	2021	2022
AMF, %	4.9a[a]	5.8a	5.2a	1.1c	3.2b
Total Biomass, ng g^{-1}	2722b	2736b	7219a	684c	1683b
Total Bacteria Biomass, ng g^{-1}	1253bc	1452b	3657a	229d	702c
Actinomycete Biomass, ng g^{-1}	251bc	279b	797a	58d	176c
Gram (-) Biomass, ng g^{-1}	487bc	546b	1494a	53d	231c
Gram (+) Biomass, ng g^{-1}	753bc	900b	2155a	179d	469c

[a]Means with different letters in the same row are statistically different ($P \leq .05$).

2.8 FIGURES

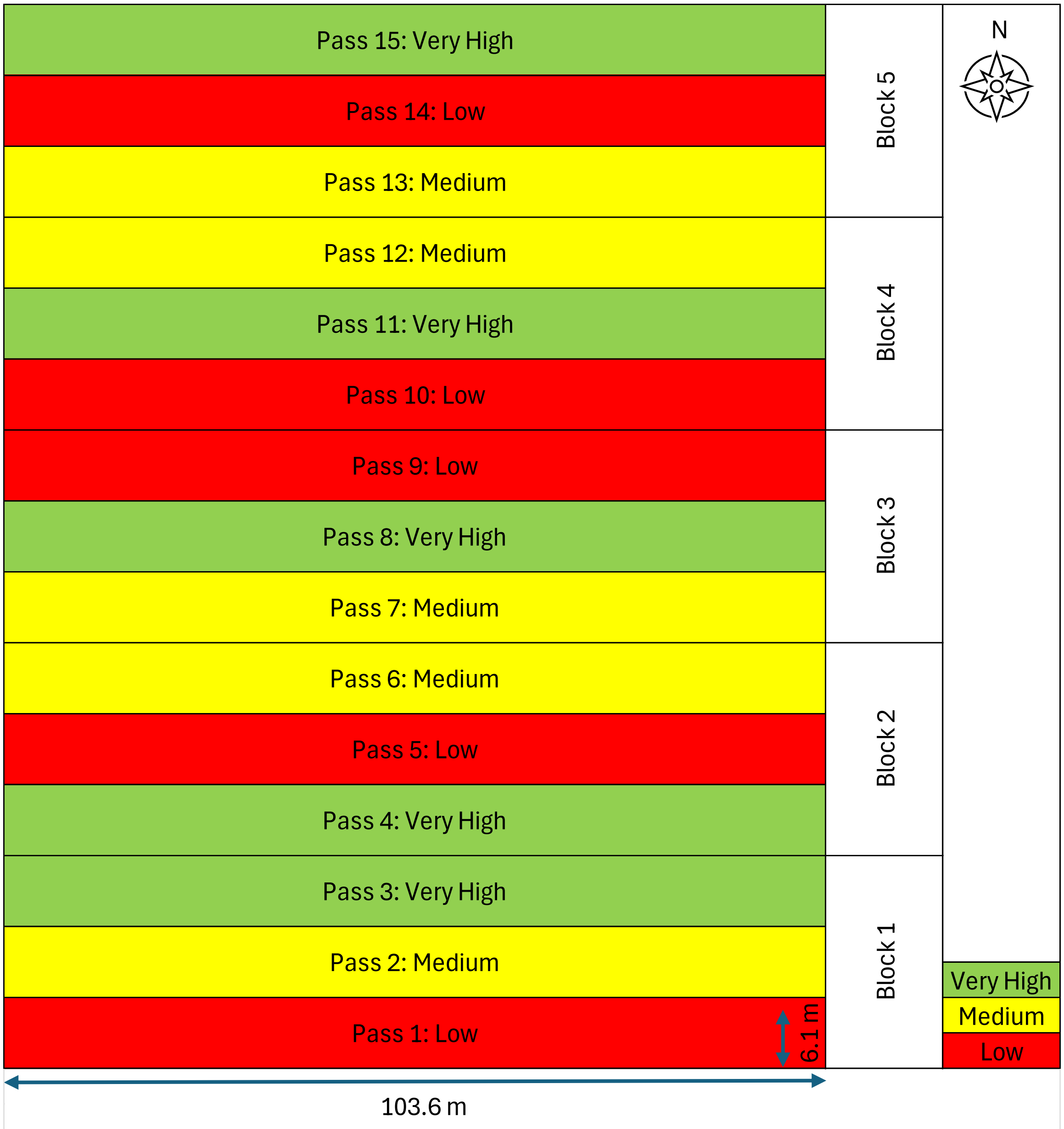

Figure 2.1. The plot map of the experimental area, including pass numbers, treatments, blocks, and experimental unit measurements.

CHAPTER 3 GENERAL DISCUSSION

3.1 ADVANTAGES OF THIS STUDY

This study had several advantages that will aid no-till producers in P fertilizer additions for crop production in SD. Firstly, the experimental area of this study has 30+ years of continuous no-till farming, which created an ideal environment to study the treatment effects of varying STP to AMF in a long-term no-till situation. Along with no-till farming practices being utilized for this length of time, the crop rotation employed by this study has been ongoing since 1994. The combination of long-term crop rotations and no-till practices is rare for agricultural experimentation stations, and finding another research area that utilizes these long-term practices would be difficult to do. This is a testament to the prolonged dedication of the farming and research management at the Dakota Lakes Research Farm. Additionally, having five years of data regarding crop yield, PLFA, and AMF MPN results was extremely advantageous to this project, allowing for the full five-year crop rotation to be analyzed. Without these data collections over this timeframe, determining the effect of STP level on these parameters would have been very difficult to do. Along with the five years of collected data, there was an added benefit to the continued maintenance of three STP levels in this study. Maintaining three different STP categories is challenging to do, and this experiment attempted to complete this.

This study also provided evidence that optimum STP level for crop yield in long-term no-till systems may need to be revisited and adjusted. A potential reduction in P fertilizer additions means that farmers would spend less money on chemical fertilizer inputs each growing season. Additionally, a reduction in STP values reduces the risk of environmental degradation from non-point sources like agricultural production near freshwater sources. In SD, a large portion of agricultural production is in close proximity to river and lake systems, which creates the need to protect these systems from P runoff. This means that there is a direct advantage for no-till farmers in reducing their P fertilizer needs, which also helps to decrease economic costs and potential environmental degradation.

Additionally, multiple measurements were taken in this study, which gave important insight to P management in no-till systems. These insights include differences in crop yield, P uptake, soil microbial life, environmental risks (soil P runoff), and soil fungi byproducts due to varying STP levels. All of the measurements collected in this study aimed to answer fundamental issues involved with P nutrient levels in agricultural fields. Although some of the results from these experiments were not published in this thesis, key differences were found that all play a role in the environment and agricultural production. The numerous measurements that were collected in this study show that the correct experimental design was utilized.

Lastly, the design of this study, both in the large-scale size and commercial management of the experiment, was intended to directly benefit SD farmers in their fertility and crop yield needs. Although many agricultural experiments have this intention, the outreach and farmer interest for this experiment was significant, due to the direct access farmers have at the Dakota Lakes Research Farm. Additionally, the location of this experiment provided significant information for farmers in central SD. A majority of the research conducted by SDSU focuses on the eastern region of the state, which varies in climatic conditions and agricultural production when comparing the western and central region of SD. This research provided farmers in central and western SD with information that is not normally provided by the state's research efforts.

3.2 LIMITATIONS OF THIS STUDY

Although this experiment was successful overall, there were some limitations to note. The most prominent limitation of this study was the maintenance of the three distinct STP levels (low, medium, and very high) in accordance with SD's fertilization guide. In order to maintain these three STP levels, P fertilization was done in 2017, 2019, and 2021. Immediately following these P fertilization events, soil test values showed that all three of these levels were achieved. However, as time went on after P fertilizer application, the soil test values showed less of a difference between treatments. Either the crops grown in the medium and very high STP treatment were taking up the excess P in the soil, or the chemical characteristics of this study site were binding P to Ca in the soil as insoluble complexes. Additionally, sampling errors may have been present in this experiment, as sampling banded layers of P is difficult to do accurately. In order to combat this, more frequent P applications or higher rates of P should have been used to maintain differences between the three STP treatments.

Another limitation of this study was the range of personnel that was involved with data collection and analysis. From 2018 to 2022, three separate researchers were collecting and analyzing data from this experimental area. Each of these researchers managed this collected data in a variety of ways, and because of that, accessing this data from Dakota Lakes archives was difficult to do. Additionally, each of these researchers had varying research objectives with this study, which means that different data was collected with these research objectives in mind. This means that there was only a select number of analyses to compare across five years. For example, from 2020 to 2022, there was more focus on soil microbial testing than in 2018 and 2019. During this 2020 to 2022 sampling period, there was gene sequencing tests (TRACE Genomics) completed on collected soil, which if collected across the entire duration of the study, would have improved the insight gained from the experiment. Furthermore, the experimental design changed over the course of the study. From 2014 to 2019, the experiment was set up as a split-plot design that also studied the effects of broadcast and banding P on crop yield and soil microbial life as tested by the AMF MPN assay. Although the STP treatments that were assigned the same as the RCBD study, the added treatment of P placement in these early years may have ultimately affected the measured STP values in the later years.

Finally, having only one location for this study limits the ability to extrapolate the results found in this study to multiple regions in SD. If more experimental sites were included with the same research objectives and experimental design, confirmation of a reduced STP level in long-term no-till systems as well as a recommended optimal STP level could have been accomplished. Furthermore, having an experimental site that was removed from the Dakota Lakes Research Farm would have the added advantage of accounting for other soil characteristics found in the central portions of the state. Additionally, the same treatment effects of this study could have been completed in a dry-land setting, which would have added more information to the research objectives as a whole, as this is a more common agricultural setting for the state.

3.3 OVERALL CONCLUSIONS

The overall objectives of this study were to 1) determine in a long-term no-till field the effect of different STP levels on crop yield and soil biological activity as measured by the PLFA and MPN assay and 2) determine if the more cost-effective PLFA test instead of the MPN test can be used to effectively identify any differences in AMF due to STP level. These objectives were accomplished using field work completed at the Dakota Lakes Research Farm, various laboratory methods to analyze soil and plant material, and several statistical tools. The ultimate goal of the study was to provide evidence that optimum STP levels need to be reevaluated for farmers using long-term no-till systems.

Crop yield results showed that there was not an economic advantage to applying P fertilizer in low testing STP soils. In four out of the five years of the study, there was no difference in yield between the low, medium, and very high STP categories. These results contradict the SDSU P fertilizer recommendations which indicate that there is upwards of an 80% chance of seeing a yield response to P fertilization in the low STP category. The results from this study provide evidence that P fertilizer recommendations in a long-term no-till system need to be revisited and reevaluated. Additionally, the difference observed in this no-till system, compared to the yield response predictions found in the SDSU P fertilization guide show that including no-till as a management consideration has an advantage for no-till farmers in the state of SD. Furthermore, other U.S. Midwest states may need to consider making this addition in their P fertilization guide to account for no-till producers in their respective states.

Results from the PLFA and MPN assays showed that the varying STP levels used in this study had an effect on several microbial parameters tested. The higher level of inorganic P in the very high and medium STP levels resulted in lower AMF activity. For the AMF MPN assay, STP level had an effect on AMF populations, where the low STP category had on average three times the AMF propagules when compared to the medium and very high STP category. These results were consistent and further validated that the level of inorganic P in the soil had a direct effect on AMF and their functionality in the soil. For the PLFA assay, results were less consistent when measuring AMF in the soil. From 2018 to 2022, differences in AMF were seen only 20% of the time, and all of the differences observed occurred in 2021, which exhibited stressful weather conditions for crop production and soil microbial life. However, there was a general trend where total fungi biomass, total fungi percentage, and AMF biomass were higher in the low STP category compared to the medium and very high STP category. Other microbial communities, such as soil bacteria and actinomycete populations were affected by the varying STP levels only 35% of the time. In the event there were differences in these soil bacteria measurements, the results were inconsistent and difficult to interpret. A similar year to year trend was observed in comparison with climatic conditions, where these bacterial measurements exhibited an average 160% reduction from 2020 to 2021, followed by a 105% increase from 2021 to 2022.

The more cost-effective PLFA assay was unable to find differences in AMF as consistently as the MPN assay. The PLFA assay was more affected by year, rather than by STP level. Meanwhile, the MPN assay was capable of showing clear differences in AMF regardless of year, indicating that this is the more effective test when approximating the density of AMF in soil. This result indicates that researchers should be mindful of what microbial assay test they use when evaluating treatment effects to soil microbial life.

Future work regarding the identification of AMF and plant symbiosis may involve testing for AMF byproducts in crop tissue and collected soil. A portion of this project involved identifying Ergothioneine (Chapter 1.3.4) in collected crop tissue, harvested seed, and soil samples collected in the spring and fall periods. This process has the advantage of a faster analysis time than the MPN assay and shows potential in consistently predicting AMF activity. However, the analysis procedure at the Biochemistry Department at SDSU was continuously delayed with malfunctioning equipment, and results from this study have not been included in this thesis. Additionally, other future work regarding these research objectives include further comparisons of varying STP levels in other locations of the state using long-term no-till systems. In conclusion, this study was able to provide substantial evidence that optimum STP levels in long-term no till systems may need to be reevaluated. Additionally, the results from this study indicate that the increased AMF activity in low STP environments, as tested by the MPN and PLFA assay, likely contributed to the lack of yield differences among the three STP categories evaluated from 2018 to 2022.