CORN TISSUE NUTRIENT RESPONSE RELATED TO SOIL HEALTH AND FERTILITY

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the entitled

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a candidate for the degree of Master of Science, and hereby certify that, in their opinion, it is worthy of acceptance.

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ABSTRACT

Nutrient uptake in corn (*Zea mays L.*) is influenced by many different factors which include properties of soil health. The push for increasing soil health has raised the question of how soil health and fertility measurements could be combined to improve nutrient management decisions. Attention to nutrient management decisions is critical for high-yielding corn production. While soil sampling for fertility testing is the generally accepted standard for making these decisions, this alone may poorly represent plant nutrient availability. Another tool used for managing plant nutrient health is tissue sampling and analysis. This tool is routinely used for some crops (e.g., especially specialty crops) for diagnosing plant nutrient needs and direct fertilizer decisions. End of season yield response to fertilizer may be improved by using both of these two diagnostic tools. Research conducted in 2019 and 2020 on 91 producer corn fields in Missouri encompassed many soil types, management practices, and landscape positions, resulting in 433 different experimental plots. Accuracy when predicting a corn tissue response to added fertilizer using established critical values were only 61%, 53%, and 55% for K, P, and S respectively. Adding soil health metrics to fertility results using random forest models improved prediction of a positive tissue response to K fertilization, but did not improve predictions for P or S. along with soil-test K, two soil health measurements emerged as important, soil respiration and beta-glucosidase. Multiple linear regression results when predicting yield response as a function of soil test and tissue test K produced mixed results. Yield response were best predicted using soil-test K, tissue-test K ($p < 0.1$). Phosphorus soil-test or tissue concentration alone or in any combination did not explain yield response. Sulfur yield responses were best explained with just tissue S

concentration alone as the only significant predictor variable. This research suggests that current tools for diagnosing K, P, and S nutrient needs in corn and making fertilizer decisions need to be improved, but current soil health measurements lack ability to do so.

INTRODUCTION

Recent research has suggested a need for an improvement in crop fertilizer recommendations (Heckman et al., 2006; Fulford and Culman, 2018). The foundational work on these recommendations began in the 1920's, with most work occurring in the 1950's and 60's, and not much work happening after the 1980's (Voss, 1998). That said, many new ideas regarding soil health have arisen over recent decades and are now at the forefront of the agriculture industry. Soil health includes the physical, chemical, and biological properties of the soil (Karlen et al., 1997). The general idea espoused is that increasing soil health will increase soil nutrient availability, and therefore decrease overall fertilizer need.

Current knowledge on how soil health affects nutrient management decisions is lacking. Farmer acceptance of soil health and adoption of soil health practices could increase if direct evidence were available, showing how improved soil health enhances nutrient uptake and therefore reduces fertilizer inputs. If links between soil health and improved fertilizer recommendations could be quantified to potentially improve profit, then farmers would be more amenable to adopting soil health practices, and adoption would be more automatic. Research supports claims that farmers who advance soil health will improve crop yields, nutrient cycling, and reduce nutrient and soil losses (Snapp et al., 2005; González-Chávez et al., 2010; Kuhn et al., 2016; Haney et al., 2018).

Crop nutrient uptake as measured through tissue sampling is one way of evaluating crop nutrient health and potential yield production. It is a diagnostic used to evaluate the performance of soil and crop management practices (Mallarino, 1996). Nutrient uptake is influenced by many different factors throughout a plant's life cycle. Genetics of the plant,

amount of soil-available nutrients, soil composition, and weather are all examples of such factors. Understanding how tissue tests along with soil fertility tests can be effectively used to identify situations where yield may be limited by nutrients is needed, and essential for improving fertilizer recommendations.

The objective of this study is to investigate early-season corn tissue nutrient content as mediated by P, K and S fertility and biological soil health metrics. A second objective of this study is to measure corn grain yield response relative to early-season corn tissue nutrient content and/or soil fertility test metrics. These two objectives are addressed Chapters 2 and 3 of this thesis, respectively.

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1. LITERATURE REVIEW

INTRODUCTION

In recent decades, farmers have been encouraged by a variety of organizations to adopt management practices that may improve soil health. In its simplest terms, soil health is "the capacity of a soil to function" (Karlen et al., 1997). A well-functioning soil will sustain microbial and plant life, cycle nutrients (e.g., carbon sequestration, nitrogen fixation), suppress disease and pests, and have high water use efficiency (Nielsen et al., 2015). Well-functioning soils typically are defined through "suitable" or "ideal" ranges of certain physical, chemical, and biological properties (Karlen et al., 1997); these properties can be compiled into quantified soil health indices, such as the Soil Management Assessment Framework (SMAF) or the Comprehensive Assessment of Soil Health (CASH) framework (Andrews et al., 2004; Moebius-Clune et al., 2016). Soil and crop management practices that enhance soil health in general include those that reduce soil disturbance, maintain soil cover, and improve biodiversity (Nunes et al., 2018; Duncan et al., 2019). Research supports claims that farmers who advance soil health will improve crop yields, nutrient cycling, and reduce nutrient and soil losses (Snapp et al., 2005; González-Chávez et al., 2010; Kuhn et al., 2016; Haney et al., 2018). For society in general, improved soil health is expected to improve water and air quality and conserve water, therefore saving renewable resources (USDA-NRCS, 2019).

Management practices used to improve planting conditions, germination uniformity, and weed control [e.g., tillage, short or no crop rotations (e.g., monoculture), and no cover crops], are implemented at the expense of soil health (Martínez et al., 2016;

Kumar et al., 2017; Nunes et al., 2018). They are popular because they are considered the most cost-effective practices. However, research has shown that farmers can still maintain their yields and minimize weed pressure by adopting soil health promoting practices such as no-till, cover crops, or adding wheat to a rotation (Fuentes et al., 2009; Pittelkow et al., 2015). For example, farmers slowly adapted to no-tillage, a soil health promoting practice, due to an initial yield drag, but that yield loss disappeared with time because of improvement in both management skills and soil properties (Karlen et al., 2013; Bavougian et al., 2019). However, many farmers are unwilling to accept the shortterm risk of reduced yields—even if sustainability increases.

Current knowledge on how soil health affects nutrient management decisions is lacking. Farmer acceptance of soil health and adoption of soil health practices could increase if direct evidence were available, showing how improved soil health enhances nutrient uptake and therefore reduces fertilizer inputs. If links between soil health and improved fertilizer recommendations could be quantified to potentially improve profit, then farmers would be more amenable to adopting soil health practices, and adoption would be more automatic. Research is needed to show producers and the general public that increasing soil health can be both cost effective and economically beneficial.

Although it is assumed that improved soil health contributes to improved plant health, little research has been conducted to demonstrate this connection. One way plant nutrient health has been evaluated historically is through plant biomass or tissue nutrient testing. Often plant nutrient concentration or content can indicate plant nutrient health before visual deficiencies are expressed (Mallarino, 1996). The concentration or content of plant nutrients at different developmental stages have been used as indices of

sufficiency (Macy, 1936; Mallarino, 1996; Stammer and Mallarino, 2018). I propose tissue sampling could be used as a diagnostic tool to better understand soil health's role in plant health and when fertilizer nutrients are needed to optimize yields. The purpose of this research is to explore how early-season corn tissue nutrient content is influenced by soil fertility and soil health metrics.

ROLES AND UPTAKE OF NUTRIENTS IN PLANTS

Role and Uptake of Potassium in Plants

Potassium is an important nutrient in plants, as it makes up anywhere from 2-10% of total dry plant biomass (Walker et al., 1996; Ragel et al., 2019). Potassium is critical in controlling ion homeostasis, protein metabolism, enzyme activity, various metabolic processes, and osmoregulation (Walker et al., 1996; Shin, 2014; Ragel et al., 2019). Potassium is absorbed from the soil solution by root epidermal and cortical cells (Thompson and Zwieniecki, 2005). There are many transporters present in root cells that allow K to get inside the roots. Examples of transporters/channels in roots include Arabidopsis K^+ Transporter 1 (AKT1) which is voltage-gated and K selective (Lebaudy et al., 2007), KT/HAK/KUP carrier proteins associated with high- or low-affinity K absorption (Ragel et al., 2019), and high-affinity K transporters (HKT) which can act as Na and K symporters depending on the plant (Shin, 2014).

Plants store K in vacuoles, where it performs osmotic functions to maintain turgor pressure and drive cell expansion (Ragel et al., 2019). Potassium is used to control stomatal opening and closing, which influences carbon dioxide flux rates (Lebaudy et al., 2007). Potassium travels from the roots to the shoots via xylem where its driving force is

negative water pressure created by evaporation from the leaves and from the shoots back down to the roots via phloem (Thompson and Zwieniecki, 2005; Ragel et al., 2019). Many different complex signaling and sensing mechanisms are involved in the transport of K (Shin, 2014).

Role and Uptake of Phosphorus in Plants

Phosphorus is an important nutrient often limiting crop growth. Phosphorus in organic matter and in mineral forms are generally stable, immobile in soil, and unusable by plants. As such, it is also resistant to leaching through the soil profile. Plants absorb solubilized P from the soil that primarily occurs as inorganic orthophosphate ions, but some soluble organic P is also available to plants (Holford, 1997). The amount of plantavailable P at any one time is very low, and that concentration drives the change of insoluble P (both inorganic and organic) to soluble P. Therefore plants drive the change of phases for P, but there must be microbes present in order for it to change forms (Richardson and Simpson, 2011).

Phosphorus enters the plant through root hairs, root tips, and the outermost layer of root cells. Uptake can be facilitated through association with mycorrhizal fungi that grow with the roots of many crops. The orthophosphate form of P mostly taken up by plants is $H_2PO_4^-$, but can also be taken up as HPO_4^{2-}. Once P enters the plant, it is stored in the root or transported to the upper portions of the plant. Phosphorus is incorporated into nucleic acids and ATP in the plant. The conversion of ATP to ADP (phosphorylation) is important as the main source of energy for many reactions taking place in plants (IPNI (International Plant Nutrition Institute), 1999). The biggest use of P in plants is in photosynthesis where ATP is the energy source driving photosynthesis.

Plants need their tissues to contain 0.2% P of total dry weight in order to grow to their full potential (McGrath et al., 2014). Phosphorus is essential in stimulating root development and overall plant growth. Phosphorus is also important in transporting nutrients throughout the plant and ensuring that all parts get the necessary amount of nutrients (Vance et al., 2003). Essential storage in seeds requires adequate P in the form of phytin, and P is required for the timing of reproductive parts of plants as well (McGrath et al., 2014).

Role and Uptake of Sulfur in Plants

Sulfur also occurs in both organic and inorganic forms in the soil. Cycling from one form to another occurs via mobilization, mineralization, immobilization, oxidation, and reduction (Scherer, 2009). Organic S is not plant available and is immobile. The amount of organic S is correlated to total N and total C in the soil. Inorganic sulfur is mobile, and sulfate is the most important form for plant uptake. Sulfate generally accounts for only 5% of the total S in the soil, and is present in the top layers of soil due to organic matter mineralization and S containing fertilizers (Scherer, 2009). There are many S soil tests available, but these do not explain the amount of S that will be mineralized during the growing season or the amount of sulfate lost by leaching. A better understanding of how S levels fluctuate in soils is needed to explain availability to plants throughout the growing season.

Sulfur plays a role in many different steps of plant metabolism, making it a crucial element for optimum plant growth. Sulfur is the major component of two main amino acids, cysteine and methionine, which are building blocks for proteins in the plant (Droux, 2004; Davidian and Kopriva, 2010). There are many other uses for sulfur in

plants, such as their presence in co-factors, glutathione, and vitamins essential for plant development (Droux, 2004). Sulfur compounds are also involved in responses to biotic and abiotic stresses, such as defenses to herbivores and other pathogens (Davidian and Kopriva, 2010).

SOIL HEALTH COMPONENTS

Soil Health Background

Soil health includes the physical, chemical, and biological properties of the soil (Karlen et al., 1997). One method to improve these soil properties is conservation tillage. This practice involves cultivating the soil less often, which leads to lower fuel consumption and less fertilizer loss from runoff, increasing farmers' potential profitability (Kuhn et al., 2016). Improving soil health using conservation tillage leads to better water infiltration and increased ecosystem services, but still uses tillage minimally instead of going to full no-tillage (Stika, 2013; Haney et al., 2018). Conservation tillage increases moisture use efficiency while decreasing flooding risks (Stika, 2013). Increased soil health provides a diverse community of organisms that perform a number of processes to improve crop production (Lopes et al., 2013; Haney et al., 2018).

Introducing no-tillage onto the farm reduces the amount of topsoil lost from erosion. No-tilled soils have better water infiltration and storage than conventional tilled soils, which leads to better nutrient uptake (Hargrove, 1985). No-tilled soils typically have a stratification of nutrients throughout the horizons, but this does not seem to limit the crop yield (Karlen et al., 2013; Veum et al., 2015; Martínez et al., 2016). Intense tillage of the soil changes the physical properties, such as decreasing the aggregate stability and increasing compaction (González-Chávez et al., 2010; Martínez et al., 2016;

Kinoshita et al., 2017). The organic matter content decreases due to greater erosion and faster decomposition of plant material by increasing oxygen concentration in the soil (Nunes et al., 2018). Greater carbon dioxide emissions are also seen in tilled soils compared to no-tilled soils (Kuhn et al., 2016). From a soil health perspective, no-tillage offers many benefits over conventional tillage.

Microorganisms are crucial in cycling major plant nutrients in the soil (Haney et al., 2018). Yao et al. (2000) showed that land use history has a significant effect on the microbial community and biomass incorporated in the soil. Soil health enthusiasts put emphasis on microbial life in the soil due to the inherent benefits of their activity (Haney et al., 2018). Scientists attempt to put a value on the impact of microbes, but have yet to quantify their importance. The lab analysis measuring enzyme activity (i.e., betaglucosidase, phosphatase and sulfatase) identifies microbial levels directly affecting crop growth (Kremer and Li, 2003; Lopes et al., 2013; Kumar et al., 2017). These enzymes are involved in the mineralization of C, P, and S respectively. Therefore, discovering the factors that affect microbial activity can potentially contribute to better decision-making for farmers. Microbial levels may be altered by diversifying the crop rotation and adding cover crops (Nunes et al., 2018).

Implementing cover crops has been shown to increase long-term soil health. Pest management, nutrient cycling, and erosion control are internal benefits to the farm. Outside the farm boundaries, cover crops decrease nitrate leaching and nutrient loss in surface runoff (Snapp et al., 2005). However, nutrients can be lost in ways other than just in surface runoff, such as leaching and volatilization. Decreasing overall nutrient loss could lead to better yield maintenance and better water quality. Cover crops can improve

water quality by decreasing nutrient loss as well as pesticide loss (Dabney et al., 2001; Roesch-Mcnally et al., 2018). Improved pest management comes in the form of weed management from the cover crops due to competition or phytotoxic compounds that they can release. This leads to a reduction of pesticide necessary to control weeds in fields (Dabney et al., 2001). Species, seeding rate, planting timing, and termination timing are all crucial to controlling weeds with cover crops (Marcillo and Miguez, 2017).

Type of cover crop determines the benefits that are available to the producer. Legumes can fix N, so choosing to incorporate a legume into the cover crop mix can be beneficial in replenishing N (Dabney et al., 2001). Legumes have been shown to have the greatest increase in yield response when compared with grasses and a mixture of grasses and legumes (Marcillo and Miguez, 2017). Other cover crops such as cereal crops (e.g., rye) can scavenge more residual soil nitrate due to faster root growth, but do not lead to a yield increase in corn (Dabney et al., 2001; Marcillo and Miguez, 2017).

Cover crops have also been proven to help maintain yield, even at lower soil test fertility (Yost et al., 2016; Conway et al., 2018). Cover crops and their impact on longterm soil health properties have not been quantified and require research to determine how yield is influenced over a longer period. Nunes et al. (2018) and Smith et al. (2008) showed that cover crops can help increase yield because of the increase in crop diversity. This study also showed that in a monoculture cropping system, cover crop introduction increases many soil health values, regardless of the tillage practice. It was also found that no-tilled soils saw a greater increase in soil health values than tilled soils when cover crops were introduced, so implementing no-tillage and cover crops proved most beneficial to the farm and decreased erosion (Nunes et al., 2018).

Studies conducted in Missouri have linked biological soil tests with soil organic matter and land management practices. Veum et al. (2014) linked microbial function, traditional measures of soil quality, and soil organic matter composition across a range of different land management practices (i.e., tillage, fertilizer type, and crop type) on various Missouri soils. Soil quality was measured using the SMAF scoring system, where a lower "score" meant the soil quality was lower. Lower scores correspond with lower active C and lower enzyme activity. Significant differences in certain soil quality indicators (soil organic C, total N, and water stable aggregates) were shown when manure was applied compared to inorganic fertilizers. However, most of the indicators, besides dehydrogenase activity, were not significant between the wheat crop and the corn crop (Veum et al., 2014). In a separate but similar study, Veum et al. (2015) showed soil quality indicators were impacted by crop diversity and no-tillage, but differences were soil depth dependent. When compared to tilled cropping systems, soil health in no-till systems improved in the 0-5 cm depth, but was poorer in the 5-15 cm depth. Tillage improved the incorporation of organic matter and nutrients to deeper depths, which is why the soil health metrics from 5-15 cm were greater in tilled soils. However, Veum et al. (2015) stated using conservation practices such as no-till, cover crops, and diversified crop rotation leads to higher soil quality overall. Hargrove (1985) also stated that even with the stratification of nutrients, no-tilled soils produced greater grain yields. Missouri has a wide variety of soils across the state, so finding out how management practices can alter the health of the soil is key to finding out how to maintain a healthy farm.

Soil Health Metrics

Many different metrics have been proposed for assessing crop and soil

management practices on soil health. Examples include: acid phosphatase activity, arylsulfatase activity, active carbon, soil respiration, soil aggregate stability, and soil total protein (Andrews et al., 2004; Moebius-Clune et al., 2016). From a producer's standpoint, the most important aspect of soil health is whether it improves crop yield performance. This perspective serves as the reasoning behind producers' exploration of new crop and soil practices; their livelihood is a business, and they seek ways to optimize return on their investment. Yield is the most direct measure of crop performance. Indirect measures of crop performance may also show the impact of soil health. For example, claims have been made that soil health improves soil nutrient availability to crops (Kuhn et al., 2016; Haney et al., 2018). Few field studies have been conducted to establish this.

Current nutrient management practices typically include grid or zone soil sampling and laboratory tests every few years to determine fertilizer recommendations (Melsted, 1967; Olson et al., 1987; Anderson-Cook et al., 1999). These soil fertility tests are chemical tests that have changed little over recent decades (Hoeft et al., 1973; Magdoff et al., 1984). These tests were calibrated to field plot trials to determine critical values on which to base recommendations (Magdoff et al., 1984; Shapiro et al., 2019). They were not designed to assess soil biological processes and activity. Thus, biological measures of soil health and soil fertility have long been thought of as separate assessments, but interactions undoubtedly occur between the chemical and physical soil fertility tests and biological tests. For example, soil microbial biomass can be correlated with soil organic matter and can be an important pool of plant-available nutrients (Pankhurst et al., 1995). Therefore, increasing the organic matter of the soil can lead to increased nutrient availability (Doran and Safley, 1997). The majority of soil health

knowledge focuses on how various practices in cropping systems can change soil properties, but lack quantification for actual management recommendations (GonzálezChávez et al., 2010; Nunes et al., 2018; Bavougian et al., 2019; Van Es and Karlen, 2019). Additional research is needed to determine how crop plants respond to fertilization under various levels of biological soil health measures.

Soil Health Tests

There are many soil health tests used in order to obtain an overall idea of the health of any one soil. Determining which measurements best represent soil health status is crucial when trying to explain crop responses to changes in these metrics. Good tests for soil health include ones that are sensitive to management practices and represent agronomically and environmentally important processes (Moebius-Clune et al., 2016). Active carbon, total protein, soil respiration, and four enzymes were all found to fit these criteria.

Active carbon is a measurement of the portion of soil organic matter that is readily available as food and energy for microbes in the soil. For this test, dark purple potassium permanganate is reduced [Mn(VII) to Mn(II)] as it reacts with and oxidizes soil organic matter, causing the solution to lose color. The amount of color change is directly proportional to the amount of active carbon in the soil sample. This can be measured using a spectrophotometer and values calculated based on known concentrations (Culman et al., 2012; Schindelbeck et al., 2016).

Total protein is an indicator of the fraction of the soil organic matter pool that is present as protein-like substances. This represents the largest pool of organically bound N in the soil. Soil proteins are determined using a sodium citrate extraction under a high

temperature and pressure (autoclave). These results are then run against a set of standards to test how much protein is present. During microbial turnover, the proteins are broken down and can provide available N to plants (Schindelbeck et al., 2016).

Soil respiration is a direct measure of the amount of microbial activity in the soil. This procedure includes rewetting 20g of 2mm sieved soil stored in an airtight jar and kept at room temperature for four days. The carbon dioxide that is released is captured in a small beaker of potassium hydroxide (the CO_2 'trap') and used as an indicator of metabolic activity. To quantify the amount of CO_2 released, electrical conductivity readings of the CO_2 trap are taken before wetting the soil and again after incubation. A higher amount of CO_2 correlates to a highly populated microbial community, and therefore a "healthier" soil (Schindelbeck et al., 2016).

Three enzyme analyses used to measure soil health are arylsulfatase, acidphosphatase, and beta-glucosidase. Arylsulfatase hydrolyzes sulfate esters with an aromatic radical, resulting in a phenol being produced by splitting the O-S bond. Sulfatases mineralize organic soil sulfur into a plant available form of sulfur. This test involves a colorimetric estimation of *p*-nitrophenol released by arylsulfatase activity when soil is incubated with a buffered sulfate substrate solution at 37 degrees C for 1 hour. The reaction is terminated with the addition of calcium chloride and sodium hydroxide (Tabatabai and Bremner, 1970). According to Maynard et al. (1985), sulfatase activity is feedback-inhibited by sulfate in soils. The phosphatase enzyme is responsible for the cycling of organic P to inorganic P in the soil. This test involves the colorimetric estimation of the *p*-nitrophenol released by phosphatase activity when soil is incubated with buffered phosphate substrate solution for one hour at 37 degrees C (Tabatabai and

Bremner, 1969; Acosta-Martínez and Ali Tabatabai, 2015). Beta-glucosidase is related to carbon cycling in the soil (Acosta-Martínez and Ali Tabatabai, 2015).

Soil Health Assessments

The Soil Management Assessment Framework (SMAF) and Cornell's Comprehensive Assessment of Soil Health (CASH) both use a set of soil health tests they find to be most influential in determining soil health (Andrews et al., 2004; Moebius-Clune et al., 2016).

The SMAF evaluation uses a series of decision rules to generate a list of suggested soil health indicators, choosing from over 80 indicators for each user's specific needs (Andrews et al., 2004). To determine which tests to run, the user answers a variety of questions pertaining to the goals of the land (i.e. maximize productivity, waste recycling, or environmental protection). Each goal leads to a different set of tests that will be used. The SMAF evaluation further narrows the tests used according to the climate, crop type, rotation, tillage, and inherent soil properties (Andrews et al., 2004). After tests have been chosen, each value from the test must be transformed into new scores using nonlinear scoring curves. A scoring algorithm changes the value into a unitless score (0 to 1) that represents the level of function within that system (Andrews et al., 2004). The last step of the SMAF is to form an index in order to form an overall soil quality value for each soil.

The CASH evaluation emphasizes biological, physical, and chemical measurements. To start, 42 potential soil health indicators are evaluated for sensitivity to changes in soil management practices, consistency and reproducibility, ease and cost of sampling, cost of analysis, and ease of interpretation for users (Moebius-Clune et al.,

2016). The physical tests found to fit these standards are available water capacity, surface hardness, subsurface hardness, and aggregate stability. The important biological tests are organic matter, total protein, soil respiration, and active carbon. Chemical tests include pH and the main plant nutrients. Each measurement is assigned a scoring value between 0 and 100 (Moebius-Clune et al., 2016). Most physical and biological tests have higher scores for higher measured values, while some have higher scores with lower measured values (hardness, root health rating). The texture of the soil can have an impact on measured values, so several indicators require a separate scoring function depending on the soil texture (Moebius-Clune et al., 2016). Different regions also require consideration when determining a higher or lower scoring function determined by CASH. Each test has a very low, low, medium, high, and very high value that is dependent on texture and the region where samples are taken.

FACTORS AFFECTING NUTRIENT UPTAKE

Nutrient uptake is influenced by many different factors throughout a plant's life cycle. Genetics of the plant, amount of soil-available nutrients, soil composition, and weather are all examples of such factors. Management factors can also influence nutrient uptake through different forms. For example, compaction can be caused when intensive tillage is used, decreasing overall root growth and therefore nutrient uptake (Miransari et al., 2009). Using the right rate, right timing, right amount, and right source of fertilizer is essential to maximizing nutrient uptake in corn plants (Johnston and Bruulsema, 2014).

A field-scale study performed by Harmel and Haney (2013) showed that reducing fertilizer rates resulted in minimal yield loss and higher profitability. Traditional fertilizer rates tend to be too high to maximize profit, and in this study, only 2 site-years out of 35

maximized profitability to the farmer. Due to increases in application costs and the push for environmental improvement, more people are becoming aware of the fertilizer rate being applied (Harmel and Haney, 2013). Results of another study show that farmers must question the amount of fertilizer applied instead of just adhering to traditional recommendations as it may lower profits and contribute to water quality degradation (Phillips et al., 2009). The way fertilizer prescriptions are currently generated only focuses on maximizing yield, while profits and pollution need to be priorities as well. A lot of fertilizer is lost in runoff, which is a common problem in Missouri due to the shallow claypan.

Central Missouri contains soils that see a rapid increase in clay concentration throughout the soil profile, known as claypan soils. Conway et al. (2018) showed that depth to claypan (DTC) influences the amount of fertilizer needed to provide sufficient nutrients (P and K) for the plant, also called buffering. A lower DTC leads to more fertilizer needed to provide enough P for optimal crop growth. However, K rates may be decreased where DTC is lower due to the K-supplying capacity of the clay. More erosion occurs when the ground is bare, leading to a shallower DTC on claypan soils. Decreasing erosion by implementing soil health practices is a focus for many producers today. Soil health practices are good at reducing sediment loss and P bound to that soil. However, the amount of dissolved P lost in runoff or leaching varies from farm to farm (Duncan et al., 2019). Lowering the amount of runoff is a start, but keeping P in the soil profile is key to improving water quality and overall field fertility. Overall, learning how to improve the availability of P and K with less pollution from fertilization is the goal.

Sulfur uptake is coordinated with the uptake and assimilation of N and C, so a N limitation leads to a decrease in S uptake. Sulfate is taken up with demand-driven regulation, meaning sulfate uptake is driven by the lack of S in the plant, which leads to an increase in transporters (Davidian and Kopriva, 2010). Sulfate uptake is increased by introducing cadmium into the system (Nocito et al., 2006). Many other compounds influence the uptake of S by plants (Davidian and Kopriva, 2010).

THE ROLE OF CORN TISSUE SAMPLING FOR ASSESSING CROP HEALTH

Crop nutrient uptake as measured through tissue sampling is one way of evaluating crop nutrient health and potential yield production. It is a diagnostic used to evaluate the performance of soil and crop management practices (Mallarino, 1996). Similarly, crop nutrient uptake could be used to evaluate the influence of soil health on crops. However, tissue sampling for many grain crops is not widely used for nutrient management because of uncertainty in the test results and high costs. Tissue test nutrient concentrations vary due to plant growth stage, plant part sampled, growing conditions, and the hybrid/variety planted (Walker and Peck, 1972; Jones et al., 1990; Mallarino, 1996). To combat these obstacles, sampling the corn early in the season has been shown to have better results across a variety of conditions (Walker and Peck, 1972; Mallarino, 1996; Stammer and Mallarino, 2018). Therefore, tissue testing corn at V6-V8 should provide better results, along with keeping the timing between samples short relative to each other.

The goal of tissue testing is to assess the nutrient status of the crop. However, Clover and Mallarino (2013) showed that K concentrations of corn at the V5-V6 growth stage have a poor capacity to assess K sufficiency and predict grain yield responses. Also,

responses to P fertilization may be apparent in early season tissue samples, but yield may not be affected. In a study by Heckman et al. (2006), anywhere between 17 and 43% of the sites that tested below the critical soil P test level showed a yield increase to P fertilizer, and 25 to 50% of the sites exhibited an increase in early season crop growth. Often, plants undergo luxury consumption, an increase in early season tissue P and K concentration, but it does not lead to a yield response. Therefore, using tissue tests as the only source of information for P and K fertilization is not adequate for maximum utility of the test.

There have been numerous studies that show "critical" concentrations for P and K in tissue tests in corn. Mallarino (2018) tested the value of tissue testing in predicting grain yield. It was shown that the stage at which the corn was sampled mattered when looking to predict yield, as corn sampled at the V5-V6 stage showed no yield increase when the P concentration was ≥ 0.48% and corn sampled at the R1 stage resulted in no yield increase at P concentration ≥ 0.25% (Fig. 2). There was also a difference between K critical levels when sampling at the different growth stages. At the V5-V6 growth stage, no yield increase was seen at K concentration ≥ 2.5% and corn sampled at the R1 growth stage showed no yield increase at K concentration ≥ 1.4%.

THESIS OBJECTIVE

A better understanding of how soil health indicators influence crop tissue and yield is needed. The main objective of this investigation is to explore how different soil health measurements (i.e., chemical, physical, and biological) can be used as indicators of crop nutrient need. For this, tissue sampling will be used to assess crop response. Chapter

2 will address how soil health and soil fertility metrics can be used to help predict a tissue response to added fertilizer. Secondarily, an assessment of yield response will be examined by adding tissue sampling to soil-test fertility sampling. Chapter 3 will address how these two tests can be used together to predict a yield response to fertilizer. Outcomes of this work will help farmers understand the role of soil health in nutrient management decisions.

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TABLES AND FIGURES – CHAPTER 1

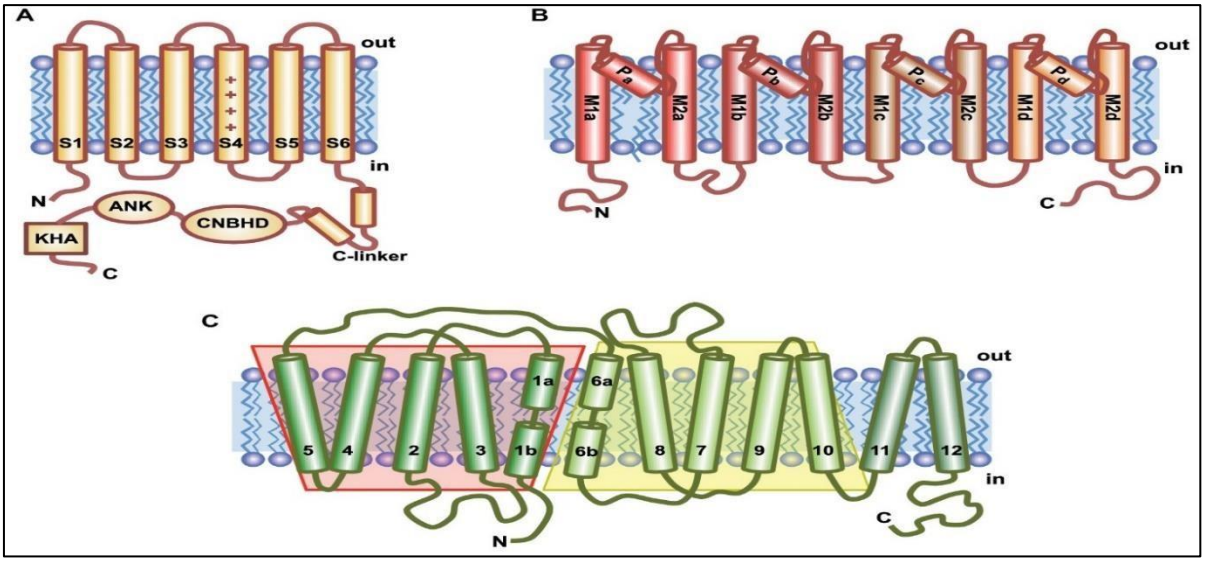

Figure 1.1. Topological models of the main ion transporters involved in K^+ nutrition. (A) Voltage-gated K^+ channels contain six transmembrane domains (S1–S6); S4 is the voltage-sensor characterized by the array of positively charged amino acids (+). The long C-terminal tail contains several conserved domains: C-linker, a cyclic nucleotide binding homologous domain (CNBHD), an ankyrin domain (ANK), and a final region rich in hydrophobic and acidic residues (KHA). (B) HKT transporters have a channel-like structure that contains four identical subunits (a–d), each comprising two transmembrane helices (M1 and M2) connected by the P-loop involved in ion selectivity. (C) KT/HAK/KUP transporters have 12 putative transmembrane domains (TMs). TM1-5 and TM6-10 are predicted to fold in the same conformation but showing inverse symmetry (Ragel et al., 2019).

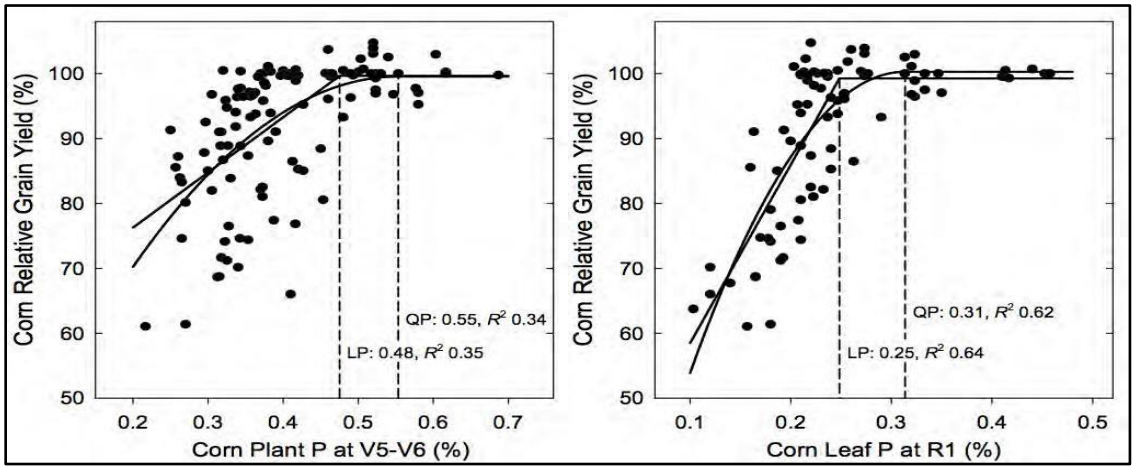

Figure 1.2. Corn tissue phosphorus concentration in relation to relative grain yield. The point where the plateau levels off is the point where the plant is thought of to have sufficient phosphorus (Mallarino, 2016).

2. CORN TISSUE NUTRIENT RESPONSE RELATED TO SOIL HEALTH AND FERTILITY

ABSTRACT

Soil health metrics, such as active carbon or soil respiration, may be important factors influencing corn (*Zea mays L.*) nutrient uptake. The push for increasing soil health has raised the question of how soil health and fertility measurements could be combined to improve nutrient management decisions. The objective of this research was to evaluate how different soil fertility and health metrics impacted early-season corn tissue nutrient uptake of potassium (K), sulfur (S), and phosphorus (P). Research conducted in 2019 and 2020 on 91 producer fields in Missouri encompassed many soil types, management practices, and landscape positions, resulting in 433 different experimental plots.Soil samples for soil health and fertility tests were collected in the spring before applying P, K, and S fertilizer treatments. Whole plant tissue samples were collected at V6-V8 and analyzed for tissue nutrient content, and response ratios were calculated. Accuracy of established fertilizer critical values were only 61%, 53%, and 55% for K, P, and S respectively. Adding soil health metrics to fertility results using random forest models improved prediction of a positive tissue response to K fertilization (>1.05 ratio), but did not improve predictions for P or S. After soil-test K, two soil health measurements also emerged as important, soil respiration and beta-glucosidase. These results indicate that additional measurements may not substantively improve the established method of determining an early season corn tissue response to added P or S.

INTRODUCTION

In recent decades, farmers have been encouraged by a variety of organizations both private and public to adopt management practices that may improve soil health. In its simplest terms, soil health is "the capacity of a soil to function" (Karlen et al., 1997). A well-functioning soil will sustain microbial and plant life, cycle nutrients (e.g., carbon sequestration, nitrogen fixation), suppress disease and pests, and have high water use efficiency (Nielsen et al., 2015). Well-functioning soils typically are defined through "suitable" or "ideal" ranges of certain physical, chemical, and biological properties (Karlen et al., 1997); these properties can be compiled into quantified soil health indices, such as the Soil Management Assessment Framework (SMAF), the Comprehensive Assessment of Soil Health (CASH) framework (Andrews et al., 2004; Moebius-Clune et al., 2016), or the new Soil Health Assessment Protocol and Evaluation (SHAPE; Nunes et al., 2021). Soil and crop management practices that enhance soil health in general include those that reduce soil disturbance, maintain soil cover, and improve biodiversity (Nunes et al., 2018; Duncan et al., 2019). Research supports claims that farmers who advance soil health will improve crop yields, nutrient cycling, and reduce nutrient and soil losses (Snapp et al., 2005; González-Chávez et al., 2010; Kuhn et al., 2016; Haney et al., 2018).

Current nutrient management practices typically include grid or zone soil sampling and laboratory tests every few years to determine fertilizer recommendations (Melsted, 1967; Olson et al., 1987; Anderson-Cook et al., 1999). These soil fertility tests are chemical tests that have changed little over recent decades (Hoeft et al., 1973; Magdoff et al., 1984). These tests were calibrated to field plot trials to determine critical values on which to base recommendations (Magdoff et al., 1984; Shapiro et al., 2019). They were

not designed to assess soil biological processes and activity. Thus, biological measures of soil health and soil fertility have long been thought of as separate assessments, but interactions undoubtedly occur between the chemical soil fertility tests and biological tests. For example, soil microbial biomass can be correlated with soil organic matter and can be an important pool of plant-available nutrients (Pankhurst et al., 1995). Therefore, increasing the organic matter of the soil can lead to increased nutrient availability (Doran and Safley, 1997). The majority of soil health knowledge focuses on how various practices in cropping systems can change soil properties, but lack quantification for actual management recommendations (González-Chávez et al., 2010; Nunes et al., 2018; Bavougian et al., 2019; Van Es and Karlen, 2019). Additional research is needed to determine how crop plants respond to fertilization under various levels of biological soil health measures.

A field-scale study performed by Harmel and Haney (2013) showed that reducing fertilizer rates resulted in minimal yield loss and higher profitability under certain conditions. Traditional fertilizer rate recommendations tend to be too high to maximize profit, and in this study, only 2 site-years out of 35 maximized profitability to the farmer. Thus, while the traditional test may have value for indicating soil nutrient supply, the associated fertilizer recommendations likely were established decades ago to guard against yield loss across a wide range of soil conditions. The result is a system that recommends over-application for many soils. Due to increases in application costs and the push for environmental improvement, more attention is focused on fertilizer rate (Harmel and Haney, 2013). For example, results from Phillips et al. (2009) showed that farmers should question the amount of fertilizer applied instead of just adhering to

traditional recommendations, as it may lower profits and contribute to water quality degradation.-The way fertilizer recommendations are currently generated only focuses on maximizing yield, while profits and environmental degradation need to be prioritized as well.

Many different metrics have been proposed for assessing crop and soil management practices on soil health. Determining which measurements best represent soil health status, and specifically the important soil function of supplying soil nutrients to crop plants, is crucial for a meaningful interpretation of soil health tests. Good tests for soil health include ones that are sensitive to management practices and represent agronomically and environmentally important processes (Moebius-Clune et al., 2016). Examples include: acid phosphatase activity, arylsulfatase activity, active carbon, soil respiration, soil aggregate stability, and soil total protein (Andrews et al., 2004; MoebiusClune et al., 2016). From the standpoint of many producers, the most important aspect of soil health is whether it improves crop yield or other elements of economic performance.

This perspective serves as the motivation behind producers' exploration of new crop and soil practices; their livelihood is a business, and they seek ways to optimize return on their investment. Since fertilizer nutrients represent one of the most significant input investments for producers, knowing soil health-nutrient supply relationships is paramount. Tissue tests, along with yield response, are the most direct measure of crop performance to fertilizer nutrients. Indirect measures of crop performance may also illustrate the impact of soil health. For example, although it has been posited that improved soil health increases soil nutrient availability to crops (Kuhn et al., 2016;

Haney et al., 2018), few field studies have been conducted to establish this.

Specific management factors can also influence nutrient uptake. Using the right rate, right timing, right amount, and right source of fertilizer is essential to maximizing nutrient uptake in corn plants (Johnston and Bruulsema, 2014), yet nutrient uptake is influenced by many different factors throughout a plant's life cycle. Genetics of the plant, amount of non-fertilizer soil-available nutrients, soil composition, and weather are all examples of such factors. For example, compaction can result from intensive tillage, decreasing overall root growth and therefore nutrient uptake (Miransari et al., 2009).

Macronutrients are important for crop growth, including K, P, and S. Each of these nutrients are unique in how they affect plant growth. Potassium is critical in controlling ion homeostasis, protein metabolism, enzyme activity, various metabolic processes, and osmoregulation (Walker et al., 1996; Shin, 2014; Ragel et al., 2019). Phosphorus is incorporated into nucleic acids and ATP in the plant. The conversion of ATP to ADP (phosphorylation) is important as the main source of energy for many reactions taking place in plants (IPNI (International Plant Nutrition Institute), 1999). Sulfur is the major component of two main amino acids, cysteine and methionine, which are building blocks for proteins in the plant (Droux, 2004; Davidian and Kopriva, 2010). There are many other uses for S in plants, such as their presence in co-factors, glutathione, and vitamins essential for plant development (Droux, 2004). Sulfur compounds are also involved in responses to biotic and abiotic stresses, such as defenses to herbivores and other pathogens (Davidian and Kopriva, 2010). Collectively, these nutrients are measured for traditional fertilizer recommendations because deficiencies will result in suboptimal yields. Assessment of their availability is often conducted via soil testing, but tissue testing could be used as well.

Crop nutrient uptake, as measured through tissue sampling, is one way of evaluating crop nutrient health and potential yield production. It is a diagnostic tool used to evaluate the performance of soil and crop management practices (Mallarino, 1996). Similarly, crop nutrient uptake could be used to evaluate the influence of soil health on crops. However, tissue sampling for many grain crops is not widely used for nutrient management because of uncertainty in the test results and high costs. With corn, tissue test nutrient concentrations vary due to plant growth stage, plant part sampled, growing conditions, and the hybrid/variety planted (Walker and Peck, 1972; Jones et al., 1990; Mallarino, 1996). To combat these obstacles, sampling the corn early in the season has been shown to provide better results across a variety of conditions (Walker and Peck, 1972; Mallarino, 1996; Stammer and Mallarino, 2018). Therefore, tissue testing corn at V6-V8 should provide better results, along with keeping the timing between samples short relative to each other.

The goal of tissue testing is to assess the nutrient status of the crop. However, Clover and Mallarino (2013) showed that K concentrations of corn at the V5-V6 growth stage have a poor capacity to assess K sufficiency and predict grain yield responses. Also, even though a response to P fertilization may be apparent in early-season tissue samples, yield may not be affected. In a study by Heckman et al. (2006), anywhere between 17 and 43% of the sites that tested below the critical soil P test level showed a yield increase to P fertilizer, and 25 to 50% of the sites exhibited an increase in early season tissue concentrations. Often, plants undergo luxury consumption, an increase in early season tissue P and K concentration that does not lead to a yield response. Therefore, using

tissue tests as the only source of information for P and K fertilization decisions is not adequate.

A better understanding of how soil health indicators influence crop tissue nutrient uptake and yield relative to fertilization is needed. As such, the main purpose of this investigation was to explore how different soil health measurements (i.e., chemical, physical, and biological) might be used as indicators of crop nutrient need. The specific objective of this research was to examine corn tissue response to K, P, and S fertilization as impacted by soil health and fertility properties. In other words, the goal was to evaluate the theory that as soil health improves, crop tissue response to added fertilizer will decline.

MATERIALS AND METHODS

Research Sites and Locations

This research was conducted as a public collaboration between the University of Missouri and Corteva Agrisciences. This project took place primarily in Missouri with additional sites in Iowa and South Dakota. Data was collected during the 2019 and 2020 growing seasons. Each year, 50-60 different fields were selected. Each field contained 3-5 spatially distributed location sites (hereafter referred to as "stamps") that were selected to capture a range of field conditions and to examine inherent in-field variability. The fields represented a wide range of soil and crop management systems to capture a range of corn tissue responses across different soil health levels. Soil and weather information can be found in Table 2.1. In total, there were 446 stamps in this study spread throughout 97 total fields (Fig. 2.1). Fields were selected in the late fall/early spring by communicating with farmers and local networks, including industry representatives MFA

and Corteva Agrisciences. Operations were conducted such that farmers avoided applying S, P, and K to the stamp sites to provide a locally representative environment with the best opportunity for response to treatments. Fields with recent grid soil sampling results allowed for selecting stamp sites that had both low and high soil fertility conditions. Stamps were placed based on differing landscape positions, soil series, and fertility levels, along with field access in mind. The goal of this experimental design was to encompass a wide range of field conditions and therefore soil health levels.

Soil Sampling, Fertilization Treatments, and Laboratory Analyses

Soil sampling and fertilization treatments were done for each stamp in the early spring of each growing season, approximately two to five weeks prior to planting. Each stamp site was a square with 12.2 m on each side, providing an area 148.8 m^2. Coordinates of each stamp center were measured using Trimble GeoXT 6000 and Geo 7x GPS devices with approximately 6-cm accuracy. With the center identified, pre-cut ropes were stretched to define the stamp area. Using a hand-held compass, the stamps were oriented on a north-south bearing regardless of where the stamp was located in the field.

For soil fertility and health assessments, eight to twelve 2.54-cm (id) cores, to a depth of 15 cm, were obtained evenly distributed within the stamp area. The cores were split into two depths (0-5 cm depth and 5-15 cm depth) and composited into buckets at the time of sampling. After gentle hand mixing, the samples were split for subsequent laboratory analysis into standard soil sampling boxes for traditional fertility analyses and re-sealable zipper storage bags for soil health analyses.

For soil profile characterization and sub-surface fertility assessment, a Giddings hydraulic soil sampling machine was used to obtain one 4.5-cm (id) profile core to an

approximate depth of 1 m. These were laid out on a processing table and visually characterized by pedogenetic horizon. The surface 0-15 cm was designated as the Ap horizon. After designating the first horizon, the rest of the core was separated into four or five total horizons determined by soil color and texture. Soil samples were bagged and removed from the stamp area before fertilization treatments were applied.

For fertilization treatments, the stamp area was sub-divided into four equal 6.1 by 6.1 m quadrats (37.2 m^2). One quadrant was designated as the control and received no fertilizer treatment. Fertilizer K, P, and S treatments were applied by hand to quadrants as shown in Figure 2.1. Fertilizer treatment rates were as follows: 1) control; 2) 112 kg ha^{-1} of K_2O applied as potash (0-0-60); 3) 112 kg ha^{-1} of P_2O_5 applied as triple super phosphate (0-45-0); and 4) 28 kg ha^{-1} of S applied as ammonium sulfate (21-0-24). So that all received equal N, an additional 25 kg N ha^{-1} as SUPER-U (46-0-0) was applied to quadrats 1-3 to balance the N applied with ammonium sulfate to treatment 4. Additionally, since both years of this study experienced above-average spring precipitation, an additional 67 kg N ha^{-1} was applied as SUPER-U to the entire stamp area at the time of tissue sampling, to guard against N deficiencies later in the growing season.

Soil samples were delivered to Ward Labs in Kearney, Nebraska for traditional fertility analyses and soil characterization/texture. Soil health samples were stored in a cooler then wet sieved through a 2.54 cm screen to homogenize the sample, then air-dried in a tin pan. Once fully dried, the soils were sieved again (2mm sieve) for downstream analysis at the USDA-ARS Soil and Water Quality Lab in Columbia, MO. Fertility and soil health analyses are summarized in Table 2.2.

Plant Sampling for Tissue Nutrient Analyses

Eight whole plants from each of the four quadrats of each stamp were harvested at the developmental growth stage of V6-V8 for nutrient content assessment. Prior to sampling, the central part of the quadrat with a uniform stand was first designated with plastic garden stakes for grain yield harvest at the end of the season. Plant samples for tissue nutrient content were then randomly collected outside of this area to avoid a "border effect" with removed plants. Harvested plants were placed into labeled brown paper bags for storage and drying. Samples were air/oven dried and ground to pass a 2-mm screen, then shipped to Ward Laboratories (Kearney, NE) for analysis of P, K, and S concentration.

Data Management and Statistical Analyses

Data were analyzed using R programming language (R Core Team, 2017). To measure relative nutrient uptake response to fertilization, tissue samples from the K, P, and S fertilized quadrats of each stamp were divided by the control nutrient content value. This ratio provided a response index for each nutrient in each stamp. Due to the lack of traditional replication with this experimental design, an alternative measure of field experimental error was developed in a companion study. Specifically, in a study of yield response across the same sites, a suite of methods were evaluated to estimate experimental error, concluding that up to an approximate 10% random error could be expected for the yield response (J. Svedin, personal communication, July 2021). However, tissue nutrient content is based on a subsample of plants selected to represent the overall stand (e.g., not an areal measurement such as yield). Therefore, it is expected that the experimental error associated with tissue nutrient content would be considerably

less. A response ratio of 5% or above was interpreted as a positive biological response to fertilization.

The response ratios were used as the dependent variables in the statistical analyses. Since there were numerous possible independent factors explored in this analysis, machine learning techniques that allow for multivariate analysis were determined to be the most useful. While several different techniques were explored, random forest analysis paired with corresponding decision tree visualizations were employed for this study. Random forest analysis allowed for variable importance to be identified and shown using mean decrease in gini and mean distance of nodes down in the decision trees formed using the random forests (Han et al., 2016). Mean decrease in gini is the average of a variable's total decrease in node impurity, weighted by the proportion of samples reaching that node in each individual decision tree in the random forest (Behnamian et al., 2017). A higher mean decrease in gini indicated higher variable importance. The most important variables were then used in a decision tree in order to best predict a tissue response to added fertilizer. Other variables used in the random forest are listed in table 2.1.

RESULTS AND DISCUSSION

This investigation included a wide range of growing conditions and soils, which led to diverse early-season corn tissue concentration response. Response was examined relative to K, P, and S fertilization, and analyzed to determine which soil and weather measurements were the most important for explaining when a response occurred. Since the response to each of these three fertilizer nutrients was unique, along with the

measurements that best characterized the response, findings are presented by each nutrient.

Corn Tissue Potassium

Corn Tissue Response Relative to Soil-Test Potassium

Corn tissue K concentration of the whole plant at V6-V8 ranged from 12.7 to 75.4 g K kg^{-1} with a mean of 38.4 g K kg^{-1}. These values are close to what other research has shown when measuring early-season corn tissue K concentration (Clover and Mallarino, 2013; Stammer and Mallarino, 2018). These concentration values when divided by the K concentration of the non-fertilized plants create a response ratio that ranged from 0.39 to 3.23. These ratios when shown relative to the soil-test K (ranging from 49 to 398 mg K kg^{-1}; Fig. 2.2) support the study objective of evaluating a wide range of growing conditions.

Figure 2.2 has four different notable features. First, the horizontal dashed line is at a ratio of 1.05 and represents a threshold above which a positive response to fertilizer was considered to have likely occurred. Second, the blue vertical band represents the range of critical values for soil-test K established by the University of Missouri Soil Fertility Testing Program. It is shown as a band because interpretation of soil-test K for fertilizer recommendations includes the variable of soil CEC. The higher the CEC, the higher the critical value. The blue band thus represents most CEC values for soils in this study (lowest and highest 5% values excluded). The lower and upper boundaries of this blue band are 132 and 160 mg K kg^{-1}. Third, the red line is a best fit linear-plateau model of the dataset, with the joint occurring at 135 mg K kg^{-1}. Collectively these features show response using early-vegetative tissue K uptake is reasonably aligned with critical values

used for soil-test values. Finally, two boxplots are shown which show the distribution of soil-test K observations both below and above 135 mg kg^{-1}. The results represented in Figure 2.2 follow a similar trend to what others have shown (Mallarino and Sawyer, 2018; Mallarino, 2016).

Site-specific critical values were calculated using CEC and were compared to the soil-test K to determine if a site should be responsive to fertilization. These were then examined relative to the tissue responses using the predetermined 5% threshold. Accuracy based on both predicted responsive and non-responsive sites for this dataset was 61%. This means that 39% of sites were either 1) above the established critical value yet were responsive, or 2) below the critical value yet un-responsive. Therefore, the standard soil fertility test alone may not be the best way to predict a tissue response to added K fertilizer. To better understand corn tissue response to added K fertilizer, other soil and weather information were examined as governing factors.

Influence of Soil and Weather Information

Using machine learning methods, all soil and weather variables (Table 2.1) were examined for their influence on corn tissue K response at the V6-V8 developmental growth stage. Variables found to be important were summarized in two different ways. First, the random forest mean decrease in Gini importance (Han et al., 2016) was applied. This method evaluates feature relevance, allowing for explicit feature elimination in high dimensional datasets and reduction of noise from the classification task. The Gini order of importance, with the most important variables on top, is presented in Figure 2.3. The second approach provided the distribution of the average minimal depth of each variable within the decision tree structure of the random forest analysis, where deeper depth

implies reduced importance (Epifanio, 2017). In addition, the number of times out of 500 runs that each variable appeared by depth in the tree analysis is represented by color coding (Fig. 2.4). In both scenarios, the ranking of the top three most important variables impacting tissue response to added K were, in the following order: soil-test K, soil respiration, and beta-glucosidase.

It is notable that soil-test K was the most important variable in the decision tree analysis (both in terms of variable order and magnitude) for understanding early-season plant K uptake. Although the points defining the relationship between soil-test K and the tissue response ratio (Fig. 2.2) exhibits some degree of scatter, of all the variables evaluated in this investigation, soil-test K emerged as the most important. This outcome thus generally supports the current approach for corn K nutrient management. However, soil CEC, also used in current recommendations, exhibited minimal influence on corn K uptake. One plausible explanation for this is that the effect of added K fertilizer on CEC takes months as soils go through multiple wetting and drying cycles (Rhoades, 1982). Thus, the fact that CEC was not helpful for explaining early-season plant K uptake is not surprising, since tissue samples were obtained within 10 weeks of fertilizer application.

After soil-test K, two soil health measurements also emerged as important. Specifically, soil respiration and beta-glucosidase were selected as important variables for explaining variation in plant tissue K content. These two measurements are related as they both deal with carbon cycling in the soil. It is interesting to note that other carbonrelated soil tests were not found to be important, and none of the weather or inherent soil characteristics were found to be important for predicting corn tissue K response.

The three variables of greatest importance were used to develop a decision tree model for early season corn tissue K content in response to added K fertilizer (Fig 2.5). As previously noted, soil-test K was most important and defined the first decision tree split. For sites with soil-test K < 119 mg K kg^{-1}, plants increased in K content with fertilization 85% of the time. This soil test value is somewhat lower than the general critical value for Missouri (as illustrated in Fig. 2.2) and suggests that some fields will not respond to fertilization using current soil test recommendations.

The next tree split for sites with soil-test K ≥ 119 mg K kg^{-1} involved soil respiration. Overall microbial activity is reflected in the soil respiration measurement (MoebiusClune et al., 2016; Haney et al., 2018; Franzluebbers et al., 1995). Only 6% of the sites (n=21) had soil respiration ≥ 230 mg kg^{-1}, but within this group of sites, the majority (84%) was non-responsive to fertilization. With the average soil respiration in our dataset of 145 mg kg^{-1}, this result shows how high the respiration has to be in order to make this split in the decision tree. When soil respiration was < 230 mg kg^{-1}, a new decision in the tree was defined that utilized beta-glucosidase enzyme activity. Then, when betaglucosidase activity was ≥ 95 mg kg^{-1} (n=72), the majority (63%) of sites were nonresponsive to fertilization. In contrast, when beta-glucosidase < 95 mg kg^{-1} (n=291), sites were generally responsive (62%). This connection between beta-glucosidase, soil respiration, K fertilization, and tissue response has not been described before and merits further research. The overall accuracy of the decision tree in predicting tissue response was 67%.

These results support the general hypothesis that microbial activity plays a quantitative role in K availability for corn plants, and aid in understanding response to added K fertilizers. This outcome validates claims that microbial soil measurements can potentially be used to refine nutrient management decisions (Snapp et al., 2005; González-Chávez et al., 2010; Kuhn et al., 2016; Haney et al., 2018). However, the added cost associated with biological measurements may not be justifiable except in high-value crops.

Following the outcome of the decision tree analysis, sites displayed in Fig. 2.2 were modified to show predicted responsive and non-responsive sites (Fig. 2.6). When below the soil-test K value of 119 mg kg^{-1}, the model always predicted a response, even in some cases when there was not a response. Above this value, the model produced mixed results with predicted responsive and non-responsive sites. However, a greater percentage of the predicted non-responsive sites were below the 1.05 response threshold (68%) than above this threshold. The opposite was also true, in that a greater percentage of responsive sites were above the 1.05 responsive threshold (70%) than below this threshold. Overall, this indicates that adding the biological measures was the most helpful when predicting responses in the range of the current soil test critical values. Normally, models based on standard fertility measurements will not predict a positive response when fertilizer is applied above a specific soil test level, but these results indicate that biological measures can improve the prediction of when corn will respond to fertilization.

Corn Tissue Phosphorus

Corn Tissue Response Relative to Soil-Test Phosphorus

Corn tissue P concentration of the whole plant at V6-V8 ranged from 1.6 to 9.6 g P kg^{-1} and averaged 4.5 g P kg^{-1}. These concentration values, when divided by the P concentration of the non-fertilized plants, create a response ratio that ranged from 0.56 to 1.51. These ratios shown relative to the soil-test P (ranging from 2 to 168 mg kg^{-1}; Fig. 2. 7), support the study objective of evaluating a wide range of growing conditions. Two features in this figure are worth description. First, the horizontal dashed line marks a ratio of 1.05 and represents the pre-determined threshold above which a positive response to fertilizer is expected. Second, the vertical dashed line lies at the current corn critical value for soil-test P determined by the University of Missouri Soil Testing Program, which is 22.5 mg kg^{-1}. Of sites below this critical value, 47% were < 1.05 response, indicating nearly half of the sites would be considered responsive based on the soil test level, but were not. Unlike the soil-test K plot (Fig. 2.2), no significant linear-plateau model was found with this relationship. The accuracy of the established critical value in predicting an early-season corn tissue response to added P was only 53%. This means soil-test P alone was not at all helpful in understanding when there was a P uptake response to fertilization at the V6-V8 corn growth development stage.

Soil and Weather Information Influencing Response

Soil and weather information was examined using random forest analysis for an influence on P uptake and variable importance was evaluated as was done for K (Figs. 2.8 and 2.9). Examining these figures, the trend in variable importance was not as strong as that exhibited by K uptake. In this case, beta-glucosidase and active carbon were

ranked most important, but there was little separation between the variables overall. This implies that no single variable emerged as most important for prediction of early-season corn tissue response to added P. The fact that soil-test P was not selected first is notable, since it is the variable currently used to determine fertilizer application rate.

Phosphorus Uptake Beyond the Soil Fertility Test

Since no variable emerged as the single most important, all variables were considered in the subsequent decision tree model (Fig. 2.10) to find the best possible prediction for corn P tissue response to added P fertilizer. The most accurate model utilized betaglucosidase, soil-test P, and sand content. For sites with a relatively high beta-glucosidase value [$\geq$ 108 mg g^{-1} (n=37)], 92% of these sites were non-responsive. If beta-glucosidase was < 108 mg g^{-1}, sand content defined the next split in the tree. If sand content was < 10%, sites most often responded to P fertilization, even though there were few sites that matched this condition (n=15). If sand content was $\geq$ 10%, another tree split was defined that invoked soil-test P. If soil-test P was < 8 mg P kg^{-1}, the site was responsive 63% of the time, and if soil-test P was $\geq$ 8 mg P kg^{-1}, the site was non-responsive 61% of the time.

The established critical value of soil-test P for corn in Missouri is 22.5 mg kg^{-1}, over twice the value found to be helpful in this decision tree analysis of 8 mg P kg^{-1}. However, it is important to note that Missouri's critical soil test values were developed based on grain yield response at the end of the growing season, not tissue sampling response. For the purposes of this study, it was presumed that early-season tissue sampling foreshadowed full season response, as in other studies (e.g., Stammer and Mallarino, 2018).

In contrast to the accuracy of the established critical value (53%), the overall accuracy of this decision tree was 61% when used to predict a tissue response to added P. Although this reflects a marginal improvement of 8%, multiple potential variables achieved a similar increase in accuracy, and the appearance and placement of variables within the decision trees was inconsistent (Fig. 2.9). These results indicate that additional measurements may not substantively improve the established method of determining an early season corn tissue response to added P.

Using the decision tree shown in Fig. 2.10, sites displayed in Fig. 2.7 were modified to show predicted responsive and non-responsive sites (Fig. 2.11). The high accuracy of this decision tree can be attributed to the abundance of sites (82%) where no response to added P was predicted. Therefore, the model is not any better at predicting a tissue response to added P than the established method of a critical value of soil-test P.

Corn Tissue Sulfur

Corn Tissue Response Relative to Soil-Test Sulfur

Corn tissue S concentration of the whole plant at V6-V8 ranged from 1.39 to 4.4 g S kg^{-1} and averaged 2.6 g S kg^{-1}. These concentration values, when divided by the S concentration of the non-fertilized plants, create a response ratio that ranged from 0.73 to 2.24. These ratios shown relative to the soil-test S (ranging from 2.5 to 14.8 mg kg^{-1}; Fig. 2.12), support the study objective by evaluating a wide range of growing conditions. Two components of this figure are worth description. First, the horizontal dashed line at a ratio of 1.05 represents a threshold above which a positive response to fertilizer occurred. Second, the vertical dashed line represents the S critical value for Missouri, which was established by the University of Missouri at 7.5 mg kg^{-1}. Of sites below the critical value,

64% of them showed > 1.05 response, indicating more sites responded when below the critical value when compared to non-responsive sites. However, when above the critical value, 69% of the sites were responsive to S fertilization. No significant linear plateau relationship was found in the S fertilization treatment. This implies that soil-test S alone is not sufficient for predicting an S uptake response to fertilization at the V6-V8 corn growth stage. The overall accuracy when using the established critical value was only 55%.

Soil and Weather Information Influencing Response

Soil and weather information was examined using random forest analysis for an influence on S uptake, and variable importance was ranked, as done for P and K (Figs 2.13 and 2.14). Similar to the P analysis, no variable stood out as being more important than the others based on these results. All variables were extremely close in mean decrease Gini values and mean minimal depth in the random forest trees. Weather information emerged higher on the list for S when compared with P or K, likely due to the effect of weather on S mineralization early in the growing season. Corn responds to added S early because little S is mineralized by microorganisms when it is cold and wet in early spring, leaving it available for plants (Scherer, 2009). Thus, weather is important when evaluating tissue response to added S when corn is at the V6-V8 developmental growth stage.

Sulfur Uptake Beyond the Soil Fertility Test

Since no variables were identified as the most important, they were all included in the decision tree analysis to predict tissue response to added S fertilizer (Fig. 2.15). The most accurate model used soil-test S and total protein. The first node in the tree was soil-test S

with a threshold value of 12 mg S kg^{-1}. Only 4% (n=14) of the sites were above this value, with 79% of them being non-responsive. If soil-test S was under 12 mg S kg^{-1}, the next node in the tree was total protein. The total protein threshold value was 2.1 mg g^{-1}, which was lower than the average total protein for the study, 3.55 mg g^{-1}. A large majority of the sites in the study fit into the category of > 2.1 mg g^{-1} (n=291). These sites had a 72% response rate.

The established critical value of soil-test S for corn in Missouri is 7.5 mg kg^{-1}, which is approximately half the value identified in the decision tree analysis of 12 mg S kg^{-1}. The overall accuracy of this decision tree was 67% when used to predict a tissue response to added S, compared with the accuracy of the established method (55%). Although this reflects an improvement of 12%, multiple variables produced similar results, and the appearance and placement of variables within the decision trees was inconsistent (Fig. 2.14). These results indicate that additional measurements may not improve the established method of determining an early season corn tissue response to added S.

Using the outcome of the decision tree shown in Fig. 2.15, sites displayed in Fig. 2.13 were modified to show predicted responsive and non-responsive sites (Fig. 2.16). The high accuracy of this decision tree can be attributed to the abundance of sites (85%) where a response to added S was predicted. Therefore, the model is not any better at predicting a tissue response to added P than the established method of a critical value of soil-test P. This makes the prediction of response/no response not useful when looking at the application of what the tree is attempting to predict. Therefore, the model is not better at predicting a tissue response to added S than the established method of a critical value of soil-test S.

CONCLUSION

This study looked at K, P, and S corn tissue nutrient concentration responses relative to soil fertility and health metrics. Random forests along with decision tree models were best when showing which soil health and fertility variables best represented the prediction of a tissue response. Beta-glucosidase and soil respiration were helpful when predicting K tissue nutrient concentration responses. These along with soil-test K provided a higher prediction accuracy than when soil-test K was used alone. The soil-test K value that was used was lower than the average value in the current Missouri recommendations used today. Together this suggests two things. One, the current recommendation for when K fertilizer should be added may be inflated. And two, improvements may be made to fertilizer recommendations in Missouri by including soil health metrics. Additional research on this finding is warranted. When it comes to P and S tissue concentration responses, soil health metrics did not help explain uptake differences. With these two nutrients, even soil test values were not very helpful. However, accuracies were slightly improved when using multiple variables within decision tree modeling. In other words, the model almost always predicted a response to fertilization, which actually occurred, but is not helpful or practical. Further as explained before, a response in tissue content is easier to detect than yield at the end of the growing season. Therefore, these findings are only intended to suggest what soil health metrics may be contributing to crop nutrient utilization, and additional studies quantifying these relationships are needed.

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TABLES AND FIGURES – CHAPTER 2

Table 2.1. Weather and MLRA's of sites with associated acronyms, methods, and citations.

Weather	Acronym	Method	Citation
Growing Degree Days	GDD	See source	McMaster and Wilhelm 1997
Corn Heat Units	CHU	See source	Bootsma et al. 2005
Abundant and well-distributed rainfall	awdr	AWDR=Rain X SDI	Tremblay et al. 2012
Precipitation from planting to sampling	Rain	Total rainfall from planting date till sample date	
Shannon Diversity Index (daily rainfall evenness)	SDI	See source	Bronikowski and Webb 1996
MLRA			
Central Claypan Soils	113		
Iowa and Missouri Heavy Till	109		
Central Mississippi Valley Wooded Slopes, Northern Part	115C		
Cherokee Praries	112		
Ozark Highland	116A		
Central Mississippi Valley Wooded Slopes, Western Part	115B		

Table 2.2. Soil fertility, soil health, and plant tissue analyses with associated acronyms, laboratories, methods, and citations.

Analyses	Acronym	Laboratory	Method	Citation
Soil Properties				
Phosphorus	Bray1	Ward	Bray 1; Mehlich-3	Bray and Kurtz 1945; Mehlich 1984
Potassium	K mg kg^{-1}	Ward	Ammonium Acetate	Warncke and Brown 1998
S-SO4-	S mg kg^{-1}	Ward	Mono Calcium Phosphate Extraction	Hoeft et al. 1973
Organic Matter	OM	Ward	Loss on Ignition	Ben-Dor and Banin 1989
Cation Exchange Capacity	CEC	Ward	Sum of Base Cations	Rhoades 1982
Soil Texture	Clay; Silt; Sand	Ward	Hydrometer Method	Gee and Bauder 1979
Soil Organic Carbon	SOC	USDA-ARS	LECO Combustion	Nelson and Sommers 1996
Active Carbon	AC	USDA-ARS	Permanganate oxidizable C (POXC)	Schindelbeck et al. 2016
Total Protein	TP	USDA-ARS	ACE Protein	Hurisso et al. 2018
Soil Respiration	Resp	USDA-ARS	4-day incubation	Schindelbeck et al. 2016
Arylsulfatase Activity	Sulf	USDA-ARS	1-hour incubation	Klose et al. 2011
Acid Phosphatase Activity	Phos		1-hour incubation	Acosta-Martínez et al. 2011
β-D-Glucosidase Activity	Beta		1-hour incubation	Deng and Popova 2011
Plant Tissue Properties				
Potassium Content	K	Ward		(Association of Analytical Chemists (AOAC), 2019)
Phosphorus Content	P	Ward		(Association of Analytical Chemists (AOAC), 2019)
Sulfur Content	S	Ward		(Association of Analytical Chemists (AOAC), 2019)

USDA-ARS = Soil and Water Quality Lab (Columbia MO); Ward = Ward Laboratories (Kearney, NE)

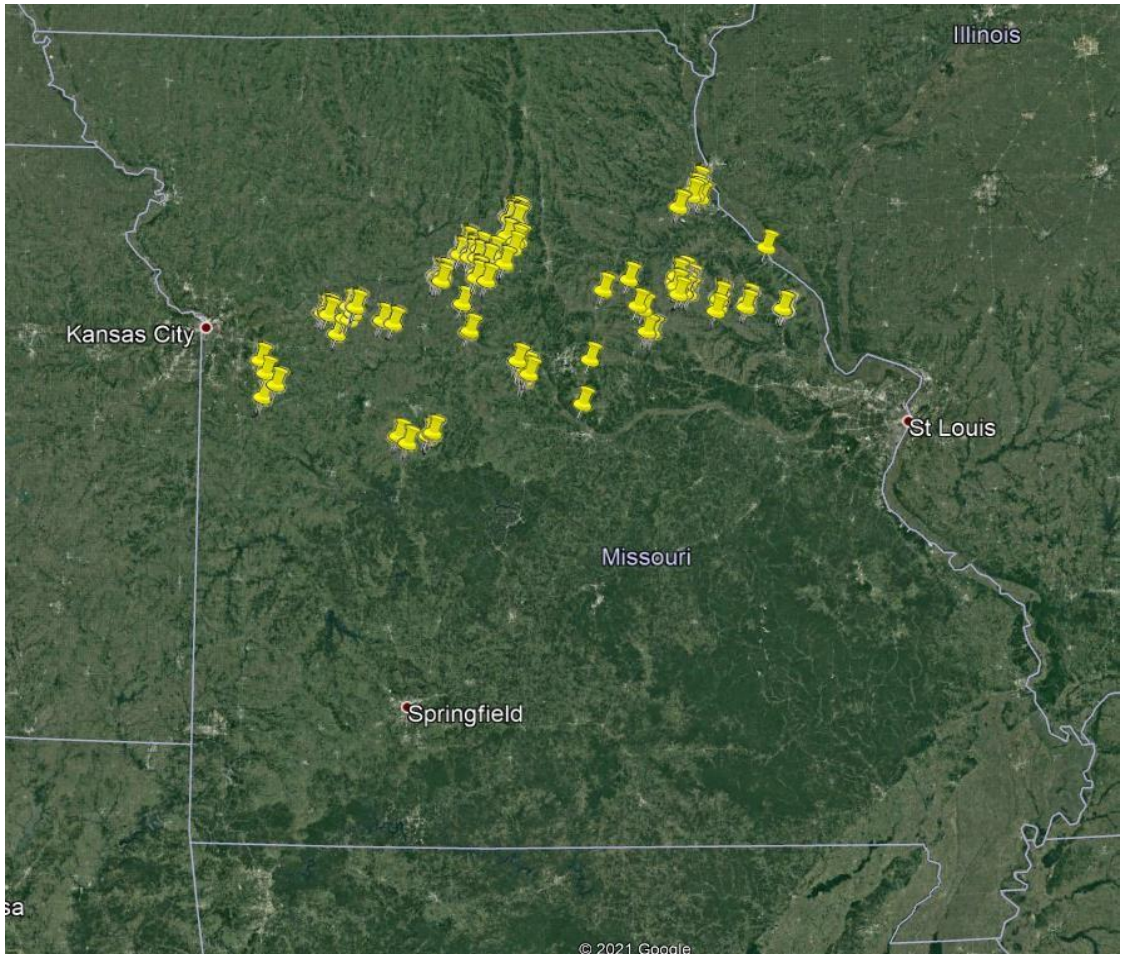

Figure 2.1. Map of locations of sites spread throughout Missouri. Each pin represents a different field used in the study, with 97 total fields used.

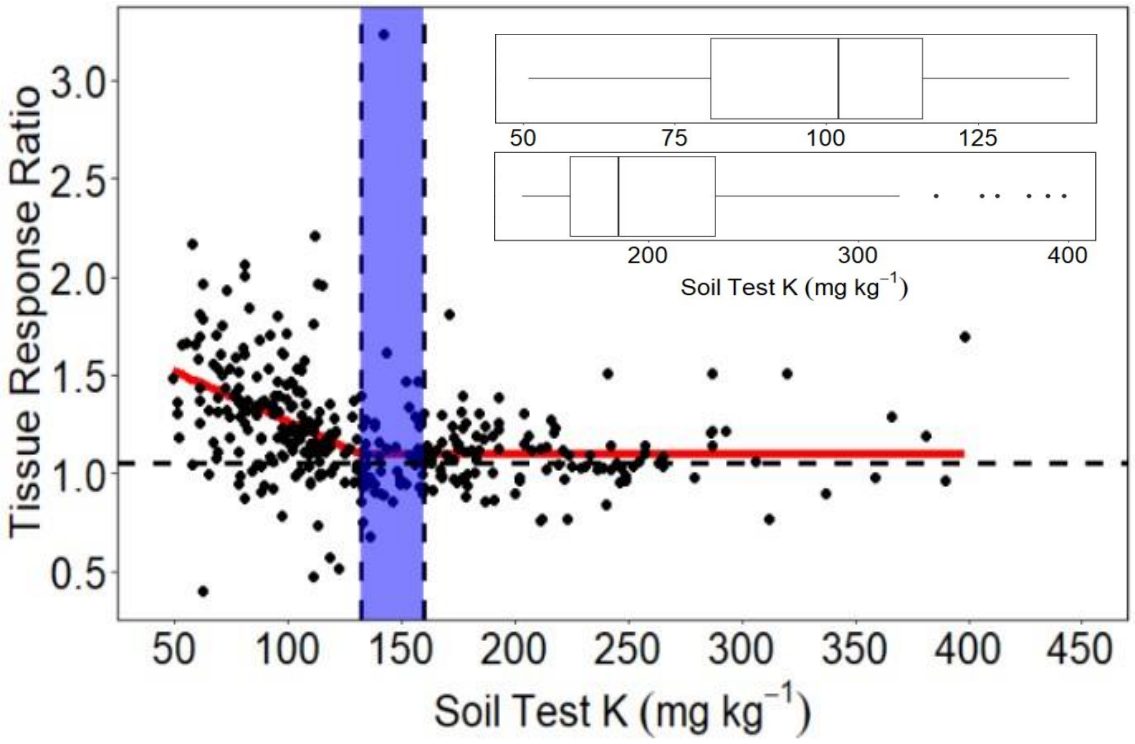

Figure 2.2. Early-season corn tissue response calculated as a ratio of potassium (K) fertilized to unfertilized relative to soil-test K. The horizontal dashed line at a ratio of 1.05 represents a threshold above which a positive response to fertilizer considered to have occurred. The vertical blue band (132 to 160 mg K kg^{-1}) represents the range of critical values for soil-test K established by the University of Missouri. The red line is a best fit linear plateau model fit to the dataset, with the joint occurring at 135 mg kg^{-1}. Boxplots in the top right show the distribution of soil-test K observations by populations below and above 135 mg kg^{-1}.

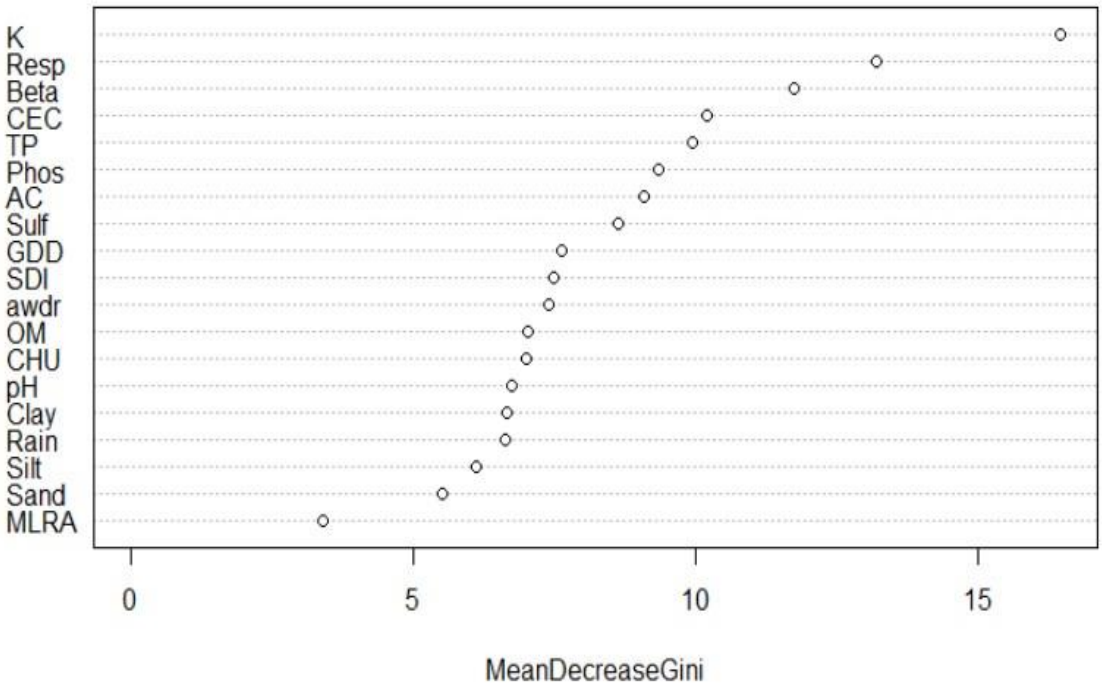

Figure 2.3. From a random forest analysis, the mean decrease in Gini importance was used to rank variable importance with added K, with the most important variables at the top of the list. In addition to soil-test K, two soil health metrics were found highly important and used further in the decision tree analysis.

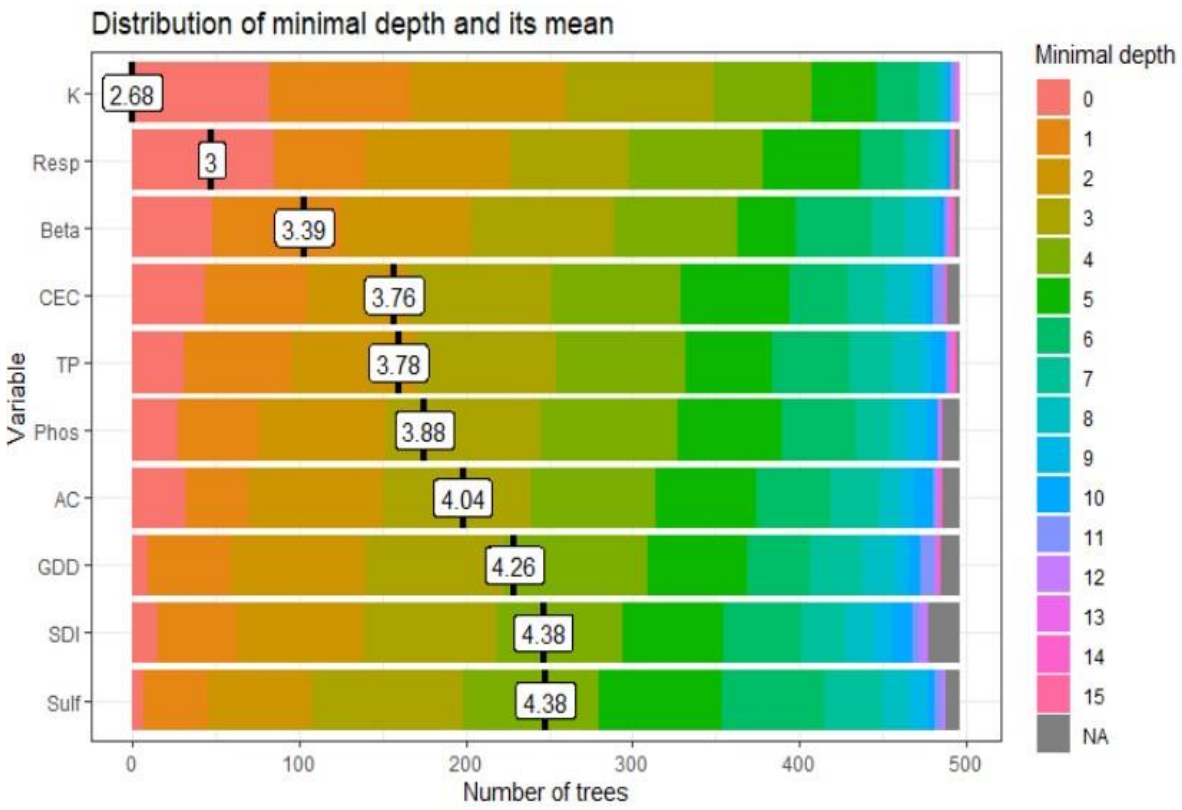

Figure 2.4. From a random forest analysis, distribution of minimal depth and its mean was used to determine variable importance with added K, with the most important variables at the top of the list. In addition to soil-test K, two soil health metrics were found highly important and used further in the decision tree analysis.

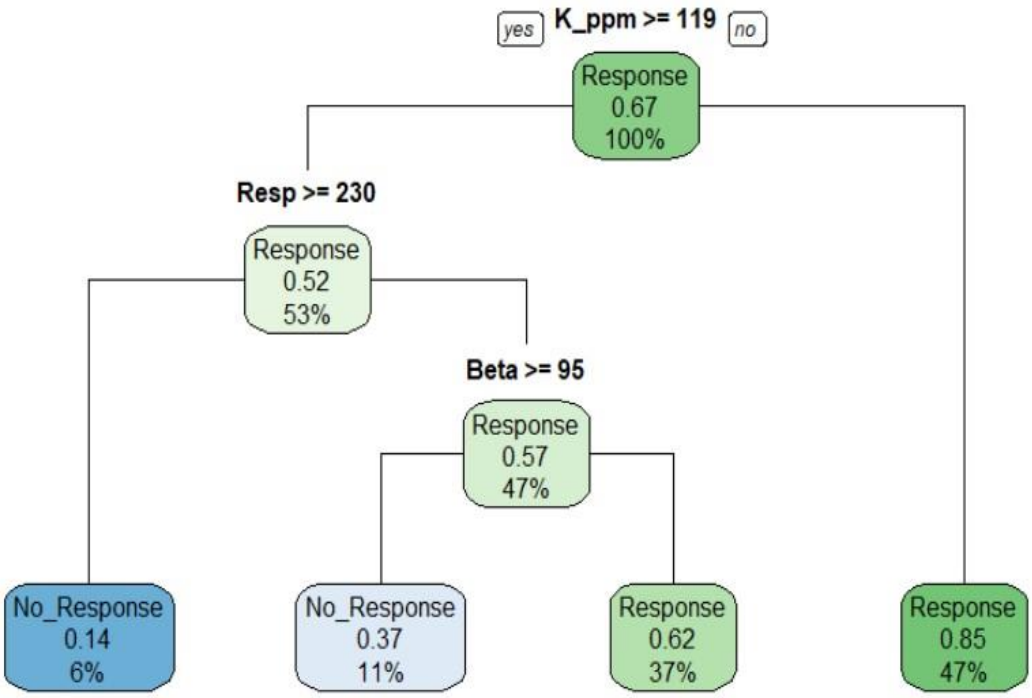

Figure 2.5. Decision tree predicting corn tissue response to added K fertilizer using the top three variables determined by random forest analysis. Potassium mg kg^{-1} was the first node with a value of 119 mg kg^{-1}. The model shows if below that value, a response to fertilizer is expected and if above that value, then it moves on to the soil respiration measurement for that site. After respiration, beta-glucosidase is used in the decision tree.

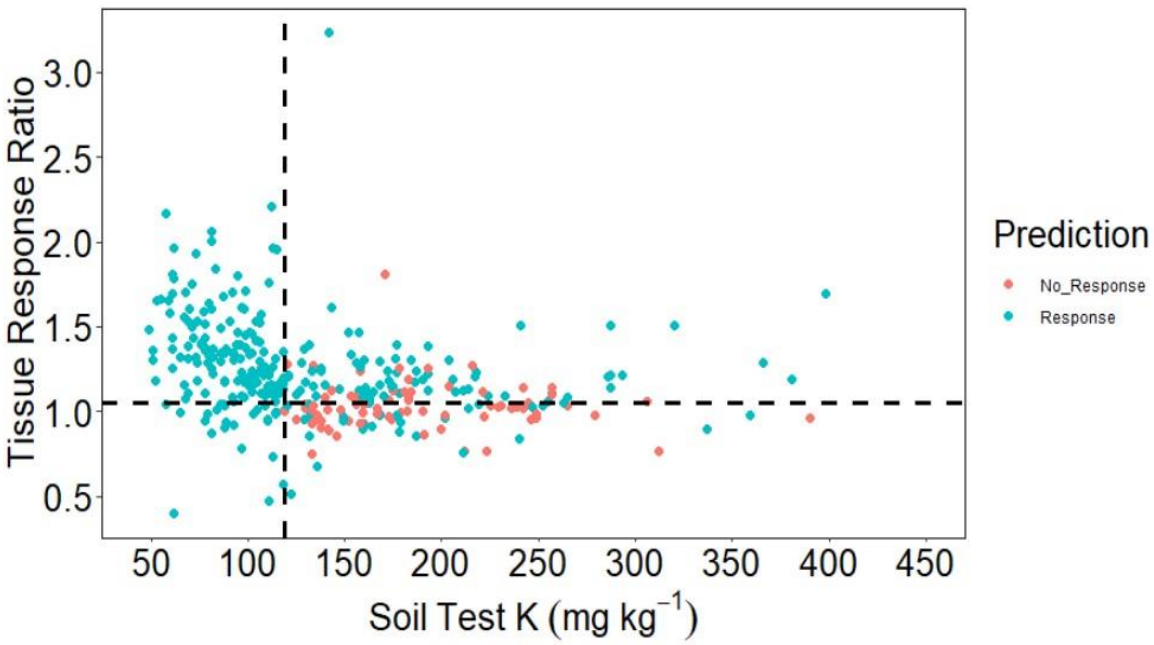

Figure 2.6. Early-season corn tissue response calculated as a ratio of potassium (K) fertilized to unfertilized relative to soil-test K. The horizontal line at a ratio of 1.05 represents a threshold above which a positive response to fertilizer occurred. The vertical line at 119 mg kg^{-1} represents the critical value for soil-test K established in the decision tree. The color of the dots corresponds to the decision tree prediction of a response or no response.

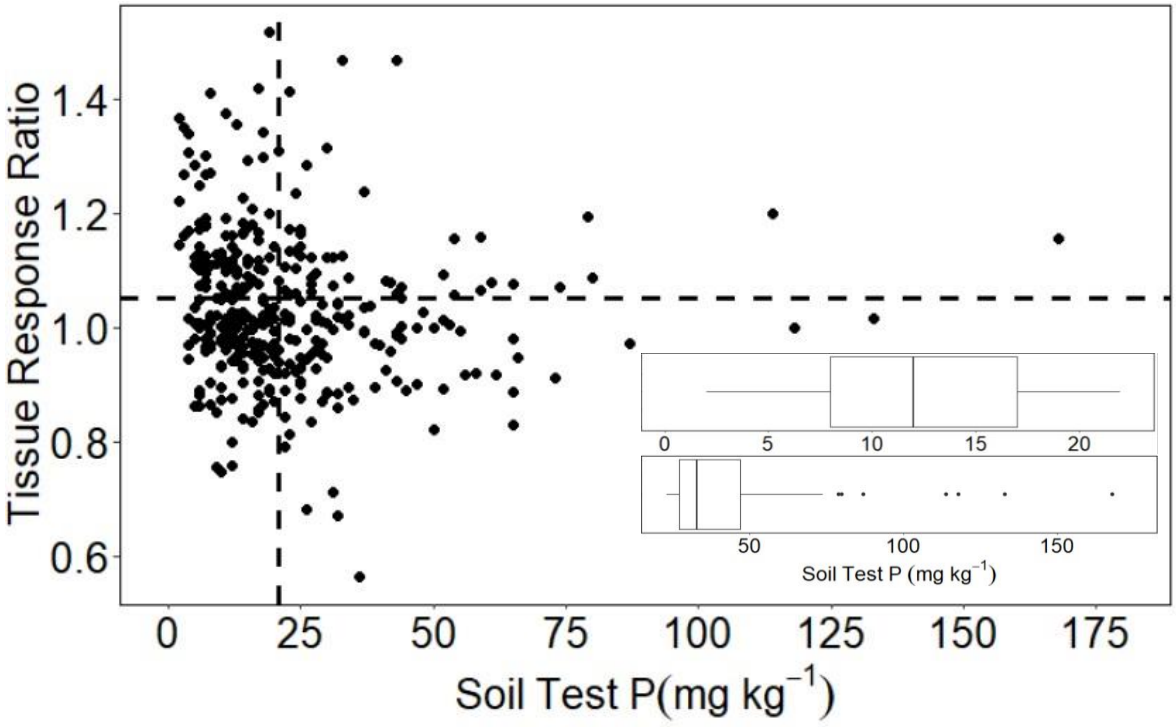

Figure 2.7. Early-season corn tissue response calculated as a ratio of phosphorus (P) fertilized to unfertilized relative to soil-test P. The horizontal line at a ratio of 1.05 represents a threshold above which a positive response to fertilizer occurred. The vertical line at 22 mg kg^{-1} represents the critical value for soil-test P established by the University of Missouri. Boxplots in the bottom right show the distribution of soil-test P observations by populations below and above 22.5 mg kg^{-1}.

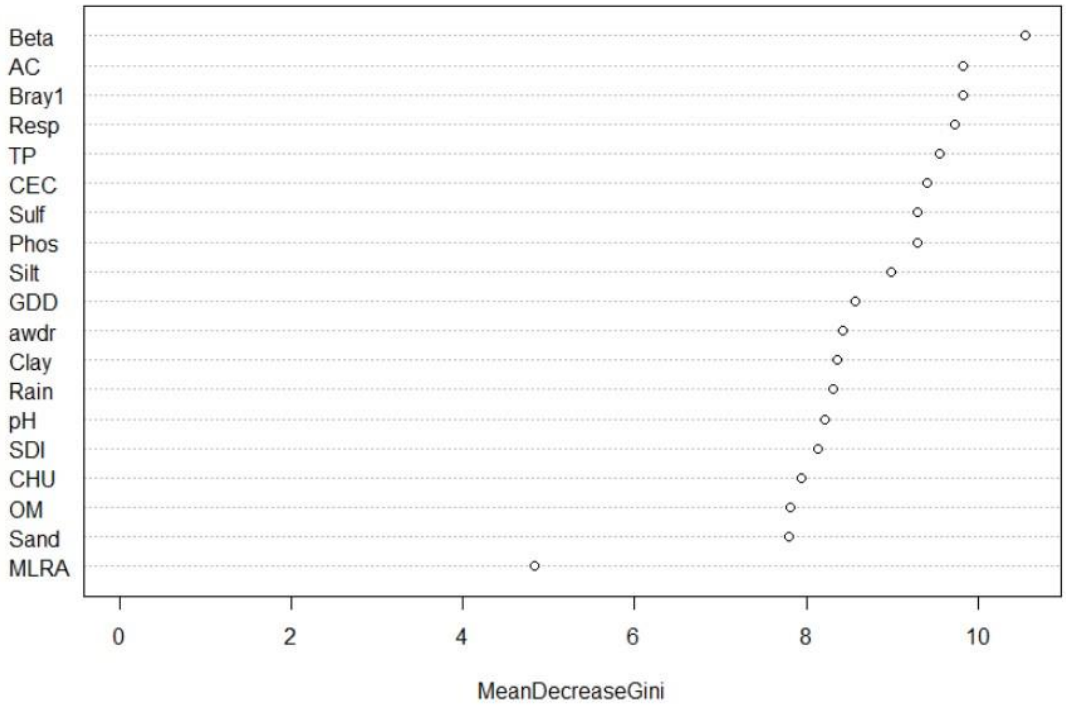

Figure 2.8. From a random forest analysis, mean decrease Gini was used to determine variable importance with added P fertilizer, with the most important variables at the top of the list. Beta-glucosidase was most important with several variables closely ranked, indicating no variables emerged as most important in predicting P tissue response.

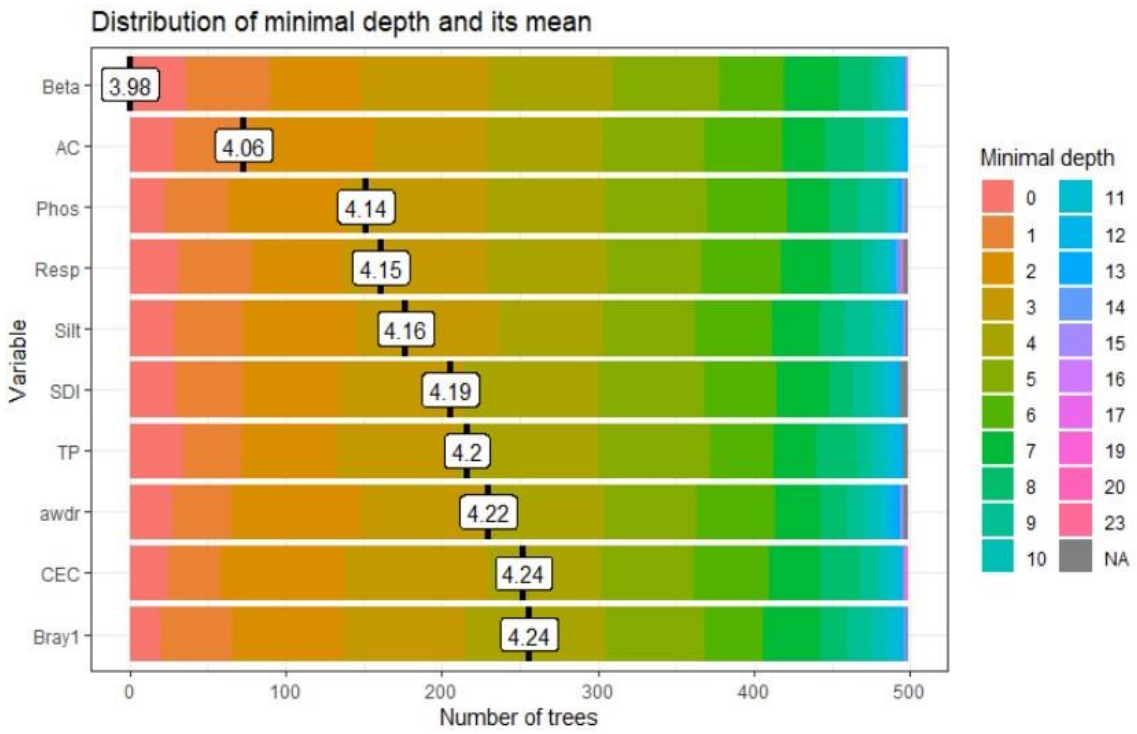

Figure 2.9. From a random forest analysis, distribution of minimal depth and its mean was used to determine variable importance for predicting tissue response to added P, with the most important variables at the top of the list. All of the variables are extremely close in their value of minimal depth. No variables stood out as being more influential than the others in the random forest analysis.

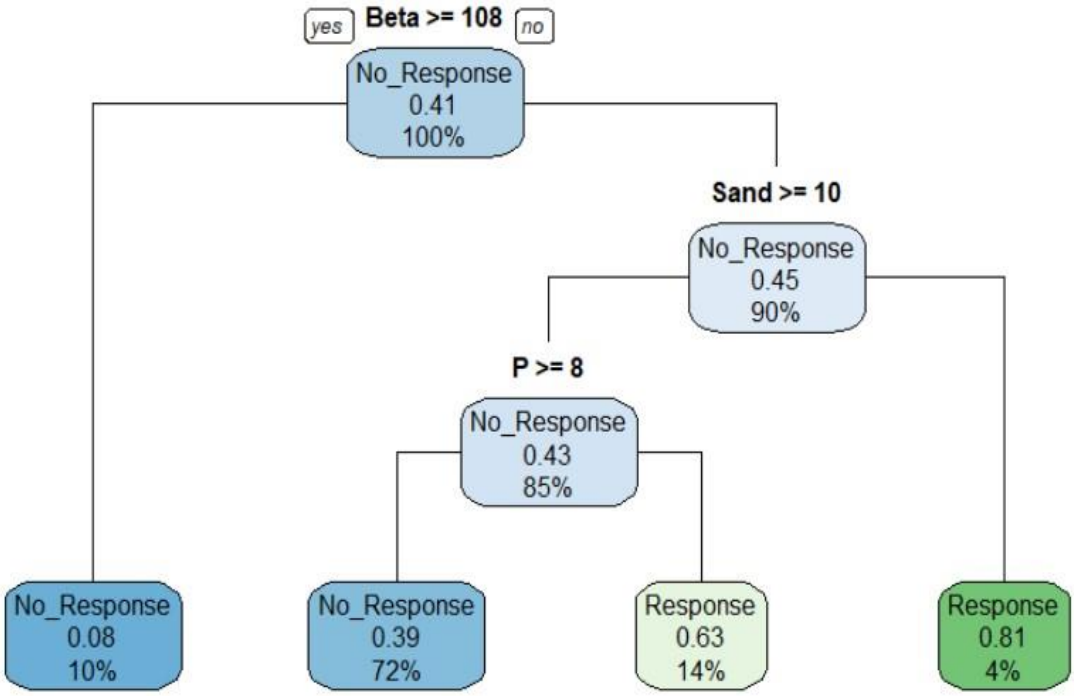

Figure 2.10. Decision tree predicting corn tissue response to added P fertilizer using the variables from the random forest model. Beta-glucosidase emerged first, with a value of 108 mg g^{-1} soil. If lower, then sand percentage was used with a value of 9.9%. The last value used in the tree was soil test P, with a value of 8 mg kg^{-1}, which is low compared to the established critical value for Missouri.

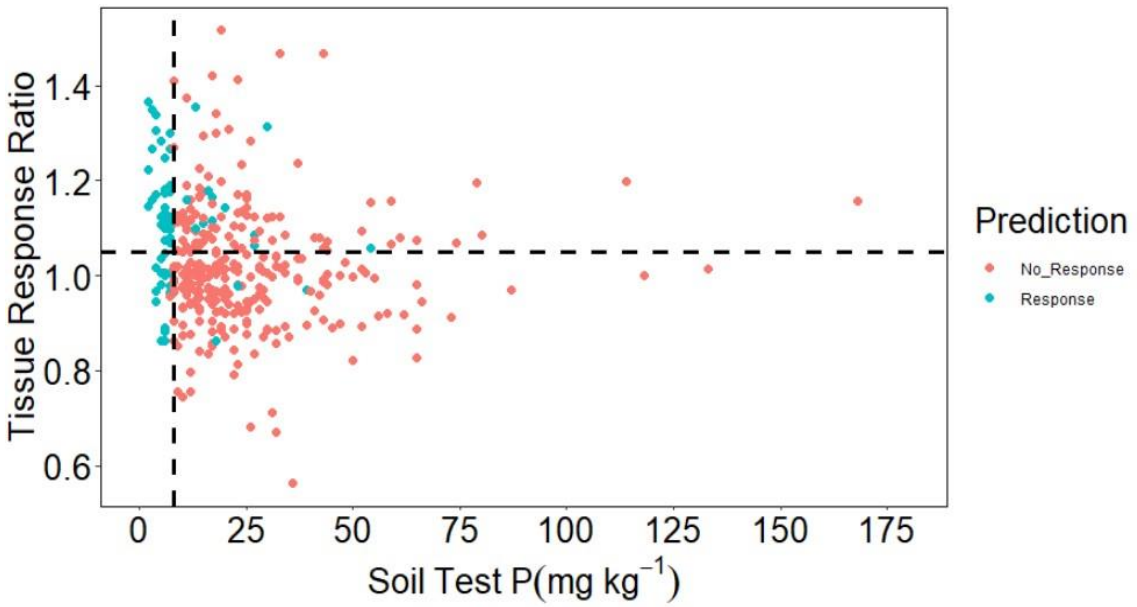

Figure 2.11. Early-season corn tissue response calculated as a ratio of phosphorus (P) fertilized to unfertilized relative to soil-test P. The horizontal line at a ratio of 1.05 represents a threshold above which a positive response to fertilizer occurred. The vertical line at 22.5 mg kg^{-1} represents. The color of the dots correspond to the decision tree prediction of a response (blue) or non-response (red).

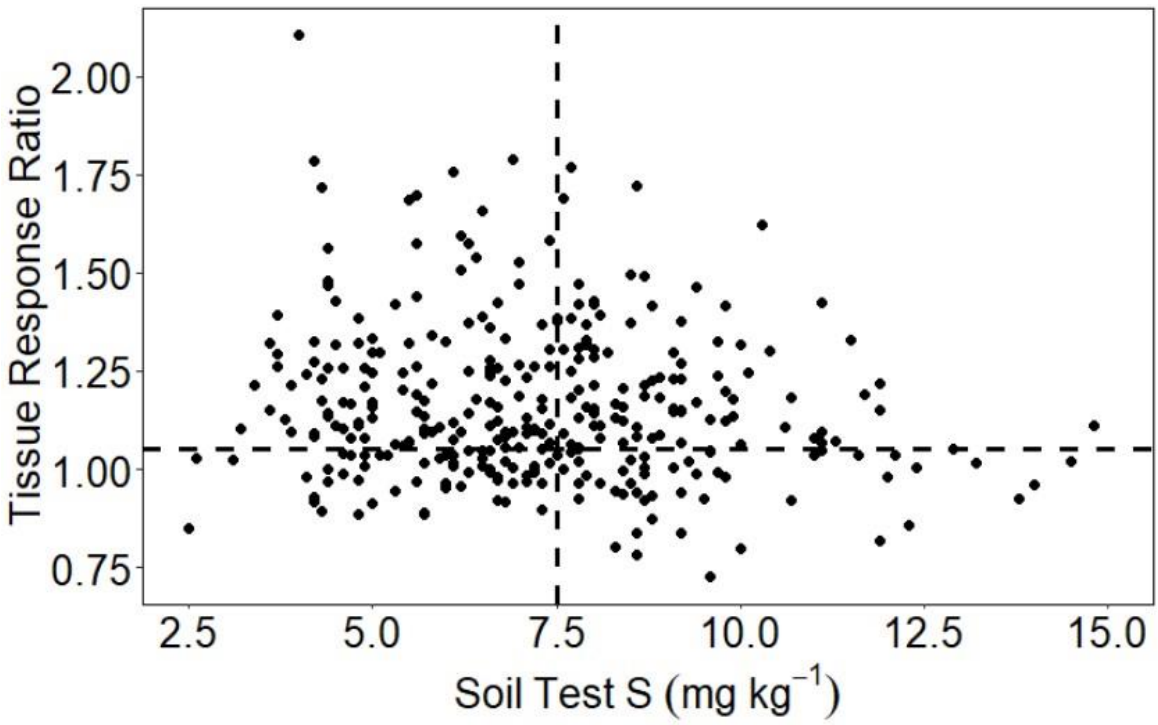

Figure 2.12. Early-season corn tissue response calculated as a ratio of sulfur (S) fertilized to unfertilized relative to soil-test S. The horizontal line at a ratio of 1.05 represents a threshold above which a positive response to fertilizer occurred. The vertical line at 7.5 mg kg^{-1} represents the critical value for soil-test S established by the University of Missouri.

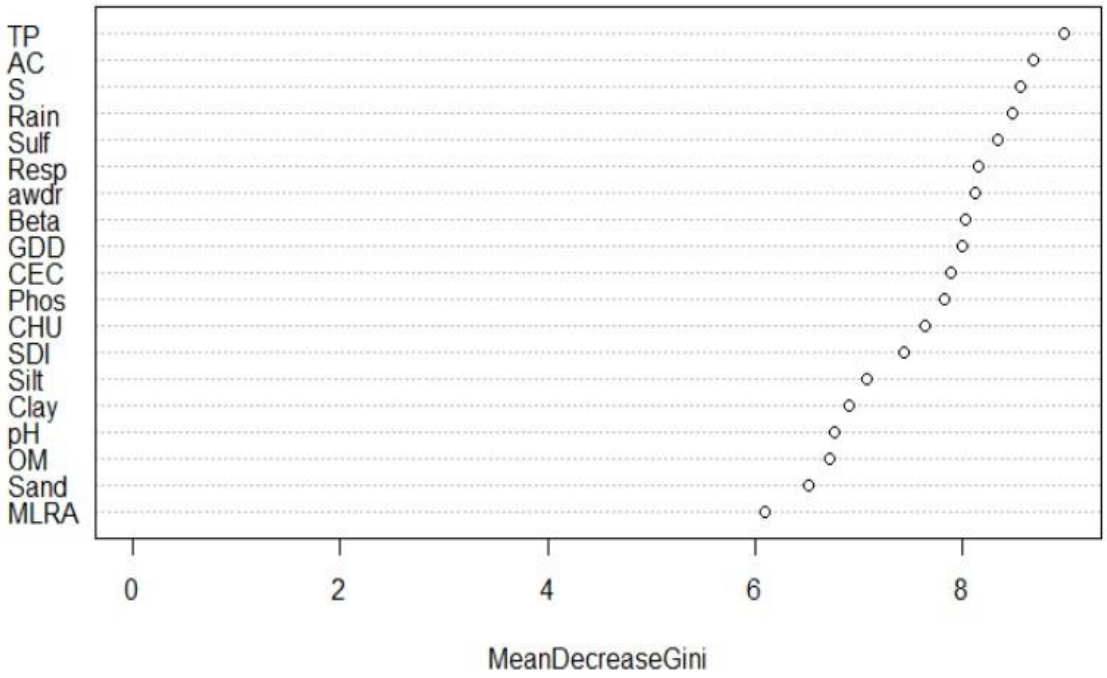

Figure 2.13. From a random forest analysis, mean decrease Gini was used to determine variable importance with added S fertilizer, with the most important variables at the top of the list. Soil-test S is near the top, along with total protein and active carbon, but there is not much separation between any of the variables at all.

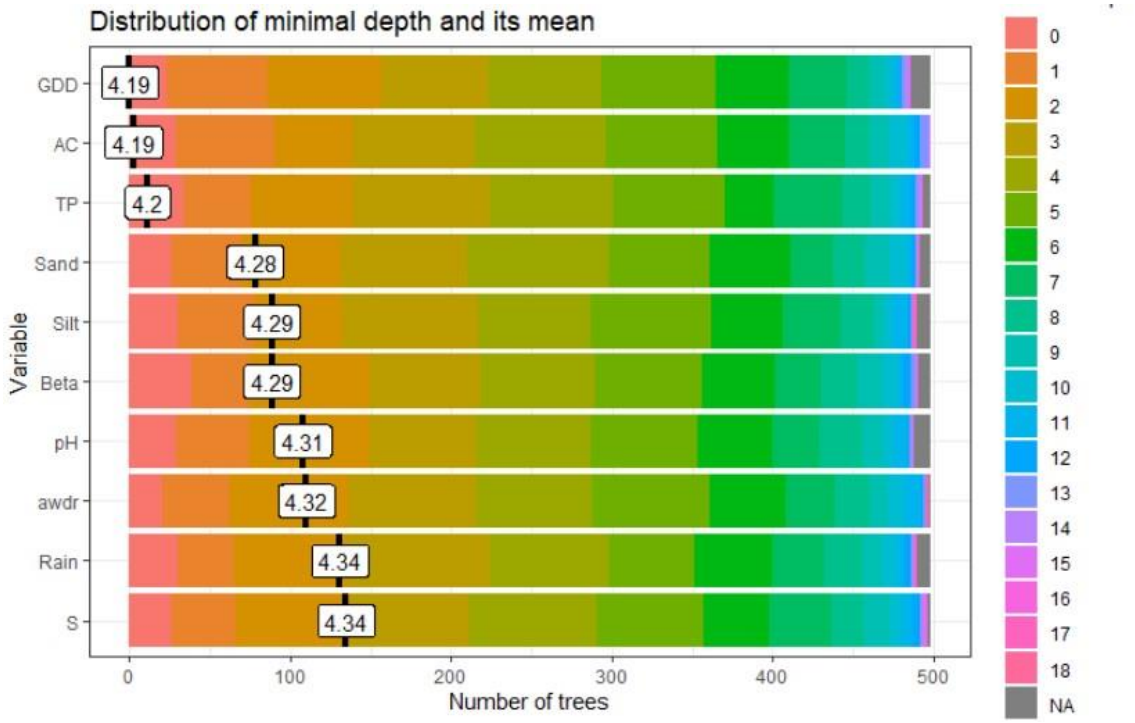

Figure 2.14. From a random forest analysis, distribution of minimal depth and its mean was used to determine variable importance for predicting tissue response to added S, with the most important variables at the top of the list. All of the variables are extremely close in their value of minimal depth. No variables stood out as being more influential than the others in the random forest analysis.

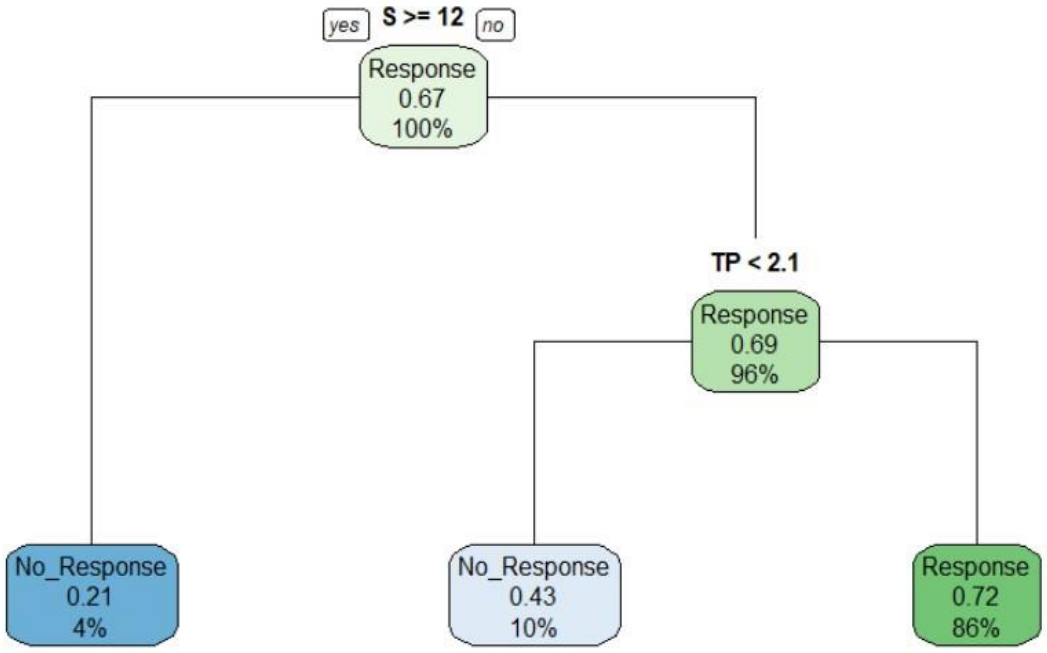

Figure 2.15. Decision tree predicting corn tissue response to added S fertilizer using all the variables in the random forest analysis. Soil-test S appeared first in the decision tree, with a value of 12 mg kg^{-1} being the cutoff. If higher than 12 mg kg^{-1}, then there no response is expected. If lower, a total protein threshold of 2.1 mg g^{-1} is used.

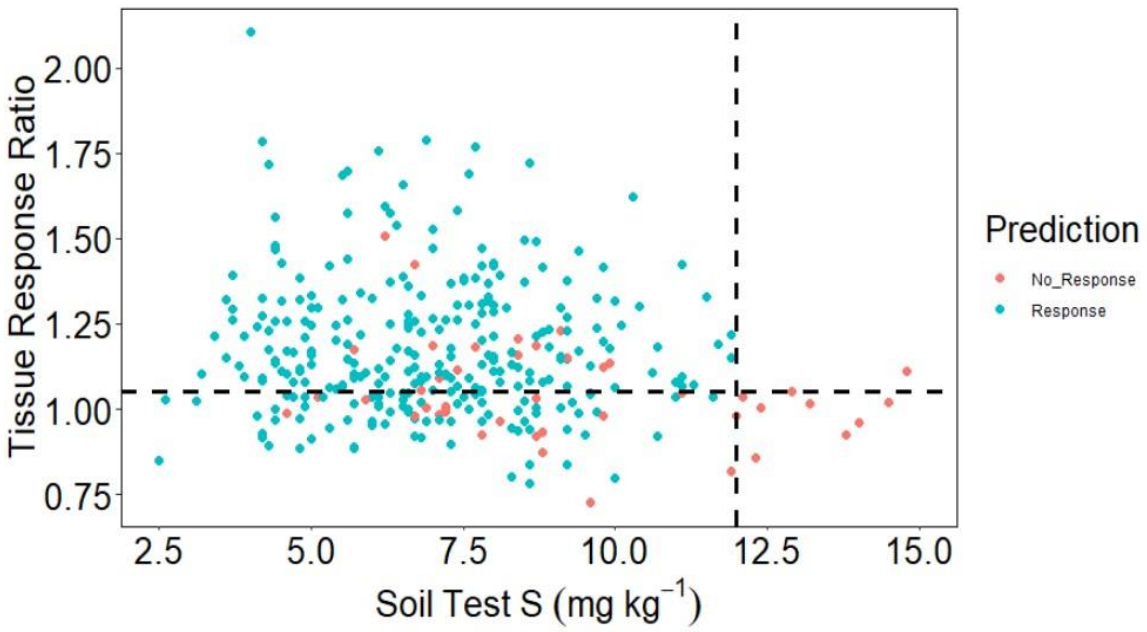

Figure 2.16. Early-season corn tissue response calculated as a ratio of sulfur (S) fertilized to unfertilized relative to soil-test S. The horizontal line at a ratio of 1.05 represents a threshold above which a positive response to fertilizer occurred. The vertical dashed line at 12 mg kg^{-1} is the value used in the decision tree using soil-test S. The color of the dots correspond to the decision tree prediction of a response (blue) or no response (red).

3. CORN GRAIN YIELD RESPONSE RELATIVE TO EARLY-SEASON TISSUE NUTRIENT CONTENT AND SOIL FERTILITY

ABSTRACT

Attention to nutrient management decisions is critical for high-yielding corn (*Zea mays L.*) production. Soil sampling for fertility testing is the generally accepted standard for making these decisions, but alone may poorly represent plant nutrient availability. Tissue sampling for nutrient content has also been used to diagnose plant needs and direct fertilizer decisions. The objective of this research was to evaluate the relationship of early-season corn tissue nutrient concentration and soil fertility tests for predicting corn grain yield responses. Research conducted in 2019 and 2020 on 91 producer fields in Missouri encompassed many soil types, management practices, and landscape positions, resulting in 433 different experimental plots, hereafter referred to as 'stamps.' Soil samples for soil fertility tests were collected in the spring before applying fertilizer. Whole plant tissue samples were collected at V6-V8 and were analyzed for tissue nutrient content. Harvest data was collected after the corn reached black layer in order to calculate the final yield. Results of multiple linear regression on each nutrient resulted in different predictor variables being significant. Potassium yield responses were best predicted using soil-test K, tissue K concentration, and the quadratic tissue K concentration, with all three variables being significant ($p < 0.1$). Phosphorus soil-test or tissue concentration alone or in any combination did not explain yield response. Sulfur yield responses were best explained with just tissue S concentration alone as the only significant predictor variable. This research suggests that current tools for diagnosing K, P, and S corn crop needs are not strong.

INTRODUCTION

Fertilizer recommendations have been around for decades now. The idea being that adding fertilizer at various soil fertility levels will increase a plant's yield, but only up to a specific nutrient level. Research began on these recommendations in the 1920's, with most work occurring in the 1950's and 60's, with not much work happening after the 1980's (Voss, 1998). A "critical value" approach arose from this research, which basically says that a plant needs a specific value of each nutrient to obtain optimum growth, where below that soil test level adding fertilizer will lead to a yield response and above that level will show no response to fertilizer (McGrath et al., 2014). Therefore, each essential nutrient must be at a plant specific sufficient level to maximize yield.

Recent research has suggested a need for an improvement in the fertilizer recommendations for soil-test K and soil-test P. In a study by Heckman et al. (2006), anywhere between 17 and 43% of the sites that tested below the critical soil P test level showed a yield increase to P fertilizer, and 25 to 50% of the sites exhibited an increase in early season crop growth. Another study showed yield responses to fertilizer at only 9 of 42 site years, along with current recommendations to build soil-test K and soil-test P being inaccurate in Ohio (Fulford and Culman, 2018). It is possible that statewide fertilizer recommendations may not be the best approach, as many different variables go into how plants uptake nutrients, such as certain soil properties and environmental conditions (Fulford and Culman, 2018). Research is needed to see how improvements can be made to these long-established fertilizer recommendations, and tissue samples may be a way to help improve them.

Nutrient uptake is influenced by many different factors throughout a plant's life cycle. Genetics of the plant, amount of soil-available nutrients, soil composition, and weather are all examples of such factors. Management factors can also influence nutrient uptake through different forms. For example, compaction can be caused when intensive tillage is used, decreasing overall root growth and therefore nutrient uptake (Miransari et al., 2009). Using the right rate, right timing, right amount, and right source of fertilizer is essential to maximizing nutrient uptake in plants (Johnston and Bruulsema, 2014).

For corn, nutrient uptake as measured through tissue sampling is one way of evaluating crop nutrient health and potential yield production. It is described as a diagnostic tool used to evaluate the performance of soil and crop management practices (Mallarino, 1996). Similarly, crop nutrient uptake could be used to evaluate the influence of soil health on crops. However, tissue sampling for many grain crops is not widely used for nutrient management because of uncertainty in the test results, high costs, and limited opportunity and ability to remediate deficiencies within the growing season. Tissue test nutrient concentrations vary due to plant growth stage, plant part sampled, growing conditions, and the hybrid/variety planted (Walker and Peck, 1972; Jones et al., 1990; Mallarino, 1996). To combat these obstacles, sampling the corn early in the season has been shown to have better results across a variety of conditions (Walker and Peck, 1972; Mallarino, 1996; Stammer and Mallarino, 2018). Therefore, tissue testing corn at V6-V8 developmental growth stage should provide better results than testing it at R1 growth stage, along with keeping the timing between samples short relative to each other (Mallarino, 1996; Stammer and Mallarino, 2018).

The goal of tissue testing is to assess the nutrient status of the crop. However,

Clover and Mallarino (2013) showed that K concentrations of corn at the V5-V6 growth stage have a poor capacity to assess K sufficiency and predict grain yield responses. Also, responses to P fertilization may be apparent in early season tissue samples, but affect yield. Often, plants undergo luxury consumption, an increase in early season tissue P and K concentration, but it does not lead to a yield response. Therefore, using tissue tests as the only source of information for P and K fertilization is not adequate for maximum utility of the test.

There have been numerous studies that show "critical" concentrations for P and K in tissue tests in corn. Mallarino (2018) tested the value of tissue testing in predicting grain yield. It was shown that the stage at which the corn was sampled mattered when looking to predict yield, as corn sampled at the V5-V6 stage showed no yield increase when the P concentration was ≥ 0.48% and corn sampled at the R1 stage resulted in no yield increase at P concentration ≥ 0.25% (Fig. 2). There was also a difference between K critical levels when sampling at the different growth stages. At the V5-V6 growth stage, no yield increase was seen at K concentration ≥ 2.5% and corn sampled at the R1 growth stage showed no yield increase at K concentration ≥ 1.4%.

One way plant nutrient health through plant tissue nutrient testing might be used is an indicator of the overall health of the soil. Although it is assumed that improved soil health contributes to improved nutrient plant health, little research has been conducted to demonstrate this connection. Often plant nutrient concentration or content can indicate plant nutrient health before visual deficiencies are expressed (Mallarino, 1996). The concentration or content of plant nutrients at different developmental stages have been

used as indices of sufficiency (Macy, 1936; Mallarino, 1996; Stammer and Mallarino, 2018).

Soil fertility levels and tissue concentrations have most always been thought of as separate independent plant nutrient diagnostic tools, but they may be able to be complimentary of one another in predicting yield response to fertilization. The objective of this research was to evaluate the relationship of both early-season corn tissue nutrient concentration and soil fertility tests for predicting corn grain yield responses.

MATERIALS AND METHODS

Research Sites and Locations

This research was conducted as a public collaboration between the University of Missouri and Corteva Agrisciences. This project took place in Missouri. Data was collected during the 2019 and 2020 growing seasons. Each year, 50-60 different fields were selected. Each field contained 3-5 spatially distributed location sites (hereafter referred to as "stamps") that were selected to capture a range of field conditions and to examine inherent in-field variability. The fields represented a wide range of soil and crop management systems in order to capture a range of corn tissue and yield responses across different soil health levels. Soil and weather information can be found in Table 3.1. In total, there were 446 stamps in this study spread throughout 97 total fields (Fig. 3.1). Fields were selected in the late fall/early spring by communicating with farmers and local networks, including industry representatives from MFA and Corteva Agrisciences. Operations were conducted such that farmers avoided applying S, P, and K to the stamp sites in order to provide a locally representative environment with the best opportunity for

response to treatments. Fields with recent grid soil sampling results allowed for selecting stamp sites that had both low and high soil fertility conditions. Stamps were placed based on differing landscape positions, soil series, and fertility levels, along with field access in mind. The goal of this experimental design was to encompass a wide range of field conditions and therefore soil health levels.

Soil Sampling, Fertilization Treatments, and Laboratory Analyses

Soil sampling and fertilization treatments were done for each stamp in the early spring of each growing season, approximately two to five weeks prior to planting. Each stamp site was a square with 12.2 m on each side, providing an area 148.8 m^2.
Coordinates of each stamp center were measured using Trimble GeoXT 6000 and Geo 7x GPS devices with approximately 6-cm accuracy. With the center identified, pre-cut ropes were stretched to define the stamp area. Using a hand-held compass, the stamps were oriented on a north-south bearing regardless of where the stamp was located in the field (Fig. 3.2).

For soil fertility and health assessments, eight to twelve 2.54-cm (id) cores, to a depth of 15 cm, were obtained evenly distributed within the stamp area. The cores were split into two depths (0-5 cm depth and 5-15 cm depth) and composited into buckets at the time of sampling. After gentle hand mixing, the samples were combined for subsequent laboratory analysis into standard soil sampling boxes for traditional fertility analyses.

For soil profile characterization and sub-surface fertility assessment, a Giddings hydraulic soil sampling machine was used to obtain one 4.5-cm (id) profile core to an approximate depth of 1 m. These were laid out on a processing table and visually

characterized by pedogenetic horizon. The surface 0-15 cm was designated as the Ap horizon. After designating the first horizon, the rest of the core was separated into pedogenic horizons (typically three or four) using soil color and texture cues. Soil samples were bagged and removed from the stamp area before fertilization treatments were applied.

For fertilization treatments, the stamp area was sub-divided into four equal 6.1 by 6.1 m quadrants (37.2 m^2). One quadrant was designated as a control and received no fertilizer treatment. Fertilizer K, P, and S treatments were applied by hand to quadrants as shown in Figure 3.2. Fertilizer treatment rates were as follows: 1) control; 2) 112 kg ha^{-1} of K_2O applied as potash (0-0-60); 3) 112 kg ha^{-1} of P_2O_5 applied as triple super phosphate (0-45-0); and 4) 28 kg ha^{-1} of S applied as ammonium sulfate (21-0-24). So that all received equal N, an additional 25 kg N ha^{-1} as SUPER-U (46-0-0) was applied to quadrats 1-3 to balance the N applied with ammonium sulfate to treatment 4. Additionally, since both years of this study experienced above-average spring precipitation, an additional 67 kg N ha^{-1} was applied as SUPER-U to the entire stamp area immediately following tissue sampling, to guard against N deficiencies later in the growing season.

Soil samples were delivered to Ward Labs in Kearney, Nebraska for traditional fertility analyses. Fertility analyses are summarized in Table 3.1.

Plant Sampling for Tissue Nutrient Analyses

Eight whole plants from each of the four quadrats of each stamp were collected at the developmental growth stage of V6-V8 for nutrient content assessment. Prior to sampling,

the central part of a treatment quadrant with a uniform stand was found and marked with plastic garden stakes for grain yield harvest at the end of the season. Outside of this designated yield area, random plants were identified for tissue nutrient content samples and collected. This sampling approach avoided a "border effect" on plants identified for grain yield. Harvested plants were placed into labeled brown paper bags for storage and drying. Samples were air/oven dried and ground to pass a 2-mm screen, then shipped to Ward Laboratories (Kearney, NE) for analysis of P, K, and S concentration.

Grain Harvest

Hand grain harvesting occurred after the corn has reached black layer, typically weeks before farmers began harvesting. Each treatment was examined closely before harvesting in order to avoid variance between the control and the other treatments due to any number of circumstances (e.g. animal damage, sprayer track damage, etc.). The four 12 foot rows were handpicked into bags and weighed with a Rapala ProGuide Digital Scale (Rapala, Minnetonka, MN). After the weight was recorded, an eight-ear sample was taken from each treatment and weighed with a digital kitchen scale (thousandths precision). These ears were then dried down and weighed again to determine grain moisture content, and then adjusted to market moisture content of 15.5% and cob weight subtracted using a grain to cob ratio of 0.89 to calculate a final yield.

Data Management and Statistical Analyses

Data were analyzed using R programming language (R Core Team, 2017). Due to the lack of traditional replication with this experimental design, an alternative measure of field experimental error was developed in a companion study. Specifically, in this study

of yield response across the same sites, a suite of methods were evaluated to estimate experimental error, concluding that approximately 10% random error could be expected for the yield response (J. Svedin, personal communication, July 2021). Due to response ratios requiring an error measurement to classify a response, yield differences were used as the response variable. These were calculated by subtracting the yield of the control from the yield of the specific treatment being discussed.

This objective was met using multiple linear regression to test yield difference as a function of two diagnostic tools, soil test fertility and plant tissue nutrient concentration. Linear, quadratic, and all possible combinations of linear and quadratic interactions of the two diagnostic tools were considered in the regression model ($p < 0.1$). There were 302, 299, and 286 data points used in the linear regression for K, P, and S respectively. Differences in observations by nutrient were due to isolated issues that varied from stamp to stamp (e.g., deer damage, tractor damage, localized depression areas with ponding and subsequent erratic stand).

RESULTS AND DISCUSSION

This study included a wide range of growing conditions and soils, leading to diverse yields as well as tissue concentrations. Overall, yields ranged from 6.4 to 19.8 Mg ha^{-1}, with an average yield of 15.1 Mg ha^{-1}. Conditions were close to optimal for Missouri growing conditions over the two years of the study, which is why yields were high in comparison to the past five years, which averaged 10.05 Mg ha^{-1} across the entire state (Garino and Mends, 2019). Yield response, calculated as a difference between the control and each treatment yield, was examined using multiple linear regression relative to K, P,

and S soil-test fertility as well as early-season tissue nutrient concentrations. Since each nutrient's response was unique, findings are presented by the nutrient.

If both diagnostic tools accurately reflect nutrient availability, one would expect that they would be positively correlated. Figure 3.3 provides the correlation of these two diagnostic tools by the three nutrients of this investigation (control treatment only). While all three nutrients show positive correlation, only the K tests are somewhat strongly correlated. Both the P and S show only slight positive correlation, which can be due to different factors that will be discussed further in the results.

Corn Yield Relative to Potassium Soil-Test and Tissue Concentration

Corn yield difference was between -2.34 and 4.45 Mg ha^{-1}, with an average yield difference of 0.33 Mg ha^{-1}. Corn tissue K concentration ranged from 10.2 to 73.3 g K kg^{-1} with a mean of 36.7 g K kg^{-1}. These tissue concentration values are close to what other research has shown when measuring early-season corn tissue K concentration (Clover and Mallarino, 2013; Stammer and Mallarino, 2018). Soil-test K values ranged from 49 to 398 mg kg^{-1}, so a large variation was represented within the dataset, providing a range of potential yield response to fertilization.

Multiple linear regression was ran with the idea of predicting yield difference using soil-test values along with tissue test values, to determine if tissue tests can aid in foreseeing end-of-season results. Yield differences were very scattered throughout the various soil-test K and corn tissue K concentrations (Fig. 3.4).

Using three contrasting different K tissue concentrations, the model showing the effect of tissue K concentration and soil test K combined is further illustrated in Figure

3.5. Yield response as shown by yield difference was greatest when tissue K was low (15 g K $kg^{-1)}$. Yield difference decreased as soil test K increased, a response approximately the same over all tissue K concentrations. Though the quadratic term of the plant tissue K was significant, the near parallel lines of the three tissue concentrations suggests the effect over the soil test values of this study was mostly linear in nature. These findings demonstrate that yield response to K fertilization was elucidated by including tissue and soil testing together. The blue line represents a linear model best fit line if just soil-test K values were used to predict a yield difference. For this line no yield difference occurs at about 225 K mg kg^{-1}; depending on the CEC, but this is considerably higher than what would be expected given current soil test K interpretations defining a critical value for when to fertilize.

Analysis results from soil samples taken before planting, sometimes months before, under the current soil test interpretation standards only weakly predicted fertilizer K corn response. Adding tissue K findings demonstrate the value of this diagnostic tool. However, K fertilization in-season may not be feasible, but such may still provide information for future seasons for the field in question or other like fields. Many factors go into plant K uptake. One notable factor is the portion of the root zone not sampled by soil sampling. Crop K uptake in some soils may have greater or less K supply because of sub-soil fertility, that soil not assessed with the standard 20-cm surface soil sample. Whereas the tissue sample uses the plant as “the sampler” and the sample depth is whatever depth of roots are at the time of tissue sampling. If farmers using tissue analysis know plants within a field do not uptake as much K as anticipated using soil testing methods, they may be able to adjust their interpretation and make a better fertilizer

management decision in the future. More research is needed to help understand how to make these adjustments, but this opens the question of the possibility of using tissue testing in a complimentary way to soil-tests.

Corn Yield Relative to Phosphorus Soil-Test and Tissue Concentration

Yield difference between the control and phosphorus treatment was between -2.21 and 3.61 Mg ha^{-1}, with an average yield difference of 0.22 Mg ha^{-1}. Corn tissue P concentration ranged from 2.4 to 7.9 g P kg^{-1}, but averaged 4.5 g P kg^{-1}. These tissue concentrations were similar to those observed in previous studies where the whole plant was taken at V6-V8 (Mallarino, 1996; Stammer and Mallarino, 2018). Soil-test P ranged from 2 to 87 mg kg^{-1} within the study, creating a diverse set of data with a wide range of outcomes. Yield differences relative to soil-test P and tissue P concentration show considerable scatter, similar to that found with K (Fig. 3.6).

Regression analysis on corn yield difference found no effect to soil-test P and/or plant tissue P for this dataset. There are many possible reasons for this to occur, with a few to follow. First, it may be that plant P uptake was not significant early in the growing season because microbes are not active in the soil due to soil temperatures and they are important for P cycling in the soil. The growing seasons that were observed were near ideal seasons, with timely precipitation in each, which may have kept microbial activity high throughout the rest of the season after tissue tests were taken. This would mean more P is available throughout the season to be taken up by the plants, which cannot be calculated by the soil fertility or tissue concentration tests. Another key reason is the fact that P is immobile in the soil. This means that early season, when root systems are relatively shallow in the soil,

corn plants are not able to reach all of the P that will be available throughout the growing season yet. As the season goes on and the roots reach deeper, more P is available to be absorbed into the plant. One last possible reason is that hybrids may be better now than they were in the past at taking up nutrients at lower concentrations. Also, this project had no replication to calculate an error with each tissue/yield measurement, there is only a yield difference taken into account in the model. This project is relying on a large population of differences to tell if there was an actual overall difference in yield, and therefore may drown out some of the possible sites with a positive response.

Corn Yield Relative to Sulfur Soil-Test and Tissue Concentration

Corn yield difference was between -2.47 and 4.30 Mg ha^{-1}, with an average yield difference of 0.27 Mg ha^{-1}. Corn tissue S concentration ranged from 1.25 to 4.4 g S kg^{-1}, but averaged 2.4 g S kg^{-1}. In this study, soil-test S ranged from 1.8 to 16.3 mg kg^{-1}. Figure 3.7 illustrates yield difference relative to these two metrics (n=286). Regression analysis on yield difference found soil test S alone was unhelpful, but plant tissue S did help explain yield difference (Table 3.3). In Missouri, the sulfate method of soil test S is the primary diagnostic tools used for recommendations. These results would support that this test is unreliable, and new methods for diagnosing corn S deficiency are needed.

Though subtle, as tissue S concentration increased, yield difference decreased (Fig. 3.8). While the relationship has poor predictive power (i.e., very low coefficient of determination), the relationship does help identify when S deficiency maybe occurring. Using the model findings, a plant tissue S test content of 1.5 mg S kg^{-1} would with S fertilization produce a 1.2 Mg ha^{-1} yield response. Once tissue S concentration reaches

~2.5 mg S kg^{-1}, no yield response to S fertilization would be expected. This means that taking a tissue sample early in the growing season can help farmers understand whether or not they will get a yield increase at the end of the season by adding S fertilizer. If not practical for the current growing season, it may help for future growing seasons on the same field. If a field is known for having a low uptake rate during the growing season, it may be practical to apply more fertilizer throughout the season in order to combat it and attain a higher yield.

CONCLUSION

This study looked at K, P, and S soil fertility and early season tissue concentrations to try to better understand how these two diagnostic tools might be used together for predicting yield response to fertilization. Potassium yield responses were best predicted using soil-test K, tissue K concentration, and the quadratic tissue K concentration, with all three variables being significant ($p < 0.1$). This suggests that current fertilizer recommendations using just soil-test K may be improved upon by including tissue K concentration tests. Additional research on this finding is warranted. Using these two tests were unhelpful when evaluating crop response to P fertilization, caused by one of many factors (e.g. subsoil fertility, microbial activity, etc.). Though weak, the sulfur plant tissue test did show promise for knowing when corn would respond to S fertilization.

This research, like many before, suggest current tools for diagnosing K, P, and S corn crop needs are not strong. There are many factors that influence the final yield of a plant, such as subsoil fertility, soil health, microbes present in the soil, and weather. Perhaps these factors often drown out the possibility of establishing simple relationships between

standard soil fertility, tissue nutrient concentrations, and yield that are only one or two dimensional.

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TABLES AND FIGURES – CHAPTER 3

Table 3.1. Soil fertility and plant tissue analyses with associated acronyms, methods, and citations.

Analyses	Acronym	Method	Citation
Soil Properties			
Phosphorus	Bray1	Bray 1; Mehlich-3	Bray and Kurtz 1945; Mehlich 1984
Potassium	K mg kg-1	Ammonium Acetate	Warncke and Brown 1998
S-SO4-	S mg kg-1	Mono Calcium Phosphate Extraction	Hoeft et al. 1973
Organic Matter	OM	Loss on Ignition	Ben-Dor and Banin 1989
Cation Exchange Capacity	CEC	Sum of Base Cations	Rhoades 1982
Soil Texture	Clay; Silt; Sand	Hydrometer Method	Gee and Bauder 1979
Plant Tissue Properties			
Potassium Content	K		(Association of Analytical Chemists (AOAC), 2019)
Phosphorus Content	P		(Association of Analytical Chemists (AOAC), 2019)
Sulfur Content	S		(Association of Analytical Chemists (AOAC), 2019)

USDA-ARS = Soil and Water Quality Lab (Columbia MO); Ward = Ward Laboratories (Kearney, NE)

Table 3.2. Multiple linear regression results containing the variable, coefficient, p-value, significance, and R^2 value of the entire model.

Variable	**Coefficient**	**p-Value**	**Significance**	**R^2**
Intercept	1.9593	1.96×10^{-7}	***	
Soil-test K mg kg^{-1}	-0.0028	0.01	**	
Tissue Test g K kg^{-1}	-0.0604	0.005	**	
(Tissue Test g K kg^{-1})^2	0.0006	0.04	**	
Overall Model		2.28×10^{-8}	***	0.11

Table 3.3. Multiple linear regression results containing the variable, coefficient, p-value, significance, and R^2 value of the entire model.

Parameter	**Coefficient**	**p-Value**	**Significance**	**R2**
Intercept	0.8346	0.01	**	
Tissue Test g S kg^{-1}	-0.2462	0.08	*	
Overall Model		0.0754	*	0.008

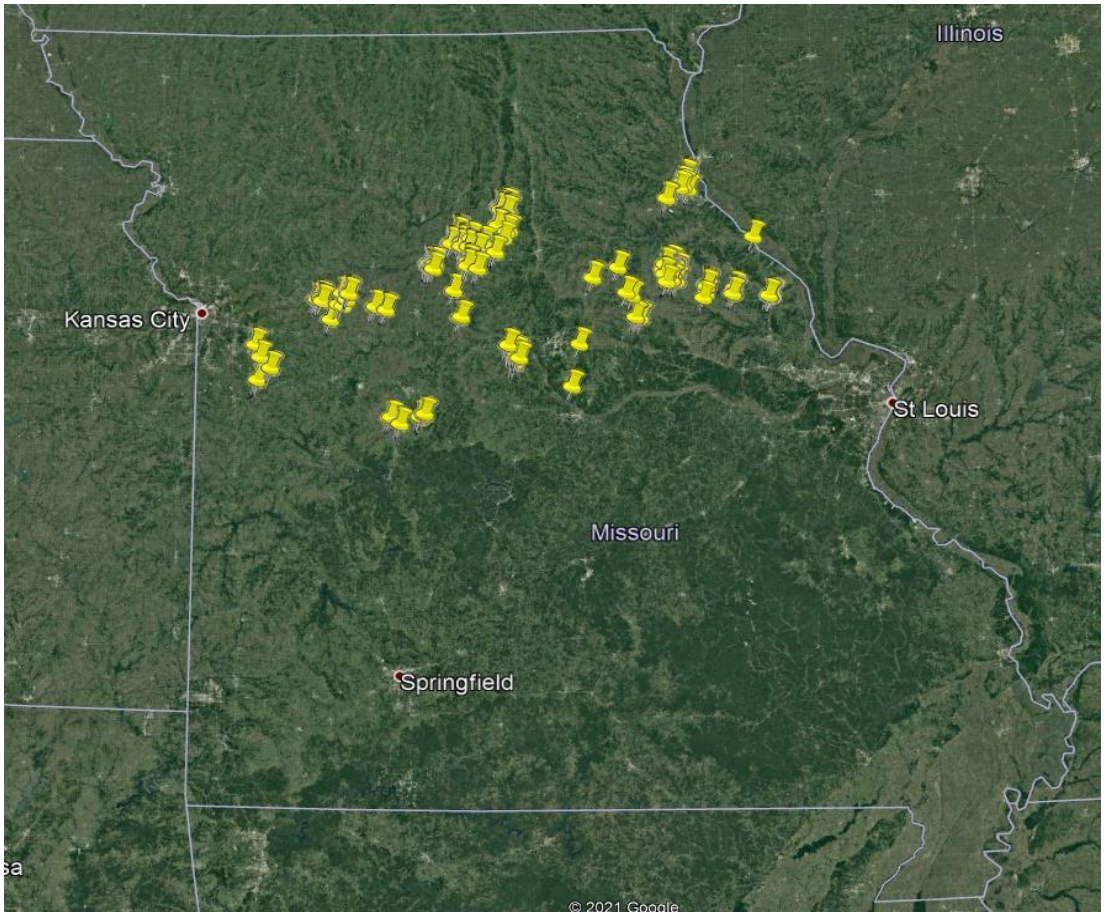

Figure 3.1. Map of locations of sites spread throughout Missouri. Each pin represents a different field used in the study, with 97 total fields used.

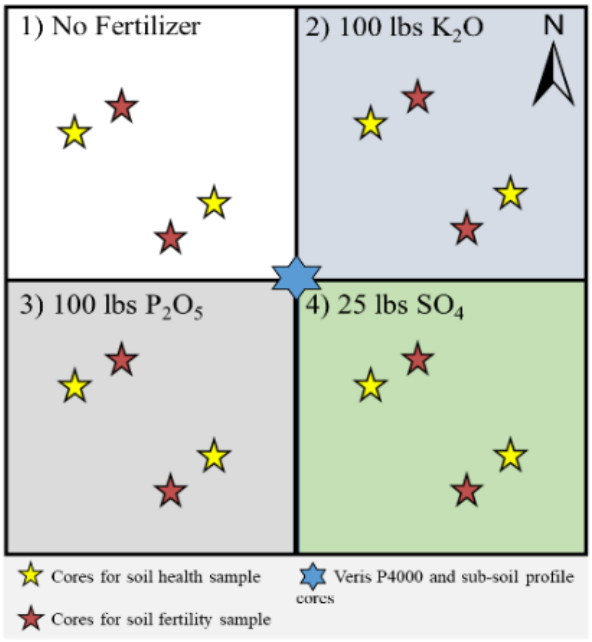

Figure 3.2. Outline of 40’ by 40’ stamp split into four 20’ by 20’ sections along with the respective fertilizer applications.

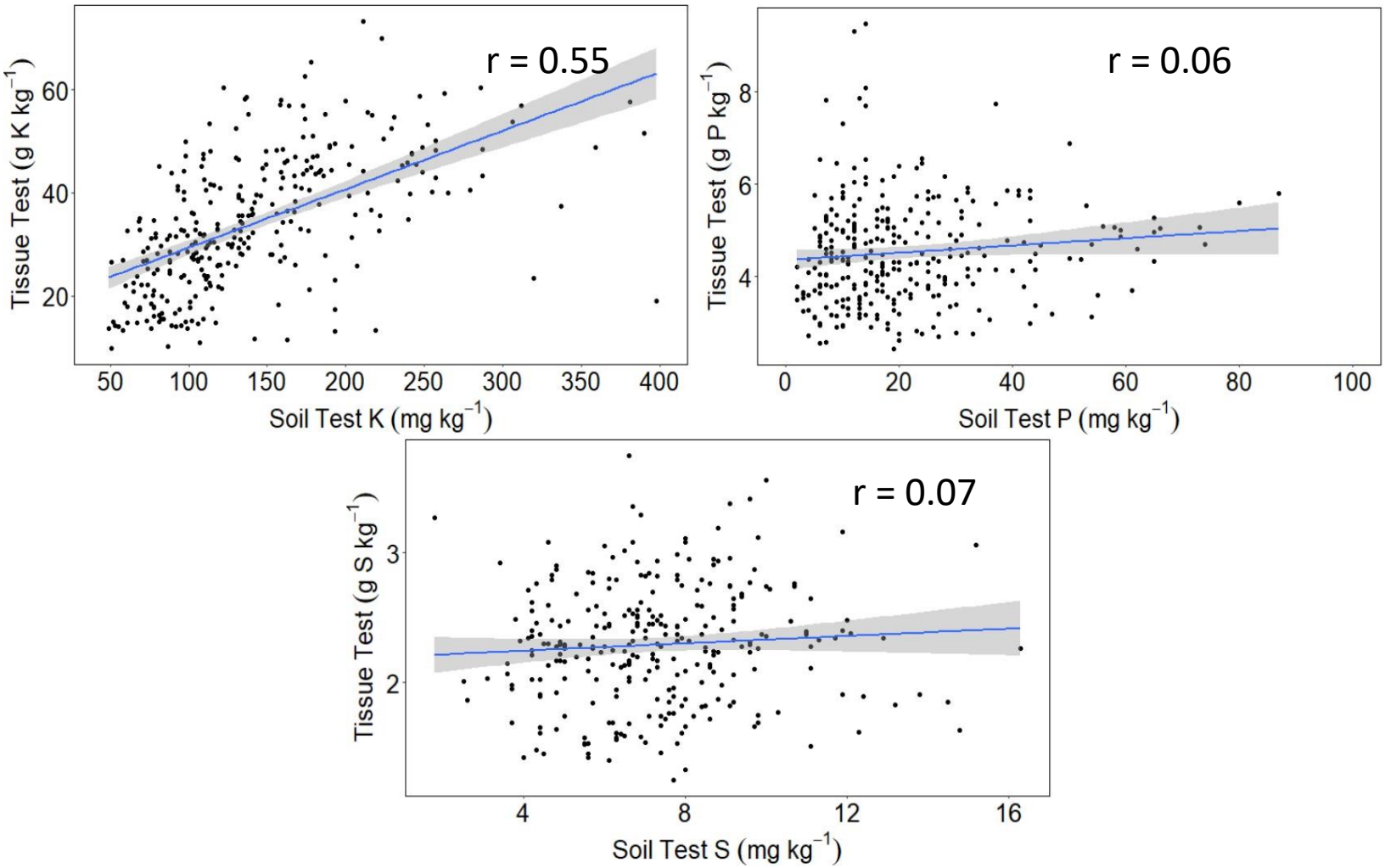

Figure 3.3. Tissue test results relative to soil test results for K, P, and S. The blue line on each graph represents the best fit linear model along with the standard error of the model. The correlation coefficient is also shown for each nutrient.

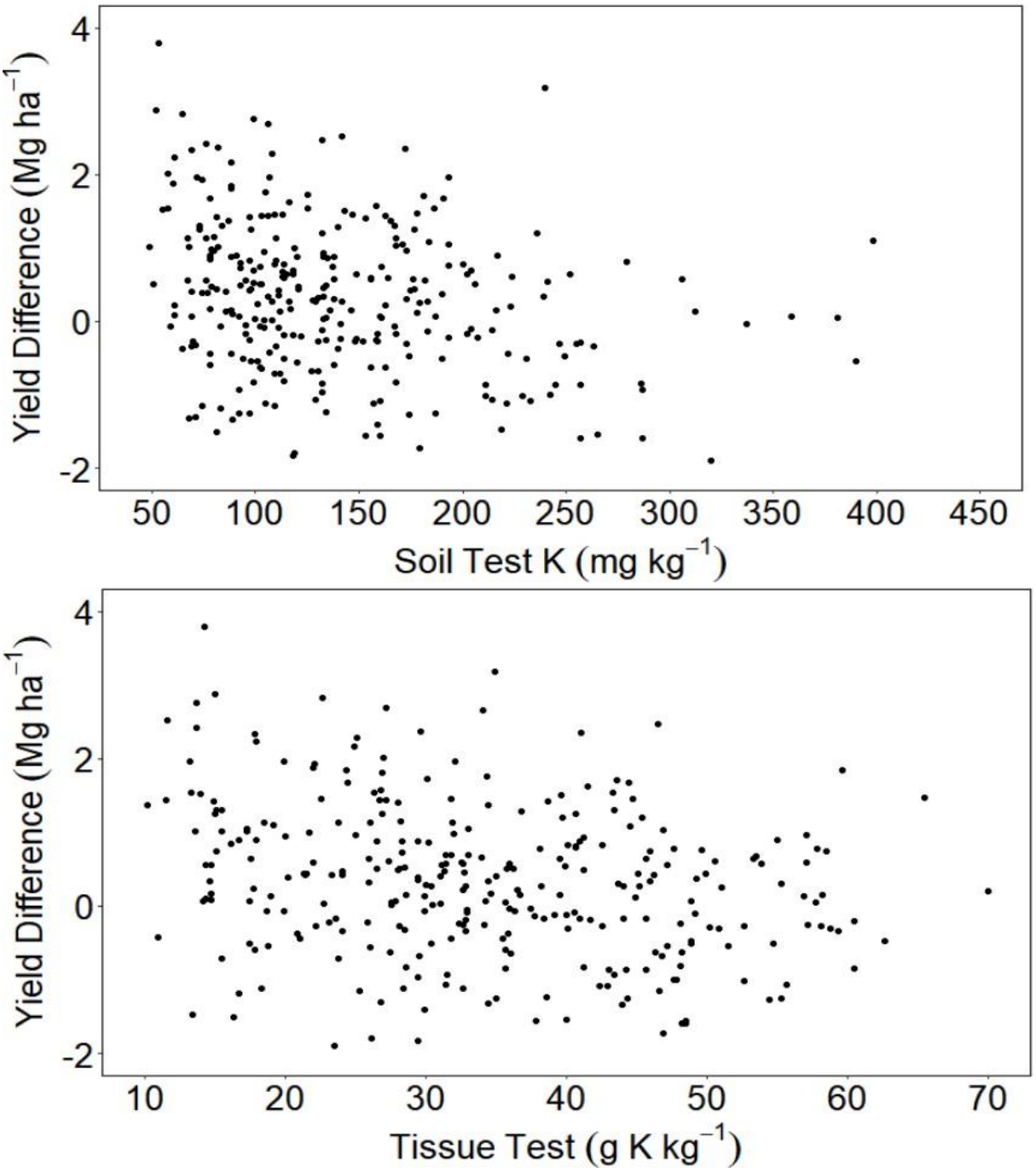

Figure 3.4. Yield difference results relative to soil-test K (top) and early-season corn tissue K concentration (bottom) where the yield difference was found by subtracting treatment 2 yield by the control yield.

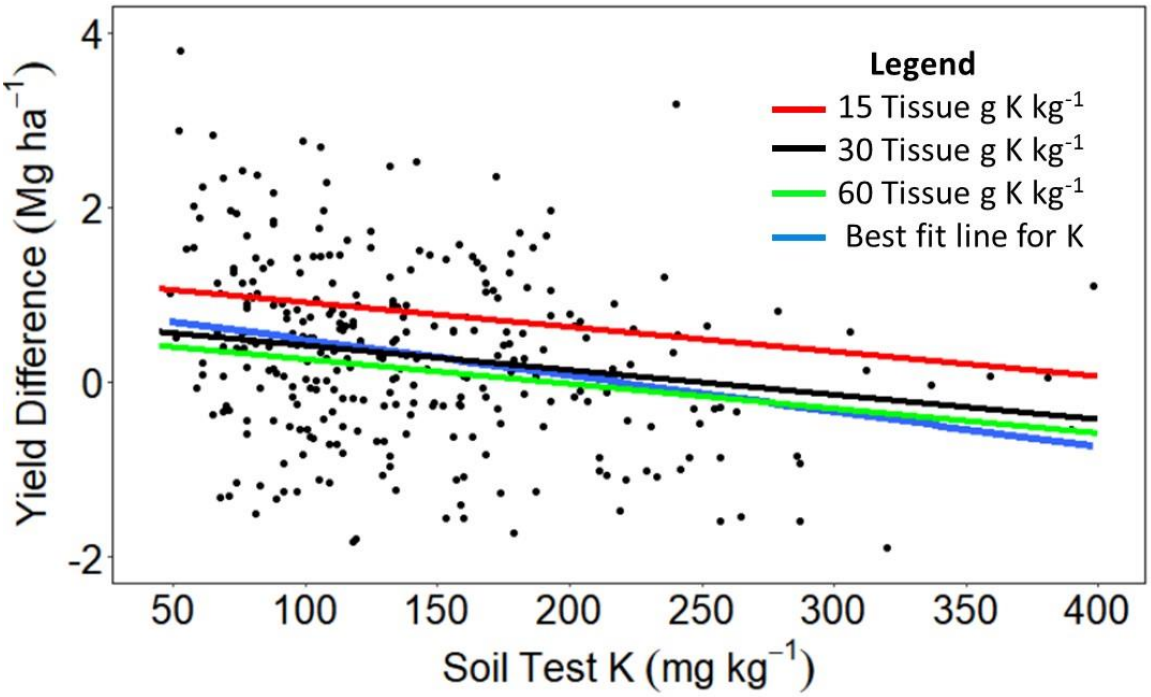

Figure 3.5. Yield difference relative to soil-test K shown with individual points. The blue line represents the linear regression best fit line only including soil-test K as the predictor variable. The red, black, and green lines represent the multiple regression model over soil-test K levels and with constant tissue concentrations of 15, 30, and 60 g K kg^{-1}.

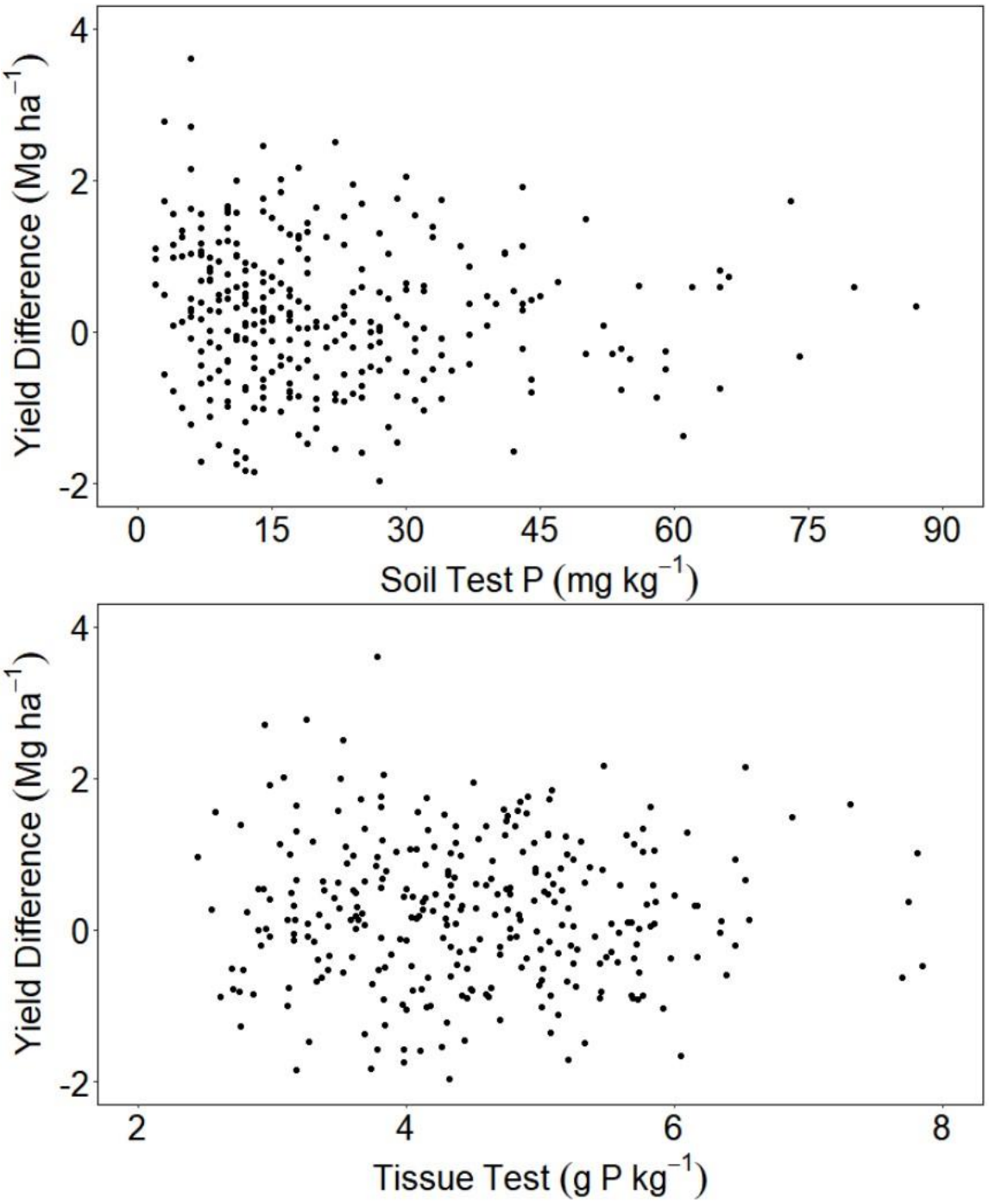

Figure 3.6. Yield difference results relative to soil-test P (top) and early-season corn tissue P concentration (bottom).

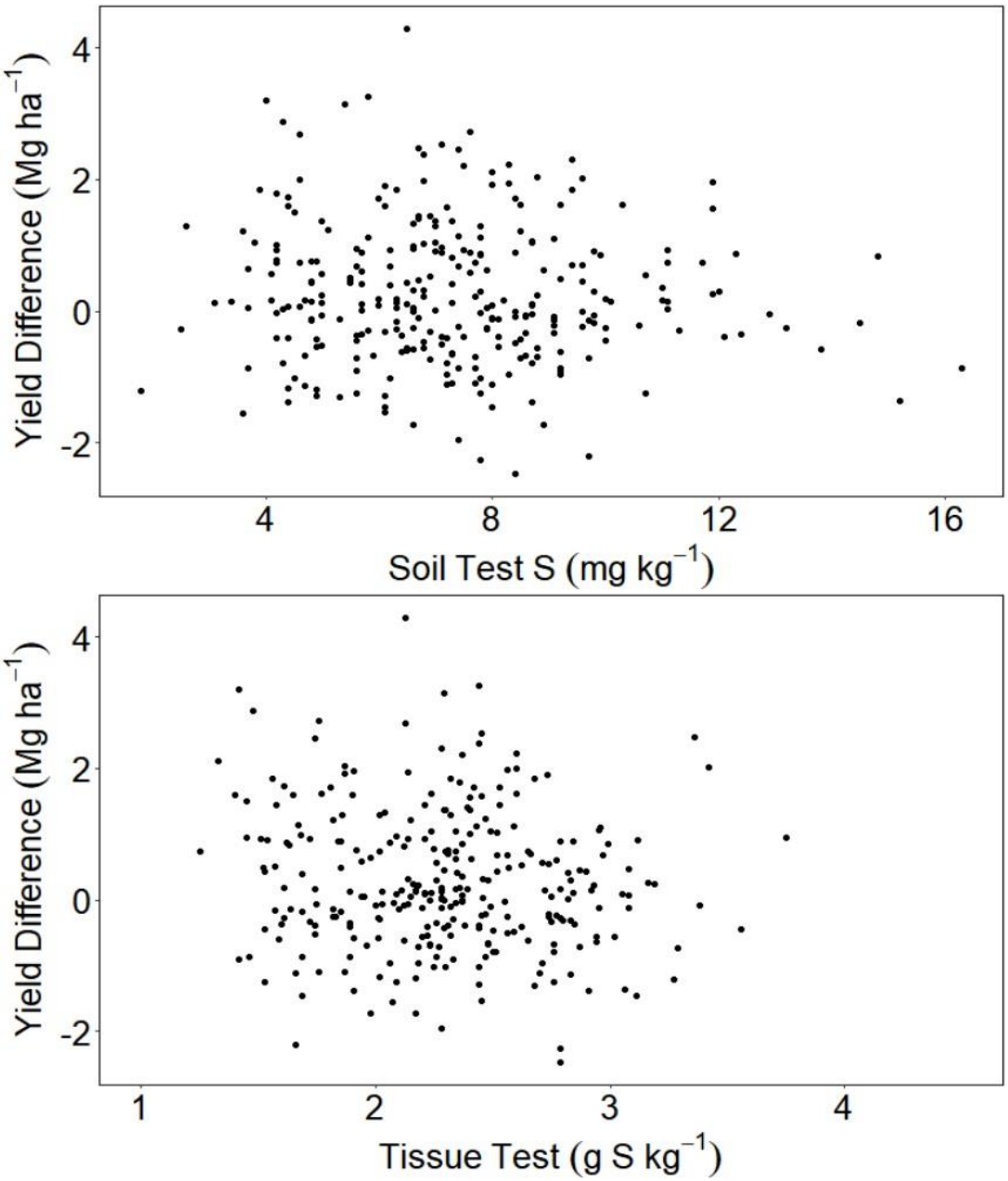

Figure 3.7. Yield difference, calculated by subtracting the S treatment yield by the control yield, relative to soil-test S (top) and early-season corn tissue S concentration (bottom).

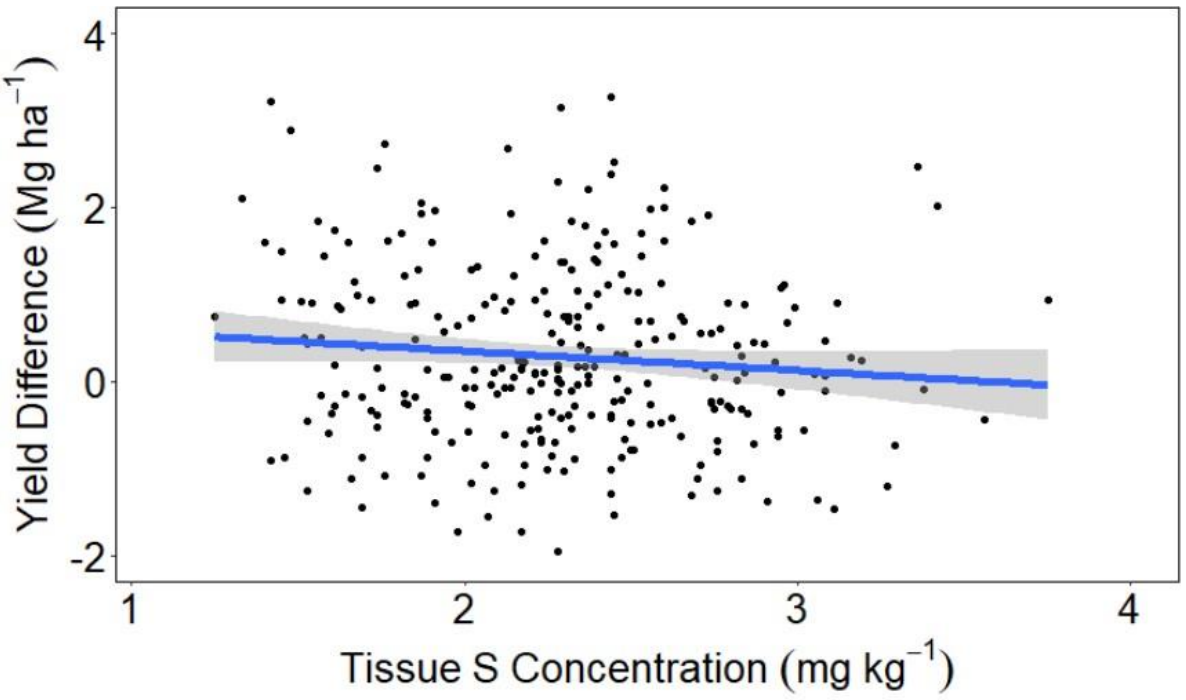

Figure 3.8. Yield difference relative to the tissue S concentration. The blue line is the best fit linear model line showing how tissue concentration is related to yield difference in corn, with standard error shown in grey (SE=1.069).