

Evaluating diagnostic tools for phosphorus and sulfur management in corn

by

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B.S., EARTH University, 2017

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2024

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Abstract

Efficient management of essential nutrients, such as phosphorus (P) and sulfur (S), is critical for maximizing corn productivity. This study aimed at enhancing corn production by evaluating diagnostic tools for nutrient management and optimizing fertilization practices, focusing on P and S nutrients. Studies were conducted across 26 sites in Kansas. The first project focuses on phosphorus nutrition. Field experiments involved varying phosphorus fertilizer rates of 34, 67, 101, and 135 kg P₂O₅ ha⁻¹, plus a control. At the V6 stage, whole plant tissue samples and normalized difference vegetative index (NDVI) readings were collected. At the R1 stage, ear leaf samples and soil plant analysis development (SPAD) readings were collected. Results indicated that the average critical P tissue concentrations (CPTC) in the whole plant at V6 and the ear leaf at R1 were 4.9 g kg⁻¹ and 3.8 g kg⁻¹, respectively. As the P tissue concentration increases, the optimum fertilizer recommendation rate decreases. Correlation shows that NDVI showed limited effectiveness in assessing phosphorus levels or yield; the SPAD correlation with yield displayed some potential for further exploration. A moderate correlation and linear relationship were found between plant P tissue concentration at V6 and leaf P tissue concentration at R1 ($r = 0.49$ and $R^2 = 0.24$). This study offers insights into phosphorus management in corn through CPTC and fertilizer calibration and highlights diagnostic tool limitations. In the second study, sulfur fertilizer rates were applied at 45 kg of S ha⁻¹, plus a control. Whole plant samples and NDVI were collected at the V6 stage, and ear leaf samples and SPAD were collected at the R1 stage. A whole plant sample at the R6 stage and grain yield were collected. Results indicated no correlation between NDVI at V6 and S tissue concentration or yield. There is a

correlation between SPAD at R1 and S tissue concentration, as well as with yield. Sulfur application increases S tissue concentration for the different stages V6, R1, R6, and grain, as well as S uptake at V6 and total S uptake across sites. A positive relationship was found between the total S uptake and yield, with an R^2 value of 0.72. The analysis revealed the mean agronomic sulfur use efficiency across sites was 1.4 kg kg^{-1} . The apparent sulfur recovery efficiency had a mean of 2.2 % across sites. This study highlights the importance of sulfur in corn. The studies provide insights into P and S nutrient management for corn production in Kansas.

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Acknowledgements

First, I am grateful for the unwavering support of my partner, family and friends throughout this journey. Their encouragement and understanding have been crucial to my success. I want to extend my heartfelt thanks to Dr. Dorivar Ruiz Diaz for granting me the opportunity to pursue my program and for his invaluable guidance and support. Additionally, I sincerely appreciate the mentorship provided by my esteemed committee members, Dr. Ganga Hettiarachchi, Dr. Nathan Nelson, and Dr. Christopher Vahl, whose expertise and insights have been instrumental in shaping my academic growth. I am also indebted to the entire soil fertility research team, including former members, visiting scholars, and student workers, for their collaborative spirit and contributions to my research endeavors. I want to express my special gratitude to my fellow graduate students for their support throughout my project. Their collaboration has been invaluable. I would also like to express my sincere appreciation to the Department of Agronomy and Research and Extension at Kansas State University, its faculty and staff, university research stations and staff, and the Kansas farmers for their generous support and provision of land for my research. Their contributions have been essential to the success of my study. I am deeply thankful to all individuals and entities who have played a role in my academic journey. I am honored to have had the opportunity to collaborate with such dedicated and inspiring individuals.

Chapter 1 - General Introduction

Corn (*Zea mays* L.) is one of the most widely cultivated cereal crops globally, playing a vital role in human and animal nutrition and various industrial applications (Ruan et al. 2019). Corn production has expanded significantly due to its adaptability to diverse climates and soil conditions, contributing to food security and economic development worldwide (Ali 2021). The United States is the biggest producer, user, and exporter of corn worldwide. On average, American farmers plant 36 million hectares (90 million acres) of corn annually. Corn is of particular significance and is a cornerstone of the agricultural economy (USDA 2023).

The successful production of corn relies heavily on the availability of essential nutrients, among which phosphorus and sulfur play pivotal roles. Phosphorus is crucial for various physiological processes in corn, including photosynthesis, energy transfer, and root development, allowing for better soil nutrient and moisture uptake (Gurmu 2023). Adequate phosphorus fertilization is essential for optimizing corn yield, particularly in soils with low phosphorus availability (Hopkins and Hansen 2019). However, an inadequate phosphorus supply can limit grain productivity in corn plants (Veloso et al. 2020). It has also been observed that increasing phosphorus fertilization rates can decrease the number of mycorrhizal spores associated with corn roots (Ortas and Islam 2018). Therefore, optimizing phosphorus fertilization is important for balancing soil fertility and supporting economic crop production in corn systems.

Similarly, sulfur is indispensable for corn growth and development, constituting amino acids, proteins, and enzymes essential for plant metabolism (Zenda et al. 2021). Sulfur deficiency can impair corn growth, reducing yields (Duke and Reisenauer 1986). Hence, sulfur fertilization has become increasingly important for ensuring optimal corn production in regions with prevalent sulfur deficiency.

In the other hand sulfur deficiency can be a complex problem due the interactions of plant-available sulfate from soil organic matter mineralization and sulfate losses to leaching (Mahal et al. 2022). The response to sulfur fertilization in corn varies depending on factors such as fertilizer type and timing. In some cases, sulfate-based fertilizers have increased corn yield, while elemental S had no effect (Fleuridor et al. 2023). However, there are also studies that suggest sulfur deficiency is not widespread in certain regions and that optimizing grain crop production does not currently require sulfur fertilization (Sutar 2017). The efficiency of applied NPK fertilizers and the economics of their use can be seriously affected under sulfur-deficient conditions, leading to lower crop yields (Ariraman et al. 2020).

The assessment of nutrient status and response in corn production can be relied on diagnostic tools such as soil tests and tissue analysis, and various in-field diagnostic techniques such as normalized difference vegetative index (NDVI) and soil plant analysis development (SPAD) measurements can be used. Understanding the interactions between nutrient availability, plant response, and diagnostic tools is essential for developing effective nutrient management practices (Srivastava and Ngullie 2009) tailored to specific crop requirements.

Optimizing phosphorus and sulfur fertilization is critical for maximizing corn yield and profitability. However, best management practices can vary across soil types, systems, and climatic conditions. Therefore, comprehensive research is needed to assess phosphorus tissue concentrations, sulfur response, and utilization efficiency and evaluate different diagnostic tools for corn production in Kansas. Through this project, executed in 26 sites across Kansas (Figure 1.1), the study goal is to enhance corn production in Kansas by evaluating diagnostic tools for nutrient management, focusing on phosphorus and sulfur.

Thesis Organization

The thesis consists of four chapters. This first chapter provides a general introduction to the thesis's contents. The second chapter, titled "Assessment of Tissue Phosphorus Concentrations as Diagnostic Tools for Phosphorus Nutrition in Corn", which aims to evaluate plant tissue analysis, NDVI and SPAD as diagnostics tools for plant phosphorus and to identify the critical phosphorus tissue concentration in two different growth stages of corn in relation to response to P fertilizer in Kansas. The third chapter, "Assessing Sulfur Response, Utilization Efficiency, and Diagnostic Tools for Corn", aims to evaluate corn response to sulfur fertilizer application, sulfur use efficiency and assess different diagnostic tools, including soil analysis, tissue analysis, NDVI, and SPAD readings, for sulfur nutrition in corn. Each chapter includes an abstract, introduction, materials and methods, results and discussion, conclusions, and references. The fourth chapter is a general conclusion of the research conducted in this thesis.

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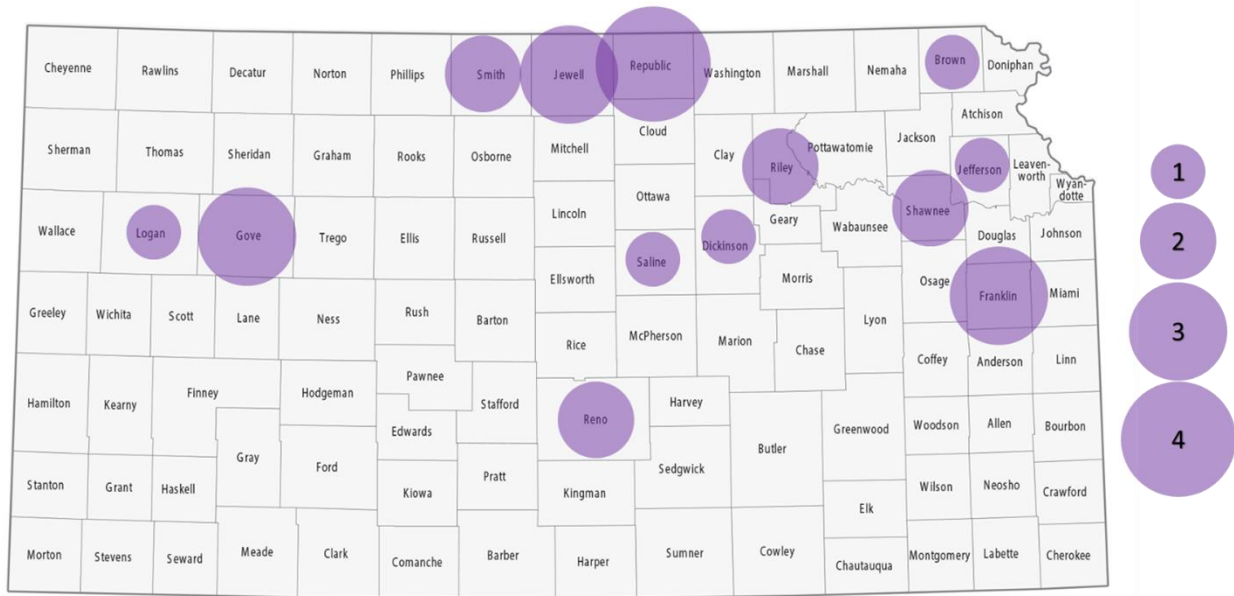


Figure 1.1. Distribution of corn study sites across various counties in Kansas. Circles denote study sites, with the size corresponding to the number of site years conducted within each county.

Chapter 2 - Assessment of Tissue Phosphorus Concentrations as Diagnostic Tool for Phosphorus Nutrition in Corn

Abstract

Phosphorus (P) is an essential macronutrient for crop growth, and plant tissue can be used to assess nutrient management. The objective of this study was to evaluate plant tissue analysis, normalized difference vegetative index (NDVI) and SPAD readings as diagnostics tools for plant phosphorus and to identify the critical P tissue concentration (CPTC) in two different growth stages of corn in relation to response to P fertilizer in Kansas. Field experiments were conducted at 26 sites. The experimental design was a randomized complete block design with four replications. Fertilizer treatments included four different phosphorus rates and a control. Whole plant tissue samples and NDVI were collected at the V6 growth stage, and ear leaf samples and SPAD were collected at the R1 growth stage. The CPTC was determined using linear plateau and quadratic plateau models, and model averaging was performed for final estimation. Results indicated that the average CPTC in the whole plant at V6 and the ear leaf at R1 were 4.9 g kg^{-1} and 3.8 g kg^{-1} , respectively. Regression analysis revealed that as the P tissue concentration increases, the optimum fertilizer recommendation rate decreases with R^2 of 0.33 and 0.25 for V6 and R1, respectively. Correlation analysis highlighted that NDVI measurements showed limited effectiveness in assessing phosphorus levels or yield; however, the SPAD correlation displayed some potential. Correlation and regression analysis showed a moderate correlation between plant P tissue concentration at V6 and leaf P tissue concentration at R1 ($r = 0.49$ and $R^2 = 0.24$). This study contributes valuable insights for phosphorus management in corn, providing CPTC and fertilizer calibration and highlighting diagnostic tools' shortcomings or potential.

Introduction

Phosphorus is a critical macronutrient that crops require in relatively large amounts. The available fraction of soil phosphorus is often low, requiring phosphorus fertilization to meet crop needs (Preston, Ruiz Diaz, and Mengel 2019). While soil analysis is the most common diagnostic tool for assessing phosphorus nutrition, plant tissue analysis can also identify P deficiencies and evaluate P fertilization management programs (Reuter and Robinson 1997).

Soil testing provides information on the available P in the soil that is based on correlation (the process of determining the relationship between yield and the amount of nutrient) - calibration (the process of determining the crop nutrient requirement at different nutrient values) with crop response; this is crucial for determining fertilizer requirements (Mali et al. 2022), and it allows for proactive soil fertility management before planting (Reijneveld et al. 2022). However, it has some disadvantages, such as soil P tests may not always be accurate (Mallarino 1996). This inaccuracy can arise from the testing method used (Menon and Chien 1995) and from relying on a single snapshot of soil P status which fails to capture variations throughout the growing season. Also, the quality depends on the soil sampling process, which can differ quickly over a large area.

Plant tissue analysis can offer a complementary approach to soil analysis by directly measuring the nutrient concentration within plant tissues. This offers insights into the current nutritional status, identifies nutrient deficiencies or excesses during the growing season, and accounts for variations. Tissue analysis is a widely used technique in vineyards to guide fertilization strategies for various nutrients (Christensen 2005; Dami and Smith 2019; Hickey et al. 2021). It is also used in pasture management and for crops like alfalfa to help with fertilization and nutritional interventions. Additionally, tissue analysis is a valuable diagnostic

tool for wheat production (Korkmaz et al. 2008; Weisz et al. 2007), also tissue analysis was incorporated in the phosphorus fertilization recommendations for bahiagrass (Silveira et al. 2011).

While tissue analysis can be a valuable diagnostic tool to evaluate crop nutrient status, it does have some limitations for P management in corn. A notable drawback is that it can only be done during the growing season when the crop is actively developing. Additionally, if deficiencies are detected, it is uncertain whether in-season P amendment will be effective for corn. This is because P is immobile in the soil, making it difficult for plants to access added nutrients quickly (Mallarino and Sawyer 2018). Interpreting P tissue concentrations can be challenging, as critical range (the nutrient concentration in the plant below which a yield response to added nutrient occurs) and sufficiency ranges (the nutrient concentration range in which added nutrient will not increase yield but can increase nutrient concentration) vary on growth stage and plant parts (Havlin et al. 2021).

Despite these limitations, in-season tissue analysis can still be a valuable tool to evaluate corn phosphorus management. Since, under certain conditions, foliar phosphorus applications in the early or mid-season can improve phosphorus use efficiency and yield in corn (Girma et al. 2007), this method can be particularly beneficial in drought conditions, as it has been shown to significantly affect yield and yield parameters in both drought-tolerant and sensitive hybrids (Ahmad et al. 2015). Tissue analysis can serve as a crucial tool for evaluating the effectiveness of a phosphorus management plan. It can guide fertilizer application for the upcoming season and identify areas of phosphorus deficiency not detected in the initial soil analysis due to sample variation. This insight allows for targeted interventions and is also helpful in determining plant uptake. Additionally, its application can extend to on-farm research, offering a method to

systematically test and refine P management strategies, ensuring nutrient availability across different field conditions.

Current guidelines for tissue analysis in corn are limited to identifying deficiency using the sufficiency range. This general sufficiency range is summarized in many textbooks (Bryson et al. 2014; Jones, Eck, and Voss 1990; Jones, Wolf, and Mills 1991; Mills and Jones 1996). Also, some regional sufficiency ranges, such as the Southern Cooperative Series Bulletin #394 (Campbell 2000), most of these sufficiency ranges are based on a collection of a wide range of studies and surveys. Also, most of the critical or sufficiency ranges for corn are based on data that is more than two decades old. Since then, there have been significant improvements in crop yields and changes in crop genetics. As a result, there is a need to establish new ranges and critical values using statistical models (Stammer and Mallarino 2018).

Research on utilizing tissue analysis for evaluating phosphorus nutrition in corn within the context of Kansas has been nonexistent, particularly for identifying critical values in relation to response to P fertilizer. Since the concentration of P in plants varies by plant part and growth stage, it's essential to establish relationships between nutrient content and yield response for each stage and part. These relationships between the nutrient concentration and yield can help to identify critical values (Munson and Nelson 1990) and make fertilizer calibrations. Other diagnostics tools and tissue analysis such as NDVI and SPAD can be useful for corn nutrition. NDVI and SPAD have been successfully used to access nitrogen in corn (Edalat, Naderi, and Egan 2019), but have been limited used in phosphorus.

The NDVI is a standardized measure that evaluates vegetation health by analyzing its light absorption and reflectance properties. It utilizes the near-infrared light reflected and red light absorbed by plants. NDVI values span from -1 to +1, where values closer to +1 signify

healthier and more vigorous vegetation, typically associated with better nutrient status (Glenn and Tabb 2019; Wang et al. 2017). The SPAD meters estimate the chlorophyll content in plant leaves by measuring the absorbance of specific wavelengths of light. Chlorophyll content is directly related to the plant's nitrogen levels, which are interlinked with phosphorus and sulfur through metabolic pathways (Nampila et al. 2018; Uddling et al. 2007). However, the NDVI and SPAD have limitations; for NDVI it can saturate dense vegetation areas and external conditions such as light variations. SPAD readings can vary with leaf age, position, and inter-leaf differences and both can be sensitive to external factors like including canopy structure, disease, water stress, other nutrient deficiencies.

Since phosphorus is a critical component of adenosine triphosphate (ATP), which is necessary for energy transfer and photosynthesis processes, sufficient phosphorus levels can ensure higher chlorophyll (Malhotra et al. 2018; Poirier, Jaskolowski, and Clúa 2022). This can be reflected in the vegetation's ability to absorb and reflect light. Healthy, phosphorus-sufficient plants will tend to reflect more light and have higher chlorophyll, which can result in a higher NDVI and SPAD reading. These tools can provide additional insights into nutrients and crop response, expanding the scope of assessment beyond traditional testing methods. The objective of this study was to evaluate plant tissue analysis, NDVI, and SPAD as diagnostic tools for plant phosphorus and to identify the CPTC in two different growth stages of corn in relation to the response to P fertilizer in Kansas.

Materials and Methods

Sites and Experiment Design

Field experiments were conducted at 26 sites in 2021-2022 across Kansas (Table 2.1). The study sites were located in farmers' fields or university experiment fields. All operations, such as nitrogen fertilization, weed management, corn hybrid, crop rotations, and others, vary depending on the region and each field or farming operation.

The individual plot size was 12.2 m in length and 3.1 m in width (4 rows), except site 5, which had a length of 10.7 m. Row spacing was 76.2 cm for all sites. The experimental design was a randomized complete block design with four replications. Fertilizer treatments consisted of four phosphorus fertilizer rates at 34, 67, 101, and 135 kg of P_2O_5 ha⁻¹ (15, 30, 44, and 59 kg of P ha⁻¹) using mono-ammonium phosphate (MAP) (11-52-0), plus a control with no phosphorus; all fertilizer was applied broadcast pre-plant. Nitrogen was balanced for all treatments by applying 62 kg N ha⁻¹ using urea (46-0-0).

Soil Sampling and Analysis

Soil samples were collected as composite soil samples of 10 to 12 cores (1.9 cm diameter) collected at the 0 to 15 cm depth by block using a hand probe, prior to planting and fertilizer application. Soil samples were then dried at 40 °C for five to ten days. After samples were dried, the soil was ground to pass through a 2-mm sieve. Soil samples were analyzed for pH 1:1 (soil:water) (Peters, Nathan, and Laboski 2012), organic matter (OM) by loss on ignition (Combs and Nathan 1998), potassium by Mehlich 3 extraction (Warncke and Brown 1998) and measured by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and soil test P by the Mehlich 3 extraction and measured by colorimetry (Frank, Beegle, and Denning

1998). Soil series and subgroup information for each site was obtained using the Web Soil Survey database (Soil Survey Staff n.d.), Table (2.2).

Plant Tissue, NDVI, SPAD, and Yield

Tissue samples were collected in the early-season as whole plants at the V6 growth stage (vary between V5 to V7) (Ciampitti, Elmore, and Lauer 2016). Six to ten whole plant samples were collected per plot from the guard rows. Mid-season tissue samples were collected as ear leaves at the R1 growth stage (varying between VT to R2) (Ciampitti, Elmore, and Lauer 2016). All tissue samples were oven-dried at 60 °C for 10 to 15 days. The weight of dry biomass for V6 samples was measured by weighing the sample (six to ten whole plants). Next, the total dry weight of the sample was divided by the number of plants to calculate the biomass in grams per plant. The dry samples were ground to pass through a 2-mm using a Thomas Wiley Cutting Mill (Thomas Scientific, Swedesboro, NJ, USA). Samples were then digested using the nitric perchloric acid digest method (Gieseking, Snider, and Getz 1935). Phosphorus concentration was determined by ICP-OES. Phosphorus concentration and biomass were used to calculate plant P uptake at V6.

The NDVI measurements were taken at V6 growth stage using an active sensor RapidSCAN CS-45 handheld crop sensor (Holland Scientific, Lincoln, NE, USA). This sensor uses a wavelength of 670 nm, red-edge light at 730 nm, and near-infrared light at 780 nm. For this study, the red and near infrared wavelengths were used to calculate the NDVI for each treatment. The sensor was held 1.4 meters above the ground, and the reading was collected for the entire length of each plot. At the end of each plot, the average measurement was recorded.

The SPAD measurements were taken at the R1 growth stage using the chlorophyll meter SPAD-502 (Konica Minolta, Japan). The SPAD stands for Soil Plant Analysis Development. This handheld device, based on two light-emitting diodes and a silicon photodiode receptor, measures leaf transmittance in the electromagnetic spectrum's red (650 nm) and infrared (940 nm) regions. The device uses these transmittance values to derive a relative SPAD meter value proportional to the amount of chlorophyll in the sample (Uddling et al. 2007). Twenty independent SPAD readings were taken around the midpoint of different ear leaves on one side of the midrib and were averaged, the reading was collected in the center of two rows for each plot.

Grain yield was determined by harvesting the center 1.5 meters of each plot (two center rows) by hand or using a plot combine. The hand-harvested sites were done on 1.5 meters width by 6.1 meters length and threshed using Almaco LPR thresher (Almaco, Nevada, IA, USA). Grain was weighed using a digital scale Ohaus Defender 5000 Series (Ohaus Corporation, Pine Brook, NJ, USA), and a subsample from each plot was analyzed for moisture and test weight using a GAC-2500-AGRI grain analysis computer (Dickey John, Auburn, IL, USA). For sites harvested with a plot combine, the yield was determined by harvesting two rows (1.5 meters in width) 11.3 meters in length (except site 5 with 9.8 meters in length), data as grain weight, moisture, and test weight were obtained from combine monitor. All grain yield was calculated and corrected to 15.5% moisture.

Relative Yield and Optimum Fertilizer Rate

The relative yield was calculated as follows:

$$RY = \frac{Y_0}{\max(Y_0 \dots Y_R)} \times 100 \quad [2.1]$$

RY = relative yield; Y_0 = mean yield of control treatment; Y_R = mean yield of maximum rate.
Numerical maximum among all treatment yield, including control.

A combination of approaches was used to determine the optimum fertilizer rate for each site. Firstly, a linear plateau model was applied to fit the yield and fertilizer rate of each site, enabling us to estimate the optimum fertilizer rate (breakpoint). However, when the linear plateau model fails to calculate the breakpoint, the optimum fertilizer rate was determined using the rate with the highest mean yield or control if the means were the same.

Statistical Analysis

Relationships between P tissue concentration and relative yield were investigated using data tissue from control plots; two different models were used to determine the CPTC. The linear plateau and quadratic plateau models were fit using nonlinear least square 'nls' regression implemented using self-starting functions from the {nlraa} R package (Miguez 2021). Model averaging using Akaike information criterion (AIC) weights was performed (Miguez & Poffenbarger, 2022), and the confidence intervals (CI) were determined using bootstrapping method ($n=5000$), using 'boot_nls' function from {nlraa} R package (Miguez 2021). The coefficient of determination (R^2) was determined using the 'rsquare' function from the {modelr} R package (Wickham 2022). The 'cor' function from {stats} R package (R Core Team 2021) was used for the Pearson correlation matrix, and the 'cor.mtest' function from {corrplot} R package (Wei and Simko 2021) with $\text{sig.level} = 0.05$. For linear regression analysis, the 'geom_smooth' function was used from the R package {ggplot2} (Wickham 2016), with method "lm" and function 'Stat_poly_eq' from {ggpmisc} R package (Aphalo 2023), was used to extract the

formula of the regression. All statistical analyses were performed in R version 4.2 (R Core Team 2021), using RStudio version 2022.12.0+353 (RStudio Team 2019).

Results and Discussion

Correlation and Relationship for Different Variables

The correlation analysis (Figure 2.1) revealed the highest correlation between biomass at V6 and plant P uptake at V6, with a strong Pearson correlation coefficient ($r = 0.94$). This strong correlation, which is expected due to the non-independent nature of these variables, indicates that as biomass at V6 increases, so does P uptake. A linear regression analysis yielded an R^2 value of 0.84 (Figure 2.2a), this regression indicates that approximately 84% of the variability in P uptake at V6 can be explained by changes in biomass at V6. The slope of the regression line reflects the rate of increase in P uptake for each unit increase in biomass. The whole plant P tissue concentration at V6 and the ear leaf P tissue concentration at R1 was found to be moderate ($r = 0.49$) and linear regression has a R^2 of 0.24 (Figure 2.2b), these findings are consistent with Stammer and Mallarino (2018), who reported an R^2 value of 0.20. The observed differences in P tissue concentrations between the V6 and R1 stages showed the changes in tissue nutrient concentration throughout the growing season, as nutrient concentration decreases with increases in plant biomass (Lemaire et al. 2019). The V6 stage is a critical period for vegetative growth, where P demand could be higher to support rapid biomass accumulation, typically higher in P tissue concentrations. In contrast, the R1 stage marks the beginning of reproductive development, where the plant's focus shifts towards flowering and ear development, which typically is lower in P concentration.

The NDVI values at V6 was found to have no significant correlation with the plant P tissue concentration at V6 or yield. Moreover, it exhibited a poor correlation with biomass at V6 and plant P uptake at V6 ($r = 0.10$). Similarly, the SPAD reading of the ear leaf at R1 showed a weak correlation with the ear leaf P tissue concentration at R1 ($r = 0.14$). It is worth noting that diagnostic tools such as NDVI and SPAD for assessing phosphorus are not commonly reported in the literature, as these tools are more commonly employed for nitrogen assessments, as evaluated by Edalat et al. (2019); and Paiao et al. (2020), or specifically focusing on NDVI as Sharma et al. (2015) or SPAD as Bullock and Anderson (1998); and Wood et al. (1992). A study conducted by Weber (2021) investigated the use of NDVI for phosphorus in winter wheat and reported a good correlation between NDVI at early jointing (Feekes 6) and yield, with an R^2 of 0.69. However, our findings highlight the limited effectiveness of NDVI as a diagnostic tool for phosphorus in corn at V6 stages. This could be due to winter wheat and corn having distinct physiological traits and canopy architectures since NDVI primarily measures the greenness and biomass of plant canopies. With its tall stature, broader leaves, and wider row spacing, corn presents a different NDVI response than winter wheat, which has a more uniform and denser canopy.

The plant P tissue concentration at V6 and SPAD at R1 showed a relatively poor correlation with yield. The ear leaf P tissue concentration does not correlate with yield. The plant P tissue concentration at V6 ($r = 0.20$) and the SPAD at R1 ($r = 0.23$) show potential for further exploration. In contrast, a study by Kovács and Vyn (2017) found a high correlation ($r = 0.72$) in experiments that included various N, P, and K application timing and rates, plant densities, and multiple tillage systems. These low correlation values indicate that yield is primarily influenced by other factors beyond P tissue concentration at those growing stages.

Critical Tissue Phosphorus Concentration

The CPTC for the whole plant at the V6 growth stage and ear leaf at the R1 growth stage were determined using two models: linear plateau and quadratic plateau. For the whole plant at the V6, the linear model has a CPTC of 3.9 g kg^{-1} with an R^2 of 0.38, while the quadratic plateau model results in CPTC of 6.4 g kg^{-1} with an R^2 of 0.40. The AIC showed slight variations between the models, and the CI ranged from 2.7 to 5.7 g kg^{-1} (Table 2.3). The average CPTC at V6 was 4.9 g kg^{-1} (Figure 2.3a).

The ear leaf CPTC at R1 was 2.9 g kg^{-1} for the linear plateau model, and 4.7 g kg^{-1} for the quadratic plateau model. The corresponding R^2 values for these models were 0.16 and 0.17, respectively. The AIC showed minimal variation among the models, and the CI ranged from 1.7 to 3.5 g kg^{-1} (Table 2.3). The average CPTC in the ear leaf at the R1 stage was 3.8 g kg^{-1} (Figure 2.3b). For the quadratic plateau model, the upper CI for both the plant P tissue concentration at V6 and ear leaf P tissue concentration at R1 could not be estimated, and as a result, it was also inestimable for the average model. This limitation stems from the quadratic model's tendency to overestimate. Specifically, the estimated value for the upper limit was excessively large and lacked practical relevance, which led to its exclusion. Furthermore, the model likely suffers from insufficient data at the higher end of the range, impairing its ability to estimate this parameter accurately. This scarcity of data points towards the upper limit, which probably resulted in the overestimation of the upper CI, justifying the decision to omit this value.

A study by Stammer and Mallarino (2018) reported CPTC of 4.8 g kg^{-1} (linear plateau) and 5.5 g kg^{-1} (quadratic plateau) for the whole plant at V6, and 2.5 g kg^{-1} (linear plateau) and 3.2 g kg^{-1} (quadratic plateau) for the ear leaf at R1, they suggest using the linear plateau model for the lower CPTC range and the quadratic model for the upper CPTC range. Limited literature

is available on model averaging using AIC weights to determine the average CPTC. The weighted model was suggested by Miguez and Poffenbarger (2022) as the best approach instead of relying on either the linear plateau (which can lead to negative bias) or the quadratic plateau (which can result in positive bias) models.

In this dataset, plant P tissue concentrations at V6 ranged from 2.0 to 6.4 g kg⁻¹; the leaf P tissue concentration at R1 ranged from 1.5 to 3.5 g kg⁻¹. While the average CPTC identified in this study is within the sufficiency range suggested by the plant analysis handbook (Mills and Jones 1996; Bryson et al. 2014), which indicates a sufficient range for whole plant P tissue concentration at V6 (3.0 to 5.0 g kg⁻¹) and ear leaf P tissue concentration at R1 (2.5 to 4.5 g kg⁻¹), the quadratic plateau model is higher than the proposed sufficiency range for both stages.

The overall model fits were relatively poor for both growth stages and plant parts (Table 2.3), having a better model fit for the V6 stage ($R^2 = 0.30$ and 0.40) than the R1 stage ($R^2 = 0.16$ and 0.17), while Stammer and Mallarino (2018) found better model fit for the R1 stage ($R^2 = 0.64$ and 0.62), but more similar results for model fit was found in older publication by Mallarino (1996) where the $R^2 = 0.18$ and 0.14 for the V6 and R1 growth stage respectively. Stammer and Mallarino (2018) suggest that their dataset included more responsive sites and a wider range of tissue P concentrations, which probably explains the discrepancy.

It is important to note that the values determining the sufficient ranges in some textbooks like Mills and Jones (1996); and Bryson et al. (2014) are often based on surveys and may not be based on fertilizer response field studies. The sufficient ranges are determined through a survey of tissue collected from numerous fields at a particular growth stage. This process generates a normal distribution curve, establishing sufficient ranges (Slaton et al. 2016). Sufficiency ranges developed through surveying nutrient concentrations are limited because it is unknown if the

addition of nutrients would have resulted in greater tissue nutrient concentrations or yield (Laboski 2016).

Our study attempts to enhance estimation accuracy by addressing biases of single-model reliance for the average CPTC. Unlike the broad sufficiency ranges proposed by Mills and Jones (1996); and Bryson et al. (2014), or the critical range proposed by Stammer and Mallarino (2018) our approach specifies precise CPTC values. This precision can advance phosphorus management guidelines by providing a clearer basis for application decisions.

Phosphorus Fertilization Calibration

A linear regression analysis was conducted to explore the relationship between the optimum fertilizer rate and the whole plant P tissue concentration at V6 of the control (Figure 2.4a) and the relationship between the optimum fertilizer rate and ear leaf P tissue concentration at R1 of the control (Figure 2.4b). The regression equation derived from the analysis for the whole plant P tissue concentration at V6 was ($y = 115 - 20.2x$), with an associated R^2 value of 0.33. Similarly, the regression equation obtained for the ear leaf P tissue concentration at R1 was ($y = 111 - 28.7x$), with an R^2 value of 0.25.

The results from both stages consistently demonstrate a negative relationship between the optimum fertilizer rate and P tissue concentrations at their respective growth stages. As the P tissue concentration increases, the optimum fertilizer rate decreases. To investigate if higher or lower yielding sites group together in terms of P tissue concentration and fertilizer rate, yield was visualized in Figures 2.4a and 2.4b. However, no clear spatial association between yield and these factors was observed.

Fertilizer recommendations based only on the tissue analysis for corn are usually not recommended (Kaiser 2018; Mallarino and Sawyer 2019). Also, it has been pointed out that phosphorus fertilization during the growing season may have a low success rate and uncertainty (Mallarino and Sawyer 2018). Nevertheless, under certain conditions, foliar phosphorus applications in the (VT) mid-season in corn can improve phosphorus use efficiency and increased yield in some of the sites, according to a study by Girma et al. (2007). Another use of it could be to implement fertilizer application for the subsequent growing season or potential for identifying a specific area with phosphorus deficiency that was not shown in the soil test due to sampling variation, where a targeted phosphorus fertilizer application can be made.

Conclusions

Based on this study's findings, the correlation analysis showed varying degrees of correlation between different variables. Moderate correlations were found between the whole plant P tissue concentration at V6 and the ear leaf P tissue concentration at R1. Diagnostic tools such as NDVI at V6 exhibited limited effectiveness, but SPAD at R1 can have some potential for assessing phosphorus in corn.

The CPTC was determined for the whole plant at V6 as early-season and CPTC at the R1 as mid-season using two different models and model averaging. The results indicate that the P tissue concentrations vary between growth stages and among models. The average CPTC in the whole plant at V6 and ear leaf at R1 were 4.9 g kg^{-1} and 3.8 g kg^{-1} , respectively. These values and the CI provide valuable insights for phosphorus management in corn.

Regarding phosphorus fertilization calibration, linear regression analyses showed that the optimum fertilizer rate is expected to decrease as the P tissue concentration is higher. However,

the R^2 of 0.33 and 0.25 for V6 and R1, respectively, indicate that the linear regression models explain only a portion of the variation in the optimum fertilizer rate based on P tissue concentrations. Future research can explore the integration of multiple diagnostics tools to improve and develop more accurate and reliable fertilizer recommendation tools.

Overall, the study provided average values of CPTC based on corn response to P fertilizer and correlations with different variables in corn production. Recommendations include considering CPTC for phosphorus management and recognizing the limitations and opportunities with other diagnostic tools.

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Tables and figures

Table 2.1. Study sites, year, county, and production system information, as previous crop, tillage system, irrigation, hybrid, or cultivar and planting date.

Site	Year	County	Previous crop	Tillage System †	Irrigation	Hybrid/Brand	Planting date
1	2021	Shawnee	Soybean	CT	Yes	P1185	29/04/2021
2	2021	Riley	Soybean	CT	No	P1151AM	04/28/2021
3	2021	Franklin	Soybean	NT	No	P1366	07/08/2021
4	2021	Republic	Soybean	NT	No	P1828AM	05/04/2021
5	2021	Republic	Soybean	NT	Yes	P1828AM	05/04/2021
6	2021	Brown	Soybean	NT	No	DKC66-17	05/05/2021
7	2021	Gove	Wheat	NT	No	Taylor8835	05/29/2021
8	2021	Gove	Wheat	NT	No	Taylor8835	05/29/2021
9	2021	Salina	Corn	NT	No	DKC29-89	07/17/2021
10	2021	Dickinson	Sorghum	NT	No	205-63STXRIB	06/26/2021
11	2021	Logan	Corn	NT	No	P0622Q	06/08/2021
12	2021	Gove	Corn	NT	No	B11V77SX	06/10/2021
13	2022	Jewell	Wheat	NT	No	P1244AM	04/20/2022
14	2022	Jewell	Wheat	NT	No	P1244AM	04/20/2022
15	2022	Jewell	Wheat	NT	No	P1548	04/21/2022
16	2022	Shawnee	Soybean	CT	Yes	DK65-95	04/22/2022
17	2022	Franklin	Soybean	NT	No	P1548	04/22/2022
18	2022	Franklin	Soybean	NT	No	P1548	04/22/2022
19	2022	Reno	Soybean	NT	Yes	A645-80	04/30/2022
20	2022	Reno	Soybean	NT	Yes	A645-80	04/30/2022
21	2022	Jefferson	Soybean	NT	No	AV8513AM	04/26/2022
22	2022	Republic	Soybean	NT	No	P1289AM	05/11/2022
23	2022	Republic	Soybean	NT	No	P1289AM	05/12/2022
24	2022	Riley	Soybean	CT	No	P1151AM	05/17/2022
25	2022	Smith	Sorghum	NT	No	213-29	06/03/2022
26	2022	Smith	Soybean	NT	No	LG62C35	05/31/2022

† CT, Conventional Tillage; NT, No Till.

Table 2.2. Study sites, predominant soil type and soil properties as pH, OM, soil test P and K. Samples were collected at 0 to 15 cm the depth before fertilizer application.

Site	Series ^a	Subgroup ^{a †}	pH	OM g kg ⁻¹	P -----mg kg ⁻¹ -----	K
1	Bismarckgrove	FL. Hapludolls	7.5	19	20	181
2	Bourbonais	FL. Hapludolls	6.2	20	46	290
3	Woodson	AB. Argiaquolls	5.8	34	11	131
4	Crete	PU. Argiustolls	6.0	27	7	429
5	Crete	PU. Argiustolls	6.5	33	5	398
6	Kennebec	CU. Hapludolls	6.2	31	47	179
7	Keith	AR. Argiustolls	6.6	27	26	629
8	Keith	AR. Argiustolls	7.1	25	21	644
9	Longford	UD. Argiustolls	5.3	29	43	235
10	Geary	UD. Argiustolls	6.0	35	23	249
11	Keith	AR. Argiustolls	6.4	28	22	555
12	Ulysses	AR. Haplustolls	6.0	31	40	601
13	Detroit	PA. Argiustolls	6.8	37	13	294
14	Hord	CU. Haplustolls	7.1	55	13	335
15	Harney	TY. Argiustolls	5.2	34	19	322
16	Eudora	FL. Hapludolls	7.0	21	2	146
17	Woodson	AB. Argiaquolls	6.1	36	4	118
18	Woodson	AB. Argiaquolls	5.7	36	5	123
19	Taver	UT. Argiustolls	7.4	28	6	262
20	Nalim	UD. Argiustolls	6.8	32	24	317
21	Grundy	AQ. Argiudolls	7.2	38	20	95
22	Crete	PU. Argiustolls	6.3	35	3	460
23	Crete	PU. Argiustolls	6.2	30	4	456
24	Wymore	AQ. Argiudolls	6.4	28	13	218
25	Harney	TY. Argiustolls	6.2	29	22	512
26	Harney	TY. Argiustolls	5.3	30	27	489

OM, Organic Matter; P, Mehlich 3 colorimetric phosphorus; K, Mehlich 3 IPC-OES.

† FL, Fluventic; AB, Abruptic; PU, Pachic Udertic; CU, Cumulic; AR, Aridic; UD, Udic; PA, Pachic; CU, Cumulic; TY, Typic; UT, Udertic; AQ, Aquertic.

^aSource: Web soil survey (<https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx>).

Table 2.3. Critical phosphorus tissue concentration for linear plateau, quadratic plateau, and average models for corn at two different growth stages.

Models	CPTC	R ²	AIC	p-value†	CI		Equation
Whole Plant at V6							
Linear plateau	3.9	0.38	180.9	<0.001	2.7	5.7	y = 57.14 + 9.078x when: x < 3.9
Quadratic plateau	6.4	0.40	180.0	0.006	3.2	-	y = 50.72 + 14.75x + -1.154x ² when: x < 6.4
Average	4.9	NA	NA	NA	2.9	-	NA
Ear leaf at R1							
Linear plateau	2.9	0.16	188.9	0.004	1.7	3.5	y = 70.5 + 7.556x when: x < 2.9
Quadratic plateau	4.7	0.17	188.7	0.578	2.1	-	y = 64.72 + 13.347x + -1.431x ² when: x < 4.7
Average	3.8	NA	NA	NA	1.7	-	NA

CPTC, Critical phosphorus tissue concentration (g kg^{-1}); AIC, Akaike information criterion; CI, Confidence intervals.

NA, Not applicable; - Not determined or excluded (for impracticality due to excessive size).

† p-value for the breakpoint (CPTC) of transition between linear or quadratic and the plateau.

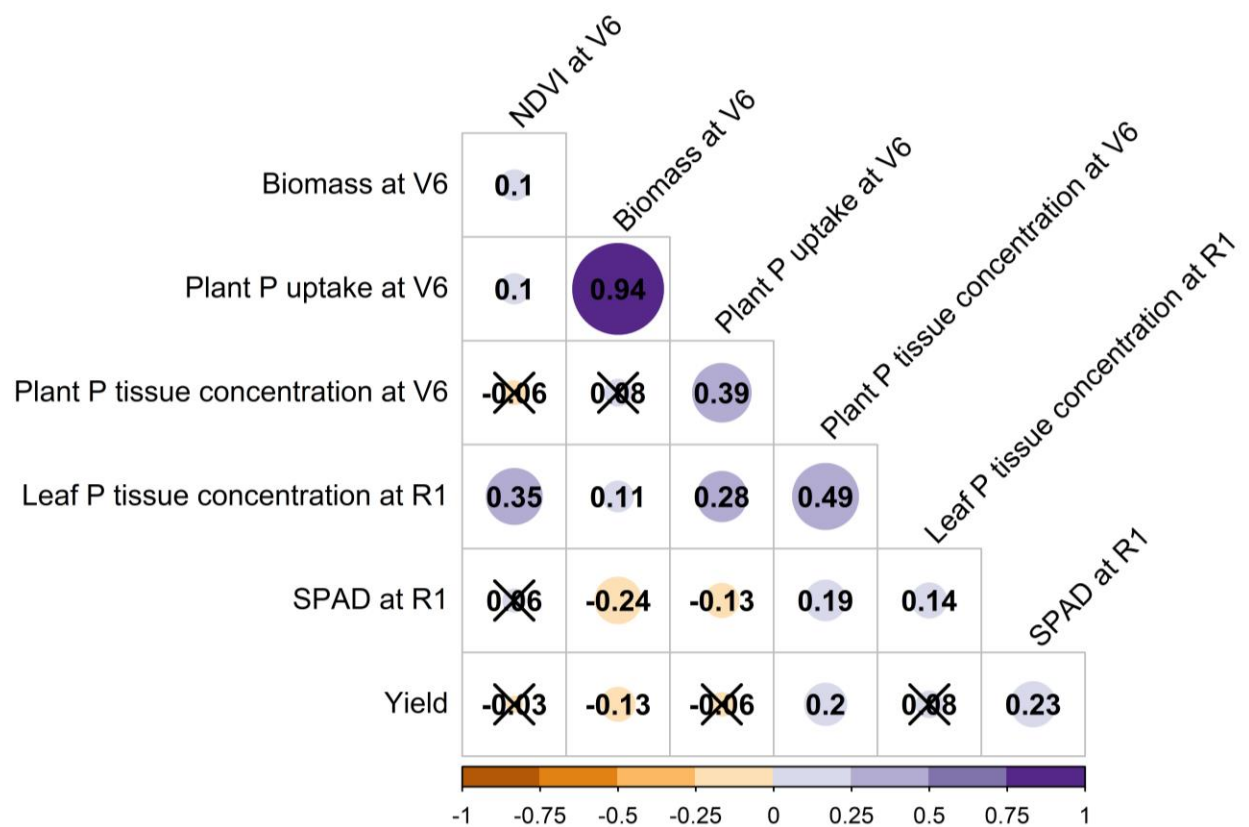


Figure 2.1. Pearson correlation matrix for biomass (g plant^{-1}), P uptake (mg plant^{-1}), NDVI, SPAD, P tissue concentrations (mg kg^{-1}) and yield (Mg kg^{-1}) ($p < 0.05$). Darker colors indicate a higher (positive or negative) correlation coefficient; non-significant correlations are indicated by an “X”.

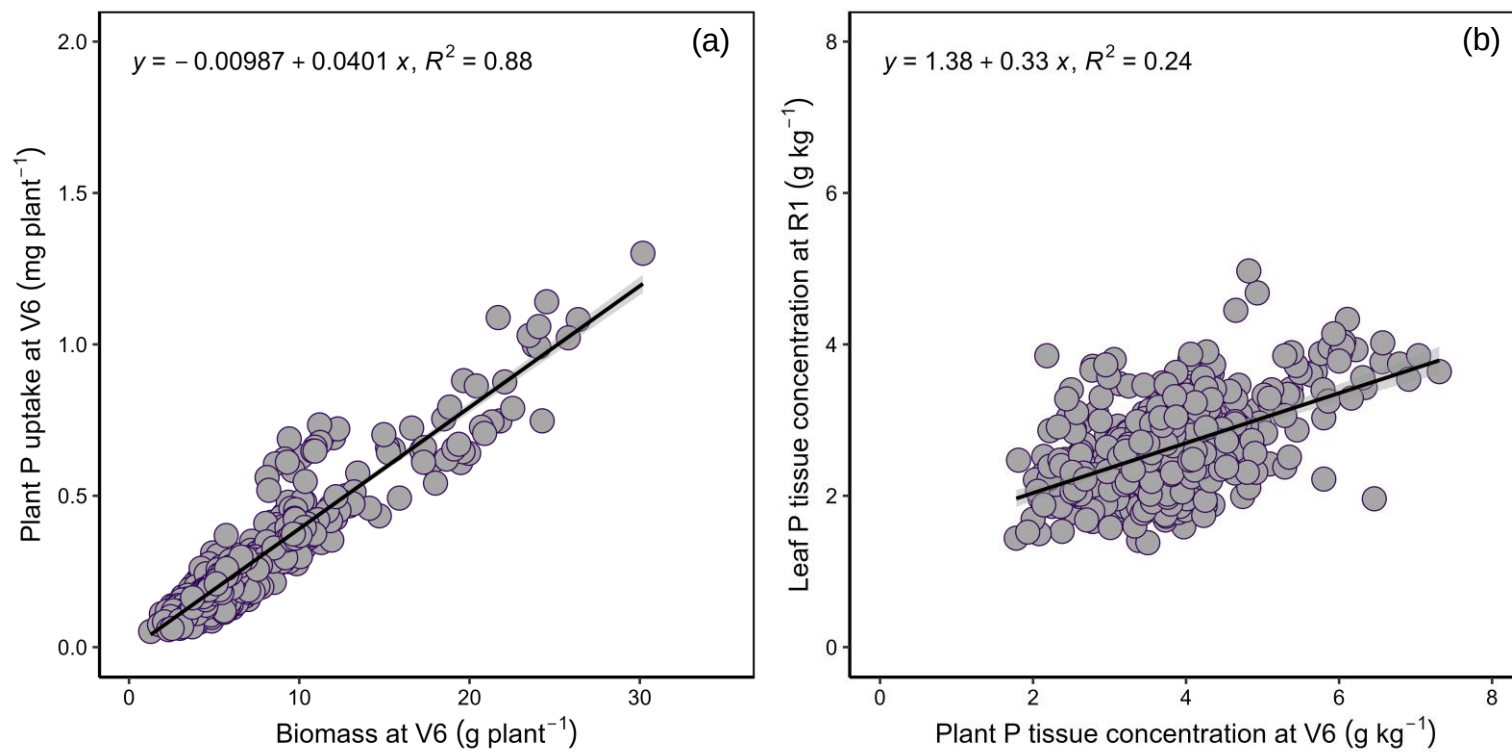


Figure 2.2. Relationship between plant P uptake at V6 and biomass at the V6 (a), and the relationship between whole plant P tissue concentration at V6 and ear leaf P tissue concentration at R1 (b).

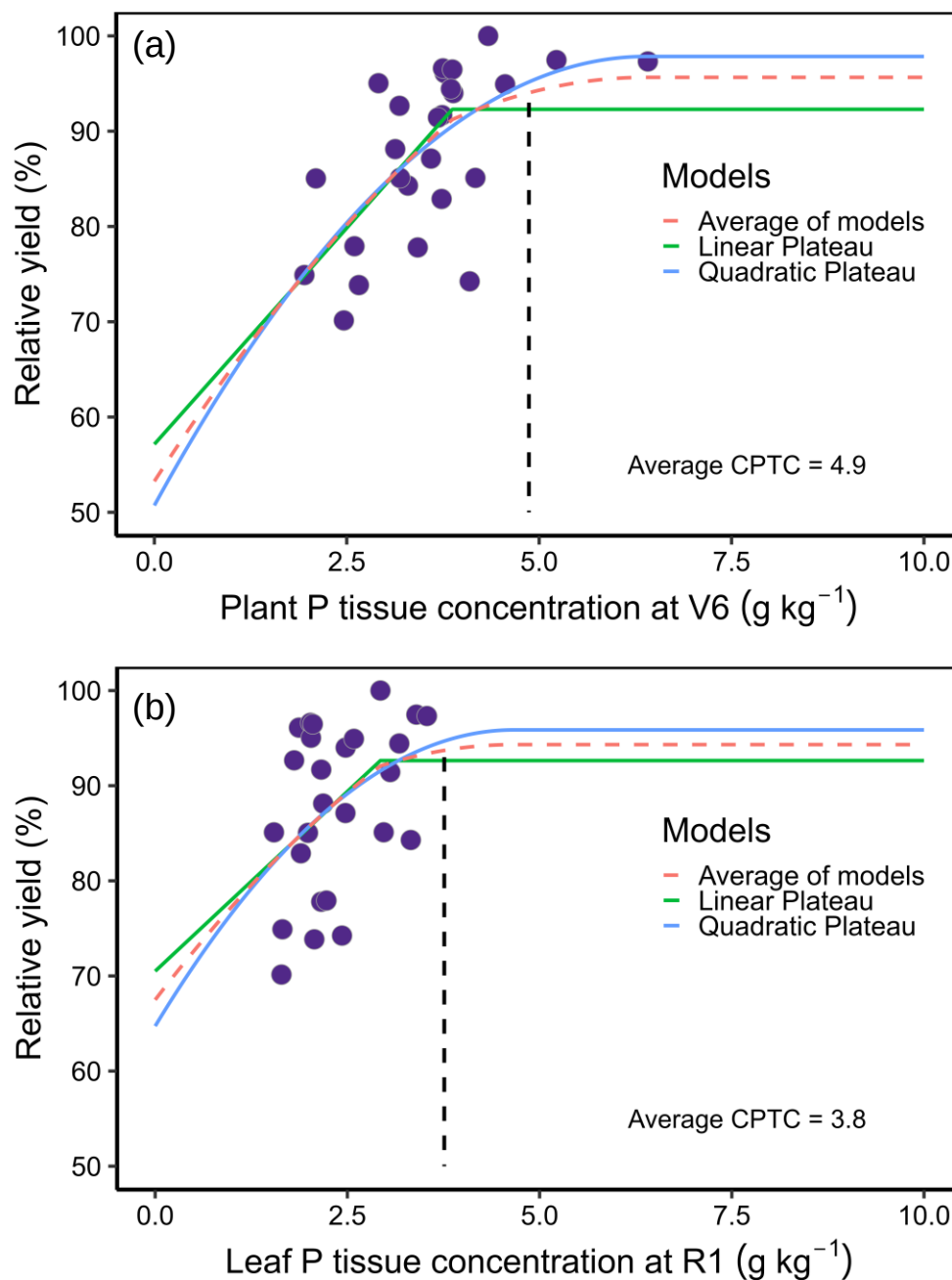


Figure 2.3. Relationship between relative yield and whole plant P tissue concentration at V6 (a) and the relationship between relative yield and ear leaf P tissue concentration at R1 (b). The vertical line indicates the average CPTC (Critical phosphorus tissue concentration) from the linear plateau and quadratic plateau models. The model and statistics are shown in table 2.3.

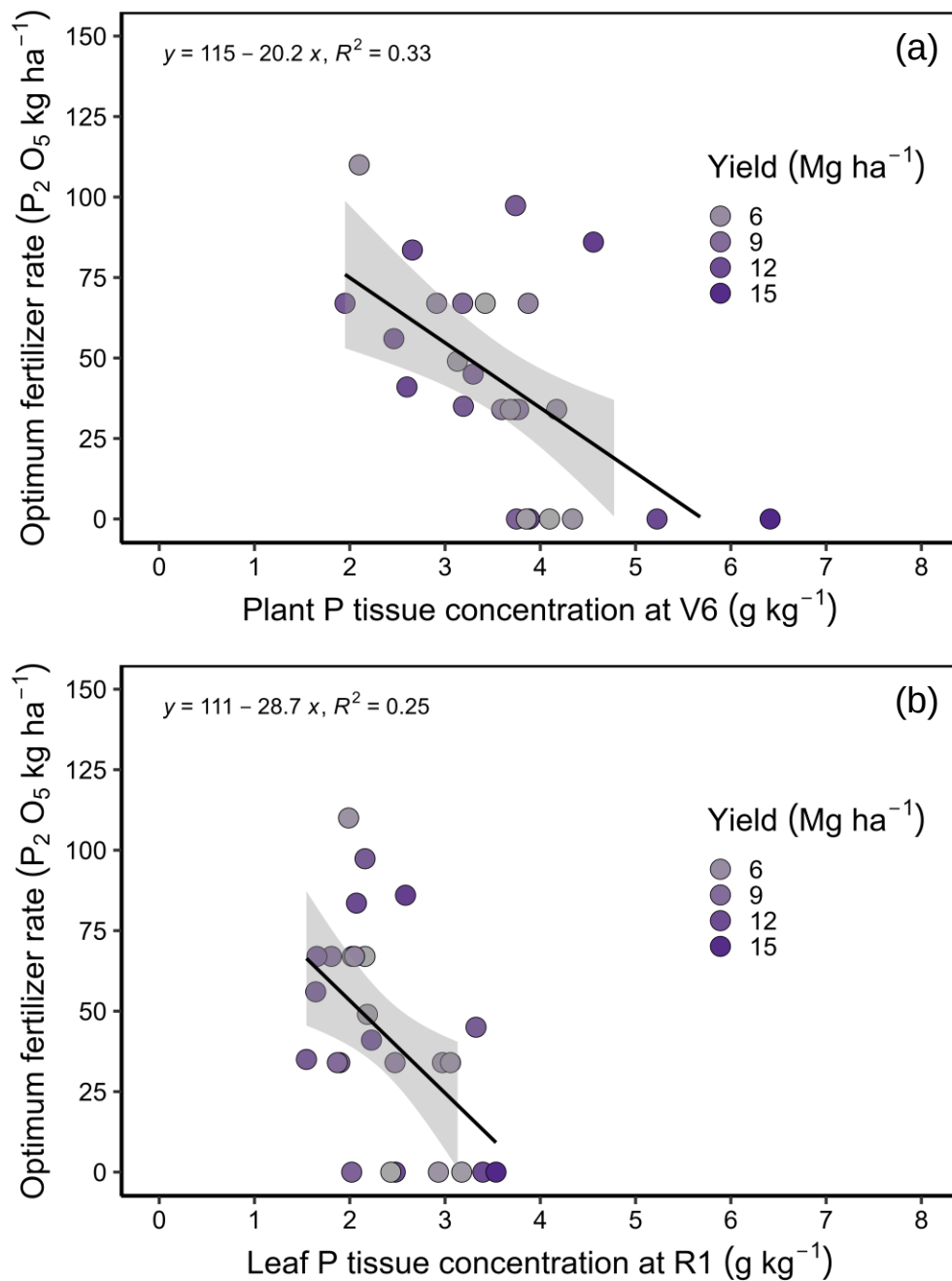


Figure 2.4. Relationship between the optimum fertilizer rate based on P response and whole plant P tissue concentration at V6 from control plots (a) and the relationship between optimum fertilizer rate based on P response and ear leaf P tissue concentration at R1 from control plots (b). The color gradient represents the yield, ranging from low (gray) to higher (purple).

Chapter 3 - Assessing Sulfur Response, Utilization Efficiency, and Diagnostic Tools for Corn

Abstract

Sulfur (S) is an essential nutrient for plant growth and development, and its deficiency has become more extensive in recent years. This study aims to evaluate corn response to sulfur fertilizer application, sulfur use efficiency (SUE) and assess different diagnostic tools (soil analysis, tissue analysis, normalized difference vegetative index (NDVI), and SPAD readings) for sulfur nutrition in corn. The study was conducted from 2021 to 2022, with field experiments carried out across 26 sites in Kansas. Soil samples were collected pre-plant. Sulfur fertilizer rates were applied at 45 kg of S ha⁻¹, plus a control without sulfur. Whole plant tissue samples and NDVI were collected at the V6 growth stage, and ear leaf tissue samples and SPAD were collected at the R1 growth stage. A whole plant biomass and tissue sample at R6 and grain yield were collected. The SUE components were then calculated as agronomic sulfur use efficiency (AE) and apparent sulfur recovery efficiency (RE). Results indicated no correlation between NDVI at V6 and S tissue concentration or yield. There is a correlation between SPAD at R1 and S tissue concentration, as well as with yield. Sulfur application increases S tissue concentration for the different stages V6, R1, R6, and grain, as well as S uptake at V6 and total S uptake across sites. A strong positive linear relationship was found between the total S uptake and yield, with an R² value of 0.72. The analysis revealed that the mean AE across all sites was 1.4 kg kg⁻¹, indicating the yield increase for each sulfur fertilizer unit applied. The RE reflects the effectiveness of S uptake, with a mean of 2.2 % across all sites. This study highlights the potential response of corn to sulfur. The correlation between different variables and AE and RE provides valuable insights into the effectiveness of sulfur utilization in Kansas.

Introduction

Sulfur is an essential nutrient for plant growth and development, playing a crucial role in various metabolic processes, including protein synthesis and enzyme activation (Narayan et al. 2023; Zenda et al. 2021). Despite its significance, sulfur deficiency in soils has become more common; there are several reasons why S deficiency appears to be becoming a more common occurrence, including reduced atmospheric S deposition, continued and increasing crop removal of S, greater use of no-tillage, and high amounts of crop residues (Camberato and Casteel 2017). Consequently, the deficiency of sulfur in agricultural soils has emerged as a limiting factor for crop production in many regions worldwide (Haneklaus, Bloem, and Schnug 2008).

Sulfur deficiency is not a recent issue in Kansas, as noted by Lamond (1997); Lamond, Whitney, and Marsh (1995). However, its increasing prevalence in recent times (Foster, Kisekka, and Golden 2017; Jaenisch et al. 2021), presents growing challenges for corn production. Addressing these challenges requires a comprehensive understanding of the effects of sulfur fertilization on corn growth and yield across various Kansas sites. Recent studies in Kansas have demonstrated the importance of sulfur fertilization in enhancing corn productivity (Husa 2021). However, the efficacy of sulfur fertilization can vary depending on soil properties, climatic conditions, and management practices (Kim, Kaiser, and Lamb 2013; Kurbondski et al. 2019).

Despite more frequent sulfur deficiency and response, prediction of the response remains a challenge because it is dynamic across space and time owing to variability in sulfate inputs from soil organic material mineralization and sulfate outputs to leaching (Mahal et al. 2022). According to several studies conducted, it has been consistently observed that the predictive capacity of soil sulfate is poor in most cases, when analyzed within a depth range of 0 to 60 cm (Franzen 2023; Pagani and Echeverría 2011; Rehm and Clapp 2008; Sawyer, Lang, and Barker

2008). This low predictive capacity can result from the spatial and temporal variability of soil sulfate (Bloem et al. 2001) or the unaccounted available S derived from mineralization during the growing season (Camberato and Casteel 2017).

On the other hand, it is generally accepted, that plant tissue analysis offers a better tool than soil testing to predict the need of S application (Pagani and Echeverría 2011; Zhao, Hawkesford, and McGrath 1999). Plant tissue analysis can reveal deficiencies in sulfur nutrition, enabling interventions that bolster the sulfur status in corn plants. Indicators of sulfur deficiency in corn include interveinal chlorosis, yellowing of younger leaves, and reddening of stems and leaves, all of which ultimately affect growth parameters and yield (Ariraman et al. 2020). Therefore, plant tissue analysis can help determine the sulfur status of corn plants and guide the application of sulfur fertilizers to optimize growth, yield, and nutritional quality (Kurbondski et al. 2019).

Other diagnostic tools include NDVI, which is a standardized measure that evaluates vegetation health by analyzing its light absorption and reflectance properties. It utilizes the near-infrared light reflected and red light absorbed by plants, and values span from -1 to +1, where values closer to +1 signify healthier and more vigorous vegetation, typically associated with better nutrient status (Glenn and Tabb 2019; Wang et al. 2017). This can be a valuable diagnostic tool for non-invasive monitoring. Studies have shown that corn with nitrogen and sulfur deficiency exhibits lower NDVI values (Henik 2012; Masoni, Ercoli, and Mariotti 1996).

Also, the SPAD provides an estimate of the chlorophyll content in plant leaves by measuring the absorbance of specific wavelengths of light. Chlorophyll content is directly related to the plant's nitrogen levels, which are interlinked with phosphorus and sulfur through metabolic pathways (Nampila et al. 2018; Uddling et al. 2007). Monitoring SPAD readings throughout the

growing season in corn has been successfully use for nitrogen (Wood et al. 1992; Bullock and Anderson 1998), with the potential use for other nutrient like sulfur.

The tissue sulfur concentration at various growth stages and in mature grain is essential for diagnosing sulfur deficiency. This information allows for a more precise understanding of sulfur uptake and utilization by the corn crop. According to Sakal et al. (2000), sulfur application elevates the average grain yield and significantly augments the total sulfur uptake. The sulfur uptake can provide valuable information on the total amount of sulfur uptake by the plant. This data can be used to determine the sulfur needs of future corn crops and optimize fertilization strategies. Research by Carciochi et al. (2020) illustrates the variability in SUE parameters like AE and RE of corn when different sulfur and nitrogen rates are applied, highlighting the interaction and influence of other nutrients.

Research on utilizing these diagnostic tools to evaluate sulfur nutrition in corn in Kansas has been limited, particularly for widespread areas throughout the state. The objectives of the study are to evaluate corn response to sulfur fertilizer application, sulfur use efficiency and assess different diagnostic tools, including soil analysis, tissue analysis, NDVI, and SPAD readings for sulfur nutrition in corn.

Materials and Methods

Sites and Experiment Design

The study was conducted from 2021 to 2022; field experiments were carried out in 26 sites throughout Kansas (Table 3.1). Different sites have management specific for each location, such as nitrogen fertilization, weed control, corn hybrid selection, crop rotation, and other factors. These methods are dependent on the region and individual farming operations. The

experimental design was a randomized complete block design with four replications. The Sulfur fertilizer rate was applied at 45 kg of S ha⁻¹, using ammonium sulfate (21-0-0-24S) as a fertilizer source and a control without sulfur fertilizer. Additionally, a uniform application of phosphorus fertilizer was applied at a rate of 101 kg of P₂O₅ ha⁻¹ (44 kg of P ha⁻¹) using mono-ammonium phosphate (MAP) (11-52-0). Nitrogen was balanced for treatments by applying 62 kg N ha⁻¹ using urea (46-0-0). All the fertilizer was applied via broadcast pre-plant. The size of each plot was 12.2 meters long and 3.1 meters wide, except for site 5, which was 10.7 meters long. All plots had a row spacing of 76.2 centimeters.

Soil Sampling and Analysis

Soil samples were collected before planting and fertilizer application as composite samples of 10 to 12 cores at 0 to 15 cm depth and 4 to 6 cores at 0 to 60 cm depth by block using a hand probe (1.9 cm diameter). The soil samples were then dried at 40 °C for five to ten days. After the samples were dried, the soil was ground to pass through a 2-mm sieve. Soil samples from 0 to 15 cm depth were used to analyze for pH 1:1 (soil:water) (Peters, Nathan, and Laboski 2012), organic matter (OM) by loss on ignition (Combs and Nathan 1998), and soil texture (particle size distribution) using a hydrometer. The soil samples from 0 to 60 cm depth were used to analyze for sulfate by the monocalcium phosphate extraction (Franzen 2015). Table (3.2).

A sample of the MAP fertilizer used in the study was collected and ground into powder using a ceramic mortar and pestle. The fertilizer powder sample were digested using the nitric perchloric acid digest method (Gieseking, Snider, and Getz 1935). The concentration of sulfur was determined by ICP-OES.

Plant Tissue, NDVI, SPAD, SUE, and Yield

During the early season, tissue samples were taken from whole plants in the V6 growth stage (range between V5 to V7) (Ciampitti, Elmore, and Lauer 2016), 6 to 10 plants were chosen from the outside rows. In the middle season, tissue sampling was done on the ear leaf in the R1 growth stage (range between VT to R2) (Ciampitti, Elmore, and Lauer 2016), for each plot, 10 ear leaf samples were collected from the center rows. During the late season, a whole plant at the R6 stage was sampled. Six plants were taken from each plot, and their ears were separated, weighed, chipped, and a subsample was taken. The tissue samples were dried in an oven at 60°C for ten to fifteen days and weighed to determine their dry biomass for V6 and R6 samples. Once dry, the samples were ground to pass through a 2-mm sieve using a Thomas Wiley Cutting Mill (Thomas Scientific, Swedesboro, NJ, USA). Next, the samples were digested using the nitric perchloric acid digest method (Gieseking, Snider, and Getz 1935). The concentration of sulfur was determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The uptake for V6 and R6 was calculated based on the concentration of sulfur and biomass.

The NDVI measurements were taken at the V6 growth stage using an active sensor RapidSCAN CS-45 handheld crop sensor (Holland Scientific, Lincoln, NE, USA). This sensor uses a wavelength of 670 nm, red-edge light at 730 nm, and near-infrared light at 780 nm. For this study, the red and near infrared wavelengths were used to calculate the NDVI for each treatment. The sensor was held 1.4 meters above the ground, and the reading was collected for the entire length of each plot. At the end of each plot, the average measurement was recorded.

The SPAD measurements were taken at the R1 growth stage using the chlorophyll meter SPAD-502 (Konica Minolta, Japan). The SPAD stands for Soil Plant Analysis Development. Based on two light-emitting diodes and a silicon photodiode receptor, this handheld device

measures leaf transmittance in the electromagnetic spectrum's red (650 nm) and infrared (940 nm) regions. The device uses these transmittance values to derive a relative SPAD meter value proportional to the amount of chlorophyll in the sample (Uddling et al. 2007). Twenty independent SPAD readings were taken around the midpoint of different ear leaves on one side of the midrib and were averaged, and the reading was collected in the center of two rows for each plot.

Grain yield was determined by harvesting the center 1.5 meters of each plot (two center rows) by hand or using a plot combine. The hand-harvested sites were done on 1.5 meters width by 6.1 meters length and threshed using Almaco LPR thresher (Almaco, Nevada, IA, USA). Grain was weighed using a digital scale Ohaus Defender 5000 Series (Ohaus Corporation, Pine Brook, NJ, USA), and a subsample from each plot was analyzed for moisture and test weight using a GAC-2500-AGRI grain analysis computer (Dickey John, Auburn, IL, USA). The sites harvested with a plot combine were made 1.5 meters in width by 11.3 meters in length (except site 5, which was 9.8 meters in length), and data on grain weight, moisture, and test weight were obtained from the combine monitor.

Sulfur use efficiency components were calculated using the agronomic sulfur use efficiency (AE) and apparent sulfur recovery efficiency (RE) described by (Fixen et al. 2015).

$$AE = \frac{Y - Y_0}{F} \quad [3.1]$$

$$RE = \frac{U - U_0}{F} \times 100 \quad [3.2]$$

Y = yield of harvested portion of crop with sulfur applied; Y₀ = yield with no sulfur applied; U = total sulfur uptake in aboveground crop biomass with sulfur applied; U₀ = sulfur uptake in aboveground crop biomass with no sulfur fertilizer applied; F = amount of sulfur fertilizer

applied. These were calculated by using the mean values for each site from the output of the linear mixed-effects model.

Statistical Analysis

For linear mixed-effects models the 'lmer' function from {lme4} R package (Bates et al. 2015) was used, and blocks were included as a random effect in the model. For analysis across sites, blocks and sites were included as random effects in the model. Linear mixed model fit by REML (restricted maximum likelihood); t-tests use Kenward-Roger's method. Statistical significance was set at $\alpha < 0.05$. The Pearson correlation matrix, was used 'cor' function from {stats} R package (R Core Team 2021) and the 'cor.mtest' function from {corrplot} R package (Wei and Simko 2021) was used with $\text{sig.level} = 0.05$. For ordinary least square linear regression analysis, the 'lm' function was used from {stats} R package; for the standardized major axis linear regression analysis, the 'sma' function from {smatr} R package (Warton et al. 2012). All statistical analyses were performed in R version 4.2 (R Core Team 2021), using RStudio version 2022.12.0+353 (RStudio Team 2019).

Results and Discussion

Correlation and Relationship for Different Parameters

The Pearson correlation analysis (Figure 3.1) shows the correlations between soil properties, plant sulfur concentration, and uptake at different growth stages. The NDVI at V6 did not show any significant correlations with the measured parameters during the V6 stage, such as biomass at V6, plant S tissue concentration at V6, and plant S uptake at V6. The significant

correlation identified was a positive association between NDVI at V6 and leaf S concentration at R1 with a correlation coefficient of $r = 0.38$.

The low or absence of correlation implies that the handheld NDVI measurements at V6 might not be directly helpful in assessing plant S or predicting yield potential. It is important to mention that studies focusing on the relationship between sulfur only and NDVI remain limited in the literature. However, Franzen et al. (2016) suggest that active-optical sensors like NDVI, typically used for nitrogen, could also help detect sulfur deficiency in corn.

Several studies have explored the potential of NDVI as a reliable predictor of crop yield. For instance, Shrestha et al. (2023) established a significant correlation ($r = 0.78$) between NDVI and corn yield. Conversely, a series of studies looking for relationships instead of correlation using handheld NDVI sensors found a low relationship. In a nitrogen study, Torino et al. (2014) found a low relationship with an R^2 value of 0.26. Similarly, Teal et al. (2006) reported an $R^2 = 0.22$. Yin and McClure (2013) found a varied relationship, with R^2 values ranging from 0.04 to 0.24 during the V6 growth stage. This generally low relationship could be due to the early stage of the crop, Maresma et al. (2020) conducted a study and their results demonstrated low relationship with an R^2 range of 0-0.07, they concluded that more accurate yield predictions for corn were obtained at the V10 growth stage or beyond. This can explain why NDVI at V6 and yield had no correlation in this study.

SPAD at R1 exhibited weak but positive correlations with several parameters like leaf S tissue concentration at R1 ($r = 0.23$), grain S uptake and total S uptake ($r = 0.20$ and 0.16 respectively), and yield ($r = 0.21$). Similarly, Kurbondski et al. (2019), found a weak correlation between SPAD and leaf S tissue concentration at a similar growth stage ($r = 0.17$). These findings suggest that SPAD measurements may provide limited information about plant S status

and performance. However, the correlations are relatively weak, indicating that SPAD alone may not be a reliable indicator of S nutrition.

Soil OM does not have a significant correlation with most parameters; however, it did positively correlate with plant S tissue concentration at R6 and grain S concentration at R6 ($r = 0.23$ and 0.16 , respectively). There is a positive correlation between soil sulfate and plant S tissue concentration at V6 ($r = 0.37$), as well as grain S concentration ($r = 0.21$), and total S uptake ($r = 0.17$). However, there is no significant correlation between OM and yield or soil sulfate and yield. A study by Kim et al. (2013), explores the relationship between OM and yield, with an R^2 of 0.57 . While our results show limited correlation between OM and yield, the positive relationship between OM and yield reported by Kim et al. (2013) underscores potential impact of soil OM on yield outcomes. However, soil sulfate and yield show no relationship, reaffirming that soil sulfate is not a good predictor of the S response (Franzen 2023; Pagani and Echeverría 2011; Sawyer, Lang, and Barker 2008).

It has been found that there is a negative correlation between the sand content in soil and the plant S tissue concentration at V6 ($r = -0.17$). The negative correlation between sand content and plant S tissue concentration at V6 indicates that higher levels of sand content in the soil are associated with lower plant S tissue concentration at V6. Additionally, a positive relationship exists between sand content and the total S uptake ($r = 0.13$) and yield ($r = 0.22$). The correlation between total S uptake and yield had a coefficient of ($r = 0.85$), which we will explore further but this strong correlation is expected due to the non-independent nature of these variables.

Response to Sulfur Fertilizer

As part of this study, a uniform rate of $101 \text{ kg of P}_2\text{O}_5 \text{ ha}^{-1}$ was applied using MAP fertilizer. It's worth noting that sulfuric acid is used during the phosphate fertilizer manufacturing process, which can result in some phosphorus fertilizers containing traces of sulfur. Our findings showed that the MAP fertilizer used in this study contained 2% $\text{SO}_4\text{-S}$, translating to a baseline of approximately 4 kg S ha^{-1} at our application rates. The application of sulfur traces with MAP fertilizer should be considered for this study as well as other field studies on sulfur fertilization.

The range of soil sulfate values in this study was between 2 and 18 mg kg^{-1} , the pH from 5.3 to 7.5, the OM range from 19 to 55 g kg^{-1} , and the sand and clay content varied from 8 to 46 % and 10 to 34% respectively (Table 3.1). Out of the 26 sites where NDVI was measured in the early season at the V6 stage, only four sites showed a statistically significant increase with S fertilizer application (Table 3.2). In terms of biomass at V6, only one site displayed a significant increase with S. However, for plant S tissue concentration at V6, 13 sites showed a significant increase with S fertilizer, while plant S uptake at V6, six sites showed a significant increase with S, and analysis across locations showed a statistically significant increase with S for plant S tissue concentration at V6 and plant S uptake at V6 (Table 3.2). Plant S tissue concentration at V6 has a mean value across sites of 2.4 mg kg^{-1} for treatment with S, and 2.1 mg kg^{-1} without S (Figure 3.2c), for plant S uptake at V6 was 0.19 and $0.17 \text{ mg plant}^{-1}$, respectively (Figure 3.2d). The sites that have significantly increased with S fertilizer in plant S tissue concentration at V6 and plant S uptake at V6, had a starting level of soil sulfate at 0 to 60 cm of 5 mg kg^{-1} or less, except for site four which had 8 mg kg^{-1} (Table 3.1).

Only one site showed a positive response in the SPAD at R1 value. On the other hand, for leaf S tissue concentration at R1, eight sites showed a statistical increase with S application

(Table 3.2). Analysis across sites demonstrated a statistically significant increase with S for leaf S concentration at R1, with 0.19 mg kg⁻¹ treatment with S fertilizer and 0.17 mg kg⁻¹ treatment without S fertilizer (Figure 3.3b); there was no statistically significant difference for S fertilizer across sites for SPAD at R1 (Table 3.2 and Figure 3.3b).

At the R6 stage, the biomass at R6 has four sites with a statistically significant increase with S fertilizer. At the same time, the plant S tissue concentration at R6 has seven sites with statistically significant increases with S fertilizer across sites. Only 3 sites showed statistically significant increases with S for plant S uptake at R6. Analysis across sites shows a statistically significant increase with S for plant S tissue concentration at R6 and plant S uptake at R6 (Table 3.2). The average plant S tissue concentration at R6 across sites was 0.9 mg kg⁻¹ for the group with S fertilizer treatment and 0.8 mg kg⁻¹ for the group without S treatment (Figure 3.4b). In terms of plant S uptake at R6, the values were 6.4 kg ha⁻¹ and 5.8 kg ha⁻¹ for the treatment with S and without S fertilizer, respectively (Figure 3.4c). No statistically significant difference for S fertilizer was found for biomass at R6 (Table 3.3 and Figure 3.4a).

Regarding the grain harvest component, only one site showed a significant increase with S for yield and grain S uptake. Specifically, site 7 was significantly increased with S application for yield, while site 13 was for grain S uptake. For grain S concentration sites 4 and 13 were significantly increased with S, and analysis across sites showed a statistically significant increase with S only for grain S concentration (Table 3.3), having 1.07 g kg⁻¹ with S and 1.04 g kg⁻¹ without S (Figure 3.5a). The total uptake, understood as the sum of the plant S uptake at R6 and grain S uptake, had sites 1, 10, 13 and 19 as significantly increased with S fertilizer application (Table 3.3).

A study by Goyal et al. (2021), found that fertilizer sulfur application did not result in a significant yield response out of 12 sites and S uptake at V6 and total S uptake varied considerably among different sites years. Other studies comparing S tissue concentration at V6 and leaf S tissue concentration at R1 showed a significant increase when comparing S fertilization to no S fertilization (Cannon et al. 2021; Steinke, Rutan, and Thurgood 2015).

However, a study conducted by Kurbondski et al. (2019) examined the significance of sulfur fertilization using different parameters. The study found that sulfur application at planting did not affect leaf S tissue concentration at R1 growth stage nor SPAD at R1 growth stage. The SPAD value range was very similar, ranging from 57.0 to 57.2, compared to our study from 55.5 to 55.7 (Figure 3.3a). The study also showed that grain yield and grain S uptake did not exhibit a significant increase. However, the grain S concentration showed a significant increase with S application, which aligns with the findings of our study (Table 3.3 and Figure 3.5a).

In a study conducted by Pagani and Echeverría (2011), during testing of the S application across different sites and years, it was observed that the concentration of S at V6, R1, and R6 varied significantly depending on the site. Similarly, our study found that some sites responded to S fertilization while others did not, and no appropriate correlation that explained it was found. This variability makes it very challenging to accurately predict the response to S fertilization.

Relationship Between Yield and Total S Uptake

In Figure 3.6a, the graph shows the relationship between yield and total sulfur uptake. The graph uses ordinary least square (OLS) regression and demonstrates the positive relationship between yield (y) and total S uptake (x). The regression equation was $y = 797 + 554x$, with an R^2 value of 0.72. However, in most cases, producers always record yield, not total S uptake. The

total S uptake is more challenging, since the sample must be collected and have the ability to dry, grind, and weigh the sample, which is more typical for research. However, we can estimate the total S uptake needs from the “expected yield”. In Figure 3.6b, the linear regression analysis is reversed, and total sulfur uptake (x) is regressed against yield (y). The resulting equation is $y = 3.42 + 0.013x$. This presents a problem because the regression analysis fails to take into account the equality between variables.

The standardized major axis (SMA) or reduced major axis regression method was utilized to estimate the relationship between two variables within the framework of allometry. This approach is used for line-fitting applications (Warton et al. 2006). The SMA regression equation was $y = -777.7 + 653.7 x$, or the derivative formula for $x = (y + 777.7) \div 653.7$. Incorporating the SMA regression by Figure 3.7 gives us a better understanding of the relationship between the two variables. SMA considers both variables equally, providing a standardized relationship perspective and allowing to solve either “y” or “x”. This approach could lead to more accurate predictions.

It has been found that the application of sulfur can significantly enhance grain yield and sulfur uptake in corn, the application of sulfur fertilizers resulted in increased yields and S uptake compared to control treatments in a long-term study (Suran et al. 2021). A study by Sakal et al. (2000) states that the application of S increased the average grain and the total S uptake from 2.58 to 9.44 kg ha⁻¹; these findings suggest that sulfur nutrition plays a crucial role in corn yield. The positive relationship between yield and total S uptake, as shown by the regressions, underscores the importance of adequate sulfur availability. The regression equations provide a quantitative measure of this relationship, offering valuable insights to estimate the need for sulfur.

Sulfur Use Efficiency

The SUE components in Figure 3.8 display the AE for 26 sites. The results shows that average AE is 1.4 kg kg^{-1} , with a 95% CI of the mean ranging from -4 to 6.7 kg kg^{-1} . The range of AE values varies significantly from -22 to 31 kg kg^{-1} . Figure 3.9 displays the RE in percentage terms. The average RE across all sites is 2.2 %, with a 95% CI of the mean ranging from 0.7 to 3.7 %. The range of RE values varies from -5.9 to 12.1 %, indicating a wide variability in AR and RE among the sites.

A study by Carciochi et al. (2020), found an average AE of 37 kg kg^{-1} using S rate of 16, 20 and 32 kg ha^{-1} , comparing our study that use a higher S rate of 45 kg ha^{-1} . The AE can vary depending on the S rate, and nitrogen rate, a study by Ahmad et al. (2016), test different S rate, in combination N rate, resulting in AE range from 14 to 46 kg kg^{-1} . In contrast, findings from Husa (2021) in Kansas indicate notably higher AE values compared to our results. Their study reported AE values ranging from 94 to 314 kg kg^{-1} across sites and suggested that AE are significantly influenced by sulfur rates of 17, 31, and 52 kg ha^{-1} .

According to Carciochi et al. (2020), an average of 9 % of RE was found using different S rates of 16, 20, and 32 kg ha^{-1} . In comparison, our study used a higher S rate of 45 kg ha^{-1} and showed a 2.2 % RE. In contrast to our results, Husa (2021) reported RE values ranging from 12% to 36%, suggesting that sulfur rates significantly influence RE values. A possible explanation for this discrepancy in our low average AE and RE across sites is that most studies tend to be conducted in fewer sites than our study. For example, Carciochi et al. (2020) and Husa (2021) both used only 5 sites, and these sites were mostly responsive sites.

Also, this variation could be due to various factors such as soil type, soil properties, weather conditions or corn varieties used. The color-coded gradient in Figure 3.7 and Figure 3.8

represents the percent of sand content, this reveals no discernible strong pattern in relation to AE or RE. This lack of a clear correlation suggests that sand content alone may not be a predominant factor influencing SUE components. To optimize sulfur application strategies, it's crucial to understand the factors contributing to differences in AE and RE. Sites with higher or lower efficiency can be examined closely to identify practices that may enhance SUE components.

Conclusions

The study examined the effects of sulfur fertilizer application on plant S concentrations and S uptake at different growth stages. The findings suggest that applying sulfur to soil can significantly increase plant sulfur concentration and uptake, the response varied across different growth stages and sites. While some sites showed significant increases in plant sulfur concentration and uptake, others did not, indicating that sulfur availability and plant response to fertilizer application can vary by site.

Notably, the application of sulfur fertilizer did not consistently lead to significant biomass or grain yield improvements across all sites. Only a few sites showed positive yield and grain S uptake responses, suggesting that other factors such as weather conditions, crop varieties or others can limit yield response to sulfur fertilization.

The study indicates that NDVI at V6 and soil sulfate levels from 0 to 60 cm depth analyzed through monocalcium phosphate extraction exhibited limited effectiveness. This suggests that these techniques may have some limitations for directly assessing sulfur nutrient needs or predicting yield response in corn. However, the SPAD at R1, tissue analysis at different growing stages and total sulfur uptake shown potential effectiveness in sulfur nutrition in corn.

AE and RE exhibit significant variability across different sites, indicating the need for site-specific sulfur management strategies. The study briefly acknowledges the presence of sulfur traces in phosphorus fertilizers, which can contribute to baseline sulfur levels in the study.

In conclusion, the study's findings underscore the complexity of sulfur dynamics in corn production, influenced by many factors. Optimizing sulfur application strategies requires a holistic understanding of site-specific conditions, encompassing soil properties, climate influences, and agricultural practices.

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Tables and figures

Table 3.1. Study sites, year, county, and soil test information from samples collected before fertilizer application. Sampling depth of 0 to 15 cm were analyzed for pH, OM, Sand, and Clay; and SO_4^{2-} at the 0 to 60 cm depth.

Site	Year	County	pH	OM	SO_4^{2-}	Sand	Clay
				g kg ⁻¹	mg kg ⁻¹	-----%-----	
1	2021	Riley	6.2	20	2	36	10
2	2021	Shawnee	7.5	19	3	46	12
3	2021	Republic	6.0	27	4	20	19
4	2021	Republic	6.5	33	8	28	15
5	2021	Brown	6.2	31	4	18	16
6	2021	Gove	6.6	27	4	21	25
7	2021	Gove	7.1	25	3	20	21
8	2021	Franklin	5.8	34	4	14	24
9	2021	Gove	6.0	31	5	21	21
10	2021	Logan	6.4	28	4	20	24
11	2021	Dickinson	6.0	35	4	22	26
12	2021	Salina	5.3	29	5	30	24
13	2022	Jewell	6.8	37	3	11	24
14	2022	Jewell	7.1	55	3	10	32
15	2022	Jewell	5.2	34	3	12	26
16	2022	Shawnee	7.0	21	2	46	11
17	2022	Franklin	6.1	36	4	12	24
18	2022	Franklin	5.7	36	4	11	27
19	2022	Reno	7.4	28	14	42	26
20	2022	Reno	6.8	32	18	31	28
21	2022	Jefferson	7.2	38	4	40	22
22	2022	Republic	6.3	35	11	14	20
23	2022	Republic	6.2	30	9	14	18
24	2022	Riley	6.4	28	4	14	28
25	2022	Smith	6.2	29	2	14	34
26	2022	Smith	5.3	30	3	8	29

OM, Organic Matter.

Table 3.2. Significance of F values for fixed effects of sulfur fertilizer on NDVI, biomass, sulfur concentration, and sulfur uptake collected at the V6 growth stage. SPAD and leaf S concentration, at the R1 growth stage.

Site	V6 Growth Stage				R1 Growth Stage	
	NDVI	Biomass	S Concentration	S Uptake	SPAD	S Concentration
----- p > F -----						
1	0.203	0.822	0.312	0.778	0.296	0.851
2	0.246	0.757	0.943	0.875	0.517	0.250
3	0.361	0.421	0.003	0.027	0.375	0.667
4	0.126	0.047	0.010	0.034	0.181	0.638
5	0.666	0.882	<0.001	0.011	0.801	0.010
6	0.513	0.517	0.420	0.637	0.569	0.390
7	0.720	0.148	0.007	0.113	0.756	0.930
8	0.628	0.839	0.309	0.567	0.293	0.473
9	0.992	0.573	0.071	0.028	0.095	0.724
10	0.382	0.416	0.020	0.364	0.375	0.437
11	0.373	0.958	0.105	0.424	0.080	0.058
12	0.326	0.793	0.227	0.941	0.072	0.018
13	0.031	0.138	0.286	0.180	0.952	0.048
14	0.255	0.894	0.184	0.751	0.799	0.649
15	0.125	0.319	0.003	0.022	0.987	0.032
16	0.840	0.613	0.003	0.163	0.612	0.380
17	0.029	0.425	0.729	0.087	0.237	<0.001
18	0.089	0.331	0.041	0.844	0.365	0.011
19	<0.001	0.242	0.922	0.865	0.579	0.636
20	0.577	0.514	0.084	0.356	0.677	0.439
21	0.539	0.452	0.349	0.961	0.029	0.036
22	0.826	0.372	0.024	0.434	0.548	0.043
23	0.822	0.408	0.008	0.045	0.903	0.858
24	0.276	0.671	0.011	0.060	0.231	0.244
25	0.015	0.064	0.003	0.143	0.922	0.068
26	0.228	0.278	0.057	0.183	0.643	0.312
Across Sites	0.273	0.735	<0.001	<0.001	0.442	<0.001

Note. The values highlighted in **bold** are less than 0.05.

Table 3.3. Significance of F values for fixed effects of sulfur fertilizer on biomass, sulfur concentration, and sulfur uptake collected at the R6 growth stage and grain S concentration, grain uptake and yield.

Site	R6 Growth Stage			Grain			Total S
	Biomass	S Con.	S Uptake	S Con.	S Uptake	Yield	Uptake
----- p > F -----							
1	0.183	0.091	0.522	0.096	0.099	0.687	0.029
2	0.767	0.215	0.887	0.920	0.582	0.925	0.760
3	0.020	0.987	0.298	0.160	0.377	0.822	0.491
4	0.581	0.778	0.359	0.002	0.139	0.298	0.077
5	0.045	0.217	0.833	0.232	0.724	0.263	0.494
6	0.042	0.944	0.248	0.676	0.689	0.172	0.385
7	0.510	0.035	0.972	0.416	0.056	0.009	0.699
8	0.120	0.003	0.063	0.344	0.145	0.487	0.158
9	0.210	0.836	0.303	0.157	0.283	0.189	0.152
10	0.151	0.046	0.102	0.635	0.178	0.156	0.012
11	0.862	0.615	0.558	0.068	0.307	0.537	0.416
12	0.277	0.248	0.269	0.821	0.642	0.469	0.699
13	0.435	0.013	0.178	<0.001	0.024	0.896	0.016
14	0.873	0.089	0.632	0.073	0.385	0.863	0.240
15	0.919	0.005	0.017	0.202	0.333	0.307	0.744
16	0.786	0.140	0.593	0.585	0.743	0.848	0.082
17	0.331	0.051	0.163	0.348	0.536	0.667	0.367
18	0.038	0.003	0.047	0.083	0.592	0.443	0.086
19	0.106	0.458	0.100	0.965	0.243	0.134	0.030
20	0.902	0.173	0.280	0.422	0.567	0.657	0.707
21	0.417	0.448	0.589	0.118	0.490	0.556	0.403
22	0.668	0.870	0.868	0.078	0.224	0.676	0.812
23	0.455	0.957	0.503	0.149	0.404	0.725	0.176
24	0.673	0.291	0.993	0.140	0.234	0.924	0.911
25	0.252	0.005	0.011	0.074	0.728	0.622	0.188
26	0.490	0.173	0.105	0.215	0.937	0.919	0.596
Across Site	0.382	<0.001	0.004	0.002	0.060	0.577	<0.001

Con., Concentration.

Note. The values highlighted in **bold** are less than 0.05.

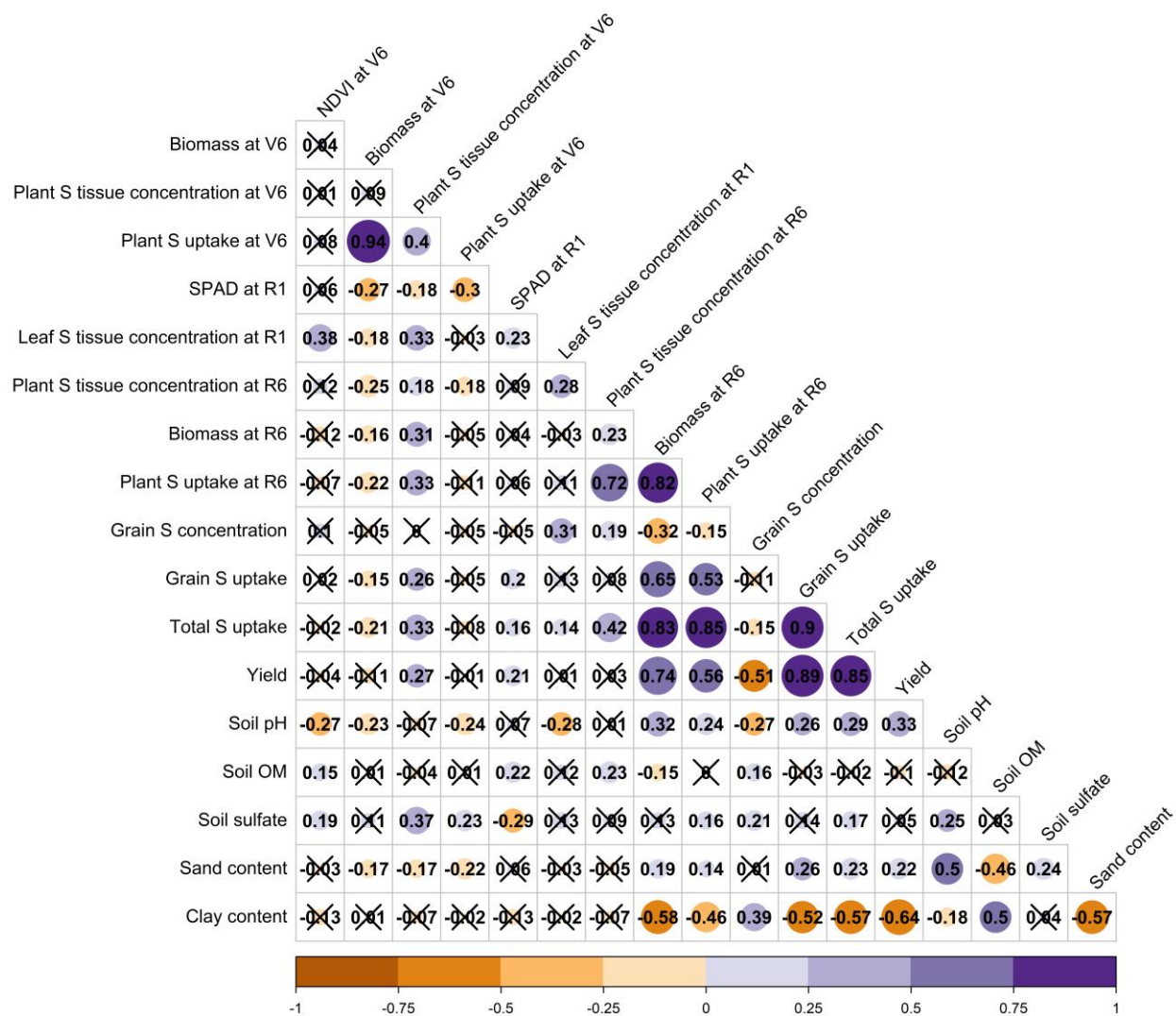


Figure 3.1. Pearson correlation matrix for different S tissue concentration, yield, and soil parameters ($p < 0.05$). Darker colors indicate a higher (positive or negative) correlation coefficient; non-significant correlations are indicated by an “X”.

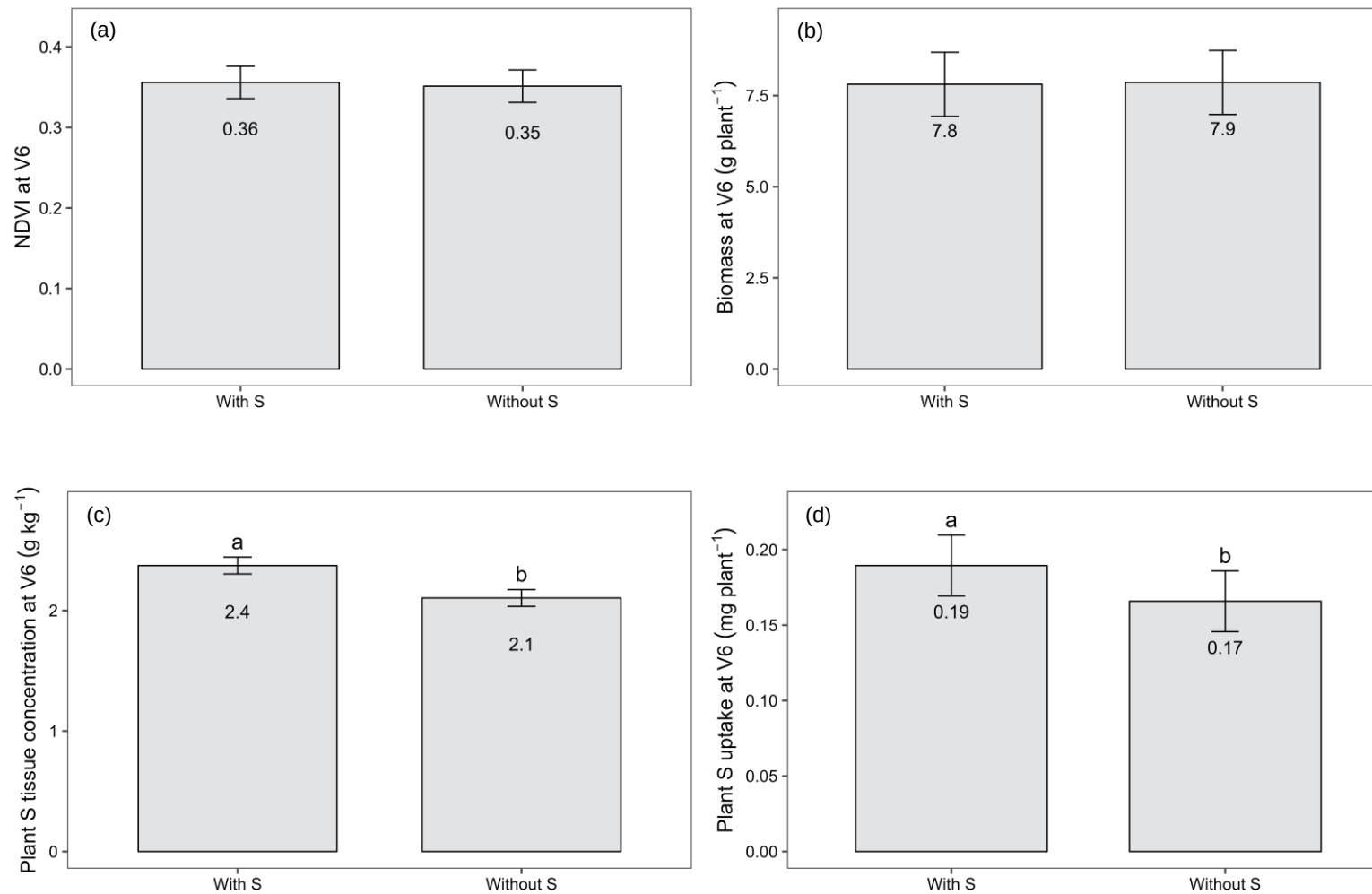


Figure 3.2. Corn at V6 growth stage response to S fertilizer and NDVI (a); S fertilizer and biomass (b); S fertilizer and plant S tissue concentration (c); S fertilizer and plant S uptake (d). Fertilizer rate application (0 and 45 kg of S ha⁻¹). The error bar stands for SE (standard error). Means with the different letters are significantly different between treatments (p<0.05).

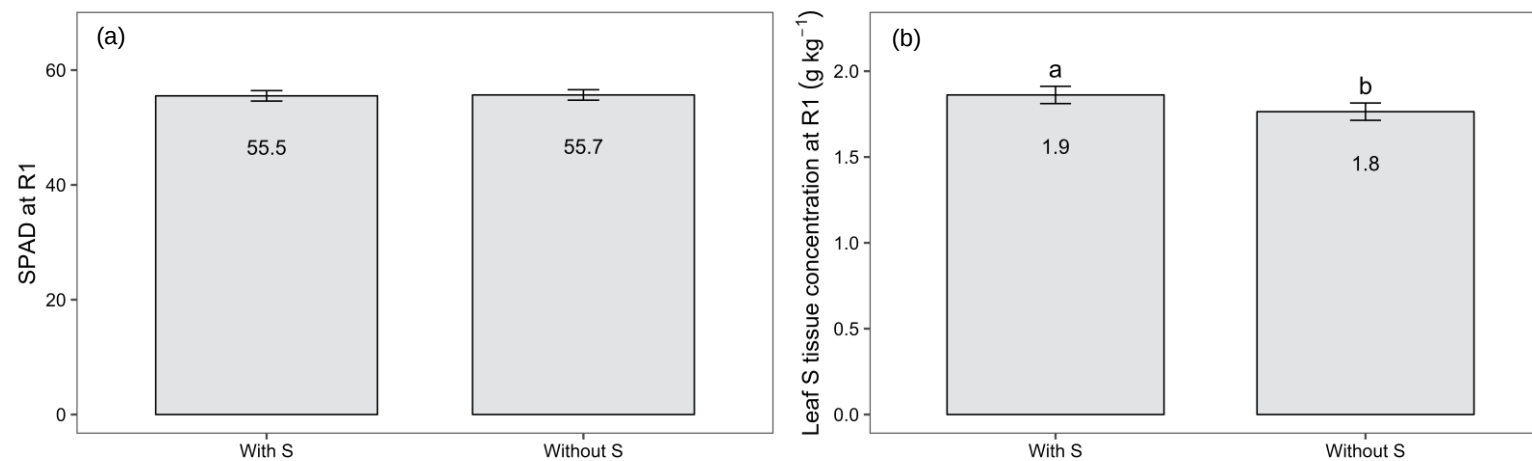


Figure 3.3. Corn at R1 growth stage response to S fertilizer and SPAD (a); S fertilizer and leaf S tissue concentration (b). Fertilizer rate application (0 and 45 kg of S ha⁻¹). The error bar stands for SE (standard error). Means with the different letter are significantly different between treatments ($p < 0.05$).

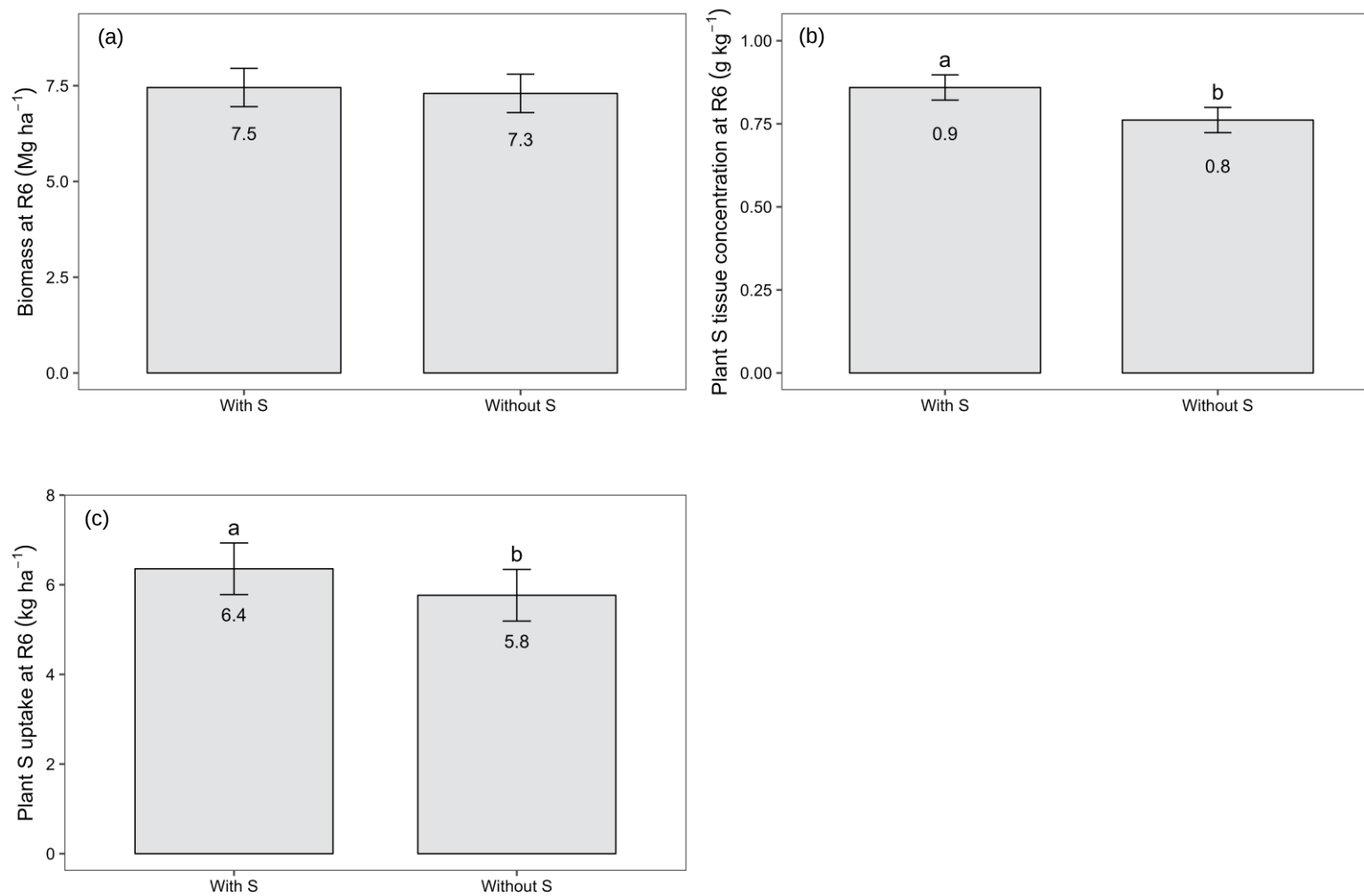


Figure 3.4. Corn at R6 growth stage response to S fertilizer and biomass (a); S fertilizer and plant S tissue concentration (b); S fertilizer and plant S uptake (c). Fertilizer rate application (0 and 45 kg of S ha⁻¹). The error bar stands for SE (standard error). Means with the different letter are significantly different between treatments ($p < 0.05$).

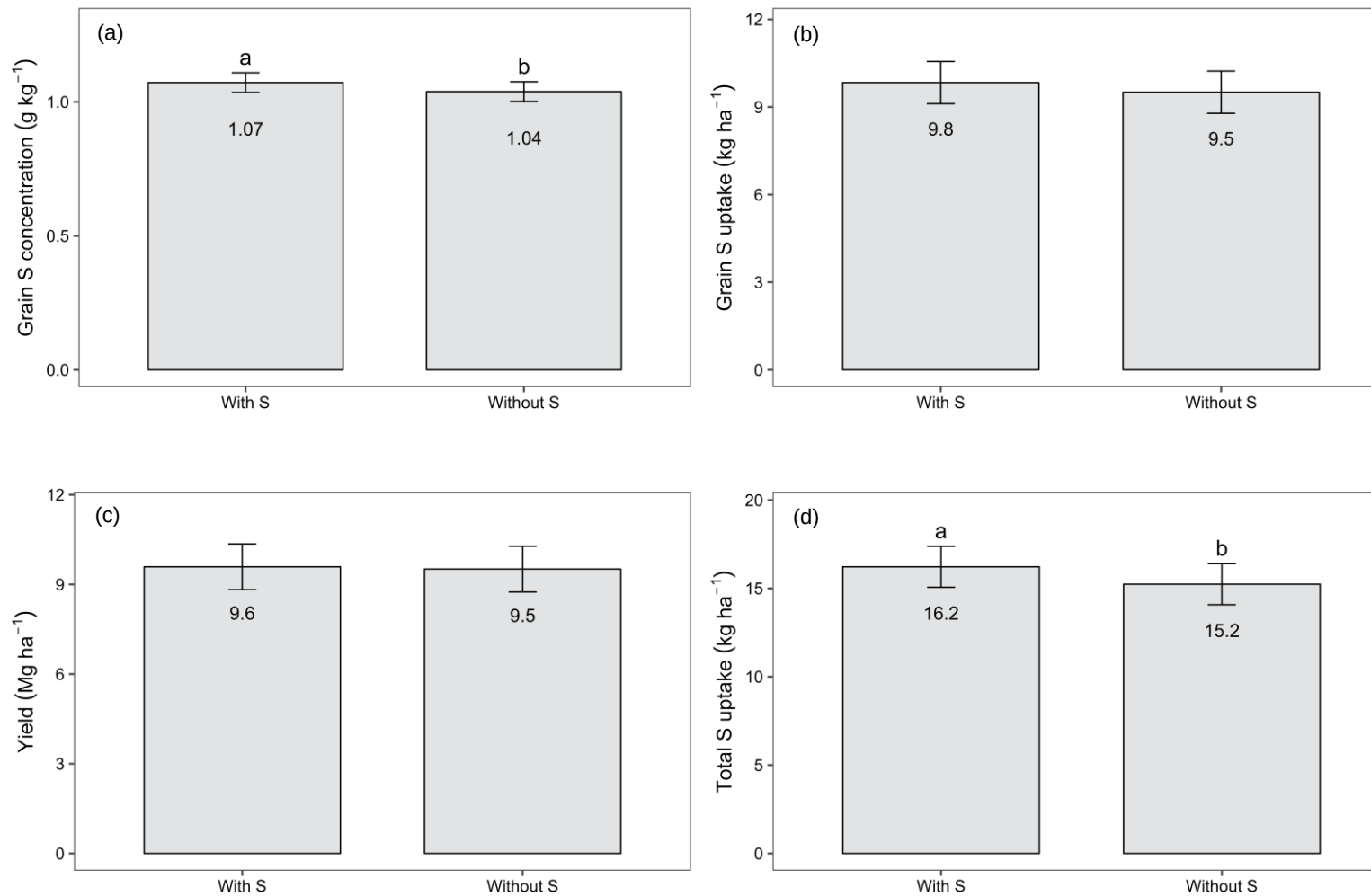


Figure 3.5. Corn response to S fertilizer and grain S concentration (a); S fertilizer and grain S uptake (b); S fertilizer and yield (c); S fertilizer and total S uptake. Fertilizer rate application (0 and 45 kg of S ha⁻¹). The error bar stands for SE (standard error). Means with the different letter are significantly different between treatments (p<0.05).

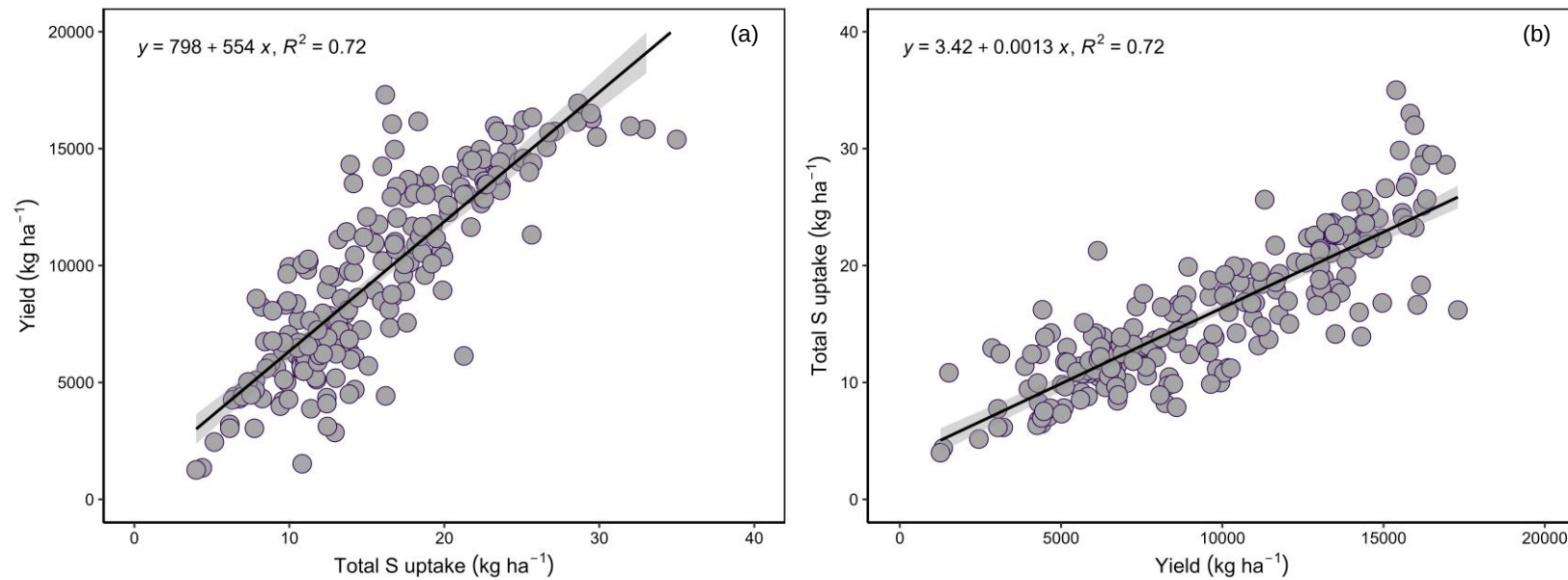


Figure 3.6. Relationship between corn yield vs total S uptake (a) and total S uptake vs yield (b) using ordinary least square (OLS) linear regression model. The OLS analysis fails to consider equality between variables. Only “y” regression formula can be used. Need two separate linear regression models.

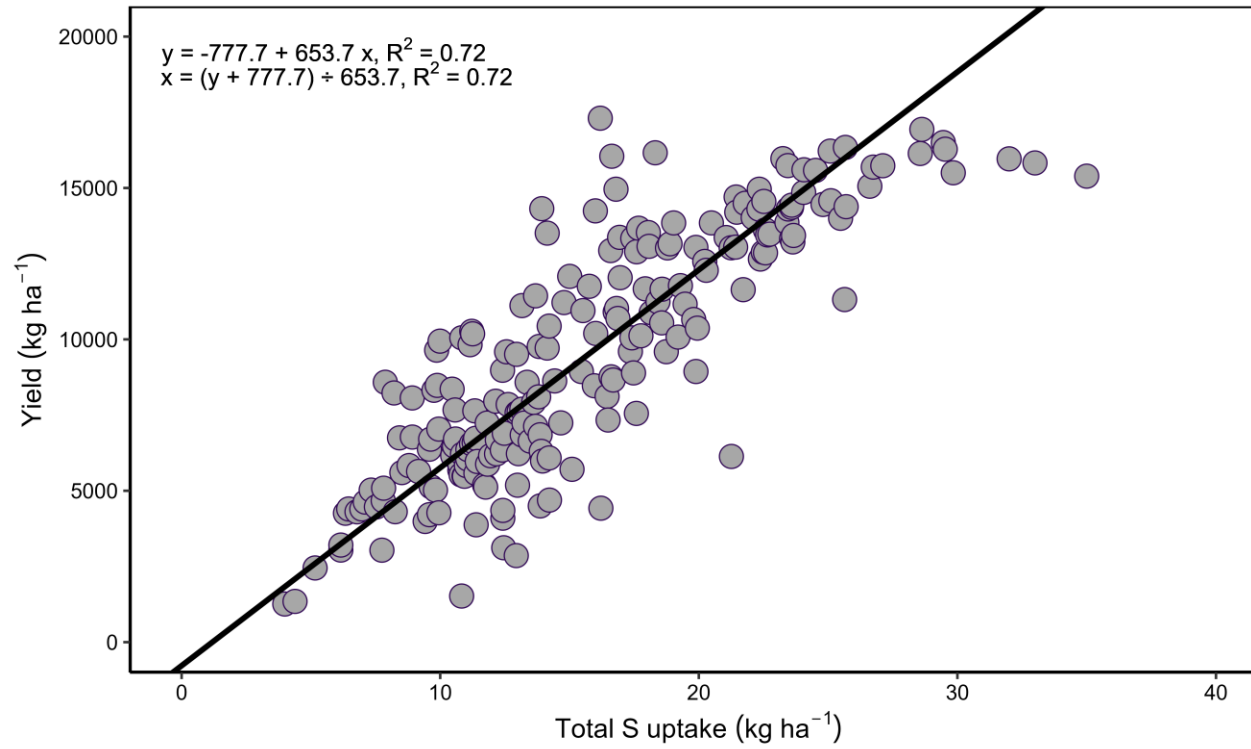


Figure 3.7. Relationship between yield and total S uptake using standardized major axis (SMA) linear regression model. The SMA estimate the relationship between two variables within the framework of allometry. Either “y” or “x” regression formula can be used. Need only one linear regression model.

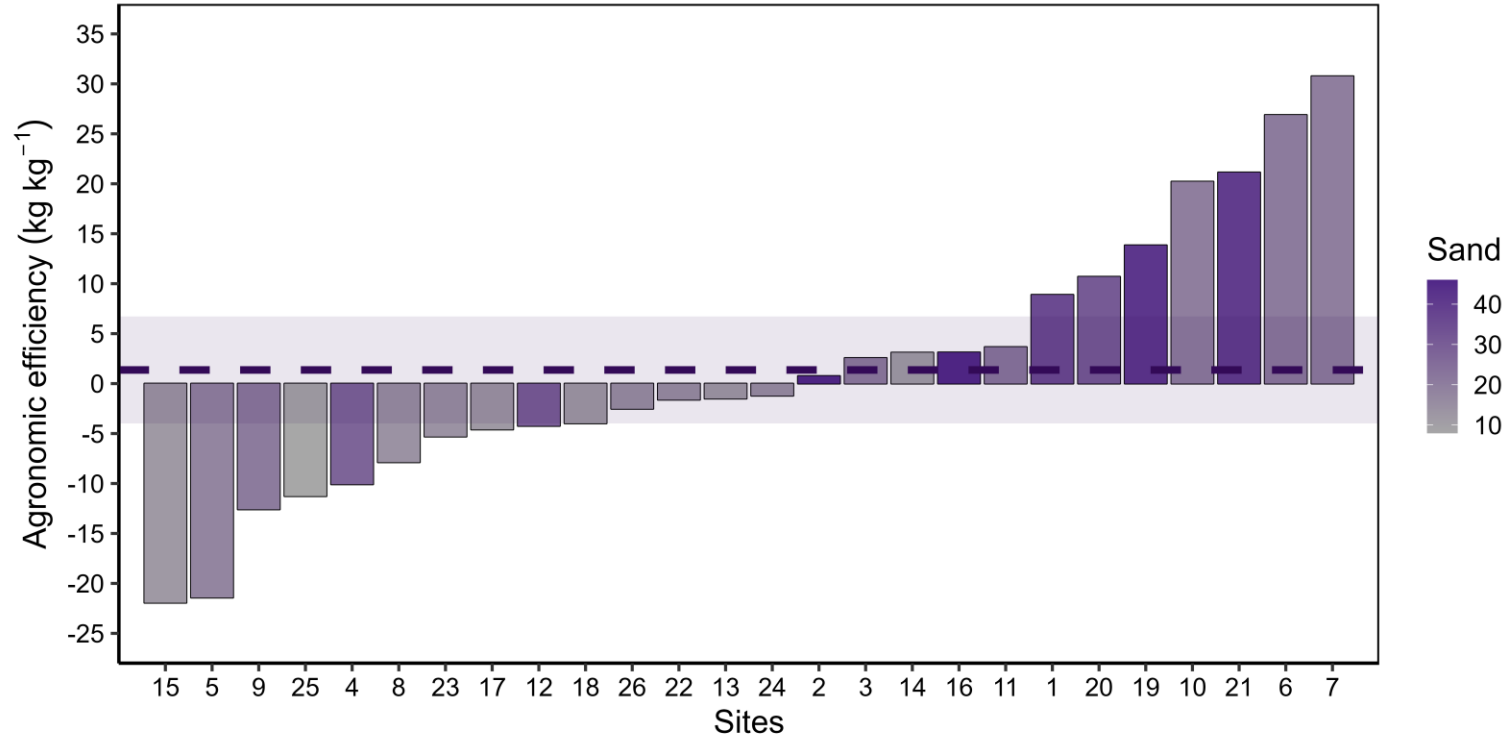


Figure 3.8. Agronomic sulfur efficiency (kg of grain per kg⁻¹ of sulfur applied), the dashed line represents the average across all sites, and the shaded area indicates a 95% CI of the mean. The color gradient represents the percentage of sand content, ranging from low (gray) to higher (purple). Sulfur fertilizer rate application (45 kg of S ha⁻¹). These values were calculated using the results from the linear mixed-effects model.

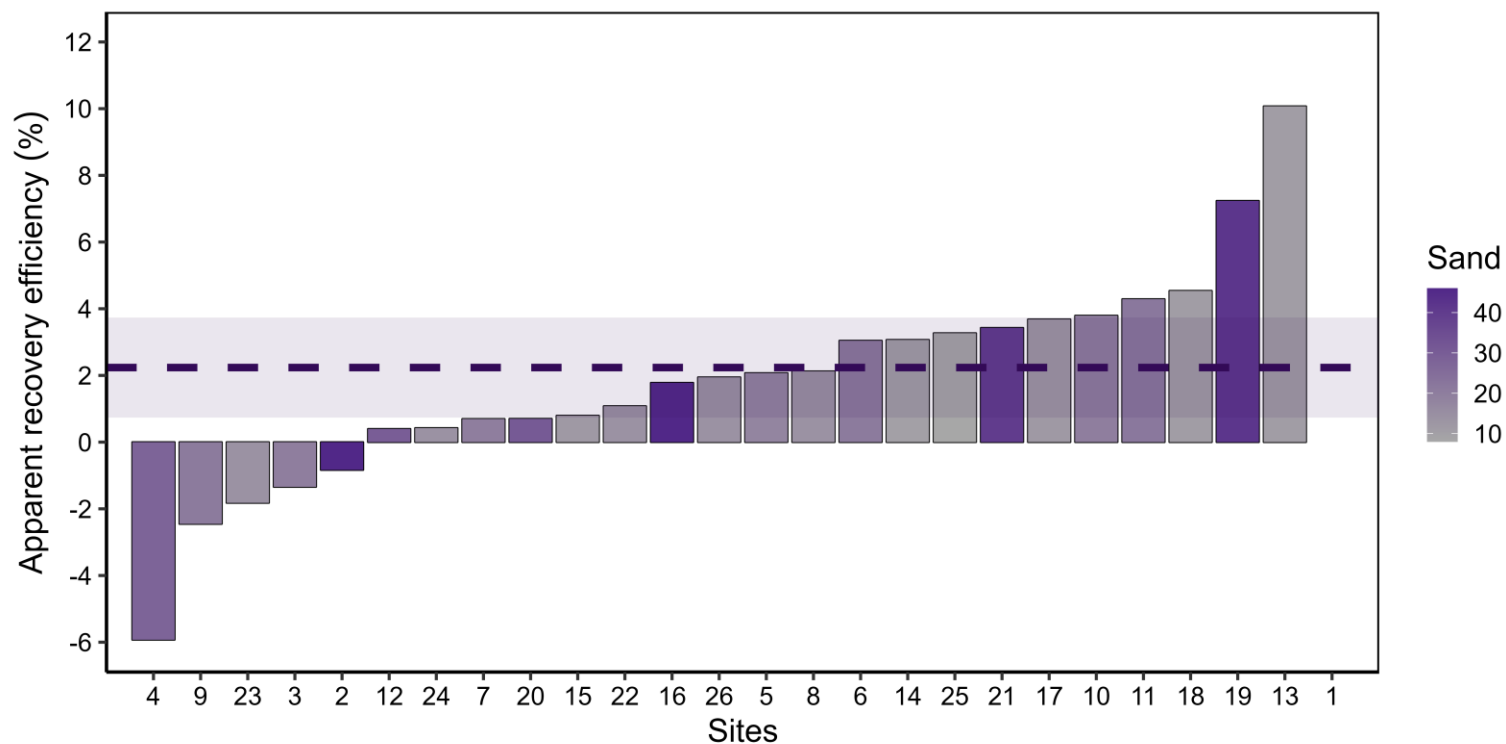


Figure 3.9. Apparent sulfur recovery efficiency (percent), the dashed line represents the average across all sites, and the shaded area indicates a 95% CI of the mean. The color gradient represents the percentage of sand content, ranging from low (gray) to higher (purple). Sulfur fertilizer rate application ($45 \text{ kg of S ha}^{-1}$). These values were calculated using the results from the linear mixed-effects model.

Chapter 4 - General Conclusions

The study on phosphorus and sulfur nutrition in corn production has provided valuable insights into nutrient status. The assessment of phosphorus tissue concentrations and diagnostic tools highlights the variability in critical P tissue concentrations across growth stages and the limited effectiveness of specific diagnostic tools, such as NDVI in evaluating phosphorus levels. The study found that the average CPTC at whole plant at V6 and ear leaf at the R1 stages were 4.9 g kg^{-1} and 3.8 g kg^{-1} , respectively, these values provide valuable insights for phosphorus management in corn.

Linear regression analyses offer insights into phosphorus fertilization calibration showing that optimum fertilizer rate decreases as the P tissue concentration increases, but the study suggests the need to incorporate additional factors to enhance the accuracy of fertilizer rate predictions. The low R^2 values suggest that other factors beyond P concentration likely influence optimal fertilizer rates recommendations. This underscores the importance of considering multifaceted interactions between nutrient availability, plant response, and environmental conditions.

The study also reveals the response of plant sulfur concentration and sulfur uptake to sulfur fertilization, influenced by various factors, including growth stage and site-specific conditions. The findings indicate that while some sites showed significant increases in plant sulfur tissue concentration and uptake in response to sulfur fertilization, others did not, highlighting the variability in sulfur dynamics across different sites.

Moreover, the inconsistent impact of sulfur fertilization on biomass and yield underscores the complexity of sulfur dynamics and the importance of considering multiple variables in optimizing sulfur application strategies. The study indicates that NDVI at V6 and soil sulfate

levels exhibited limited effectiveness. However, the SPAD at R1, tissue analysis at different growing stages and total sulfur uptake shown potential effectiveness in sulfur nutrition in corn.

Furthermore, agronomic sulfur use efficiency and apparent sulfur recovery efficiency exhibit substantial variability across sites, further emphasizing the need for tailored management strategies. These significant variability in agronomic sulfur use efficiency and apparent sulfur recovery efficiency across different sites, highlighting the importance of optimizing sulfur application rates based on site-specific conditions.

Overall, these studies provide valuable insights into optimizing phosphorus and sulfur fertilization practices in corn production, recognizing the multifaceted interactions between nutrient management and plant response. Addressing the limitations of diagnostic tools and considering the complexities of nutrient dynamics are essential steps toward developing sustainable and efficient nutrient management strategies tailored to the diverse agricultural landscape of Kansas and beyond.