

Evaluation of soil parameters and diagnostic tools to assess nitrogen and sulfur response in corn
and wheat

by

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Abstract

Sulfur fertilization is receiving more interest, with sulfur deficiencies showing up in crops including corn and wheat. Decreased atmospheric deposition and higher yields have driven the need for sulfur fertilization. Two studies were conducted to evaluate nitrogen (N) and sulfur (S) responses. The objectives of the first study were to assess the effects of nitrogen sources and sulfur rates on yield, nutrient uptake, and nutrient use efficiency terms on corn (*Zea mays* L.) production. Nitrogen and S concentrations in corn biomass and grain were evaluated, and N and S use efficiency indicators were calculated at five locations in 2019 and 2020. The experiment was set up in a randomized complete block design with seven treatments and four replications. Treatments included a control with no fertilization, three fertilizer treatments evaluating sulfur rates, and three evaluating N sources. Fertilizer treatments were balanced to 202 kg N ha⁻¹. Fertilizer treatments were sidedress applied on the soil surface before V6 growth stage. Organic matter greatly impacted nutrient use efficiency indicators, including agronomic efficiency (A.E.), apparent recovery efficiency (R.E.), and internal efficiency (I.E.). The impact of S rate and N source on yield was not statistically significant across locations, but yields at locations 1 and 2 trended higher with added sulfur fertilizer at increments of 17, 31, and 52 kg S ha⁻¹. Nitrogen fertilizer resulted in a significant increase in post-harvest soil nitrate compared to the control, but there was no significant difference between N source and S rate.

The second study focused on wheat (*Triticum aestivum* L.) response to S affected by soil characteristics. The objectives of this study were to evaluate plant uptake of S and N with added S fertilizer and the effect of soil parameters on wheat response to S. A greenhouse study was conducted utilizing 15 soils from Kansas and Nebraska. Fertilizer treatments included Urea Ammonium Nitrate (UAN) (28-0-0; N-P₂O₅-K₂O) at 0.99 mL UAN pot⁻¹ (300 kg N ha⁻¹) to all

pots, and half of those pots had micronized elemental sulfur (0-0-0-50S; N-P₂O₅-K₂O-S) at 0.19 mL (100 kg S ha⁻¹). All fertilizers were topdress applied using a metered pipet. A randomized complete block design was utilized using three replications for each N and N plus S fertilizer treatment. Pre-fertilization soil samples were analyzed for properties including Soil Organic Matter (S.O.M.), Cation Exchange Capacity (C.E.C.), pH, soil texture, phosphorous, potassium, zinc, total carbon, total nitrogen, and soil test sulfur. The average whole plant wheat sulfur uptake (WPU-S) was increased with the application of sulfur fertilizer for many soils in this study. The main soil variables determining plant response to S fertilizer were soil test phosphorus, soil organic matter, cation exchange capacity, and sand content. Higher soil test phosphorus (STP) and sand content contributed to greater plant response and uptake of sulfur from applied S fertilizer.

In contrast, higher soil organic matter and cation exchange capacity resulted in smaller plant response to applied S fertilizer. Thus, the traditional soil test S showed a low correlation with plant response in our study. These results suggest that a more accurate prediction of wheat response to S fertilizer may be accomplished considering multiple soil parameters.

Table of Contents

List of Figures	vii
List of Tables	viii
Acknowledgments.....	ix
Dedication	x
Chapter 1 - Introduction.....	1
Thesis organization	2
References.....	3
Chapter 2 - Nitrogen and sulfur use efficiency affected by fertilizer sources and sulfur fertilization in corn	5
Abstract.....	5
Introduction.....	6
Materials and methods	9
Treatments, Experiment Design, and Implementation.....	9
Field Measurements and Efficiency Calculations.....	10
Statistical Analysis.....	11
Results and discussion	12
Grain Yield.....	12
Treatment by Location Interaction.....	13
Nutrient Use Efficiency Indicators	13
Post-Harvest Soil Test Nutrients.....	15
Total S and N Uptake Regression	16
Organic Matter, Sulfur Availability, and Uptake.....	16
Conclusion	17
References.....	18
Tables and figures	24
Chapter 3 - Evaluation of soil factors determining wheat response to sulfur fertilizer	38
Abstract.....	38
Introduction.....	39
Materials and methods	41

Treatments, Experiment Design, and Implementation.....	41
Measurements and Statistical Analysis.....	42
Results and discussion	43
Total Biomass	43
Sulfur Whole Plant Uptake	43
Tissue Sulfur Analysis	44
Plant Uptake, Plant Dry Weight, and Tissue S Concentration.....	44
Correlation for Various Soil Test Parameters	45
Relative Importance of Soil Parameters on WPU-S	46
Conclusion	47
References.....	48
Tables and figures	52
Chapter 4 - General conclusions	59

List of Figures

Figure 2.1. Average corn yield response to nitrogen source and sulfur rate fertilizer treatments across all locations in Kansas. Error bars indicate standard error of the mean and mean values followed by the same letter are statistically different ($P < 0.05$).....	33
Figure 2.2. Nitrate (a), ammonium (b), and sulfate (c) concentrations from the 0-60 cm soil depth after corn harvest across all locations in Kansas. Error bars indicate standard error of the mean, and mean values followed a different letter are statistically different ($P < 0.05$).	34
Figure 2.3. Total N uptake (stover + grain) (a) and total S uptake (stover + grain) (b) relation to yield across all locations in Kansas.	35
Figure 2.4. Total S availability (profile + applied) (a) and S uptake (grain + stover) (b) across all locations in Kansas	36
Figure 2.5. Initial profile S (kg ha^{-1}) in relation to soil organic matter (%) (a) and initial profile S (kg ha^{-1}) relation to sand content (g kg^{-1}).....	37
Figure 3.1. Percent change in whole plant sulfur uptake (WPU-S), plant dry weight (DW), and tissue S concentration (Tissue S) with the application of S in wheat.	55
Figure 3.2. Pearson correlation matrix for various soil test parameters. DW, dry weight; STP, soil test phosphorus; TS total sulfur; WPU-S, whole plant sulfur uptake.	56
Figure 3.3. Relative importance of soil parameters on WPU-S ($\text{WPU-S} = -5.01 + 0.09 \text{ OM} + 0.05 \text{ STP} + 0.006 \text{ Sand} - 0.04 \text{ SO}_4$; $R^2 = 0.74$; $p\text{-value} = 0.001$).....	57
Figure 3.4. Conditional inference tree for the effects of sand, OM, STP, and soil SO_4 on the increase in whole plant S uptake with S fertilizer application.	58

List of Tables

Table 2.1. Locations, predominant soil type, precipitation, and soil analysis	24
Table 2.2. Monthly precipitation and average monthly temperature for all five site-years in Kansas.	25
Table 2.3. Treatment, Location, and Treatment by location interaction for corn response parameters by locations.....	26
Table 2.4. Yield, total N uptake, and total S uptake at five locations in Kansas and across locations.	27
Table 2.5. Sulfur use efficiency indices with added sulfur fertilizer by and across five locations in Kansas.	28
Table 2.6. Nitrogen use efficiency indices† with nitrogen fertilization alone and with added sulfur by and across five locations in Kansas.	29
Table 2.7. Nitrate (NO ₃), ammonium (NH ₄), and sulfate (SO ₄) soil test values from 0-60 cm soil depth post-harvest.	30
Table 2.8. Grain and Stover Sulfur Uptake at five locations in Kansas and across locations.	31
Table 2.9. Contrasts between S rate, N source, and Control for yield at. five locations in Kansas and across locations.	32
Table 3.1. Location information, predominant soil type, GPS coordinates, and previous crop..	52
Table 3.2. Soil test results for 15 soils across Kansas and Nebraska.....	53
Table 3.3. Sulfur whole plant uptake, plant dry weight, and Tissue Sulfur at and across 15 locations in Kansas and Nebraska.....	54

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Dedication

I dedicate this thesis to the small, family farmers. Who not only operate their farms as businesses but pursue farming with an unwavering passion. Instilled at a young age, this passion originates from working and connecting with livestock and the land. Farm life is an experience that cannot be taken away or have a monetary value assigned to it.

“I will never put my name on a product that does not have in it the best that is in me.”

—John Deere – blacksmith

Chapter 1 - Introduction

With the current volatility in corn commodity prices, producers are looking to optimize nutrient inputs to enhance returns. Until recent years, sulfur is a nutrient that has often been overlooked. Increasing crop removal due to higher yields, decreased atmospheric deposition, and greater crop residues have increased the likelihood of sulfur (S) deficiency (Camberato and Casteel, 2017). Kim et al. (2013) state that decreases in the indirect S application can be traced back to the Clean Air Act in 1970. The eastern United States saw a decrease of 80% of sulfur dioxide (SO₂) from 2005 to 2014 (National Aeronautics and Space Administration, 2015). With decreasing soil test sulfur (STS) comes the need to replenish S; however, the uptake and yield response of sulfur needs further research and is receiving increased interest from producers. Sulfur deficiencies are common in soils that have sandier texture and lower organic matter levels. Soil tests have been the standard indicator for sulfur testing; however, sulfate levels do not necessarily have a good correspondence to grain yield or critical sulfur concentrations (Steinke et al., 2015).

Nitrogen application rates play a significant role in response to sulfur application rates (Steinke et al., 2015). Fuentes-Lara et al. (2019) state that approximately 80% of sulfur and nitrogen in plant organic compounds are found in proteins where both sulfur and nitrogen are in adequate proportions. Sulfur concentrations are closely associated with nitrogen Tabatabai (1984), having an optimal N:S ratio at 16-25:1 (Steinke et al., 2015). From a whole-plant perspective, the distribution, uptake, and assimilation of sulfur need to be balanced (Hawkesford and De Kok, 2006).

Higher nitrogen fertilizer application rates tend to increase sulfur use efficiency, and increased application rates tend to improve nitrogen use efficiency. Further, Carciochi et al.

(2020) reported a 42% increase in sulfur use efficiency with increased nitrogen fertilization across all sites. In contrast, the interaction of both nitrogen and sulfur resulted in an increase in nitrogen use efficiency and sulfur use efficiency; Biswas and Ma (2016) found an increase in N application resulted in not only a decrease in N-uptake efficiency but a decrease in agronomic N use efficiency as well. Except for the nitrogen harvest index, Wortmann et al., (2011) noted decreases in internal efficiency, biomass efficiency, agronomic efficiency, recovery efficiency, partial factor productivity, physiological efficiency, total nitrogen in the aboveground biomass at physiological maturity, and residual soil nitrate when nitrogen rate was increased. Xu et al., (2015) found management practices, such as one-time applications of fertilizer and high nitrogen application rates, decreased nitrogen use efficiency. Gao et al., (2012) suggested that the yield ceilings need to be increased to simultaneously achieve both high nitrogen use efficiency and high yields. Timsina et al., (2001) noted different factors, such as water availability, can contribute to variations in both sulfur use efficiency and nitrogen use efficiency.

Thesis organization

This thesis is organized into four chapters. The first chapter is an introduction that provides an overview of the material covered in this thesis. The second chapter, “Fertilizer source and rate impact on sulfur and nitrogen use efficiency in corn,” investigates the impact of the rate of sulfur and the source of nitrogen on nutrient use efficiency indicators in corn for two growing seasons. The experiments were conducted at two locations in North Central Kansas and three locations in Northeast Kansas. The third chapter, “Analysis of sulfur mineralization and the uptake of nitrogen and sulfur in winter wheat,” analyzes the mineralization of micronized elemental sulfur and the impact it has on the uptake of sulfur, nitrogen, and total

carbon in the plant. Chapter four is a general conclusion and summary of the results of the research conducted in this thesis.

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Chapter 2 - Nitrogen and sulfur use efficiency affected by fertilizer sources and sulfur fertilization in corn

Abstract

With sulfur deficiencies being found throughout Kansas, the evaluation of nitrogen and sulfur fertilization on plant uptake, yield response, and nutrient use efficiency indicators are vital to optimize corn (*Zea mays* L.) production. The objectives of this study were to evaluate the effects of nitrogen sources and sulfur rates on yield, nutrient uptake, and nutrient use efficiency indicators in corn. Nutrient concentrations in corn, biomass, and grain were evaluated, and nitrogen and sulfur use efficiency indicators were calculated at five locations in 2019 and 2020. Seven treatments were assessed in 2019 and 2020, including control with no sulfur and no nitrogen, three with different nitrogen sources, and three fertilizer treatments evaluating sulfur rates of (17, 31, and 52, kg S ha⁻¹). All treatments, except for the control, were balanced to 202 kg N ha⁻¹. Fertilizer treatments were sidedress applied on the soil surface before V6 growth stage. Whole corn plant biomass and grain samples were taken at R6 physiological maturity, dried, and ground to pass through a 2 mm sieve and analyzed for nitrogen and sulfur concentrations. Corn was harvested utilizing a plot combine at four of the five locations, and one location (location 3) was hand-harvested from the twenty feet of the center, two rows. Grain weight, moisture, and test weight were recorded, and yield was calculated, and moisture was corrected to 15.5% moisture. Locations with different soil organic matter greatly impacted nutrient use efficiency indicators, including agronomic efficiency (AE), apparent recovery efficiency (RE), and internal efficiency (IE). The impact of N (Nitrogen) source and added sulfur (S) on yield were not statistically significant across locations. Nitrogen fertilizer provided

a statistically significant increase in soil test nitrate compared to the control, but there was no significant difference between N source and S rate.

Introduction

Corn is one of the most important crops in Kansas, with over 6.10 million acres planted in 2020 alone (United States Department of Agriculture, 2020). Nitrogen and sulfur are two critical nutrients for corn, and understanding the dynamics between these two nutrients is imperative for optimizing production in Kansas. Increased nutrient removal by higher-yielding hybrids and reduced atmospheric deposition of sulfur have led to sulfur deficiencies (Steinke et al., 2015). Due to these deficiencies, sulfur amendments to the soil have received much attention. In addition, there is further interest in understanding how nitrogen, with a supply of sulfur, impacts yield, nutrient use efficiency indicators, and the uptake of nitrogen and sulfur in the plant. Producers are interested in the uptake and yield response from sulfur applied to corn; hence further research is needed to understand these parameters. Not all fields respond to sulfur application, but sulfur application response occurred in fields with loam, silt loam, fine sandy loam, loamy fine sand, and sandy loam textural classes (Sawyer et al., 2011).

Sulfur is available for plant uptake from multiple combinations of sulfur; however, most sulfur is taken up in the sulfate form dissolved in soil solution (Fuentes-Lara et al., 2019). Plants require sulfur for the synthesis of proteins and amino acids, certain coenzymes and vitamins, physiologically and structurally linkages, and for the activation of certain enzymes (Tabatabai, 1984). A close relationship between sulfur and nitrogen exists in corn regarding uptake, reduction, and assimilation (Tabatabai, 1984). Variation in the pools of nitrogen and sulfur often results in a disproportion of nitrogen and sulfur in the plant (Bouranis et al., 2008).

Uptake, distribution, and assimilation need to be coordinated and balanced in the entire plant. (Hawkeford and De Kok, 2006).

Moreover, the assimilation of S within corn is highly coordinated with the assimilation of nitrogen and carbon (Fuentes-Lara et al., 2019). Sulfur is taken up by the roots as sulfate but must be further reduced to sulfide before it is metabolized (Bimbraw, 2008). Additionally, sulfur is stored in the vacuoles within the plant from which it is distributed to the rest of the plant (Fuentes-Lara et al. 2019). The effectiveness of the mobilization of the vacuolar sulfur pools vary but reflects on the remobilization processes in accordance with growth rate (Bimbraw, 2008).

The plant requirement and demand for sulfur are closely related to nitrogen supply (Reuveny et al., 1980). Corn typically responds to nitrogen fertilizers more so than sulfur fertilizers. However, Pagani et al. (2012) noted that sulfur yield responses are generally found more often in sandier soils. Low sulfur content in sandy soils can be attributed to high leaching rates due to the sulfate failing to bind to elements like iron in the soil (Camberato and Casteel, 2017). In soils with less sand and higher organic matter, sulfur mineralization is expected to increase.

Nitrogen use efficiency and sulfur use efficiency tend to increase with the combined application of nitrogen and sulfur (Fismes et al., 2000). Contrastingly, a later study done by Wortmann et al. (2011) found that an increased N application rate decreased nitrogen use efficiency. Even though increased N fertilization rate lowered N use efficiency, increased S application improves N use efficiency and increased N application increases S use efficiency (Carciochi et al., 2020). Sulfur fertilization increased NUE by approximately 13% and at one location, Carciochi et al.,(2020) found an increase of 69% in SUE due to the application of N.

The combined application of nitrogen and sulfur resulted in increased biological NUE and SUE (Qahar & Ahmad, 2016) in corn, where yields increased numerically from 19783 kg ha⁻¹ with the control to 20038 kg ha⁻¹ with the 60 kg S ha⁻¹ application rate. However, Rasheed et al., (2004) found that nitrogen and sulfur applications significantly increased dry weight per plant at tasseling as well as grain number per ear. The increase was attributed to grain number per row and an overall increase in cob length. Pagani et al. (2012) found no significant interaction between nitrogen and sulfur accumulation in any of their sampling data for sulfur accumulation in grain; however, they found that an increased rate of nitrogen increased the sulfur accumulation in grain. Moreover, Pagani et al. (2012) found that nitrogen had the statistically significant effect of increasing corn grain yield; however, sulfur application was not significant.

Moreover, Qahar & Ahmad (2016) also found that sulfur levels did not significantly impact grain yield. Pagani et al. (2012) attribute the response or lack of response to sulfur fertilizer to the sulfur status of the soil, amount of available nitrogen, and amount of precipitation. Although sulfur did not provide a significant response, varying nitrogen rates proved to be statistically significant in yield response in corn (Pagani et al., 2012; Qahar & Ahmad, 2016). N and S are imperative to biological functions in corn and must be both adequate to support optimum growth. The objective of this study was to assess the effects of nitrogen sources and sulfur rates on yield, nutrient uptake, and nutrient use efficiency indicators on corn production in Kansas.

Materials and methods

Treatments, Experiment Design, and Implementation

Five locations (Table 2.1) were established (two in 2019 and three in 2020), including three sites under irrigation and two sites under rain-fed conditions (Table 2.1). Rainfall amounts and average temperature for all five site-years are located in (Table 2.1) and monthly precipitation and average monthly temperature are given in (Table 2.2). A linear sprinkler irrigation system was used at all locations. Locations 1 and 5 received supplemental irrigation six times during the 2019 and 2020 growing seasons for a total application rate of 122 mm and 106 mm, respectively, and location 2 utilized irrigation three times, applying 95.2 mm. Plot size was 12 m in length by 3 m in width (36 m²) for all locations in 2019 and 2020 except for Location 1, which was 10 m in length by 3 m in width (30 m²). Row spacing was 76 cm for all the locations. Seven treatments were evaluated, including a control with no sulfur and no nitrogen, three nitrogen fertilizer sources, and three sulfur fertilizer treatments evaluating sulfur rates of 17, 31, and 52 kg S ha⁻¹ set up in a randomized complete block design. All treatments, except for the control, were balanced to 202 kg N ha⁻¹. Granulated fertilizers were broadcast by hand while liquid fertilizers were applied using a backpack sprayer with streamer nozzles spaced at 76 cm on a 1.53 m boom at the V3-V4 growth stage. The nitrogen sources included Urea Ammonium Nitrate (UAN) (28-0-0; N-P₂O₅-K₂O), Urea (46-0-0; N-P₂O₅-K₂O), and Calcium Ammonium Nitrate (CAN) (15.5-0-0; N-P₂O₅-K₂O). Sulfur sources included Ammonium Thiosulfate (ATS)(12-0-0-26s; N-P₂O₅-K₂O-S) plus UAN for the 17 kg S ha⁻¹ treatment, Urea Calcium Sulfate (UCS33) (33-0-0-5s; N-P₂O₅-K₂O-S) for the 31 kg S ha⁻¹ treatment, and Urea Calcium Sulfate (UCS27) (27-0-0-7s; N-P₂O₅-K₂O-S) for the 52 kg S ha⁻¹ treatment. UAN was used as the N source to balance ATS, while UCS27 and UCS33 were

balanced to 202 kg N ha⁻¹ utilizing each respective fertilizer. The N fertilizer sources did not receive any S fertilizer application, serving as sulfur control treatments.

Field Measurements and Efficiency Calculations

Composite soil samples (12 cores, 1.9 cm in diameter) from 0-15 cm and ten cores from 0-60 cm depth were collected from each replication prior to fertilization. Soil samples were dried in an oven at 40°C for one week and ground to pass through a 2 mm sieve. The 0-15 cm samples were analyzed for pH using a 1:1 (soil:water) method (Watson and Brown, 1998), organic matter by loss on ignition (Combs and Nathan, 1998), soil test P with the Mehlich-3 extraction (Frank et al., 1998), K by the ammonium acetate extraction (Warncke and Brown, 1998), and Fe, Cu, Zn, and Mn by use of DTPA extraction (Whitney, 1998). The summation method was utilized to measure cation exchange capacity (C.E.C) (Warncke and Brown, 1998). Soil texture was analyzed using a hydrometer (Gee and Bauder, 1986). 0-15 cm soil analyses can be found in (Table 2.1) and (Appendix A). The samples collected at 0-60 cm were analyzed for NO₃-N and NH₄-N using the KCL extraction (Gelderman and Beegle, 1998), and SO₄-S using a monocalcium phosphate extraction (Combs et al., 1998) and determined with an Inductively Coupled Plasma (ICP) spectrometer.

Six corn plants (three from each border row), as well as grain samples from the ears on each plant, were gathered from each plot at physiological maturity in the corn crop to attain whole plant biomass. Whole plant samples were collected, weighed, and dried at 60°C and then reweighed to attain dry matter content. Dried whole-plant biomass samples and grain samples were ground to pass through a 2 mm sieve, and then analyzed for total N by dry combustion using a LECO C:N analyzer as described by (Thomas et al., 1967). Sulfur analysis was performed using the nitric perchloric digest Gieseking et al., (1935) and determined with an

Inductively Coupled Plasma (ICP) spectrometer. At location 3 in 2020 (Table 2.1), corn was hand-harvested from twenty feet of the center two rows. Grain weight, moisture, and test weight were calculated at the other locations using the plot combine. Grain yield was calculated and corrected to 15.5% moisture. After corn harvest, soil samples (6 cores per plot at a depth of 0-60 cm per core) were collected and dried in an oven at 40°C and ground to pass through a 2 mm sieve. Nitrate and ammonium were measured utilizing a 1N KCl extraction (Gelderman and Beegle, 1998), and sulfur was measured by a monocalcium phosphate extraction (Combs et al., 1998). Efficiencies of N and S use were calculated according to the following equations proposed by (Dobermann, 2007): Partial factor productivity (PFP; kg grain / kg nutrient) = G_y / N_a , Agronomic efficiency (AE; kg grain / kg nutrient) = $(G_y - G_{yu}) / N_a$, Recovery efficiency (RE; %) = $(U - U_0) / N_a$, and Internal efficiency (IE; kg/kg) = G_y / U . Where G_y = grain yield with nutrient applied; N_a = amount of nutrient applied; G_{yu} = grain yield without nutrient applied; U = total nutrient uptake in aboveground crop biomass with nutrient applied; U_0 = nutrient uptake in aboveground crop biomass with no nutrient applied.

Statistical Analysis

All statistical analyses were completed in SAS using the generalized linear mixed model GLIMMIX (SAS Institute, 2013) procedure for analysis of variance (ANOVA). For analysis across locations, location was considered as a random effect in the model, with replication nested within location. For analysis by location, replication was included as a random effect. Single-degree of freedom contrasts were performed to compare specific treatments including UAN vs. UAN + ATS, N source vs. control, and N source vs. S application.

Results and discussion

Grain Yield

Grain yield for all site years and locations is displayed in (Table 2.4). There was a statistically significant difference between the control and baseline nitrogen source applications of UAN, CAN, and Urea for all sites (Table 2.4). Location 1 exhibited a marginal yield increase with each level increase in sulfur application (Table 2.4). This likely resulted from the extremely low concentration of sulfur in the pre-plant soil test analysis (Table 2.1) and the high sand, low clay content, and low organic matter (Table 2.1). Similarly, Carciochi et al. (2019) observed grain yield and aboveground biomass responses in sandy soil texture, low organic matter, and soil S below 31 to 49 kg S ha⁻¹ as proposed by (Carciochi et al., 2016). Location 4 did not exhibit a statistically significant difference among fertilizer treatments by N source or by S rate except over the control (Table 2.4). This is likely due to the elevated level of organic matter in the soil increasing mineralization of both S and N compared to the other locations (Table 2.1). This parallels the findings of a previous study by Sawyer et al. (2011), who had only 14% of their locations respond to sulfur fertilization in silty clay loam and clay loam soils.

Our results were similar to those of (Nibau et al., 2008), who reported optimal yield and growth in soils with higher concentrations of nitrate, ammonium, and sulfur from the pre-plant soil analysis (Table 2.1), which likely resulted in enhanced root growth and greater exploration of nutrients promoting sufficient S uptake for optimal yield and growth. Locations 3 had the numerically highest yields with 17 kg S ha⁻¹ treatment suggesting that the 17 kg S ha⁻¹ application rate was sufficient for optimal growth at that location. By location (Table 2.4) and across locations (Figure 2.1), N source did not have a statistically significant impact on yield between Urea, UAN, and CAN. Furthermore, the yield differences by S rate were not

statistically significant from one another and not significantly different compared to N source applications across locations (Table 2.4).

Treatment by Location Interaction

A test for treatment by location interaction was run where treatments were fixed effect in the model and location was treated as a fixed effect (Table 2.3). For yield, Total Uptake of Sulfur (TU-S), and Total Uptake of Nitrogen (TU-N), there were significant treatment by location interactions, and both treatment and location each were significant as well for each source of variation (Table 2.3). This means that for yield, TU-S, and TU-N, response to each treatment varied by location and was likely impacted by soil and environments at each location. Partial Factor Productivity for Sulfur (PFP-S), Partial Factor Productivity for Nitrogen (PFP-N), Agronomic Efficiency for Nitrogen (AE-N), and Recovery Efficiency of Nitrogen (RE-N) did not exhibit a significant treatment by location interaction .

Nutrient Use Efficiency Indicators

Sulfur use efficiency indicators are given in (Table 2.5), nitrogen use efficiency indicators are represented in (Table 2.6), and nitrogen and sulfur total plant uptake are given in (Figure 2.3). All 5 locations and across locations exhibited the highest PFP for S with the lowest rate of S of 17 kg S ha⁻¹ with a decrease in PFP with the higher S rates (Table 2.5). Sulfur PFP for all three sulfate fertilizer treatments was the highest at locations 1 and 5 (Table 2.5). Fixen et al. (2015) stated that elevated partial factor productivity indicates that soils are more responsive to added fertilizer. Moreover, locations 1 and 5 contained elevated levels of sand, lower organic matter, and less sulfur than locations 2 and 4 (Table 2.1)and, therefore, would inherently be more responsive to sulfur fertilization. Agronomic Efficiency RE, and IE were also most elevated for the rate of 17 kg S ha⁻¹ by and across all locations (Table 2.5). AE,

RE, and IE were also most elevated for the 17 kg S ha⁻¹ treatment by and across all locations. Nitrogen PFP was the highest with the application of 31 kg S ha⁻¹ at locations 1 and 2 (Table 2.6). Only Location 1 exhibited a statistically significant difference between the 31 kg S ha⁻¹ treatment and CAN fertilizer treatments (Table 2.6). Location 5 did not exhibit any statistically significant difference in Nitrogen PFP among treatments (Table 2.6).

As S-rate increased, SAE was significantly reduced at all locations except location 2, which did trend lower. Agronomic efficiency was lowest at locations 2 and 4 at 80.0 and 158.1 kg grain per kg sulfur for the 17 kg S ha⁻¹ treatment (Table 2.5). This suggests that the margins of yield increase between added sulfur fertilizer compared to the yield without added sulfur were narrower, likely caused by a sufficient supply of sulfur from the soil (Table 2.1). It is important to note that grain (kg grain) weight for agronomic efficiency is the difference between the yield with and without sulfur fertilizer applied over the applied fertilizer rate (Fixen et al., 2015). There was a significant difference at location 5 and across all locations suggesting that these soils were responsive to the increase in sulfur fertilization (Table 2.4). Results for the nitrogen agronomic efficiency were mixed with no distinctive pattern except that location 5 exhibited no significant difference among any of the treatments and that location 2 exhibited the lowest AE relative to the other locations (Table 2.5).

Further, RE followed a similar pattern to AE. As more sulfur was applied, yield and uptake (for AE and RE, respectively) did not significantly change causing a decrease for both sulfur AE and sulfur RE across locations. Uptake and yield were not gained despite additional sulfur fertilizer. Locations 1, 3, and 5 had higher apparent recovery efficiencies for UAN + ATS, UCS33, and UCS27 compared to locations 2 and 4 (Table 2.5). This may indicate a greater difference in total sulfur uptake in aboveground biomass between fertilized crops and

the unfertilized control for locations 1, 3, and 5, suggesting that more fertilizer applied was taken up. For Nitrogen RE, no distinctive patterns were observed among treatments.

For UAN +ATS, UCS33, and UCS27, location 5 saw the highest internal efficiency of all 5 locations and across locations. The elevated internal efficiency indicates a deficiency of sulfur (Fixen et al., 2015) which is reflected in the pre-fertilization soil test sulfur level of 1.4 mg kg⁻¹ (Table 2.1). Results were mixed for the fertilizer applications for Nitrogen IE (Table 2.6) with no distinctive patterns.

Post-Harvest Soil Test Nutrients

Fertilizer treatments exhibited statistically significant effects on soil nutrient concentrations for nitrate and sulfate across locations (Figure 2.2ac), but no significant response was observed for soil ammonium (Figure 2.2b). Locations 2 and 3 exhibited significantly higher amounts of nitrate with the CAN application compared to the Urea and UAN, and location 3 exhibited the highest content of nitrate with CAN application over the other fertilizer treatments (Table 2.7). Added nitrogen fertilizer provided a significant increase over the control for soil test nitrate; however, there wasn't a statistically significant increase with added sulfur fertilizer across locations (Figure 2.2a). CAN exhibited a statistically significant increase in soil test nitrate over Urea and UAN across locations (Figure 2.2a). . Location 5 did not exhibit any statistical differences among fertilizer treatments either, but ammonium test results were relatively low compared to other locations (Table 2.7). Further, soil test ammonium was relatively unaffected by fertilization, with only marginal trends being exhibited (Figure 2.2b). Soil test sulfur was relatively unaffected by nitrogen fertilization application (i.e., N fertilizer source) but increased significantly with the application of 17 kg S ha⁻¹ and 52 kg S ha⁻¹ (Figure 2.2c). Single degree of freedom contrast was utilized to analyze the average fertilizer

treatments against one another. For UAN vs. UAN+ATS, location 3 showed statistical significance at $P=0.013$ (Table 2.9). For N source vs. Control, all locations exhibited statistical significance as well as across all locations. For N source vs. S rate, locations 2 and 3 showed statistical significance at $P=0.005$ and $P=0.001$.

Total S and N Uptake Regression

For both N and S total uptake, most points were concentrated at approximately the 8-14 Mg ha⁻¹ (Figure 2.3). Sulfur total plant uptake for optimized yield was approximately the same as the sufficient application rate of 17 kg S ha⁻¹ across locations (Table 2.4). A strong, curvilinear relationship was observed between S total uptake and grain yield. Similarly, Kaur et al. (2019) observed a significant relationship between tissue S and grain yield for V6 and V12 growth stages in 2016 and V6 and R1 growth stages in 2017 but had low R² values in their study.

A strong curvilinear relationship occurred between N total uptake and grain yield, with 70% of the variability in grain yield being explained by N total uptake (Figure 2.3). Moreover, Nyiraneza et al. (2009) observed a strong positive correlation of 0.97 between total N uptake and grain yield in their study.

Organic Matter, Sulfur Availability, and Uptake

Profile samples (0-60cm) in depth pre-fertilization indicated that there was approximately 3.16 kg S ha⁻¹ available to the corn crop across locations. Sulfur was added at 17, 31, and 52 kg S ha⁻¹ (Figure 2.4a). The control exhibited the lowest uptake of sulfur for both grain and stover at 5.82 and 4.98 kg S ha⁻¹, respectively (Figure 2.4b; Table 2.8). Nitrogen fertilization enhanced the uptake of stover marginally over the control as well as did the respective sulfur applications (Table 2.8). Grain uptake of sulfur increased numerically with the

addition of nitrogen fertilization and trended upward with the application of sulfur (Figure 2.4b; Table 2.8). The sulfur taken up by the grain and stover where none was applied likely mineralized from the soil organic sulfur (Carciochi et al., 2018).

(Figure 2.5a) represents the comparison of initial profile S compared to organic matter from the control treatment at 5 locations. From the equation, there is a strong correlation with a coefficient of determination of $R^2=0.93$ or approximately 93% of the variability in initial profile S being explained by SOM. From the check plots of our five locations, it can be concluded that SOM can serve as a strong indicator of profile sulfur. Additionally, initial profile S, when regressed against sand content, exhibited a coefficient of determination of $R^2=0.61$ (Figure 2.5b).

Conclusion

Organic matter content and soil texture greatly affected nutrient use efficiency indicators, total S and N uptake, grain yield, and post-harvest soil nutrients. Further, our regression (Figure 2.5) shows initial profile S is strongly correlated to SOM with a $R^2=0.93$. 93% of the variability in SOM can be explained by initial profile S, and for every 1 kg ha⁻¹ increase there was a 0.052% increase in SOM. Initial profile S was correlated with sand content (g kg⁻¹) with a coefficient of determination $R^2=0.61$. Thus, initial profile S resulted from the combination of both sand content and SOM for our study. Moreover, results from this study indicate that low levels of organic matter and elevated levels of sand, such as that at locations 1, 3, and 5, increased RE (Table 2.5). For both total S uptake and total N uptake, curvilinear relationships were observed between total S and total N uptake and grain yield (Figure 2.3ab). By location and across locations, N source did not have a statistically significant difference on grain yield (Figure 2.1) (Table 2.4). Yield statistical differences, by S rate or N source, were

not significantly different from one another across locations (Figure 2.1). Grain yield increased significantly with increased nitrogen fertilization over the control, and 17 kg S ha⁻¹ appears to be sufficient to optimize corn growth based on a trend (Figure 2.1).

None of the 5 locations exhibited a statistically significant difference in soil test ammonium between fertilizer treatments (Table 2.7). Added nitrogen fertilizer gave a statistically significant increase in soil test nitrate over the control. Still, there wasn't a significant difference between S rate and N source on soil test nitrate (Figure 2.3a). Agronomic efficiencies that were lower were indicative of a sufficient supply of sulfur due to the margins of yield increase being smaller at locations 2 and 4 (Table 2.5).

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Tables and figures

Table 2.1. Locations, predominant soil type, precipitation, and soil analysis

Location	County	Predominant Soil		Precip.††	Irrig.§	Soil analysis¶										
		Series†	Subgroup‡			CEC	pH	P	K	OM	Sand	Silt	Clay	NO ₃	NH ₄	SO ₄
				— mm —		meq 100g ⁻¹		—mg kg ⁻¹ —		— g kg ⁻¹ —				— mg kg ⁻¹ —		
1	Shawnee	Eudora L	F. Hapludolls	945	122	7.0	6.5	30.5	147.5	15.0	550	360	90	7	2.1	1.3
2	Republic	Crete SIL	P. Argiustolls	732	95	17.2	6.2	6.0	441.8	34.0	155	645	200	6	4.0	6.2
3	Riley	Belvue SL	T. Udifluvents	827	—	7.8	6.4	24.5	134.0	14.0	675	245	80	10	3.1	2.3
4	Republic	Crete SIL	P. Argiustolls	777	—	24.5	4.8	49.7	323.6	28.0	140	615	245	11	7.3	4.3
5	Shawnee	Eudora L	F. Hapludolls	945	106	12.3	5.4	49.3	152.4	15.0	400	495	105	7	3.4	1.4

† Soil Series: SIL, silt loam; SL, sandy loam; L, loam.

‡ P, Pachic; F, Fluventic; T, Typic.

†† Precip., Precipitation; mean annual precipitation from 30-yr norm from weather station within 20 km of each study location.

§ Irrig., Irrigation; supplemental irrigation only for irrigated locations 1, 2, and 5.

¶ Sampling depth of 0-15 cm for cation exchange capacity (CEC), organic matter (OM), Sand, Silt and Clay; and 0-60 cm for NO₃, NH₄, and SO₄

Table 2.2. Monthly precipitation and average monthly temperature for all five site-years in Kansas.

Location	Month						
	April	May	June	July	August	September	October
precipitation (mm)							
1	73	284	170	75	229	49	65
2	15	179	143	79	119	45	68
3	61	189	128	257	45	66	8
4	11	64	63	157	9	41	1
5	74	106	110	219	25	68	12
temperature (°C)							
1	14	17	23	25	24	24	10
2	12	15	22	24	23	23	8
3	12	16	25	26	24	19	11
4	10	16	25	25	23	18	10
5	12	17	25	26	24	19	11

Table 2.3. Treatment, Location, and Treatment by location interaction for corn response parameters by locations

Sources of Variation	yield	TU-S	TU-N	PFP-S	PFP-N	AE-S	AE-N	RE-S	RE-N	IE-S	IE-N
		<i>P</i> > <i>F</i>									
Treatment (T)	<0.001	<0.001	<0.001	<0.001	0.050	<0.001	0.104	<0.001	0.709	0.092	<0.001
Location (L)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
T x L	<0.001	<0.001	<0.001	0.116	0.063	<0.001	0.196	0.008	0.241	0.004	0.013

TU-S, total sulfur plant uptake; TU-N, total nitrogen plant uptake; PFP-S, sulfur partial factor productivity; PFP-N, nitrogen partial factor productivity; AE-S, sulfur agronomic efficiency; AE-N, nitrogen agronomic efficiency; RE-S, sulfur recovery efficiency, RE-N, nitrogen recovery efficiency; IE-S, sulfur internal efficiency; IE-N, internal nitrogen efficiency.

Table 2.4. Yield, total N uptake, and total S uptake at five locations in Kansas and across locations.

Location	Fertilizer Source				Fertilizer rate (kg S ha ⁻¹)		
	0	Urea	UAN	CAN	17	31	52
	Yield (Mg ha ⁻¹)						
1	5.7c	15.6ba	14.6ba	13.9b	14.6ba	16.1ba	17.1a
2	9.6c	10.4bc	10.4bc	10.8b	10.9b	11.0ba	11.8a
3	4.2d	8.6bc	9.2bc	7.8c	11.0a	9.2bc	9.7ba
4	10.4b	12.7a	13.1a	12.2a	13.1a	12.5a	12.8a
5	7.4b	13.3a	14.3a	14.3a	14.3a	13.8a	13.0a
All locations	7.5b	11.9a	12.3a	11.8a	12.7a	12.2a	12.6a
	Total N uptake (kg ha ⁻¹)						
1	61.0c†	204.0ba	194.3ba	178.3b	213.7ba	194.7ba	225.3a
2	135.0d	167.3bcd	155.0cd	205.3a	169.5bc	189.0ba	171.3bc
3	67.5c	136.5b	135.8b	140.0ba	163.5a	129.5b	132.5b
4	153.0d	239.3a	231.8ba	216.3bac	210.0bc	204.5c	213.5bac
5	97.3c	165.8b	190.0ba	215.0a	186.3ba	175.0b	177.0b
All locations	105.0b	181.4a	181.0a	191.3a	187.3a	177.7a	181.8a
	Total S uptake (kg ha ⁻¹)						
1	7.3c	18.0ba	18.3ba	16.0b	20.3a	19.3ba	21.7a
2	17.8a	19.3a	18.0a	21.3a	19.3a	19.8a	19.8a
3	7.0c	12.3ba	10.5b	10.0bc	13.8a	13.5a	14.5a
4	12.5b	16.5a	17.3a	15.3a	15.3a	15.3a	16.0a
5	8.8c	13.8b	15.5ba	17.4a	15.0ba	15.0ba	14.5b
All locations	10.8b	15.8a	15.9a	16.1a	16.5a	16.3a	17.0a

†Letters represent significant differences between treatments within a row (location) at $P < 0.05$

Table 2.5. Sulfur use efficiency indices with added sulfur fertilizer by and across five locations in Kansas.

Location	Sulfur rate (kg S ha ⁻¹)		
	17	31	52
	PFP (kg grain/ kg S)		
1	867.0a†	524.3b	326.3b
2	649.0a	360.5b	224.5c
3	653.5a	301.5b	186.3c
4	775.3a	409.0b	244.5c
5	852.0a	448.8b	247.5c
all locations	759.0a	399.9b	239.0c
	AE (kg grain/ kg S)		
1	529.4a	339.1b	217.8b
2	80.0a	48.1ba	41.7b
3	403.1a	163.9b	105.5b
4	158.1a	70.3b	46.1b
5	412.6a	207.5b	106.5c
all locations	313.5a	152.4b	93.7c
	RE (%)		
1	0.76a	0.39b	0.27b
2	0.11a	0.08a	0.04a
3	0.41a	0.21b	0.14b
4	0.18a	0.09ab	0.07b
5	0.38a	0.21b	0.12c
all locations	0.36a	0.18b	0.12b
	IE (kg/kg)		
1	721.3a	829.3a	793.7a
2	580.5a	567.3a	606.3a
3	790.0a	689.8a	683.3a
4	851.0a	827.3a	800.8a
5	948.3a	920.0a	888.5a
all locations	787.3a	749.6a	460.2a

†Letters represent significant differences ($P < 0.05$) between treatments across locations
PFP, partial factor productivity; AE, agronomic efficiency; RE, apparent recovery efficiency;
and IE, internal efficiency

Table 2.6. Nitrogen use efficiency indices† with nitrogen fertilization alone and with added sulfur by and across five locations in Kansas.

Locations	Nitrogen source			Sulfur rate (kg S ha ⁻¹)		
	Urea	UAN	CAN	17	31	52
	PFP (kg grain/ kg N)					
1	77.4ba‡	72.2ba	68.8b	72.2ba	79.5ba	84.6a
2	51.4b	51.6b	53.3b	54.0ba	54.7ba	58.2a
3	42.5bc	45.5bc	38.5c	54.4a	45.7bc	48.2ba
4	63.1ba	64.6a	60.5b	64.6a	62.0ba	63.3ba
5	65.8a	70.8a	70.7a	71.0a	68.1a	64.2a
all locations	59.0b	60.8ab	58.5b	62.9a	60.5ab	62.0ab
	AE (kg grain/ kg N)					
1	49.3ba	44.1ba	40.7b	44.1ba	51.4ba	56.4a
2	6.2b	4.2b	5.9b	8.3ba	7.3ba	10.8a
3	21.6bc	24.6bc	18.6c	33.6a	24.9bc	27.3ba
4	11.7ba	13.2a	9.1b	13.2a	10.7ba	11.9ba
5	29.2a	34.2a	33.6a	34.4a	31.5a	27.6a
all locations	21.9b	23.6ab	21.3b	25.6a	23.0ab	24.6ab
	RE (%)					
1	0.7ba	0.7ba	0.6b	0.8ba	0.7ba	0.8a
2	0.2b	0.1b	0.3a	0.2b	0.3ba	0.2b
3	0.3ba	0.3ba	0.4ba	0.5a	0.3b	0.3b
4	0.4a	0.4ba	0.3bac	0.3bc	0.3c	0.3bc
5	0.3b	0.5ba	0.6a	0.4ba	0.4b	0.4b
all locations	0.4ab	0.4ab	0.4a	0.4ab	0.3b	0.4ab
	IE (kg/kg)					
1	78.0bc	75.7bc	78.0bc	68.7c	82.7ba	76.0bc
2	62.5a	67.0a	52.5b	64.8a	61.3ba	68.5a
3	63.3ba	68.3ba	56.3b	67.5ba	71.8a	73.5a
4	53.5d	56.5cd	56.8bcd	62.0b	61.5cb	60.0cb
5	80.8a	75.8ba	66.5b	77.3a	79.5a	74.8ba
all locations	66.9bc	69.0ab	62.2c	68.2ab	70.0ab	69.5ab

†PFP, partial factor productivity; AE, agronomic efficiency; RE, apparent recovery efficiency; and IE, internal efficiency

‡Letters represent significant differences between treatments at $P < 0.05$

Table 2.7. Nitrate (NO₃), ammonium (NH₄), and sulfate (SO₄) soil test values from 0-60 cm soil depth post-harvest.

Location	Control	N Fertilizer source			S fertilizer rate (kg S ha ⁻¹)		
	0	Urea	UAN	CAN	17	31	52
NO ₃ mg kg ⁻¹							
1	1.2b†	2.4a	2.0ba	2.1a	2.4a	2.2a	2.1a
2	2.8b	4.5b	3.7b	8.3a	3.8b	4.2b	4.1b
3	1.8b	2.7b	2.0b	12.7a	2.4b	2.3b	2.4b
4	1.2c	4.9bc	8.0ba	10.8a	7.3ba	8.5ba	5.5bac
5	2.7b	3.6ba	3.3ba	4.2a	2.9ba	2.9ba	3.9ba
NH ₄ mg kg ⁻¹							
1	2.3a	2.6a	2.2a	2.5a	2.5a	2.6a	2.4a
2	5.2a	5.3a	6.0a	5.1a	5.4a	5.9a	5.5a
3	2.5a	2.5a	2.5a	2.3a	2.5a	2.6a	2.7a
4	7.8a	6.8a	7.7a	6.9a	7.4a	7.5a	7.2a
5	3.0a	3.8a	3.2a	2.9a	3.2a	2.8a	3.2a
SO ₄ mg kg ⁻¹							
1	1.8b	1.6b	1.5b	1.9ba	1.7b	1.7b	2.5a
2	5.9b	6.1b	6.1b	5.8b	6.4b	9.2a	8.9a
3	1.7c	2.0c	1.6c	1.0c	1.8c	4.5b	6.1a
4	2.9dc	2.7dc	2.9dc	2.3d	3.8c	5.4b	7.3a
5	1.5bc	1.1c	1.4bc	1.4bc	1.4bc	2.8ba	3.4a

†Letters represent significant differences between treatments at $P < 0.05$

Table 2.8. Grain and Stover Sulfur Uptake at five locations in Kansas and across locations.

Location	Fertilizer Source				Fertilizer rate (kg S ha ⁻¹)		
	0	Urea	UAN	CAN	17	31	52
Grain Sulfur Uptake (kg ha ⁻¹)							
1	4.13c†	13.4ab	13.1ab	11.3b	13.8ab	14.3ab	14.7a
2	8.28c	9.08bc	9.08bc	10.6a	9.35abc	9.98ab	10.3ab
3	3.25c	6.33b	6.55b	5.36b	9.23a	8.00a	8.47a
4	7.70b	9.63a	9.93a	9.30a	9.70a	9.55a	10.2a
5	5.38c	9.85b	11.3ab	11.8a	10.9ab	10.3ab	10.4ab
All locations	5.82c	9.45b	9.90ab	9.68ab	10.5ab	10.1ab	10.5a
Stover Sulfur Uptake (kg ha ⁻¹)							
1	3.05d	4.90bcd	5.37abc	4.50cd	6.37ab	5.07bc	6.83a
2	9.33a	10.3a	8.75a	10.6a	9.63a	9.73a	9.25a
3	3.83a	5.78a	3.78a	4.40a	4.78b	5.60a	5.95a
4	4.65b	6.78a	7.13a	6.10ab	5.68ab	5.73ab	5.80ab
5	3.40c	3.83bc	4.20bc	5.69a	4.20bc	4.75ab	4.40abc
All locations	4.98b	6.38a	5.88ab	6.41a	6.12a	6.22a	6.41a

†Letters represent significant differences between treatments at $P < 0.05$

Table 2.9. Contrasts between S rate, N source, and Control for yield at. five locations in Kansas and across locations.

Location	UAN vs. UAN+ATS†	N source vs. Control§	N average vs. S average¶
	<i>P</i> > F		
1	1.000	<0.001	0.110
2	0.249	0.010	0.005
3	0.013	<0.001	0.001
4	1.000	<0.001	0.643
5	0.973	<0.001	0.562
All Locations	0.391	<0.001	0.082

† Single-degree of freedom contrast comparing UAN vs. UAN+ATS

§ Single-degree of freedom contrast comparing nitrogen source to control (no fertilizer)

¶ Single-degree of freedom contrast comparing nitrogen source to sulfur rate

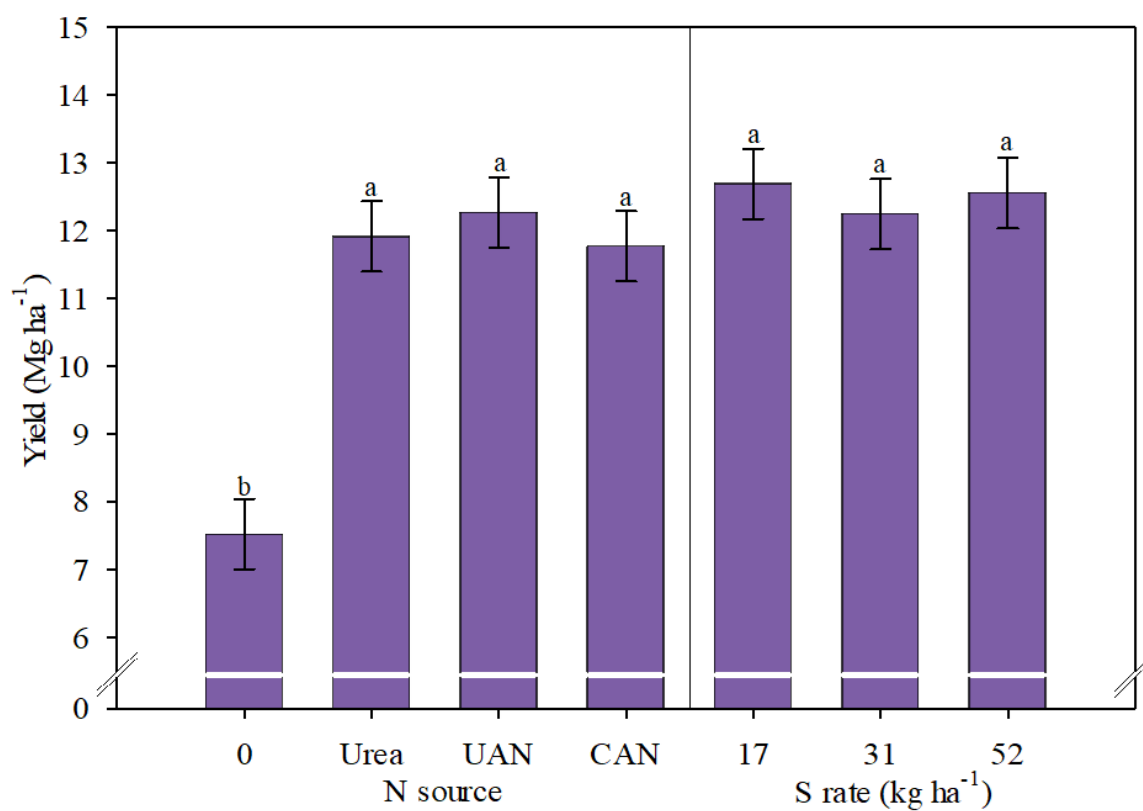


Figure 2.1. Average corn yield response to nitrogen source and sulfur rate fertilizer treatments across all locations in Kansas. Error bars indicate standard error of the mean and mean values followed by the same letter are statistically different ($P < 0.05$).

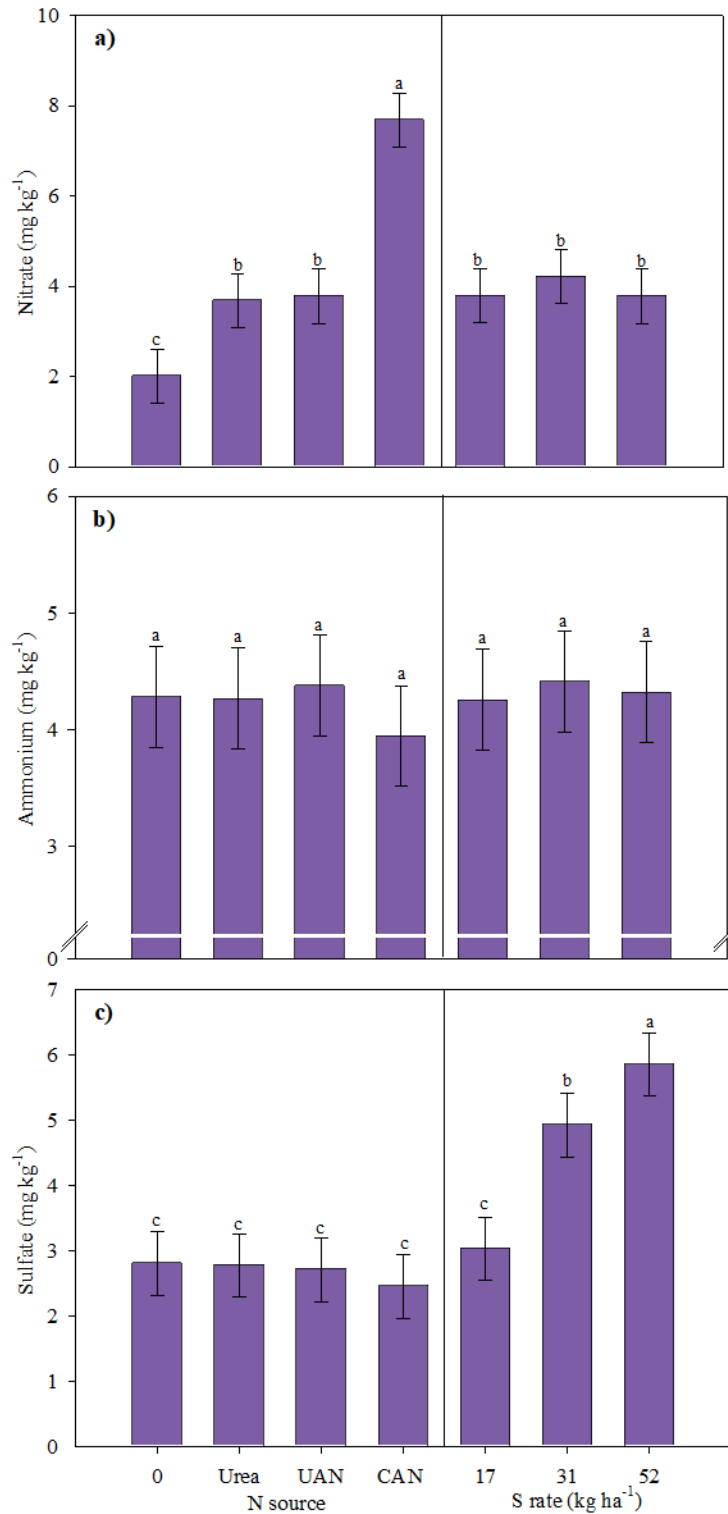


Figure 2.2. Nitrate (a), ammonium (b), and sulfate (c) concentrations from the 0-60 cm soil depth after corn harvest across all locations in Kansas. Error bars indicate standard error of the mean, and mean values followed a different letter are statistically different ($P < 0.05$).

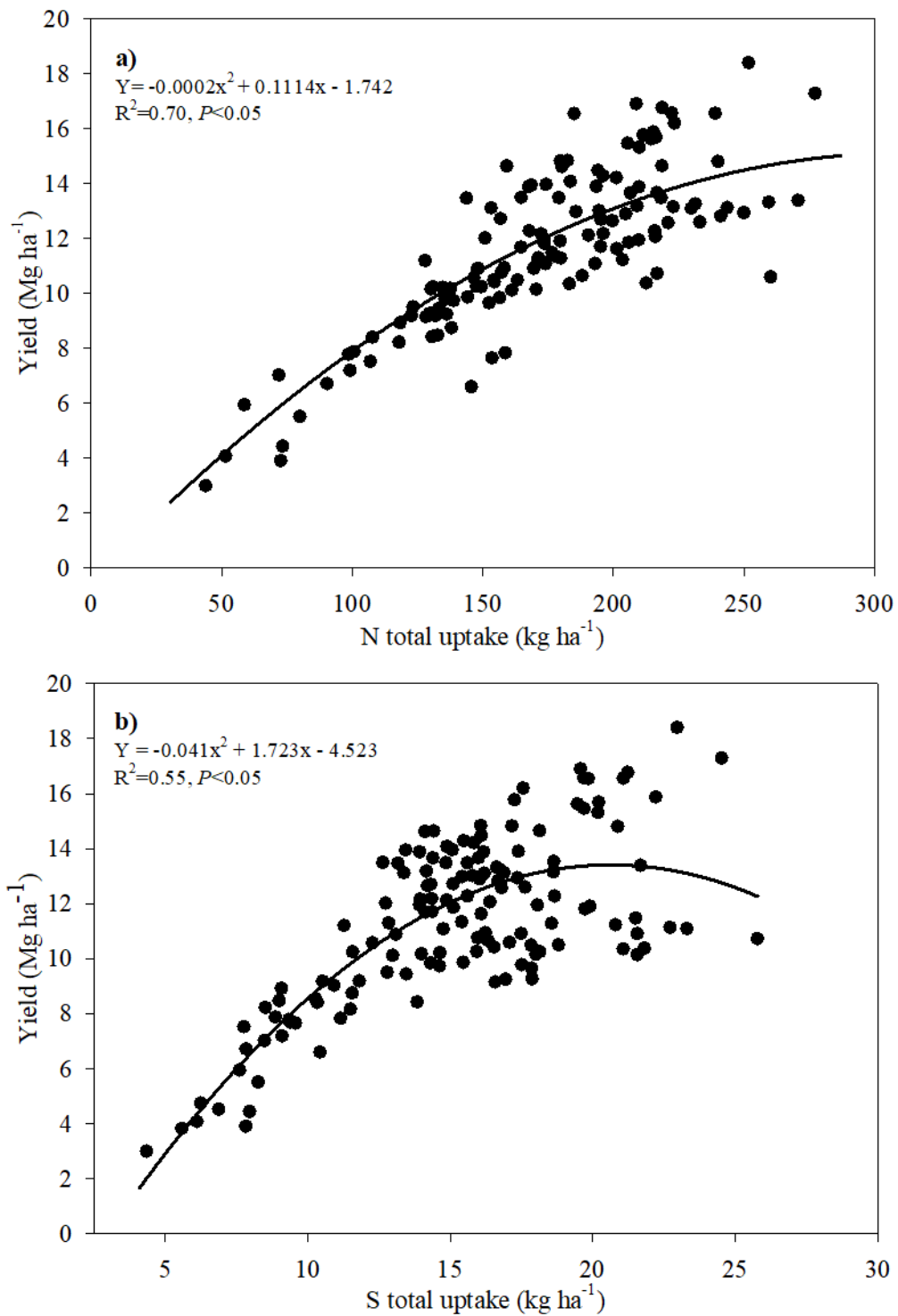


Figure 2.3. Total N uptake (stover + grain) (a) and total S uptake (stover + grain) (b) relation to yield across all locations in Kansas.

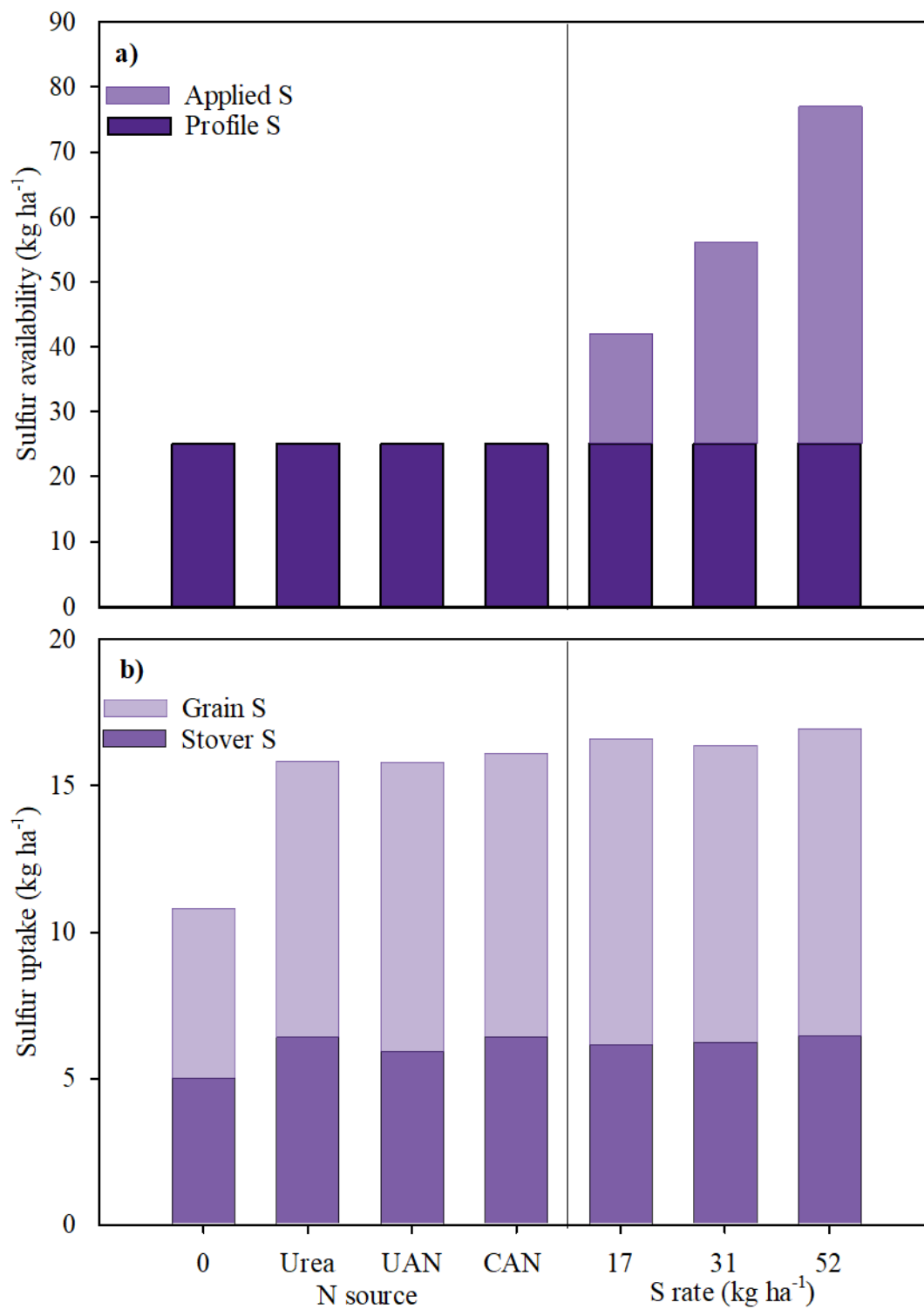


Figure 2.4. Total S availability (profile + applied) (a) and S uptake (grain + stover) (b) across all locations in Kansas

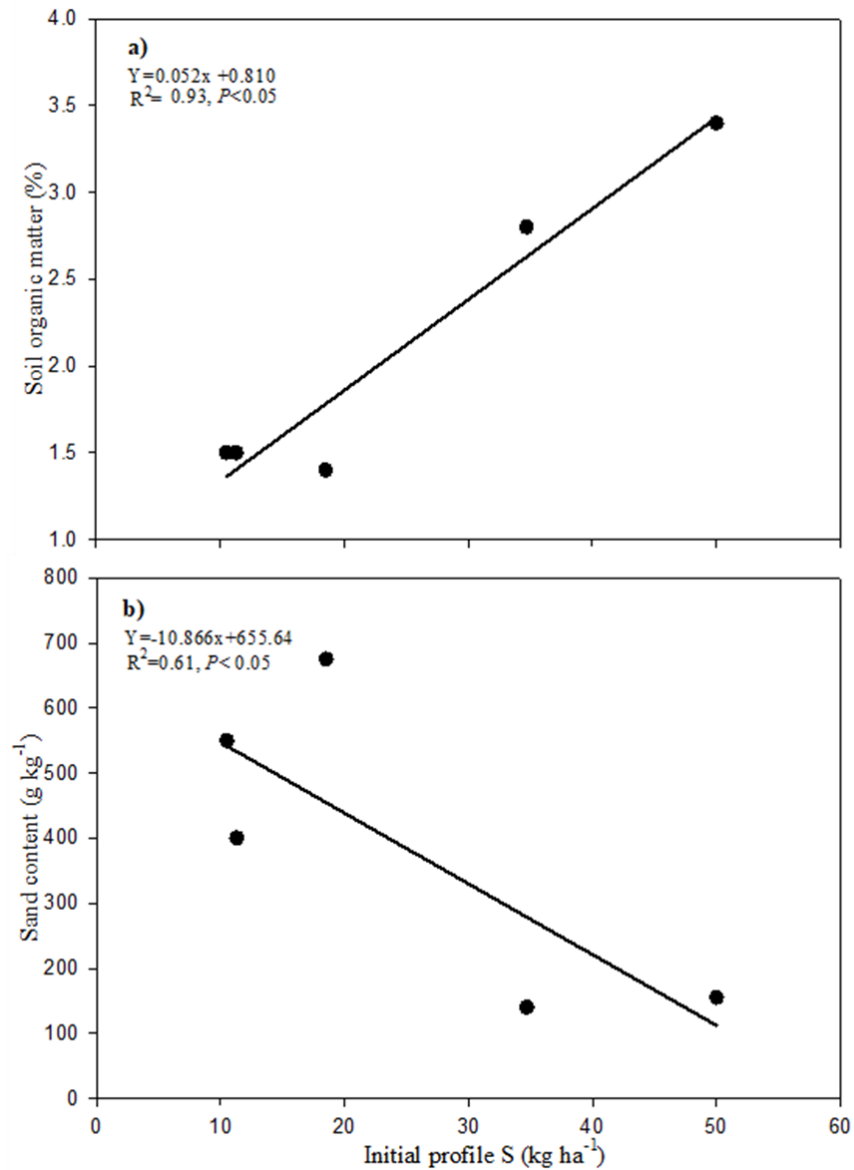


Figure 2.5. Initial profile S (kg ha⁻¹) in relation to soil organic matter (%)(a) and initial profile S (kg ha⁻¹) relation to sand content (g kg⁻¹)

Chapter 3 - Evaluation of soil factors determining wheat response to sulfur fertilizer

Abstract

Identifying how sulfur (S) fertilization and soil parameters influence wheat (*Triticum aestivum* L.) growth and response to S is of great interest to producers. The objective of this study was to evaluate the response of wheat to S fertilizer and identify the main soil parameters affecting the response. A greenhouse study was conducted utilizing 15 soil types from Kansas and Nebraska. Fertilizer treatments included Urea Ammonium Nitrate (UAN) (28-0-0; N-P₂O₅-K₂O) at 0.99 mL UAN pot⁻¹ (~154 mg N kg⁻¹) to all pots, and half of those pots had S (0-0-0-50S; N-P₂O₅-K₂O-S) at 0.19 mL (~51 mg S kg⁻¹). All fertilizers were topdress applied using a metered pipet. A randomized complete block design was implemented utilizing three replications for each nitrogen and nitrogen plus sulfur fertilization for each soil where each table was the blocking factor. Pre-fertilization soil samples were analyzed for properties including Soil Organic Matter (SOM), Cation Exchange Capacity (CEC), pH, soil texture, phosphorous (P), potassium (K), zinc (Zn), total carbon (C), total nitrogen (N), and (S). Biomass samples were harvested at Feekes 11.1 (milk) and analyzed for total nitrogen, total carbon, and sulfur concentration. Increases in plant S uptake with S fertilizer application vary in magnitude by soil type. The main soil parameters contributing to explain the variability in plant response were sand content, soil organic matter, and soil test P (STP). Higher sand content and STP contributed to a greater plant increase in S uptake, while lower SOM contributed to greater response, particularly on soils with low sand content. Results from this study suggest that STS should be interpreted in

combination with other soil parameters, specifically sand content, SOM, and other limiting nutrients such as P.

Introduction

Sulfur (S) deficiency in wheat has become more prevalent over the past decade, and the ability to properly diagnose S deficiency in the soil is a challenge. Sulfur deposition has decreased the amount of sulfur available in the sulfur soil pool. Sulfur (S) deposition was reported to be 18 pounds of S per acre (20 kg S ha^{-1}) prior to the Clear Skies Act was reduced nearly 50% to less than 10 pounds per acre (11 kg S ha^{-1}) across Indiana (Camberato and Casteel, 2017). The decreasing atmospheric sulfur deposition has resulted in inadequate soil test S (STS) to meet the demand of many crops like wheat. In addition, the advent of highly concentrated and cleaner fertilizers has decreased the amount of S that was otherwise inadvertently applied (Scherer, 2001). Decreasing the sulfur application with more pure fertilizers in combination with reduced atmospheric S deposition over time can have a considerable effect. Although this decrease does not seem substantial, S is required in smaller quantities compared to other nutrients like nitrogen (N), phosphorus (P), or potassium (K) (Hagin, 1982). Even small changes in S can have a big impact on the supply of S for winter wheat throughout the growing season. Recognizing S deficiencies are difficult since they resemble that of nitrogen; sulfur deficiency symptoms appear as chlorosis and firing on the younger leaves of the plant, while nitrogen deficiency symptoms appear on the older leaves (Ruiz Diaz and Mengel, 2018).

Although decreased atmospheric deposition and decreased sulfur content in fertilizer are ubiquitous, sulfur deficiencies are irregular. In Kansas, S deficiencies patterns have developed in north-central and northeastern Kansas in wheat (Ruiz Diaz and Mengel, 2018). Evaluating soil test sulfur (STS) has been the primary tool for sulfur evaluation in soils. There are several soil

tests currently being utilized for sulfur testing, including monocalcium phosphate (Combs et al., 1998), Mehlich-3 (Frank et al., 1998), and ammonium acetate (Warncke and Brown, 1998). It is important to note that STS may poorly estimate the actual sulfur available to the crop during the growing season. However, Brennan and Bolland, (2006) suggested that tissue analysis in early plant development may not properly account for sulfur levels due to the crop not having a sufficient root system to access sulfur deeper in the profile. Gupta (1976) found that S concentrations in wheat grain were not good indicators of S deficiency and that when S was sufficient in the soil; increasing levels of S did not affect S concentration in the grain. Soil test sulfate (SO_4^{2-}) is known not to be a good indicator of the actual sulfur represented in the soil. Soil sulfate-sulfur must be mineralized before it can become available to crops, and due to this it is not a good predictor of the maximum S (Pagani and Echeverria, 2011).

Sulfur is available to plants in an inorganic form, and Camberato and Casteel, (2017) state that organic matter must be mineralized into the sulfate form of sulfur to be taken up by the crop's roots. With this, sulfur deficiency is more likely to be exhibited by soils with lower soil organic matter (Camberato and Casteel., 2017). Much of the sulfur available to the plants is mineralized into an inorganic form of S (i.e., SO_4^{2-}). Further, sulfur oxidation hinges upon temperature and moisture since this process is performed by microorganisms in the soil (Wainwright, 1984). Further, sulfur is typically treated like nitrogen due to having comparable cycles and experiencing similar losses such as through leaching. Economic feasibility and applying an adequate amount of S are key in crops like winter wheat in Kansas. Better management through understanding the efficiency of how the crop uses S and how it is taken up from the soil is imperative for agricultural and environmental sustainability in agriculture. The

objective of this study was to evaluate the response of wheat to S fertilizer and identify the main soil parameters affecting the magnitude of response.

Materials and methods

Treatments, Experiment Design, and Implementation

The greenhouse study was conducted utilizing 15 soils (12 soils sourced throughout the state of Kansas and three from southeast Nebraska, Table 3.1). Soil collections had coordinates obtained and the previous crop recorded. Each soil sample had the top residue scraped off, and the soil sample was attained from 15cm deep to fill up a 19-liter bucket. The soil was thoroughly mixed and sieved to pass through a 1 cm screen. Soils were placed in 15 cm diameter by 15 cm deep pots for a total of 2.3 kg of soil per pot. Tatanka variety of wheat was sown in potting soil and vernalized in growth chambers for six weeks at 8° C. 4 wheat plants were then transplanted in each pot, and one plant was rogued, leaving three plants in each pot. Plants were watered to achieve optimum growth with a shower head garden hose attachment.

The experimental design was a randomized complete block with three replications. Across soil types, treatment was treated as a fixed effect, with replication nested within soil type being treated as the random effect and treatments were treated as fixed effects with replication being treated as the random effect in the model by soil type Urea Ammonium Nitrate (UAN) (28-0-0; N-P₂O₅-K₂O) was applied at 0.99 mL UAN pot⁻¹ (~154 mg N kg⁻¹) to all pots, and half of the pots had sulfur (0-0-0-50S; N-P₂O₅-K₂O-S) at 0.19 mL (~51 mg S kg⁻¹) one week after transplanting. The UAN and sulfur fertilizer treatments were mixed in with water as a carrier for an application rate of 1mL per pot topdress applied with a pipet. Sevin® insecticide, Carbaryl (1-naphthyl N-methylcarbamate), was applied at the equivalent rate of 4.7 L ha⁻¹ at Feekes 9 to control an aphid infestation.

Measurements and Statistical Analysis

Pre-plant soil analysis was completed on oven-dried soils at 40° C for five days and ground to pass through a 2 mm sieve. Soils were analyzed for soil chemical and physical properties, including Soil Organic Matter (SOM), Cation Exchange Capacity (CEC), pH, soil texture, phosphorous, potassium, zinc, calcium, total carbon, total nitrogen, and sulfur (Table 3.2). Organic matter was measured by loss on ignition (Combs and Nathan, 1998), Cation Exchange Capacity by summation technique (Warncke and Brown, 1998), and pH was analyzed using a 1:1 (soil:water) method (Watson and Brown, 1998). Soil texture was analyzed using a hydrometer (Gee and Bauder, 1986), soil test P with the Mehlich-3 extraction (Frank et al., 1998), Ca, Mg, Na, and K by ammonium acetate extraction (Warncke and Brown, 1998), and Fe, Cu, Zn, and Mn by use of DTPA extraction (Whitney, 1998). and sulfur (sulfate) was measured using a monocalcium phosphate extraction (Combs et al., 1998)

Three wheat plants were gathered at Feekes 11.1 (milky stage), and biomass was measured. Whole plant samples were gathered, weighed, dried at 60°C, and then reweighed to obtain dry matter content. Dried whole-plant biomass samples were ground to pass through a 2 mm sieve, and then total N was analyzed using a LECO FP-528 CN combustion analyzer to acquire both total N on a weight percent basis (LECO Co., St. Joseph, MI) (McGill and Figueiredo, 1993). Sulfur measurements were taken using a nitric perchloric digest Gieseking et al., (1935) with the use of an Inductively Coupled Plasma (ICP) spectrometer to analyze the digestion.

Statistical analyses were performed in SAS and R. The PROC CORR procedure in SAS was utilized for correlation analysis for soil parameters and PROC REG for multiple regression analysis to understand the influence of soil parameters on Whole Plant Uptake-Sulfur (WPU-S),

and the generalized linear mixed model GLIMMIX procedure (SAS Institute, 2013) for the analysis of variance (ANOVA).

Results and discussion

Total Biomass

Initial soil test results for the locations across Kansas and Nebraska (Table 3.2) and N and S whole plant uptake and total biomass are listed in (Table 3.3). Total biomass exhibited no statistically significant differences at the $P < 0.05$. Locations 1 and 14 showed a statistically significant change in total biomass with the application of S (Table 3.3). These findings show the large variability in plant response to S application depending on soils. Salvagotti and Miralles (2008) found increases in biomass production from 7-19% and from 20-35% due to the added S at 5 kg S ha⁻¹ and 35 kg S ha⁻¹, respectively. Interestingly, locations 1 and 14 exhibited very low phosphorus levels at 9.2 mg kg⁻¹ and 2.5 mg kg⁻¹, respectively (Table 3.2), suggesting a possible limiting factor from low STP. Moreover, the Mehlich-3 results for phosphorus provided a strong and positive correlation with total biomass (Figure 3.2). Locations 3, 6, 8, 10, 11, 12, and 13 trended upward in total biomass production with added sulfur over nitrogen fertilization alone (Table 3.3). Furthermore, the addition of sulfur allowed for an upward trend in total biomass production across locations.

Sulfur Whole Plant Uptake

Results for Sulfur Whole Plant Uptake (WPU-S) were mixed with multiple locations only showing trends (Table 3.3 and Figure 3.1). One location out of 15, location 13, demonstrated a statistically significant increase from 9.6 mg pot⁻¹ to 12.6 mg pot⁻¹ with the addition of sulfur at the $P < 0.05$ (Table 3.3). Even though location 9 had elevated levels of sulfate sulfur when compared to the other locations (Table 3.2), it did not exhibit a statistically significant increase in

WPU-S (Table 3.4). Blake-Kalff et al. (2000) found fewer changes in total S concentration in oilseed rape when comparing the sulfate concentration against the increased availability of S. Location 14 had a statistically significant decrease in WPS-U (Table 3.4) with the addition of sulfur fertilizer from 2.5 mg S pot⁻¹ to 0.9 mg pot⁻¹ (Table 3.3). An upward pattern in WPU-S was observed at 11 out of 15 locations (Table 3.3). Lastly, across locations, the addition of sulfur fertilizer increased from 6.2 mg pot⁻¹ to 6.9 mg pot⁻¹ for WPU-S (Table 3.3).

Tissue Sulfur Analysis

Changes in tissue sulfur analysis vary by location; however, analysis across locations showed a statistically significant increase in sulfur concentration in the tissue with the addition of sulfur (Table 3.3), locations 1-15 trended upward with applied sulfur. Calvo et al. (2008) found the significance for the change in S concentration in the wheat plants varied among growth stages.

Plant Uptake, Plant Dry Weight, and Tissue S Concentration

Results were mixed for whole plant sulfur uptake (WPU-S), plant dry weight (DW), and tissue S concentration (Tissue S) for the 15 locations. Out of these 15 locations, 13 locations exhibited numerical increases in tissue S; seven locations showed a positive numerical increase for the change in DW, and 12 locations a positive trend in WPS-U.

Location 12 exhibited the largest decrease out of all 15 locations for whole plant uptake of sulfur WPU-S and DW with added sulfur fertilizer at 60.1% and 40.3%, respectively (Figure 3.1). This is likely due to the relatively high sand content of 920 g kg⁻¹ (Table 3.2). Camberato and Casteel (2017) state that sulfate is easily leached from sandier soils. This would lead to a greater crop response from the wheat. Location 2 exhibited the greatest change in tissue S with an increase of 18.8%. Location 1 had the second-highest magnitude of change in Tissue S at

16.6% increase over the no sulfur fertilizer treatment (Figure 3.1). Interestingly, both these soils came from Gage County, NE but are two vastly different soil types, with location 1 being a Malmo, eroded Pawnee Complex, and Location 2 being a Wymore silty clay (Table 3.1). Soil Test Sulfur levels were low at these two locations, with Location 1 containing 1.9 mg kg^{-1} and Location 2 containing 2.1 mg kg^{-1} (Table 3.2). These levels are well below the 10 ppm (mg kg^{-1}) that is sufficient (Sawyer et al., 2011).

Location 4 did not follow the same response pattern and had one of the lowest responses among WPU-S, DW, and Tissue S at 8.5%, 0.0%, and 7.6%, respectively (Figure 3.1). Contradictory to Location 12, Location 4 also had an elevated sand content of 580 g kg^{-1} but is a silt loam (Table 3.1). Location 14 showed the largest decrease in DW and WPU-S at a decrease in the magnitude of 67.9% and 65.1%, respectively.

Correlation for Various Soil Test Parameters

Soil test P had a strong positive correlation with the magnitude of response for DW and WPU-S (Figure 3.2). Overall, this agrees with the data with location 14 having a very low soil test phosphorus of 2.5 mg kg^{-1} (Table 3.2) and also a much lower DW compared to the other locations (Table 3.3). Furthermore, STP had a strong, positive correlation between STP and the magnitude of response in WPU-S. This aligns with the study by Assefa et al., (2021), who found sulfur uptake to be significantly influenced by the main effect of P in wheat grain and straw.

Further, the magnitude of change in DW had a strong, positive correlation to the magnitude of change and the magnitude of change in WPU-S. Similarly, Resurreccion et al., (2001) found an increase in dry weight with applied sulfate fertilizer for both shoot and root of rice in their study. A strong negative correlation between sand and OM occurred for this study. As sand content goes up, we can expect OM to go down thus also expecting a decline in CEC as

well. This is evidenced at locations (2, 4, and 12) with higher sand content, causing lower CEC and decreased OM. The magnitude of response in whole plant uptake of S (WPU-S) was negatively affected by soil organic matter, total carbon, and CEC (Figure 3.2)

Relative Importance of Soil Parameters on WPU-S

Multiple regression analysis (Fig. 3-3), given by the equation $WPU-S = -5.01 + 0.09 OM + 0.05 STP + 0.006 Sand - 0.04 SO_4$, indicated that four soil test parameters explained 74% of the variability in $\Delta WPU-S$ (STP, Sand, OM, and SO_4) (Figure 3.3). STP accounted for a large portion for the prediction of $\Delta WPU-S$ at 47.8%. It is likely that phosphorus for many of the soils in the study to be a limiting factor for wheat production. Ruiz Diaz and Mengel (2014) claim that 20 ppm ($mg\ kg^{-1}$) is the Critical P Soil Test level. Five soil locations for this study fell below that threshold and likely limited the wheat growth and development. Further, sand was another substantial predictor for $\Delta WPU-S$ at 34.4%. Many sandy soils have low sulfate retention due to lower anion exchange capacity, and therefore sulfate molecules are not retained in the soil (Riley et al., 2006).

Organic matter is another major component in the prediction of $\Delta WPU-S$. Organic matter accounted for 15.8% normalized prediction of WPU-S. Organic matter holds and releases sulfur when decomposition occurs, and the sulfur in organic matter is primarily comprised of organic S (Edwards, 1998). Soil test S was not a good predictor of WPU-S, with sulfate contributing only approximately 2% to the prediction of WPU-S. Sawyer et al. (2009) found from soil samples taken at several locations that the soil test sulfur levels did not correspond well to growth/coloration differences in the field, S concentrations in plant analyses, or yield responses to applied S.

A conditional inference tree analysis of our data indicated that sand content is one of the main predictors of plant response (Figure 3.4). Therefore, a combination of sand content, soil test P, OM, and SO₄ seems to provide the best prediction of potential plant response.

Conclusion

Results from our study showed that STP was significantly correlated to DW and WPU-S and that soil test sulfate alone was a poor indicator of the potential wheat response to S fertilizer. If not in adequate supply, phosphorus could be limiting for plant growth and limit response to S regardless of the application rate. Further research is necessary for how wheat responds to S fertilization to identify more test methods to help accurately diagnose and correct sulfur deficiency.

For plant dry weight at Feekes 11.1, results were mixed between S applied and no S applied treatments. The two locations showed a decrease in plant dry weight with added S fertilizer. Both locations were severely deficient in STP (well below 20 mg kg⁻¹) and produced plants with the greatest numerical reduction in dry weight. However, eight of the 15 locations exhibited a numerical increase in dry weight with added sulfur fertilizer. Additionally, tissue sulfur analyses were affected by added sulfur fertilization, exhibiting various levels of upward trends. Two sites with greatly different soils in Gage County, Nebraska, had the greatest change in tissue S. Further research is needed to identify and quantify better, more accurate methods for analyzing soil test sulfur.

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Tables and figures

Table 3.1. Location information, predominant soil type, GPS coordinates, and previous crop.

Location	Location description	Predominant soil		GPS coordinates	previous crop
		Series†	Subgroup‡		
1	Gage Co., NE	Malmo, eroded Pawnee C.	A. Hapludalfs	40°05'34"N; 96°35'9"W	soybean
2	Gage Co., NE	Wymore SC.	A. Argiudolls	40°05'37"N; 96°34'54"W	soybean
3	Gage Co., NE	Kipson-Sogn-rock outcrop C.	UD & L. Haplustolls	40°05'30"N; 96°34'45"W	soybean
4	Shawnee Co., KS	Eudora SIL.	F. Hapludolls	39°7'1"N; 95°55'40"W	corn
5	Republic Co., KS	Crete SIL.	P. Argiustolls	39°48'10"N; 97°50'33"W	soybean
6	Riley Co., KS	Belvue SIL	T. Udifluvents	39° 8'32"N; 96°37'52"W	soybean
7	Riley Co., KS	Smolan SIL.	P. Argiustolls	39°12'28"N; 96°35'40"W	corn
8	Ellis Co., KS	Harney SIL.	T. Argiustolls	38°50'34"N; 99°19'05"W	sorghum
9	Osage Co., KS	Osage SIL.	T. Epiaquerts	38°37'24"N; 95°50'57"W	soybean
10	Reno Co., KS	Ost L.	U. Argiustolls	37°55'38"N; 98°01'37"W	wheat
11	Finney Co., KS	Ulysses SIL.	TO. Haplustolls	38° 1'12"N; 100°49'24"W	wheat
12	Stafford Co., KS	Farnum L.	P. Argiustolls.	37°57'25"N; 98°44'58"W	wheat
13	Sheridan Co., KS	Keith SIL.	AR. Argiustolls	39°14'2"N; 100°35'15"W	wheat
14	Labette Co., KS	Parsons SIL.	M. Albaqualfs	37°21'37"N; 95°17'35"W	soybean
15	Brown Co., KS	Kennebec SIL.	C. Hapludolls	39°50'33"N; 95°28'59"W	soybean

†Soil series: SIL, silt loam; SL, sandy loam; L, loam; SC, silty clay; C, complex

‡ A, Aquertic; AR, Aridic; C, Cumulic; P, Pachic; F, Fluventic; L, Lithic; M, Mollic; T, Typic; TO, Torriorthentic; U, Ustic; UD, Udorthenic.

Nebraska

Table 3.2. Soil test results for 15 soils across Kansas and Nebraska.

Location	pH	Buffer pH	P	S	K	CEC Sum.	OM LOI†	Sand	Silt	Clay	Total N	Total C
			mg kg ⁻¹			-meq 100g ⁻¹ -	g kg ⁻¹					
1	5.5	6.3	9.2	1.9	298.2	24.2	32.0	140	480	380	1.5	9.9
2	4.8	6.2	37.6	2.1	177.2	17.2	18.0	580	260	160	1.8	16.2
3	4.8	6.3	15.4	3.0	248.5	20.0	35.0	260	580	160	2.2	18.8
4	7.9	7.5	15.7	2.0	138.0	7.5	16.0	580	340	80.0	1.0	8.9
5	5.9	7.0	14.4	5.0	450.1	13.3	28.0	180	600	220	1.7	16.5
6	6.1	7.1	42.3	1.8	262.6	10.2	27.0	320	560	120	1.4	14.2
7	5.8	6.9	29.4	4.8	259.0	16.7	34.0	180	540	280	1.7	18.9
8	8.1	7.5	32.2	2.0	637.4	17.9	27.0	200	580	220	1.5	14.9
9	7.5	7.4	41.2	29.8	104.5	15.0	35.0	200	640	160	1.8	21.7
10	6.5	7.1	50.4	1.1	218.4	10.3	19.0	260	580	160	1.0	10.6
11	8.1	7.5	48.0	4.3	685.0	18.2	23.0	260	520	220	1.4	12.2
12	5.4	7.2	41.5	0.6	88.9	1.9	6.0	920	60.0	20.0	0.4	2.6
13	5.2	6.3	81.9	4.2	592.1	18.9	26.0	220	560	220	1.5	14.0
14	6.1	6.8	2.5	3.1	49.9	12.3	25.0	160	660	180	1.3	13.1
15	6.2	6.9	66.3	2.4	146.0	13.8	32.0	120	660	220	1.8	18.0

†OM LOI, organic matter loss on ignition

Table 3.3. Sulfur whole plant uptake, plant dry weight, and Tissue Sulfur at and across 15 locations in Kansas and Nebraska.

Location	WPU-S‡		Plant dry weight		Tissue Sulfur	
	+ S	-S	+ S	-S	+ S	-S
	mg pot ⁻¹				g kg ⁻¹	
1	3.3a†	3.6a	1133b	1433a	2.85a	2.43a
2	4.2a	3.8a	1333a	1467a	3.06a	2.57a
3	4.2a	3.6a	1667a	1633a	2.37a	2.21a
4	2.6a	2.4a	1167a	1167a	2.30a	2.13a
5	10.2a	12.0a	4800a	5367a	2.13a	2.22a
6	7.5a	5.7a	3767a	3200a	1.96a	1.77a
7	1.0a	0.9a	500a	500a	2.05a	1.83a
8	7.7a	6.2a	3433a	3167a	2.26a	1.95a
9	8.0a	7.7a	4533a	4633a	1.78a	1.66a
10	9.2a	8.5a	4500a	4033a	2.05a	2.09a
11	6.9a	6.4a	3467a	3433a	2.01a	1.87a
12	13.5a	8.4a	5033a	3500a	2.68a	2.33a
13	12.6a	9.6b	7500a	6067a	1.68a	1.59a
14	0.9b	2.5a	300b	933a	2.74a	2.65a
15	12.2a	11.4a	6467a	6667a	1.90a	1.72a
All locations	6.9a	6.2a	3307a	3147a	2.25a	2.07b

†Letters represent significant differences between treatments at $P < 0.05$

‡ WPU-S, Whole Plant Uptake-Sulfur

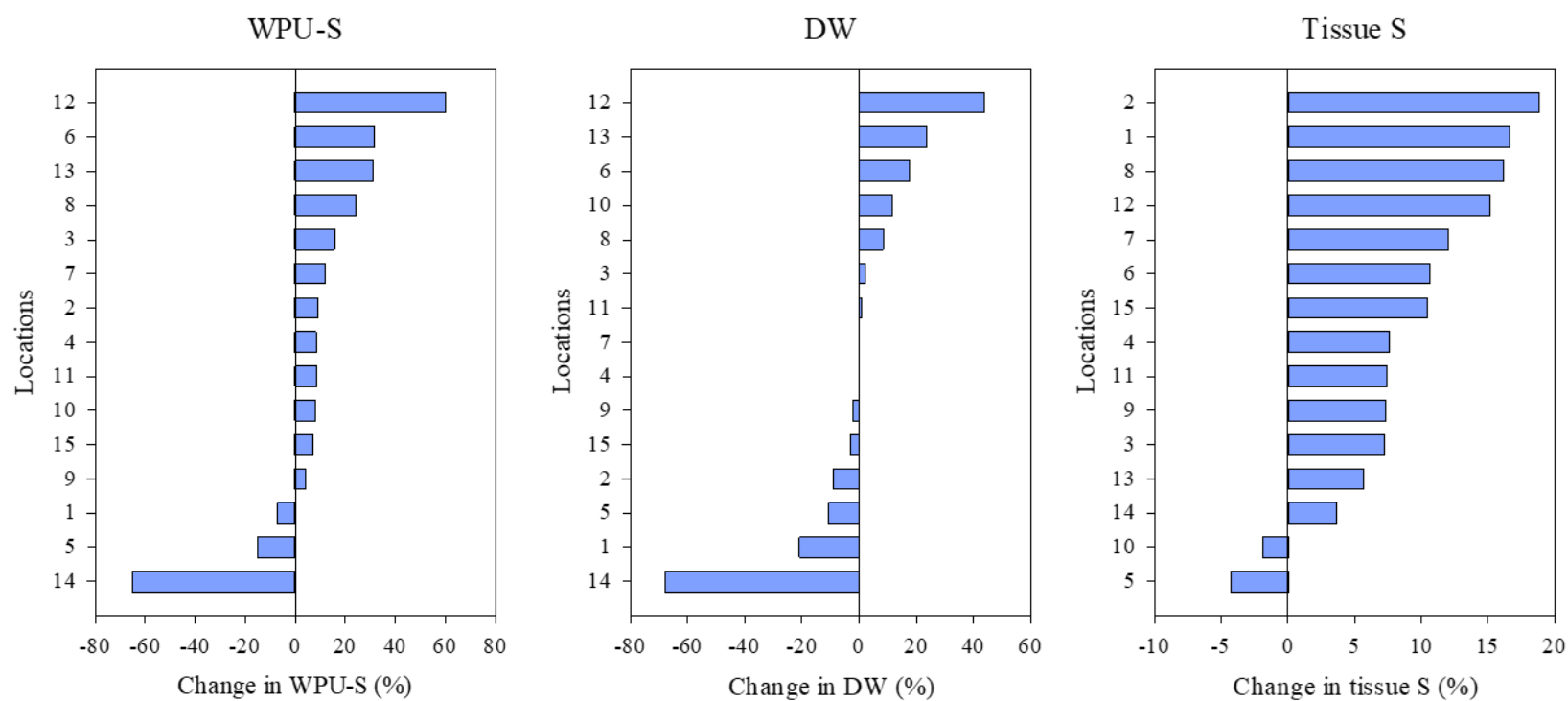


Figure 3.1. Percent change in whole plant sulfur uptake (WPU-S), plant dry weight (DW), and tissue S concentration (Tissue S) with the application of S in wheat.

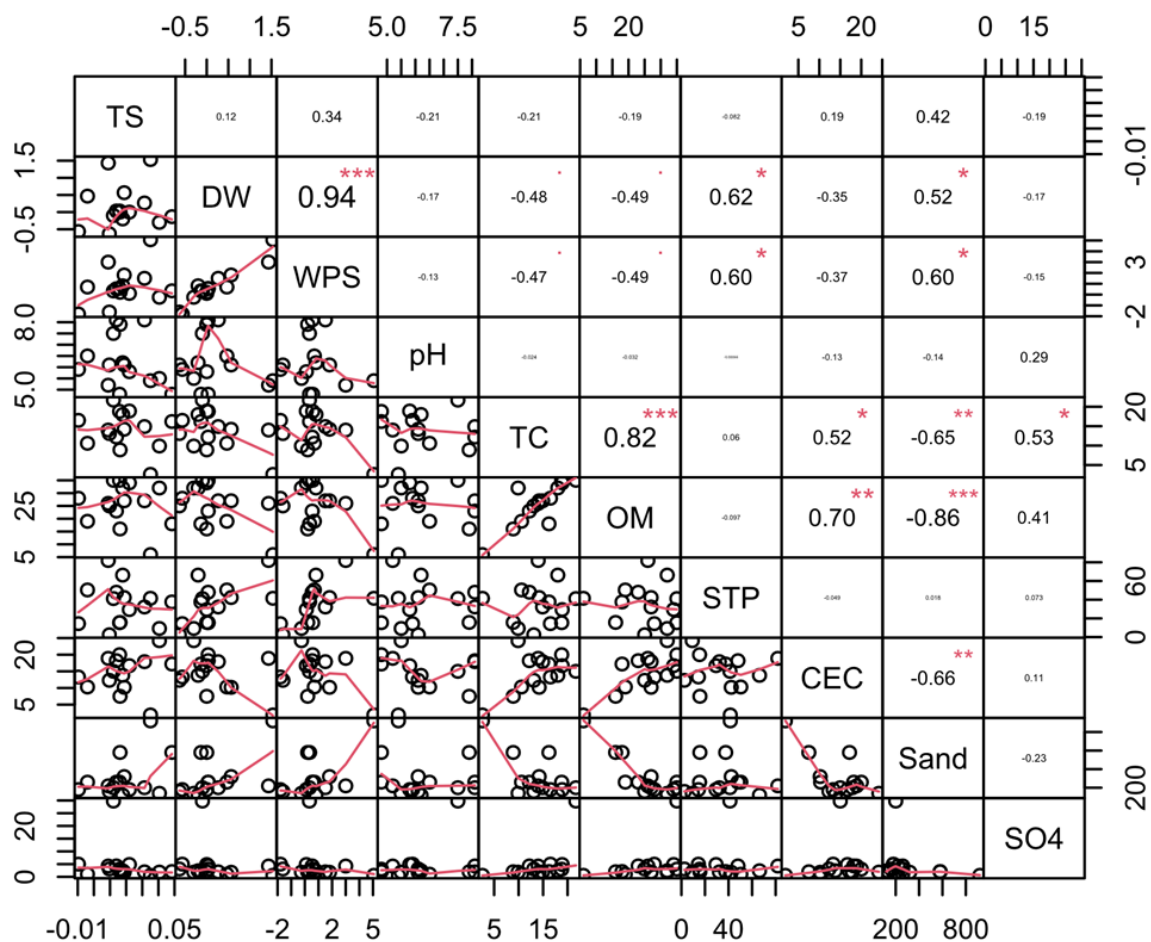


Figure 3.2. Pearson correlation matrix for various soil test parameters. DW, dry weight; STP, soil test phosphorus; TS total sulfur; WPU-S, whole plant sulfur uptake.

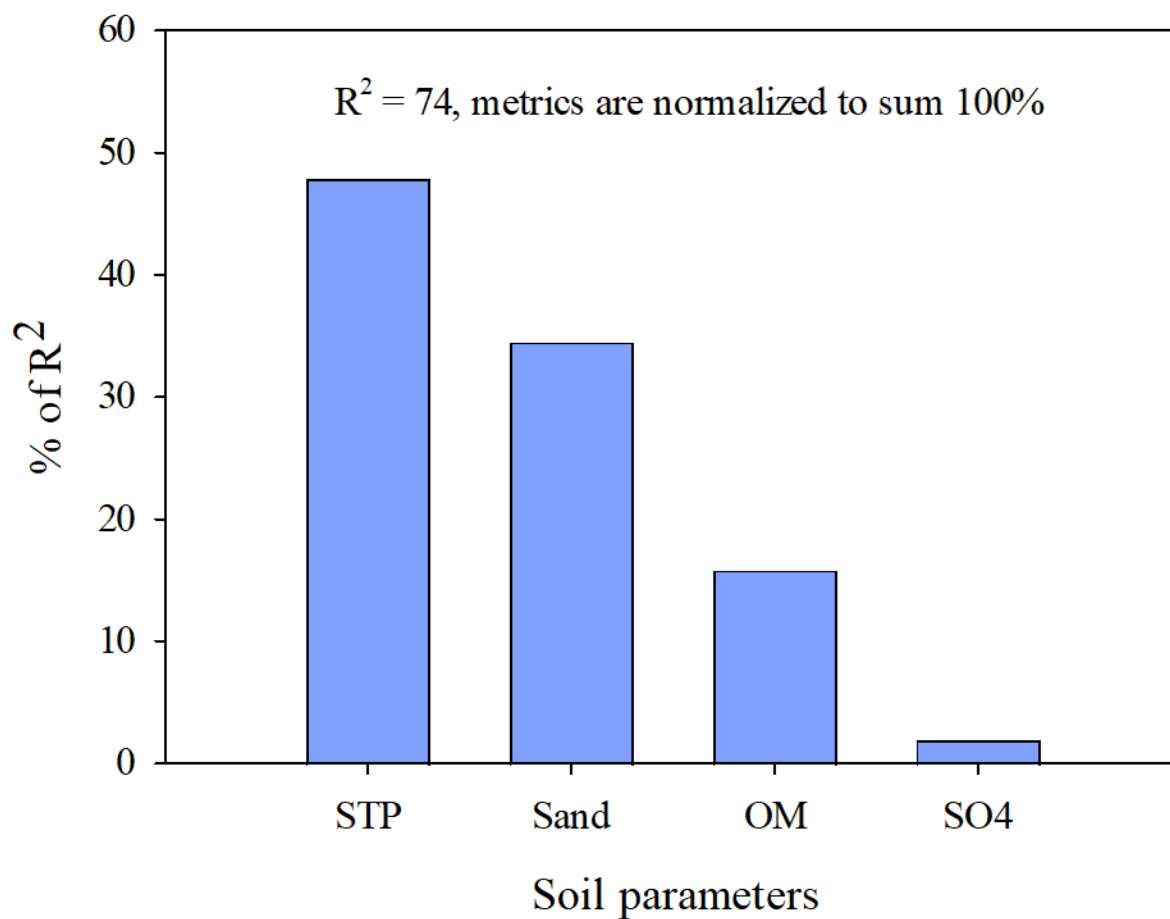


Figure 3.3. Relative importance of soil parameters on WPU-S (WPU-S = $-5.01 + 0.09 \text{ OM} + 0.05 \text{ STP} + 0.006 \text{ Sand} - 0.04 \text{ SO}_4$; $R^2 = 0.74$; p-value = 0.001)

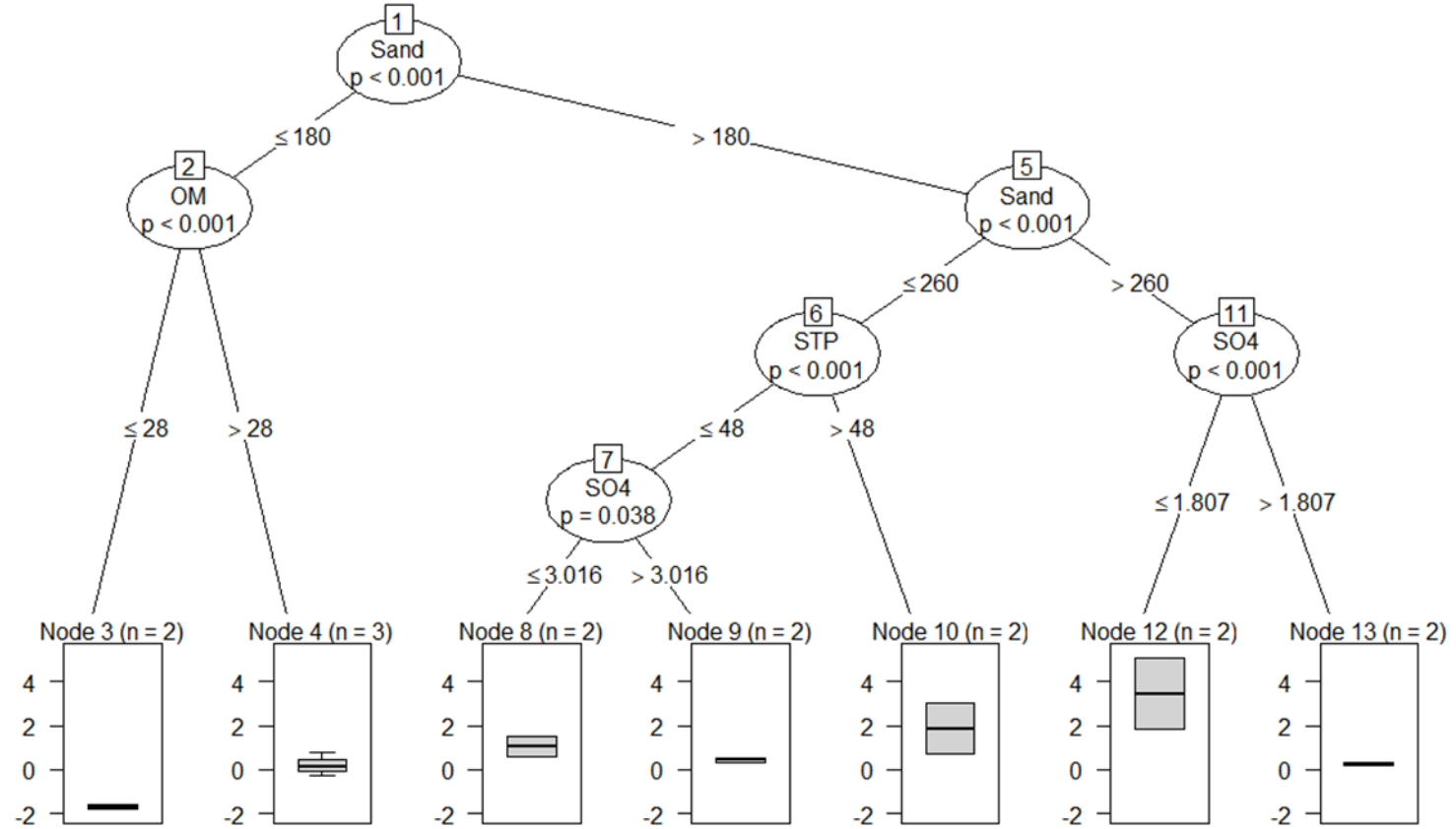


Figure 3.4. Conditional inference tree for the effects of sand, OM, STP, and soil SO₄ on the increase in whole plant S uptake with S fertilizer application.

Chapter 4 - General conclusions

Understanding different soil parameters and responses to those parameters by given inputs is critical for economically feasible corn and wheat management practices in Kansas. Both corn and wheat exhibit a great sulfur response in soils with high sand content and low organic matter levels. Results from the corn study indicate that yield differences by S rate or N source were not statistically significant across locations. However, across all locations, nitrogen fertilization over the control generated a statistically significant response grain yield. Based on the lack of statistically significant differences for yield across locations, 17 kg S ha⁻¹ appears to be a sufficiency threshold.

Total N uptake and total S uptake plotted against yield is a tool to help estimate yield based on a given fertilizer uptake. With an R² of 0.70, 70% of the variability in yield can be explained by total N uptake and with an R² of 0.55, 55% of the variability in yield can be explained by total S uptake. The graph indicates with a lower band of the graph plateauing that sulfur may not be the limiting nutrient for these locations. Moreover, added nitrogen fertilizer had a statistically significant increase over the control for post-harvest soil test nitrate, but sulfur failed to show a statistically significant increase over the nitrogen fertilizers across locations. None of the five locations demonstrated a significant change in post-harvest soil test ammonium. Post-harvest soil test sulfur in the grain and stover was higher than the initial soil test where no sulfur was added. Much of the sulfur came from the mineralization of sulfur from the OM. And accounting for this sulfur requires knowledge of outside influences in sulfur such as sulfur applied prior to planting and sulfur from irrigation water.

Results from the wheat study suggest that sulfur may not be the limiting nutrient for some soils. Although not statistically significant, 7 locations trended upward with the application

sulfur over nitrogen fertilization alone. An upward trend in DW was exhibited with added sulfur fertilization. High sand content at one of the locations likely contributed to large increase for WPU-S and DW at 60.1% and 40.3%, respectively. The correlation of various soil parameters were a factor in WPU-S and DW. Soil Test P and sand content influenced DW and WPU-S. Soil Test P demonstrated a strong, positive correlation for the difference in magnitude of response for WPU-S and DW. For locations 1 and 14, extremely low phosphorus levels at 9.2 mg kg^{-1} and 2.5 mg kg^{-1} may have been the limiting nutrient. Further research is needed to better identify and utilize soil test parameters and tools for improved soil diagnostics.