IS SULFUR LIMITING SOYBEAN YIELD IN NEW YORK?

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ABSTRACT

In New York, soybean (*Glycine max* (L.) Merr.) yields have not yet reached their genetic potential. Reduced ambient sulfur (S) deposition, combined with increased crop yields over time and a change from S-containing nitrogen (N) and phosphorus (P) fertilizers to urea, monoammonium phosphate (MAP) and diammonium phosphate (DAP) which do not contain S, has contributed to increased occurrence of S deficiencies in forage and grain crop such as alfalfa (*Medicago sativa* L.) and corn (*Zea mays* L.) in recent years. Given a reduction in S deposition from an estimated 10 kg SO_4-S in 1991 to 3 kg SO_4- S ha^{-1} currently, combined with an increase in yield over time from 2,690 kg ha^{-1} to greater than 4,708 kg ha^{-1}, farmers are now asking the question if S could be limiting soybean production in New York.

Sulfur is especially important for legumes like soybeans as it plays a critical role in N_2 fixation. Plants use S to regulate photosynthesis and build proteins and enzymes. In soybean plants there are two key amino acids that contain S, cysteine and methionine. Insufficient S supply can result in a reduction of both yield and crude protein (CP) content in soybeans, impacting crop production and grain quality. Thus, it is important to ensure sufficient S is available, especially given the increasing yield potential of soybeans.

Chapter 1 summarizes the literature on S needs for soybeans. In particular, we identify historical trends in soybean production versus decline in atmospheric S deposition, discuss factors affecting soybean supply of S for growth, yield and nutritional value, and present studies on tools used for S management. Modern soybean varieties now produce more biomass (photosynthetic activity) with improved nutritional value thanks to a combination of breeding and agronomic management improvements. This translates into higher S uptake per cropland unit. Reduced S supply by deposition combined with greater S removal in harvest explains the

increasing incidence of S crop deficiencies and, thus, the need for tools to assist in S management decisions. Tissue testing can be used to assess S deficiencies of plants. Soil S testing can identify potential for crop response to the application of S fertilizer earlier in the season but additional work is required to establish critical values for soybean soil testing methods. Field S balances can also be viewed as a management method for end-of-season assessment.

Chapter 2 presents the result of on-farm S response trials, conducted to understand the impact of S fertilization on soybean grain yield, crude protein (CP), S content, N:S ratio, and crop S balances. On-farm, replicated, trials were performed in thirteen locations in western New York comparing two S sources ($CaSO_4$ and $(NH_4)_2SO_4$) versus a no-S control. The lack of response to S addition in tissue analyzes conducted at early bloom and full maturity were consistent with results of soybean grain yields and CP levels, and in N:S ratios and did not suggest an S limitation. The negative partial S balances of the control treatments (crop removal – S supply by S deposition) showed a significant contribution of soil S to total crop supply. Soil organic matter (SOM) measured by the loss-on-ignition, however, was not a good soil S supply predictor. Therefore, critical soil test S levels could not be determined given none of the thirteen trials showed a crop response to the addition of S. We conclude that S at this time does not limit yield potential but continued monitoring is important as partial balances indicate that S may become deficient over time.

BIOGRAPHICAL SKETCH

Jodi Lynn Letham is a Field Crops Specialist for Cornell University Cooperative Extension Northwest New York Dairy, Livestock and Field Crops Team, where she provides interdisciplinary knowledge and applied research on forage livestock systems, nutrient management, and soil health in her respective region. Growing up on a family dairy farm in western Pennsylvania, Jodi quickly developed a strong passion for agriculture and an eagerness to learn. She began her academic career at The Pennsylvania State University majoring in Agricultural Sciences, minoring in Agronomy. While attending Penn State, Jodi worked for the USDA Pasture Management and Environmental Research Laboratory where she assisted in data collection and statistical analysis to determine forage quality for pastures utilized in rotational grazing for cattle. During her senior year, she accepted an internship with Growmark FS, LLC as a research associate in Hershey, Pennsylvania. Her summer internship project consisted of evaluating fourteen soybean seed treatments to determine efficacy and crop performance under biotic and abiotic stress conditions. Upon graduation in December, 2012, Jodi obtained fulltime employment with Brandt Consolidated Inc., consulting and selling plant nutrition products in the Northeast. She joined Cornell Cooperative Extension in March of 2017, where she gained experience in field, crop production, plant nutrition, nutrient management, soil fertility, integrated pest management, consultation, data analysis interpretation, and business development. While working full-time Jodi's desire to learn, grow and help farmers has led to her pursuit of a Master's of Science degree, first through the distance education MS Agronomy Program at Iowa State University where she completed her course work, and later transferring into the employee degree program at Cornell University where she conducted her research to evaluate if sulfur is limiting soybean yield in New York.

This thesis work is dedicated to my husband, Josh, who has been a constant source of support and encouragement during the challenges of graduate school and life. I am forever and truly thankful for having you in my life. This work is also dedicated to my parents, Lee and Judy, sisters, Jamie and Jenna, and grandparents, Jim, Sylvia and Mary, who have always supported and loved me unconditionally and have taught me to work hard for the things that I aspire to achieve.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1:

Importance of Sulfur for Soybean Production; a Review

Abstract

Soybean (*Glycine max* (L.) Merr.) is of high importance worldwide for oil, biodiesel and protein production. Reduced atmospheric sulfur (S) deposition, coupled with increased crop yields over time and a shift away from S-containing nitrogen (N) and phosphorus (P) fertilizers to urea, monoammonium phosphate (MAP), and diammonium phosphate (DAP), contributed to an increased occurrence of soil S deficiencies for crops. The objective of this literature review was to summarize literature on S management of soybeans. Specifically, we describe historic trends in soybean production versus decline in atmospheric S deposition, discuss factors affecting the supply of S for growth, yield, and nutritive value of soybeans, and present studies on tools for S management. Soybean production has increased over time in the United States. Through a synergy of breeding and agronomic management improvements, modern soybean varieties now produce more biomass (photosynthetic activity) with increased nutritive value. This translates into higher S uptake per unit of cropland. Reduced S supply through deposition combined with greater S removal in harvest explains the increasing occurrence of S deficiencies in crops and hence the need for tools that aid in decisions about S management. Tissue testing can used to determine S deficiencies of growing plants. Soil S testing can identify potential for crop response to S fertilizer addition earlier in the season, but additional research is needed to develop critical values for various soil testing methods for soybeans. Field S balances can be considered as an end-of-season evaluation tool for management as well.

Introduction

In 2019, farmers in the United States grew 108,864,000 metric tons of soybeans (*Gycine max* (L.) Merr.), taking the lead as top producer of soybeans internationally. Out of the total production, 25% were exported (27,463,600 million metric tons), with China importing the largest amount of U.S. grown soybeans. About 82% of the soybeans exported are sold whole, with soybean meal representing 15% and soybean oil 3% of exports. Soybean production has seen an increase over time (USDA NASS, 2019) (Figure 1.1). Unfortunately, prices have not followed the same upward trend and producers have experienced rising costs of input variables across the board (USDA NASS, 2020) (Figure 1.1). The prolific production of soybeans reflects the multitude of uses for protein (both in human food and animal feed), oil and biodiesel.

Production of high-yielding soybeans requires that farmers pay attention to selection of the best varieties, timely planting, appropriate row width, weed control, disease and insect scouting and management, proper soil management including pH management, and nutrient management. While according to the USDA-NASS (2019), soybean yields in the United States averaged 3,470 kg ha^{-1} in 2019, yields exceeding 6,725 kg ha^{-1} have been obtained in yield competitions (Frankenfield, 2017), and in 2019 a Georgia farmer set a new world record, producing 12,793 kg ha^{-1} (Latzke and Scott, 2019). Thus, yield potential of soybean seed can far exceed yield typically obtained.

As do other plants, soybeans require essential nutrients for growth and production, including the primary macronutrients nitrogen (N), phosphorus (P) and potassium (K), secondary macronutrients calcium (Ca), magnesium (Mg) and sulfur (S), and each of the eight essential micronutrients. Soybean response to nutrient addition depends on the initial nutrient status, crop growth and yield, and the weather during the growing season.

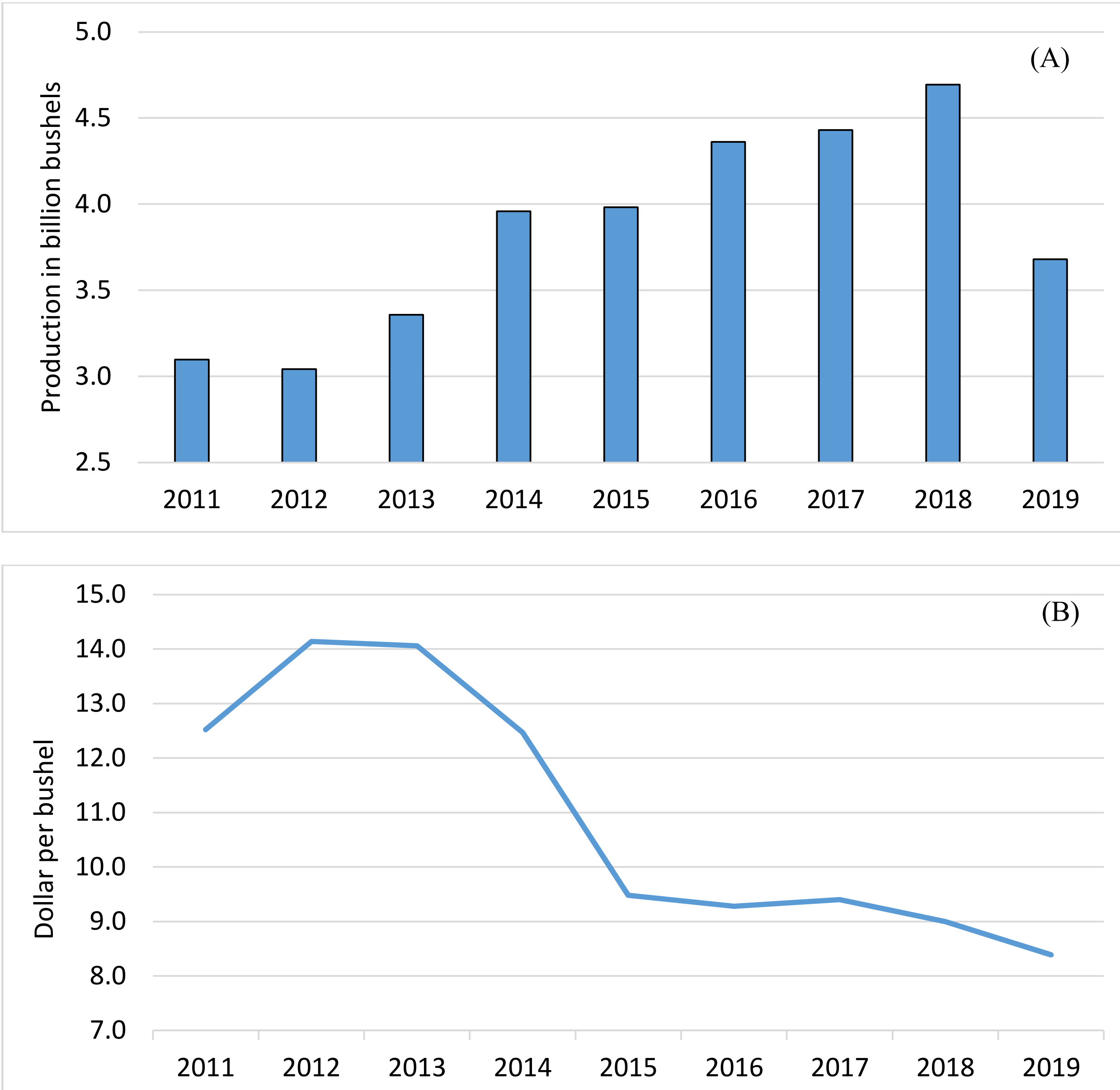

Figure 1.1: Total soybean production (A) and average prices received for soybean by month (B) in the United States (Source: USDA-NASS, 2020).

Of the macronutrients, S is unique in the sense that its availability for crop growth has changed dramatically throughout the world. In the United States, the passing of the Clean Air Act in 1970 as well as changes in fertilizer composition away from S containing sources, resulted in a considerable reduction in S availability for crops overall, contributing to a growing occurrence of S deficiencies in some areas and for some crops, including soybeans. Prior to the 1970 Clean Air Act, global atmospheric S deposition was an estimated 43 Tg S yr^{-1} (Andreae, 1986). The combustion of fossil fuels accounted for 80 to 85% of the total anthropogenic S emissions (Whelpdale, 1992). Since then, S deposition has declined over time (Figure 1.2).

Field research conducted in the 1980's in New York showed no yield increase with addition of S for alfalfa, corn, or wheat, indicating sufficient S supply with deposition and S mineralization from soil organic matter (Klausner et al., 1982; 1984). Beegle and Bowersox (2000) showed S supply from soil organic matter and S deposition in Pennsylvania was sufficient to meet corn S requirements as well. However, reports of S deficiencies have increased in more recent years. In Wisconsin, S responses have been reported on soils where they would not have anticipated deficiencies in the last 20 years (Collamer et al., 1999). Kelling and Speth (1998) measured an alfalfa yield increase with S addition in years 3 and 4 of a 4-year experiment across 19 counties in Wisconsin. Ketterings et al. (2012) showed S deficiencies in alfalfa in New York, reflecting a decline in S deposition since studies by Klausner et al. (1982; 1984).

Between 1989 and 2016, total S deposition in the eastern United States decreased 40% on average (NADP, 2018; USEPA, 2018) (Figure 1.3). Most atmospheric S deposition studies have been in forested watersheds with acidified lakes that were already sensitive to acid rain (e.g. Adirondack Mountains in New York, Appalachian Mountains). Reduced deposition of S in these areas has not yet resulted in full restoration of water quality (Shao et al., 2020).

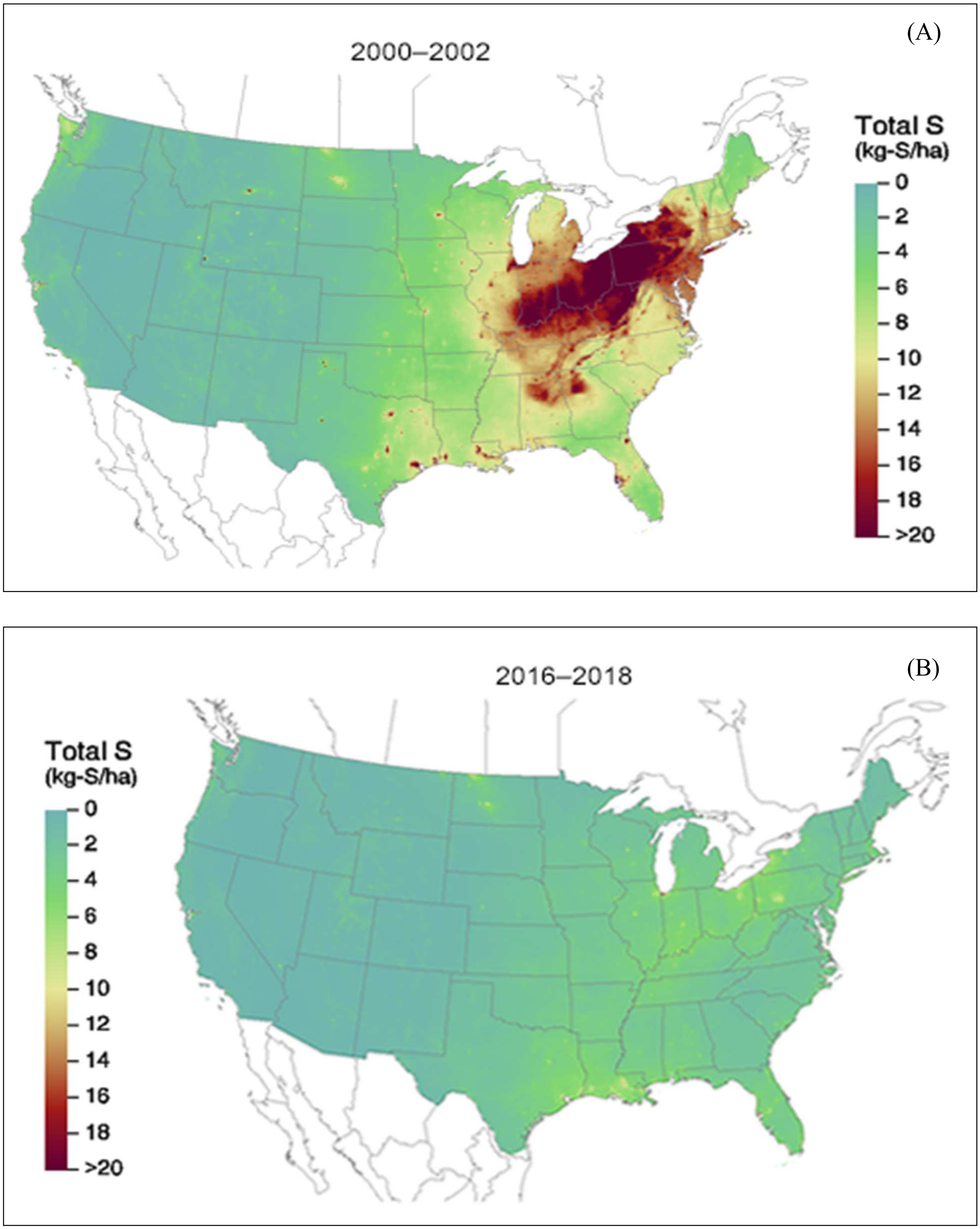

Figure 1.2: Three-year average total S deposition in 2000-2002 (A) compared to 2016-2018 (B) (Source: CASTNET/CMAQ/NADP, USEPA, 2019).

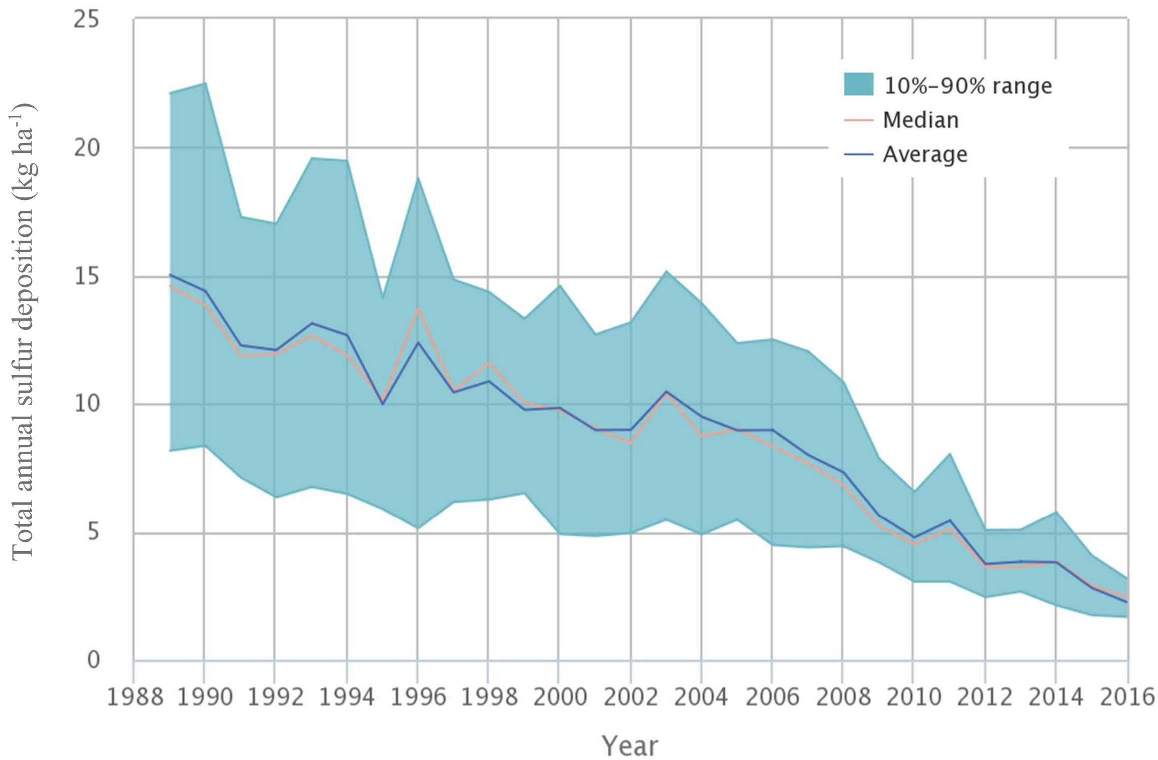

Figure 1.3: Total S deposition in the eastern US from 1989 to 2016. (Source: NADP, 2018; USEPA, 2018).

Sulfur deposition in New York amounted to about 10 kg SO_4 S ha^{-1} in 1991 (National Atmospheric Deposition Program, 2016), which could explain the lack of a response to S addition in studies by Klausner et al. (1982; 1984). Since then, SO_2 emissions from the combustion of fossil fuels decreased by 37% from 2002 to 2010, with the greatest reduction occurring in the northeastern US and the Ohio River Valley (Schwede and Lear, 2014). Currently, S deposition in New York amounts to 3 kg S ha^{-1} based on data from USEPA (2019). Given the increase in yield over time and reduction in S deposition, we can no longer assume that sufficient S is available for crop growth. Research is needed to evaluate crop response to S.

The objective of this review is to summarize current understanding of S needs for soybean production, with focus on the role of S in the plant, crop S uptake and removal with harvest, tools to determine S needs, including tissue testing, soil testing, field S balances, and S sources, including manure and fertilizers.

Role of Sulfur

Sulfur is an essential macronutrient for crop growth (Marschner, 1995). It is especially important for legumes like soybeans as S plays a critical role in N_2 fixation, the process where rhizobia bacteria in soybean roots fix atmospheric N (Scherer, 2001). Plants use S to regulate photosynthesis and build protein and enzymes. The two critical amino acids (building blocks of proteins) that contain S are cysteine and methionine (Krishnan et al., 2018). Because S is essential for N_2 fixation, S deficiency can result in the reduction of both yield and crude protein (CP) content in soybeans, impacting crop production and grain quality. Understanding nutrient accumulation, partitioning and remobilization is critical for optimal soybean production, but extensive research, including secondary macronutrients and micronutrients, is limited.

Crop Removal and Uptake

Soybean yields in New York have increased drastically since the 1960s but yields have leveled in the past 5-10 years, with the exception of higher yields in 2018, an exceptionally good soybean growing season (Figure 1.4). Since 2013, yields have averaged 3,026 kg ha^{-1} (45 bu/acre), ranging from less than 2,690 kg ha^{-1} (40 bu/acre) to greater than 4,708 kg ha^{-1} (70 bu/acre) for individual fields.

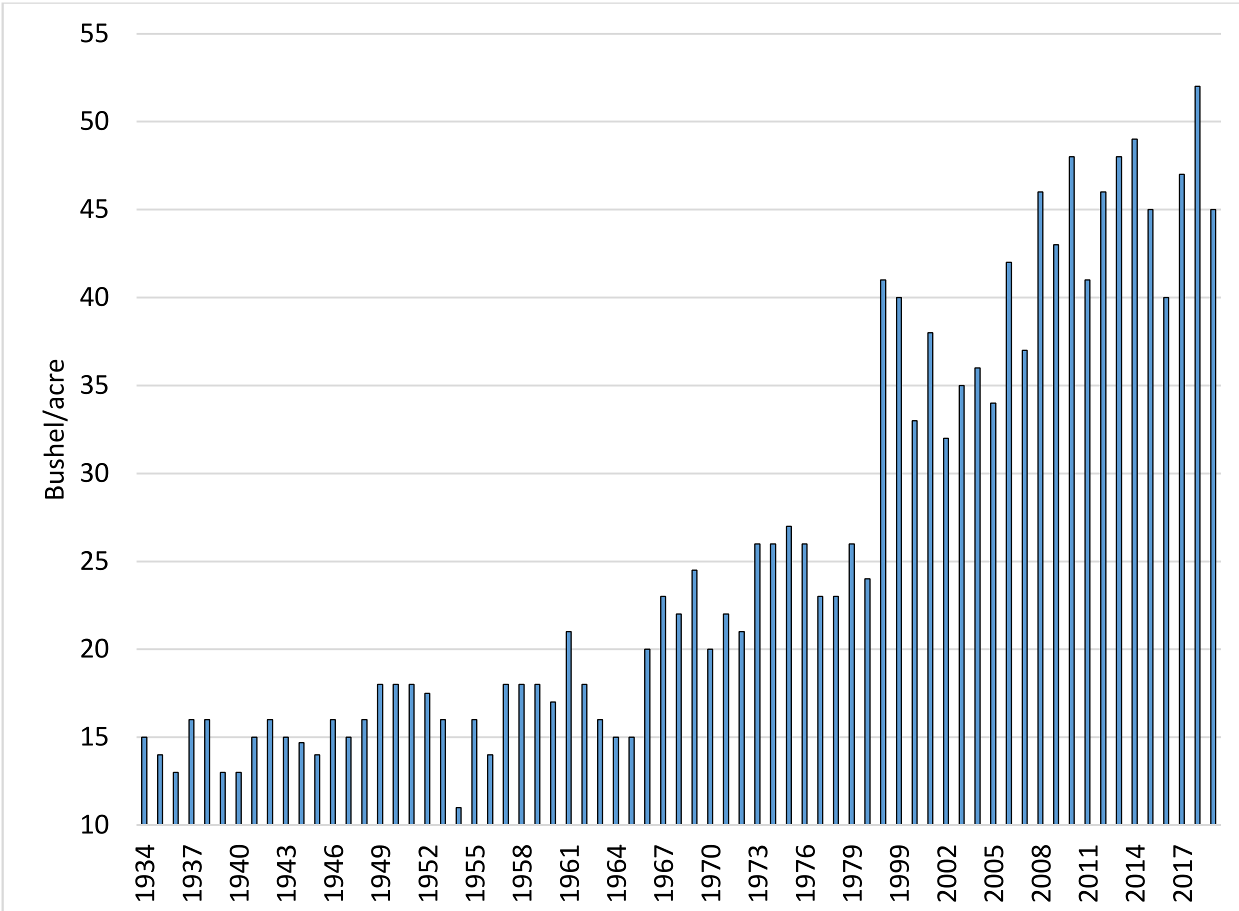

Figure 1.4. Trends in soybean production in New York (Source: USDA-NASS, 2020).

Bender et al. (2015) and Tamagno et al. (2016) showed that through the interaction of improved breeding and better agronomic management, newer modern lines respond favorably to timely (earlier) planting and N addition, if N is limiting growth. Leaf N concentrations and leaf N accumulation increased consistently throughout the entire growing season, reflecting the ability of improved varieties to retain leaf nutrient concentrations longer and increase photosynthetic activity (Bender et al., 2015; Tamagno et al., 2016). A similar response has been seen with S and other essential nutrients. The newer varieties are better at taking up and retaining nutrients through the R4-R6 growth stages, followed by reallocation of nutrients at pod fill (Figure 1.5).

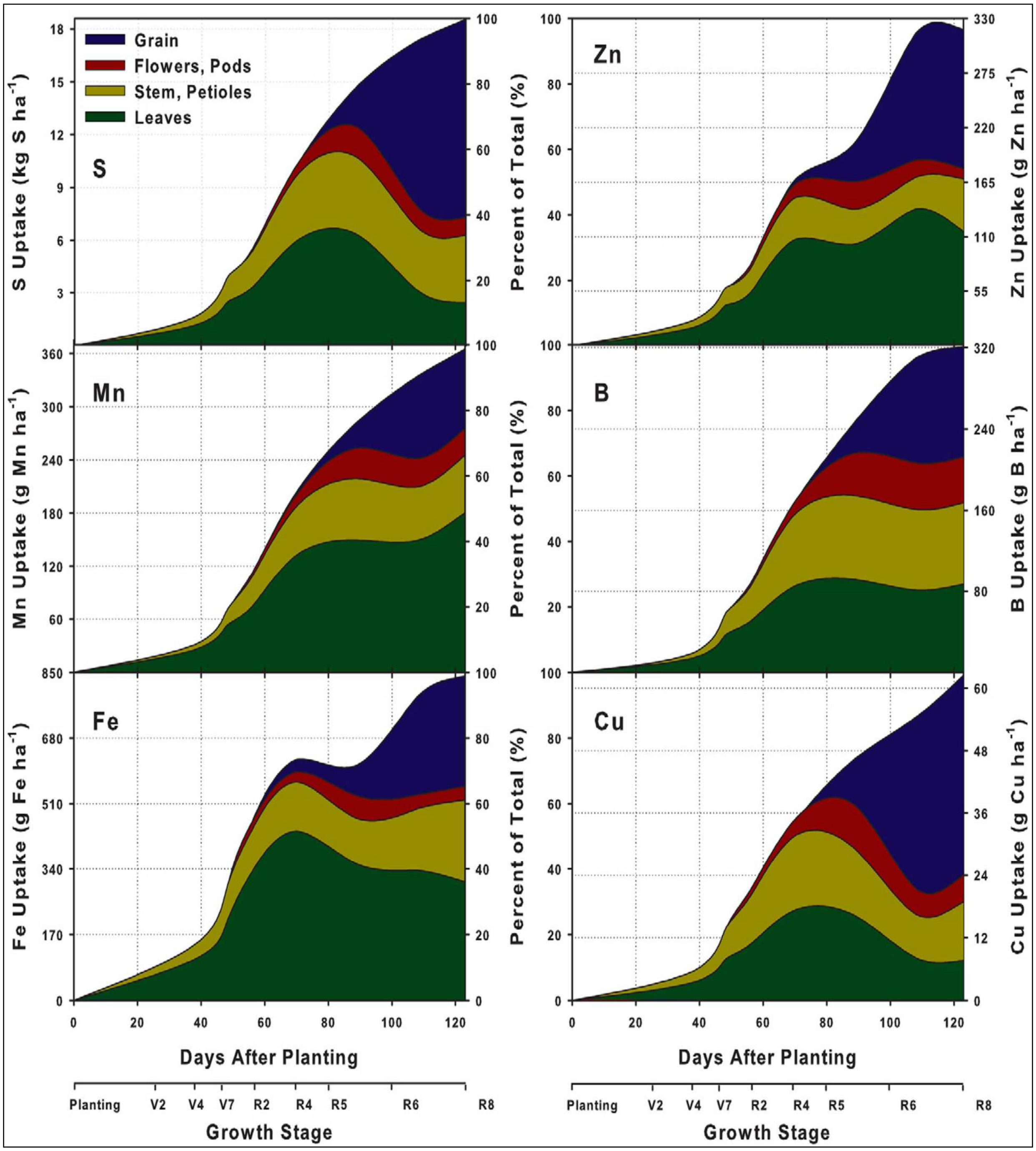

Figure 1.5. The seasonal accumulation and partitioning of sulfur (S), zinc (Zn), manganese (Mn), boron (B), iron (Fe), and copper (Cu) averaged over two soybean varieties and fertility regimes at 3 site-years during 2012 and 2013. All parameters were measured on a dry weight basis and produced an average grain yield of approximately 3480 kg ha^{-1} (Bender et al., 2015).

Sulfur Supply by Soils

Proper S nutrition of plants requires a sound understanding of the soil S cycle that influences the ability of a soil to supply plant-available S over different periods of time in the growing season. These processes include biological, chemical, and physical transformations of sulfur. Sulfate (SO_4^{2-}) is the plant available form of S in the soil.

Soil microbes are instrumental in turning soil organic matter (SOM) and crop residues into available nutrients, including sulfate, through a process called mineralization. Mineralization rates are impacted by temperature and moisture, with greatest mineralization when soil temperatures are between 20 and 40^{o}C and moisture content is just below field capacity for optimal moisture and oxygen availability (Weil and Brady, 2017). A higher and more active microbial population results in faster breakdown of SOM and thus greater S release. The carbon (C) to S ratio (C:S ratio) of SOM can be used to estimate S release but total S content is not typically stated on soil test reports. In general, 0.45 to 1.4 kg of S are released for every 1% SOM into the soil (Weil and Brady, 2017). According to Schoenau et al. (2008), within the SOM fraction, the organic sulfates are considered readily mineralizable because they are not directly bonded to C but rather linked to C via an oxygen or N atom ($C\text{-}O\text{-}SO_3$, $C\text{-}N\text{-}SO_3$). Organic sulfates make up 30-70% of the absolute organic S in soils. The remainder is organic S that is C-bonded in the form of S-containing amino acids (cysteine and methionine) in proteins, sulfonic acids, and complex heterocyclic mixes. Even though the amino acids might be readily mineralized by soil microorganisms, a significant portion of the C-bonded S can be very stable (Schoenau et al., 2008).

Organic S in crop residues mainly takes the form of C-bonded S. Decomposition of crop residue in the soil results in conversion of C-bonded S compounds (amino acids and vitamins) into microbial biomass and SOM rich in organic sulfates. Crop residue type and C:S ratio, placement and level of incorporation, soil temperature, moisture and oxygen level affect microbial activity and production of enzymes which in turn affect the release of plant available sulfate by mineralization (Scherer, 2001; Schoenau et al., 2008).

Soil pH can regulate nutrient availability as well. Whether a soil is acidic, neutral, or basic has much to do with the solubility of various compounds, the relative bonding of ions to exchange sites, and the activity of various microorganisms in the soil system (Alam et al., 1999). A pH between 6.8 and 7.0 is optimal. Several studies have shown that solution pH greatly affects the absorption of inorganic nutrients by plants (Islam et al., 1980; Jariel et al., 1991, Jensen, 2010; Casteel et al., 2019). Nutrient solution pH will affect the availability of certain elements, particularly the micronutrients, stimulating excessive uptake at a lower pH as was shown in studies by Casteel et al. (2019), where soybean responded to S addition in a sandy soil with a pH of 6.4. The solubility and plant availability of micronutrient cations in soils generally decreases with increasing pH due to adsorption-precipitation reactions (Alam et al., 1999).

Sandy soils are more prone to S deficiencies than medium- to heavy-textured soils because (1) negatively charged sulfate can leach out of the root zone of course-textured soils, and (2) sandy soil is typically lower in SOM and drier, resulting in reduced microbial activity (Weil and Brady, 2017).

Manure histories of fields can impact S supply as well. Manure contains S in both organic and inorganic forms. It is generally in reduced forms (e.g. sulphide), versus an oxidized form (e.g. sulfate) that can be used by plants. Any organic S in the manure must be converted to sulfate-S by soil microorganisms before it can be used by the plants. As much as 95% of the total S in manure may not be readily available to plants, depending on manure source (Eriksen, 2009). This raises questions about S supply in manured fields. Ketterings et al. (2011) studied soil S trends with time as impacted by fertility management and found that, during corn years, S concentrations of the top 30 cm of the soil profile increased over time with annual addition of compost or manure for corn year, averaging 12 mg S kg^{-1} across four manure and compost treatments versus 9 mg kg^{-1} in plots that had received inorganic N fertilizer only. During the alfalfa years that followed the first five years of the study, soil S content declined each year at a rate of about 2 mg S kg^{-1} yr^{-1} (Ketterings et al., 2011), quicker than buildup of S in the corn years. Ketterings et al. (2011) concluded that manure fields are not likely to be S responsive during the year of manure application or within a year of the last application of manure, but corn and alfalfa rotations should be monitored for S over time, given the rapid decline in soil test S when S is no longer supplied with manure and crops with a high S removal rate such as alfalfa are grown.

Combining all factors discussed here, soybeans grown on coarse-textured, low organic matter (OM) soils are most likely to be S deficient, while S is unlikely to be limited on high organic matter soils and when soybeans are planted on fields with a recent manure history (Weil and Brady, 2017).

Plant and Soil Tests to Determine Sulfur Levels

Predicting S requirements for plants has become increasingly important for crop production around the world. Common diagnostic practices such as plant tissue analyses, seed analyses, harvest index (HI), and soil analysis have been used to make additional S application recommendations. All of these methods have their advantages and disadvantages.

According to Black Kalff et al. (2002), plant tissue analyses are better at predicting the necessity of S fertilizer than soil testing. Plant tissue analysis can provide a means of testing whether the S requirements of the crop are actually being met, and appears to be more closely related to the actual amount of S available to the crop at the time of sampling (Melsted et al., 1969). Tissue testing is recommended by most land grant universities for determining S deficiencies (Vitosh et al., 1995; Pennsylvania State University Extension, 2010). Tissue analyses of the newest, fully developed trifoliate at pre-bloom, early bloom, or before pod set can be used to determine if the soybean plant is S deficient. It is recommended to collect 25 trifoliate leaves per field or sampling area (Dairy One Forage Laboratory, 2017). Normal S sufficiency levels range (for fully developed trifoliate soybean leaves) from 0.20 to 0.40% (Dairy One Forage Laboratory, 2017). Work by Ketterings et al. (2012) indicated a critical tissue test of 0.27% S in the top 15 cm of alfalfa at early bloom, prior to third cutting in trials conducted in 2008 in New York, consistent with 0.25% S typically reported for the crop.

Some studies suggest plant tissue N:S ratio can be used as a diagnostic tool (Guillermo et al., 2015). Guillermo et al. 2015 reported that N and S vary concomitantly because both nutrients are mainly related to protein content in plants. As a result, concomitant variation of N and S content of lamina caused low variation of the N:S ratio. In contrast, the decrease of N:S in stem was more pronounced as S content increased. Guillermo et al. (2015) showed these patterns were also evident in the low dispersion of N:S in lamina compared to N:S in stem. The high responsiveness of S content and N:S ratio in stem to S addition makes these variables good candidates for diagnosing the S status of soybean.

Seed analyses have also been utilized to successfully diagnose the S status of different crops (Randall et al., 1981, 2003; Reussi Calvo et al., 2011). Hitsuda et al. (2004) reported that S concentration in soybean seed was better correlated to the relative seed yield than leaf S concentration in a pot experiment. During seed filling, grain tissues required remobilization and season long nutrient accumulation. However, work by Salvagiotti et al. (2012) indicated the S concentrations or N:S in seed did not correctly identify sites responsive to S in field experiments. Chlorophyll concentration associated with leaf greenness intensity is connected to S concentration in the shoot (Hoefgen and Nikiforova, 2008; Pagani and Echeverria, 2012). Hitsuda et al. (2004) found this index to be associated with the seed yield of plants grown in pots under gradient S rates. Recent field work under diverse experimental conditions (i.e. pots or fields) evaluated some indices (plant tissue and stoichiometry) of S sufficiency in soybeans (Salvagiotti et al., 2012; Kaiser and Kim, 2013). However, the analysis of the patterns of N and S concentration and therefore the definition of critical thresholds for S deficiency were limited as only two S treatments were tested (no S and one S rate).

Research conducted by Tamagno et al. (2016) found that the yield to uptake relationship for N was primarily explained by the nutrient harvest index (NHI) and that plant nutrient ratios were primarily governed by the nutrient content of the stover fraction, acting as a nutrient reservoir or supply depending on nutrient demand for the seed. Therefore, the NHI index equation could explain whether a response to fertilizer addition is likely. Research is needed to evaluate the NHI for use as a S assessment tool.

The literature shows a variety of soil S test methods (Westermann, 1974; Warman and Sampson, 1992; Ajwa and Tabatabai, 1993; Tabatabai, 1996; Ketterings et al., 2011). The study by Ketterings et al. (2011) showed that of six soil tests evaluated, the 0.01 *M* $CaCl_2$ extraction was best correlated with S addition and showed the greatest consistency between analysis methods tested (Ketterings et al., 2011). Field trials with this test (Ketterings et al., 2012) showed a critical value of 8 g kg^{-1} beyond which alfalfa response to S addition was unlikely. Additional research under a broader variety of soils, field histories, and environmental and climatic conditions is needed to test the accuracy of current soil-test based management tools and practices for making decisions on S management of soybeans.

Sulfur Fertilizer

If seed, plant, and/or soil testing suggest that an S deficiency is likely, S addition can be supplied with fertilizer. Fertilizer sources of S may be categorized as inorganic or organic (Table 1.1). Similar to S from crop residues and SOM, elemental S needs to be converted to plant-available sulfate first through a biological process carried out by several types of microorganisms. The rate of conversion is determined by physical properties (particle size) of the elemental S source, microbial population, and environmental conditions. The other four S sources listed are highly soluble (contain sulfate), so S from these sources is directly available for plant uptake (Dick et al., 2008). There is little to no carryover of sulfate applied from one year to the next (Ketterings et al., 2012).

Table 1.1: Inorganic sources of sulfur fertilizers.

Fertilizer sources	N-P_2O_5-K_2O	S
	----------------------%	----------------------
Elemental S	0-0-0	99.5
Gypsum	0-0-0	24
Ammonium thiosulfate	12-0-0	26
Ammonium sulfate	21-0-0	24
Superphosphate	0-9-0	11-12
Magnesium sulfate	0-0-0	14
Potassium magnesium sulfate	0-0-18.2	22
Potassium sulfate	0-0-50	18
Calcium sulfate	0-0-0	15-18

Sulfur Balances as Diagnostic Tools

Sulfur balances can be derived in different way. An equation describing all sources and sinks for plant available S in the soil can focus attention on those components that are most important in controlling crop S nutrition. The starting point for a material balance on a component within a system is the equation (Felder and Rousseau, 2000):

$$\text{Input} + \text{Generation} = \text{Output} + \text{Accumulation} \quad [1]$$

The input term includes atmospheric deposition of S, typically as sulfate in acid rain, and S addition with fertilizer or manure. The generation term represents plant available S produced by mineralization of SOM and by weathering of sulfur-containing soil minerals. The primary outputs or losses from the soil are leaching of sulfate and crop removal of S as harvested grain or forage. The accumulation term represents a change in plant available S in the soil and could be positive, negative, or zero. Substituting these terms into the general equation yields (Kost et al., 2008):

$$S_{\text{deposited}} + S_{\text{fertilizer}} + S_{\text{mineralized}} + S_{\text{weathering}} = S_{\text{leached}} + S_{\text{removed by crop}} + S_{\text{accumulated}} \quad [2]$$

or:

$$S_{\text{deposited}} + S_{\text{fertilizer}} + S_{\text{mineralized}} + S_{\text{weathering}} - S_{\text{leached}} = S_{\text{removed by crop}} + S_{\text{accumulated}} \quad [3]$$

If the amount of S needed for good crop growth is greater than that made available in the soil by atmospheric deposition, fertilizer application, organic matter mineralization, and mineral weathering, the deficit in S could be satisfied, at least in the short term, by a negative S accumulation which implies a decrease in plant available S in the soil.

We usually lack information on the annual accumulation of plant available S in the soil. If we assume, when deriving balances, a steady state situation (S accumulation = zero), it leads to the following equation that may be used to evaluate soil terms for their abilities to supply sufficient sulfur for good crop growth (Kost et al., 2008):

$$S_{deposited} + S_{fertilizer} + S_{mineralized} + S_{weathering} - S_{leached} = S_{removed\ by\ crop} \qquad [4]$$

If the value of the left side of equation [4] is less than the S required by the growing crop, the crop will suffer some degree of S deficiency.

Yet, in equation [4], there are still three terms that are difficult to quantify: weathering, mineralization, and leaching. Characterizing soil nutrient flows is an important tool for net negative or positive nutrients budget predictions (Sanchez et al., 1997) but for most soils, S input due to weathering of minerals is small and inconsequential. For soils high in SOM, soil mineralization can be significant and in wet climates and sandy soils, S lost by leaching can be large too.

A balance approach to predicting crop responses to S fertilizer additions was developed by McGrath and Zhao (1995) for conditions in the United Kingdom and production of corn and alfalfa in Ohio as well. The risk of S deficiency in cereals in Britain was assessed using a qualitative model and for each of the 6301 soil data points, a risk index was generated by considering the inputs of S from atmospheric deposition, the content of soil organic matter and factors influencing the potential leaching of sulfur, i.e. soil type, texture, pH and annual precipitation (McGrath and Zhao, 1995). Based on this risk index and the assumption of 15 Mg DM ha^{-1} yield of alfalfa and 2 kg S per ton of dry matter (DM) removal of S with yield, Kost et al. (2008) classified 43% of soils in Ohio as variably deficient in S for alfalfa, while 50% were classified as moderately or highly deficient.

A complete balance would be useful, but lack of information on several of the pools in the equations above make this challenging. Evaluation of partial budgeting as an approach to S management and evaluation is more realistic. Partial budgets can be developed by taking into account S removed by the crop (in grain) or S accumulated in the crop biomass, compared to S supplied by fertilizer, manure, and S deposition. Such partial balances can be quantified more accurately (less “estimation”) and they can still be meaningful for assessment of long-term sustainability of cropping systems. Such partial budgets can be derived using the following equations:

$$\text{Partial } S_{grain} \text{ balance} = S_{deposited} + S_{fertilizer} - S_{removed\ by\ crop\ grain\ yield} \quad [5]$$

$$\text{Partial } S_{biomass} \text{ balance} = S_{deposited} + S_{fertilizer} - S_{removed\ taken\ up\ by\ crop\ biomass} \quad [6]$$

Partial balances for grain are typically easier to derive as farmers typically know seed yield, while S content can be measured or derived from averages reported by analytical laboratories. For crop biomass balances, whole plant S content and biomass values are required. Where biomass is not measured, assumptions about HI can be used to derive total plant biomass. Petersen and Lauer (2004) evaluated the response of soybean yield components to management systems and planting date in an upper Midwestern field study. In their study, the measurement of crop yield (HI) was impacted by both planting dates, with higher HI at the earlier planting and management system, and greater HI for no-till compared to conventional tillage. However, across treatments, the HI still showed a relatively small range, from 56 to 58%. Thus, while introducing uncertainty, a S biomass balance can be derived using information on seed yield, total plant S content, and HI. While not commonly reported in the literature, combining tissue testing with soil testing and S balances, can give growers greater confidence in the need for S management.

Summary/Conclusions

As atmospheric S emission has decreased in recent decades, farmers are questioning the need for S management for crops. Soybeans need sufficient S to assist in N fixation and production of two essential amino acids, cysteine and methionine, that help build protein and enzymes. Tissue, seed and soil testing can aid in diagnosing a S deficiency and are common practices utilized to make seasonal S management decisions. In addition, field S balances can be used to identify the sources and sinks that are most important in controlling crop S nutrition and predict crop responses to S fertilizer additions. Due to the lack of a response to S in studies conducted in the 1980s, research on crop response to S is limited, especially for soybeans. However, current S balances where no S is supplied with fertilizer suggest such research is needed.

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Chapter 2:

Impact of Sulfur Application on Soybean Yield and Quality in New York

Abstract

Sulfur (S) fertilizer is not widely used for soybean (*Glycine max* (L.) Merr.) production in the Northeastern United States while responses to S have occurred more frequently in other crops. The objectives of this study were to determine the impact of S fertilizers ($CaSO_4$ and $(NH_4)_2SO_4$ at 168 kg S ha^{-1}) on soybean grain yield, crude protein (CP), S content, N:S ratio, and crop S balances. On-farm, replicated, trials were conducted in 2017-2019 using a randomized complete block design in three to six replications across thirteen trials in western New York. Tissue analysis for each trial at early bloom and full maturity did not suggest an S limitation, consistent with the lack of a response to S addition in soybean grain yields and CP levels, and in N:S ratios which were in sufficiency ranges of 14:1 to 18:1. The negative partial balances (crop removal – S supply by S deposition) of the control treatments showed a considerable contribution of soil S to total supply for the crop. However, soil organic matter (SOM) measured by loss-on-ignition was not a good indicator of soil S supply. Given none of the thirteen trials showed a crop response to S addition, critical soil test S levels could not be determined. Studies on S deficient sites are needed to determine critical values for soil test S for soybeans. We conclude S is not limiting soybean yield at this time, but continued monitoring is needed as partial balances suggest S may become deficient over time.

Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the largest cash crops in the United States, dominating a full one-third of the country's agricultural land (USDA-NASS, 2019). Soybeans are a prime export crop, reflecting their use for human food, animal feed, and biodiesel, as well as a host of other products. The majority of the crop is processed for oil. On average the production of 5 kg of crude oil requires 27 kg of soybeans (USSEC, 2015), and also results in the production of 22 kg of protein-rich soybean meal that can be used as animal feed.

Sulfur (S) is a vital macronutrient that plants use to build protein and enzymes (Marschner, 2012). The two critical amino acids (building blocks of proteins) that contain S are cysteine and methionine (Krishnan et al., 2018). A S deficiency reduces the production of both of these amino acids and can result in yield loss and lower protein content of soybeans (Sweeney and Granade, 1993). Thus, it is important to ensure sufficient S is available to sustain increasing yields over time.

The reduction of SO_2 emissions from industry to the atmosphere resulting from environmental legislation to limit such emissions (National Atmospheric Deposition Program, 2018) has had a major impact on reducing S deposition from air to soil. In New York, S deposition is now estimated to be 3 kg SO_4- S ha^{-1} compared to 10 kg SO_4-S in 1991 (USEPA, 2019). In addition, agriculture has shifted to use of N and P fertilizers such as urea, mono-ammonium phosphate (MAP), diammonium phosphate (DAP), and triple superphosphate (TSP), which contain little or no S. Research in New York on S needs for alfalfa [*Medicago sativa* L.], corn [*Zea mays* L], and wheat [*Triticum aestivum* L.] showed no response to S addition in the mid-1980s (Klausner et al., 1982; 1984). However, S deficiency has become a major problem for crop production in many countries (Chien et al., 2011) and research on the impact of S addition on yield and quality of crops is needed. A review by Hitsuda et al. (2008) of several S sources indicated that, while the S needs of legume crops are high per unit of yield produced, their need for S fertilizer to meet the demand is relatively low compared to other crops, suggesting considerable capacity by legumes to take up S from the soil.

Reports of yield responses to S have been mixed (Ham et al., 1975; Sawyer and Barker, 2002; and Gutierres Boem et al., 2007). Diagnostic factors such as soil, plant tissue, or grain tests have been used to make additional S application recommendations. Soil testing has been a reliable method for determining phosphorus (P) and potassium (K) fertilizer application for soybeans (Kaiser et al., 2013) but soil testing for S is not a common practice. Ketterings et al. (2011) evaluated six soil tests for use as a soil S test for alfalfa. Among the methods tested, the 0.01 *M* $CaCl_2$ extraction was best correlated with S addition and showed the greatest consistency between analytical methods tested (Ketterings et al., 2011). Much of the organic sulfates in SOM are carbon-bonded and not readily available to plants. Soil fertility textbooks suggest that 0.45 to 1.4 kg of S is released in the soil for every 1% SOM of the soil (Weil and Brady, 2017). However, field research is needed to determine a critical soil test S value beyond which soybeans are unlikely to respond to the addition of S.

Plant tissue analysis is a better way to predict the necessity of S fertilizer than soil testing, according to Melsted et al. (1969) because plant tissue can provide a means for testing whether the crop's S requirements are actually being met, and appears to be closer to the actual amount of S available to the crop at the time of sampling. Hitsuda et al. (2008) suggested an optimum concentration of leaf S at early bloom for satisfactory yield of 2.0 to 3.1 g S kg^{-1}; however, these values were not determined using S response trials in the field but rather in a greenhouse under a controlled environment. Guillermo et al. (2015) found N:S ratio to be a better diagnostic of the S status than simple S content in the leaf and shoots. Hitsuda et al. (2004) determined that the concentration of grain S was more closely related to yield than that of tissue S. Yet, in more recent work Salvagiotti et al. (2012) indicated that the concentrations of S or N:S in grain did not correctly identify trials responsive to S in their field experiments. In addition, samples taken later in the season, while good for diagnostic purposes, prevent remedial applications (too late in the season) and may thus not be useful if differences for a given site within a given year are related to specific environmental factors.

Nitrogen, P, K, calcium (Ca), magnesium (Mg) and S are all essential macronutrients for growth and development of crops. If one nutrient affects the ability of plants to use another nutrient, the application of such nutrients may create synergistic relationships (Black, 1993). Fertilizer addition in the right amounts can improve crop production and quality. Sexton et al. (1998), for example, reported that N and S affect the level of composition of protein stored in soybean seeds. Kim et al. (1999) stated that during assimilation to N and S, SO_4^{2-} and NO_3^- are linked to protein synthesis. Many researchers have noted that although total protein content may not be affected by the application of S, protein quality through increasing the levels of amino acids in the grain may still have potential benefits (Sexton et al., 1998; Krishnan et al., 2005; Naeve and Shibles, 2005). Hitsuda et al. (2004) explained that, even if soybean yield reductions are not occurring, S can still be considered deficient if grain protein levels are improved through fertilizer application.

Partial balances can be established by taking into account S removed with harvest of the crop (in grain) or accumulated in the crop biomass (whole plant), compared to S provided by fertilizer, manure, and S deposition. Partial balances can be quantified more accurately (reduced estimation) than complete nutrient balances that take into account S supply through mineralization and S loss through leaching and other loss pathways. A partial balance, when no S is supplied with fertilizer and manure, can also be used to estimate soil S supply through soil organic matter (SOM) mineralization. Such partial balances are a valuable way to evaluate the long-term viability of crop systems and management (Sanchez et al., 1997).

The objectives of this study were to evaluate the impact of S fertilizers ($CaSO_4$ and $(NH_4)_2SO_4$) on soybean grain yield, crude protein, S content, and N:S ratio. In addition, we evaluated partial S balances to determine S supply by soil across three years and several trials. We hypothesize that S addition, independent of S source ($CaSO_4$ and $(NH_4)_2SO_4$), will increase yield, crude protein, S content, and N:S ratio of soybean grain yield.

Materials and Methods

On-farm S response trials were conducted at thirteen farms in western New York from 2017 to 2019. In 2017, the treatments included $CaSO_4$ (gypsum; 20% S) versus a no-S control. In 2018 and 2019, a second S source, $(NH_4)_2SO_4$ commonly known as ammonium sulfate (AMS; 24-26% S) was added. Each S source was applied at a rate of 34 kg S ha^{-1}. The two S sources were chosen to separate N or Ca response from an S response. Treatment strips were arranged using a randomized complete block design. Strip width was 21.3 m at all trials with the exception of trial thirteen which had a strip width of 9.1 m. The length of each strip and number of blocks (replications) were determined by field size and shape. As a result plot length for trials one to twelve ranged from 152 to 682 m resulting in plot sizes ranging from 3,246 m^2 to 14,526 m^2, and from 3 to 7 replications. For trial thirteen the plot length was 99.1 m resulting in a plot size of 902 m^2, and had 4 replications. The width of the actually harvested plot areas varied from 5 to 12 m determined by the combine header width (Table 2.1). The previous crop at each trial was either corn harvested for grain, corn harvested for silage, or alfalfa. Soybean varieties were determined by the farmers and differed from trial to trial. The seeding rate ranged from 303,810 to 419,900 seeds ha^{-1} depending on the drill and row spacing of the planting equipment at each farm. For all trials, primary tillage took place in the fall followed by spring field cultivation for seedbed preparation prior to planting (Table 2.1).

Soil cores were collected from the whole field at each site before S application. Composite samples from each field (15-20 cores per 8 ha^{-1}, 0-20 cm depth) were air-dried, crushed to pass a 2-mm sieve, and oven-dried at 50°C before analysis of Mehlich-3 extractable nutrients. Soil samples were analyzed for soil pH (1:1 soil/water; Watson and Brown, 1998), SOM (loss-on-ignition at 500°C; Wang and Anderson, 1998), and Mehlich-3 extractable P, K, Ca, and S (Mehlich, 1984). Extractions were analyzed using inductively coupled plasma-optical emission spectroscopy (ICP-AES) in 2017 and 2018. In 2019 soils were analyzed for available S using the 0.01 mol L^{-1} $CaCl_2$ extractable S method described in Ketterings et al. (2011). Based on Mehlich-3 results, one trial was classified as low in soil test P while five trials were low in soil test K (Table 2.2), according to Pennsylvania State University Agricultural Analytical Services Laboratory (2016). In addition, for seven trials the soil pH was below 6.7, the minimum pH below which lime addition is recommended. In fields where fertility was very low or low, farmers made a fall and/or spring applications. At trial 11, where soil test P was low, 68 kg ha^{-1} of 9.8-28-3-5.2 (N-P_2O_5-K_2O-S) starter fertilizer was applied for a total P application of 19 kg P_2O_5 ha^{-1}, to address potential P deficiencies. At trials 4, 6, 8, 10 and 13, where soil test K was low, K was applied at planting at the rate of 113.5 kg ha^{-1} using muriate of potash (0-0-60) for a total K application of 68 kg K_2O ha^{-1} to overcome any potential K deficiencies.

Tissue samples of the newest fully developed trifoliate was taken at early bloom and consisted of sampling 25 trifoliate leaves within each plot. The tissue samples were dried using a 60ºC forced-air oven for a minimum of 24 h, ground to pass through a 0.5-mm sieve, extracted with acid digestion (Dairy One Forage Laboratory, 2019) and then analyzed for total P, K, Ca, Mg and S with ICP-AES following USEPA (1996). Tissue P content ranged from 4.2 mg kg^{-1} in trial 2 to 6.5 mg kg^{-1} in trial 5 indicating sufficient P availability across all sites. Tissue K content ranged from 15.9 mg kg^{-1} in trial 4 to 29.1 mg kg^{-1} in trial 1 exceeding 17.0 mg kg^{-1} at all trials, the critical value according to Pennsylvania State University Agricultural Analytical Services Laboratory, except trial 4. While close to the critical value, tissue results of trial 4 suggest K could have been yield limiting. Based on the tissue test results, P and K were not limiting for the trials where soil test P and K levels suggested a possible deficiency, indicating the additional P and K supplies to those trials met crop needs.

Table 2.1. Site and field information.

Year	Site	County	Variety†	Gypsum	AMS±	Previous Crop	Planting Date	Row Width	Plant Tissue	Harvest Date	Combine Head	Planting Pop.
				-----kg ha^{-1}-----				--cm--			--m--	seed ha^{-1}
2017	1	Wayne	AG520-J5X	168	.	Corn grain	6/03/17	38	7/25/17	10/15/17	9	370,500
2018	2	Wayne	AG06X7	197	128	Corn grain	5/24/18	38	7/20/18	9/24/18	9	358,150
2018	3	Livingston	AG2431	197	128	Corn grain	5/30/18	76	7/10/18	12/12/18	11	303,810
2018	4	Orleans	ND	197	128	Corn grain	5/24/18	38*	7/19/18	10/24/18	11	345,800
2018	5	Genesee	AG2035	197	128	Corn grain	6/01/18	76	.	10/19/18	8	358,150
2018	6	Livingston	2108R2	197	128	Corn grain	6/01/18	76	7/30/18	10/26/18	8	303,810
2018	7	Livingston	S20-T6	197	128	Corn grain	6/11/18	38	7/19/18	10/22/18	8	360,620
2018	8	Ontario	SG2125	197	128	Corn silage	6/12/18	76	7/17/18	10/24/18	9	407,550
2018	9	Wayne	P19A14X	197	128	Corn grain	6/08/18	38	7/17/18	10/30/18	9	345,800
2018	10	Livingston	NK14J7	197	128	Corn grain	5/26/18	76	.	10/22/18	12	345,800
2019	11	Genesee	AG20x9	197	140	Corn grain	6/19/19	76	8/20/19	10/29/19	12	345,800
2019	12	Cayuga	HS 21X80	197	140	Corn grain/Alf§	5/27/19	38*	7/31/19	10/29/19	5	419,900
2019	13	Livingston	SG2253x	197	140	Corn grain	6/08/19	38*	7/29/19	10/24/19	12	370,500

†AG, ASGROW; 2108R2, Channel; S and NK, Syngenta; SG, Seedway; P, Pioneer; HS, HiSOY.
±AMS, ammonium sulfate treatment; this treatment was not included in 2017.
§Alf, alfalfa
*Twin row planter on 38 cm centers.

Table 2.1: Soil series and fertility status prior to implementation of soybean S-response trials.

								Mehlich-3-extractable nutrients§										CaCl$_2$*
Year	Site	County	Soil Series	Texture	Tax†	pH$_{H20}$	OM±	P		K		Ca		Mg		S		S
							g kg^{-1}	----------------mg kg^{-1}----------------										
2017	1	Wayne	Wallington	SiL	AF	6.2	37	193	H§	117	O	1457	S	264	VH	14	M	.
2018	2	Wayne	Channery	SiL	U	6.6	25	167	H	142	O	1309	S	273	VH	9	L	.
2018	3	Livingston	Conesus	SiL	MGH	7.3	30	159	H	196	H	1149	S	249	VH	19	O	.
2018	4	Orleans	Appleton	SiL	MAE	6.2	21	91	H	59	L	581	S	145	VH	13	M	.
2018	5	Genesee	Lima	SiL	GH	6.6	25	59	H	126	M	996	S	245	VH	13	M	.
2018	6	Livingston	Lima	SiL	MGH	6.8	15	53	H	64	L	650	S	223	VH	10	L	.
2018	7	Livingston	Cazenovia	SiL	GH	6.5	18	55	H	135	O	963	S	190	VH	18	O	.
2018	8	Ontario	Canandaigua	SiL	MOH	6.9	19	93	H	88	L	750	S	220	VH	20	O	.
2018	9	Wayne	Phelps	GrL	GH	6.0	15	48	O	114	M	455	S	258	VH	9	L	.
2018	10	Livingston	Eel	SiL	FE	8.0	15	41	O	64	L	1335	S	220	VH	11	M	.
2019	11	Genesee	Retsof	SiL	AOGH	6.4	32	13	VL	124	M	1349	S	248	VH	.	L	7
2019	12	Cayuga	Kendaia	SiL	MAE	7.0	55	46	O	97	M	2897	S	165	VH	.	L	9
2019	13	Livingston	Rhinebeck	SiL	MAO	6.9	32	41	O	75	L	2004	S	210	VH	.	L	6

†AF, Aeric fragicquepts; U, Udorthents; MGH, Mesic, Glossic Hapludalfs; MAE, Mesic, Aeric Endoaqualfs; GH, Glossoboric Hapludalfs; MOH, Mesic, Oxyaquic Haplodalfs; FE, Fluvaquentic Eutrochrepts; AOGH, Aeric Ochraqualfs, Glossic Hapludalfs; MAO, Mesic Aeric Ochraqualfs.

±Organic matter (OM) as determined by loss-on-ignition at 500^{o}C for 2 h (Storer, 1984).

§H, high; O, optimum; VL, very low; M, medium; L, low; S, sufficient; VH, very high determined by Mehlich-3 Extraction (Mehlich, 1984). Interpretations based on Penn State University Agriculture Analytical Services.

*In 2019, soil sulfur levels were determined using the 0.01 mol L^{-1} CaCl$_2$ extraction (Cornell Sulfur Test) (Ketterings et al., 2011).

Whole-plant samples were collected during harvest at each location in 2018 and 2019 (data not available for 2017) and consisted of sampling 12 to 15 individual plants within each plot. Samples were dried using a 60°C forced-air oven for a minimum of 48 h to determine moisture content, ground to pass a 2-mm sieve, and analyzed for total S using acid digestion and ICP-AES following USEPA (1996). Subsamples were analyzed for CP (AOAC, 2000a) using a LECO FP-528 (LECO Corp., St Joseph, MI). Total biomass and S content were used to determine realistic S removal rates and assess S management needs of soybean across locations.

Grain yield measurements were taken from the center of each plot for each treatment strip using a commercial combine. Soybean grain yield was adjusted to 13% moisture. Grain subsamples were collected from each harvest area and analyzed for total crude protein (corrected to a dry-weight basis) by near-infrared (NIR) spectroscopy analysis on unground samples using Foss NIRSystems Models XDS and 6500 with ISIScan v.4.6.1. Following the NIR analysis, the grain was analyzed for total S by ICP following wet digestion (Gavlak et al., 2003). Partial S balances were calculated for grain yield and for whole plant biomass:

$$\text{Partial } S_{\text{grain}} \text{ balance (kg ha}^{-1}) = S_{\text{deposited}} + S_{\text{fertilizer}} - S_{\text{removed by crop grain yield}} \quad [1]$$

$$\text{Partial } S_{\text{biomass}} \text{ balance (kg ha}^{-1}) = S_{\text{deposited}} + S_{\text{fertilizer}} - S_{\text{removed taken up by crop biomass}} \quad [2]$$

The quantity of S removed with grain harvest or taken up in the plant biomass at harvest (grain plus stems and leaves) was subtracted from the quantity of nutrient added with S deposition and fertilizer application. It is recognized that there are other processes that may affect the availability of S, including leaching, erosion, immobilization, root uptake, and gaseous losses. Nevertheless, partial balances derived here for the control treatments are useful to determine soil S supply, and to evaluate if an S response was likely per site in the first place.

To evaluate if S fertilization impacted yield, quality, and tissue S, the AOV model (ANOVA with TukeyHSD) in RStudio was used. Yield, CP, N, S, and N:S ratio of tissue and whole plant biomass were analyzed per trial location with S treatment as a fixed effect and block as a random effect. Mean separations were done using the TUKEYHSD comparison to adjust for a $P \leq 0.05$. Means were considered significantly different when $P \leq 0.05$. Data were also analyzed across all farms with S treatment, farm and year as a fixed effect and block as a random effect.

Results and Discussion

Sulfur and Nitrogen Content Early Bloom

Tissue S content in the control plots at early bloom ranged from 3.10 to 4.36 g kg^{-1} (Table 2.3). Tissue S content was not affected by S application within any of the trials but was significantly increased with S addition when all trials were analyzed together (Table 2.3), averaging 3.90 g kg^{-1} in the AMS and gypsum treatments and 3.80 g kg^{-1} in the control treatments (Table 2.3). If we assume 2.0 to 5.0 g S kg^{-1} to be optimal for soybean production (Dairy One Forage Laboratory, 2017), tissue S results suggest response in yield to S addition was unlikely. The N content at early bloom was not affected by S application. Tissue N averaged 58.1 g kg^{-1} in the AMS and gypsum treatments and 57.7 g kg^{-1} in the control. Given the optimal range of 42.5 to 55.0 g N kg^{-1} (Dairy One Forage Laboratory, 2017), the tissue N results were sufficient and did not suggest a N deficiency.

In the plant, S is a component of two amino acids and occurs in protein in a N:S ratio of 15:1. The lower the S content and the higher the N:S ratio the more likely S is deficient in the plant. In our study, the greatest N:S ratio, 19:1, was observed in the AMS and gypsum treatments of trial 6 (versus 17:1 in the control treatment in this trial) while for all other trials, the ratio was between 14:1 and 17:1, averaging 16:1 across control treatments and 15:1 where S had been added (Table 2.3). An ideal range of 14:1 to 18:1 has been reported in literature (Jamal et al., 2010; Casteel et al., 2019; Ibanez et al., 2020), consistent with the lack of a yield response to S addition across trials in our study.

Sulfur and Nitrogen Content at R8

Whole plant S and N content and N:S ratio at R8, close to harvest, were not significantly affected by S application (Table 2.3). The S content ranged from 2.25 to 3.35 g kg^{-1}, averaging 2.70 g kg^{-1} for the control treatments and 2.80 g $kg^{-}1$ when S had been added. The N content ranged from 32.8 to 46.3 g kg^{-1}, averaging 39.7 g kg^{-1} for the control treatments, 39.2 g kg^{-1} for the AMS treatments and 38.6 g kg^{-1} for the gypsum treatments. The N:S ratio of the whole plant ranged from 11:1 to 17:1, somewhat lower than the N:S ratio at early bloom for seven out of the thirteen trials, similar to the findings in Sweeney and Grande (1993). Across trials, the average N:S ratios for whole soybean plants was 14:1 for the AMS and gypsum treatments, and 15:1 for the control (no-S). Both ratios are considered optimum for protein formation and suggest no S deficiency (Dev and Saggar, 1974), consistent with the findings of N:S ratios at R1.

Table 2.3. Effect of S application on soybean tissue levels at R1 (early bloom) and R8 (full maturity) for each site-year.

Year	Site and County	Treatment	--------Plant Tissue-------- S	N	N:S Ratio	--------Whole Plant-------- S	N	N:S Ratio
			----g kg^{-1}----			-----g kg^{-1}-----		
2017	1. Wayne†	AMS	.	.	.	.	.	.
		Gypsum	3.50	56.2	16	.	.	.
		Control	3.57	52.7	15	.	.	.
		P-value	0.18	0.16		.	.	
2018	2. Wayne	AMS	3.50	55.0	16	2.43	36.4	15
		Gypsum	3.55	55.5	16	2.60	38.8	15
		Control	3.53	56.3	16	2.26	35.5	16
		P-value	0.72	0.63		0.15	0.42	
2018	3. Livingston	AMS	4.36	63.2	14	2.50	42.5	17
		Gypsum	4.15	60.9	15	2.30	38.1	17
		Control	4.18	60.3	14	2.50	40.8	16
		P-value	0.05	0.03		0.46	0.19	
2018	4. Orleans	AMS	3.43	51.4	15	2.90	36.7	13
		Gypsum	3.36	52.7	16	2.60	34.1	13
		Control	3.10	51.1	16	2.25	35.1	16
		P-value	0.35	0.62		0.03	0.04	
2018	5. Genesee†	AMS	.	.	.	3.00	39.4	13
		Gypsum	.	.	.	3.03	40.6	13
		Control	.	.	.	2.96	38.9	13
		P-value	.	.		0.95	0.81	
2018	6. Livingston	AMS	3.70	70.1	19	3.06	40.5	13
		Gypsum	3.50	64.8	19	3.13	45.2	14
		Control	3.73	65.0	17	2.96	45.0	15
		P-value	0.25	0.08		0.26	0.11	
2018	7. Livingston	AMS	3.55	53.3	15	2.90	43.1	15
		Gypsum	3.70	55.6	15	2.55	34.8	14
		Control	3.70	56.4	15	3.00	42.9	14
		P-value	0.68	0.60		0.61	0.65	

Year	Site and County	Treatment	--------Plant Tissue--------			--------Whole Plant--------		
			S	N	N:S Ratio	S	N	N:S Ratio
			----g kg^{-1}----			-----g kg^{-1}-----		
2018	8. Ontario	AMS	3.73	57.7	15	3.35	43.3	13
		Gypsum	3.73	59.2	16	3.30	46.3	14
		Control	3.76	59.2	16	2.97	45.4	15
		P-value	0.93	0.81		0.47	0.62	
2018	9. Wayne	AMS	3.60	56.0	16	2.30	32.8	14
		Gypsum	3.70	57.2	15	2.63	35.2	13
		Control	3.40	56.9	17	2.36	37.2	16
		P-value	0.08	0.86		0.22	0.13	
2018	10. Livingston†	AMS	.	.	.	2.90	39.2	14
		Gypsum	.	.	.	2.80	38.9	14
		Control	.	.	.	2.60	38.0	15
		P-value	.	.		0.46	0.86	
2019	11. Genesee	AMS	3.73	56.0	15	3.03	33.9	11
		Gypsum	3.65	59.0	16	2.93	33.4	11
		Control	3.60	56.6	16	2.78	34.7	12
		P-value	0.18	0.64		0.52	0.92	
2019	12. Cayuga	AMS	4.58	58.8	13	3.09	40.5	13
		Gypsum	4.50	58.5	13	2.91	39.1	13
		Control	4.40	59.1	13	3.08	40.5	13
		P-value	0.10	0.85		0.22	0.67	
2019	13. Livingston	AMS	3.93	57.3	15	2.95	40.9	14
		Gypsum	3.98	57.5	14	2.85	38.8	14
		Control	3.80	57.2	15	2.95	41.7	14
		P-value	0.39	0.93		0.84	0.57	
All farms		AMS	3.90	58.1	15	2.80	39.2	14
		Gypsum	3.80	58.1	15	2.80	38.6	14
		Control	3.80	57.7	16	2.70	39.7	15
		P-value	0.01	0.57		0.06	0.35	

†., no data. In 2017, AMS was not a treatment and whole plant samples were not collected. Plant tissue samples were not collected for trials 4 and 9 in 2018 due to weather.

Grain Yield and Crude Protein

Grain yield at the 2017 trial averaged 3,568 kg ha^{-1} with gypsum addition, not different from 3,682 kg ha^{-1} without S (Table 2.4). The lack of a response to S ($P = 0.53$) was not unexpected, given the S content and N:S ratios in the plant tissue results (Table 2.3), combined with a high level of SOM at this location (Table 2.2).

Similar yields were seen across trials in 2018 and 2019, with yields ranging from 2,417 kg ha^{-1} at trial 11 to 5,400 kg ha^{-1} at trial 5 (Table 2.4). Within trials as well as across trials, grain yields did not increase with S addition, independent of S source, with the exception of trial 4 where S addition decreased yield. Trial 4 results could have been impacted by a potential K deficiency. A similar negative trend was seen in trial 10 as well. This suggests neither a N or S limitation to soybean yields across these trials. These results were expected given mid-season tissue S and N:S results as well (Table 2.3).

Our results are consistent with soybean S trials conducted by Ham et al. (1975) in St. Paul, Minnesota, by Brown et al. (1981) in Columbia, Missouri, by Matheny et al. (1981) in Florence, South Carolina, by Klausner et al. (1982, 1984) in Aurora, New York, by Chandel et al. (1989) in Buenos Aires, Argentina, and by Sweeney et al. (1993) in Parsons, Kansas. In all these studies in the late 1970s to mid-1990s, researchers concluded that the availability of S in the untreated control plots was adequate for optimum yields and that there was no need for S fertilization. Similarly, our current research in New York suggest that even for higher yielding trials under reduced S atmospheric deposition, S is not limiting yield.

Research in the Brazilian Cerrado region conducted by Hitsuda et al. (2004) suggested that if grain CP increased with S fertilization, even if yield did not respond, the crop could still be considered deficient in S. In our study, N content of the grain was not impacted by S addition

(Table 2.4). Crude protein (6.25 x N content, Table 2.4) of the grain averaged 3.93 g kg^{-1} for the gypsum, 3.94 g kg^{-1} for the AMS and 3.95 g kg^{-1} for the control treatments, demonstrating grain CP was not impacted by S addition. The lack of a response of N content of grain to S addition was not unexpected given the lack of a yield response, and was consistent with studies by Brown et al. (1981), Matheny et al. (1981), Chandel et al. (1989) and Sweeney et al. (1993).

The N:S ratio for grain was 16:1 across all trials (Table 2.4), with no impact of S addition on this ratio. The ratios in this study are lower than the critical total N:S ratio of 18:1, beyond which a S deficiency is likely based on work by Casteel et al. (2019), suggesting that S was not limiting yield, consistent with lack of a response in yield, grain protein content and S content.

Table 2.4. Effect of S application for each site-year on soybean yield, S content, N content and N:S ratio. Sulfur was applied at a rate of 34 kg S ha^{-1}.

Year	Site and County	Treatment	Grain yield	S content	N content	N:S ratio
			kg ha^{-1}	g kg^{-1}	g kg^{-1}	
2017	1. Wayne†	AMS	.	.	.	
		Gypsum	3568	3.97	63.6	16
		Control	3682	3.94	63.0	16
		P-value	0.53	.	0.64	
2018	2. Wayne	AMS	4170	3.82	61.1	16
		Gypsum	4257	3.77	60.3	16
		Control	4201	3.79	60.6	16
		P-value	0.40	0.44	0.49	
2018	3. Livingston†	AMS	4456	.	.	.
		Gypsum	4595	.	.	.
		Control	4616	.	.	.
		P-value	0.76	.	.	
2018	4. Orleans	AMS	3923	3.86	61.8	16

Year	Site and County	Treatment	Grain yield	S content	N content	N:S ratio
			kg ha^{-1}	g kg^{-1}	g kg^{-1}	
		Gypsum	3811	3.87	62.0	16
		Control	4041	3.91	62.5	16
		P-value	0.04	0.41	0.88	
2018	5. Genesee	AMS	5400	4.13	66.1	16
		Gypsum	5304	4.08	65.3	16
		Control	5380	4.06	65.0	16
		P-value	0.17	0.87	0.62	
2018	6. Livingston	AMS	5334	4.06	65.0	16
		Gypsum	5277	4.10	65.6	16
		Control	5257	4.22	67.5	16
		P-value	0.26	0.39	0.15	
2018	7. Livingston	AMS	3733	3.86	61.8	16
		Gypsum	3749	3.75	59.9	16
		Control	3807	3.87	61.9	16
		P-value	0.64	0.25	0.44	
2018	8. Ontario	AMS	3782	4.09	65.5	16
		Gypsum	4205	4.12	65.9	16
		Control	3980	4.17	66.6	16
		P-value	0.28	0.61	0.66	
2018	9. Wayne	AMS	4003	3.71	59.4	16
		Gypsum	4120	3.64	58.2	16
		Control	4073	3.57	57.1	16
		P-value	0.60	1.00	0.23	
2018	10. Livingston	AMS	2951	4.06	64.9	16
		Gypsum	3047	4.04	64.5	16
		Control	3058	4.12	66.0	16
		P-value	0.06	1.00	0.48	
2019	11. Genesee	AMS	3059	3.57	57.1	16
		Gypsum	2417	3.71	59.3	16

Year	Site and County	Treatment	Grain yield	S content	N content	N:S ratio
			kg ha^{-1}	g kg^{-1}	g kg^{-1}	
		Control	3125	3.66	58.5	16
		P-value	0.12	0.77	0.27	
2019	12. Cayuga	AMS	4433	4.04	64.7	16
		Gypsum	4446	3.99	63.8	16
		Control	4452	3.97	63.5	16
		P-value	0.95	0.78	0.49	
2019	13. Livingston	AMS	4472	4.19	67.1	16
		Gypsum	4394	4.17	66.7	16
		Control	4488	4.19	67.1	16
		P-value	0.70	0.19	0.86	
All farms		AMS	4143	3.94	62.2	16
		Gypsum	4091	3.93	62.1	16
		Control	4166	3.95	63.2	16
		P-value	0.82	0.40	0.89	

†., no data. The AMS treatment was not included in 2017. Subsample for grain analyses could not be obtained for trial 3 in 2018.

Initial Mehlich-3 soil test S ranged from 9 to 20 mg kg^{-1} in 2019 (Table 2.2). In 2019, results of the 0.01 mol L^{-1} $CaCl_2$ extraction showed values below 8.0 mg S kg^{-1}, the critical value determined for this test for alfalfa in New York (Ketterings et al., 2014). As S was not limiting yield in any of the thirteen trials, calibration of either of the two soil test methods for soybean production in New York could not be done. The results do suggest calibration studies are needed as current interpretation for Mehlich-3 extractable S by Pennsylvania State University would have suggested the potential for a S deficiency at trials 2, 6 and 9, while the 2019 $CaCl_2$ test data suggest that the critical value for this test will be lower for soybean than the critical value of 8 mg kg^{-1} established for alfalfa in New York (Ketterings et al., 2014).

Partial Sulfur Balances

The partial balances for grain S and biomass S were positive (more S applied than taken up or removed with harvest) when S fertilizer was applied (Figure 2.1). Partial balances were negative with no addition of S (control treatment), ranging from -3 to -15 kg S ha^{-1} for partial grain balances and from -9 to -25 kg S ha^{-1} for whole plant biomass partial balances (Figure 2.1). The negative balance without S additions combined with the lack of a yield response to S addition (Table 2.4) suggest that S supplied by the soil was sufficient to meet plant requirements.

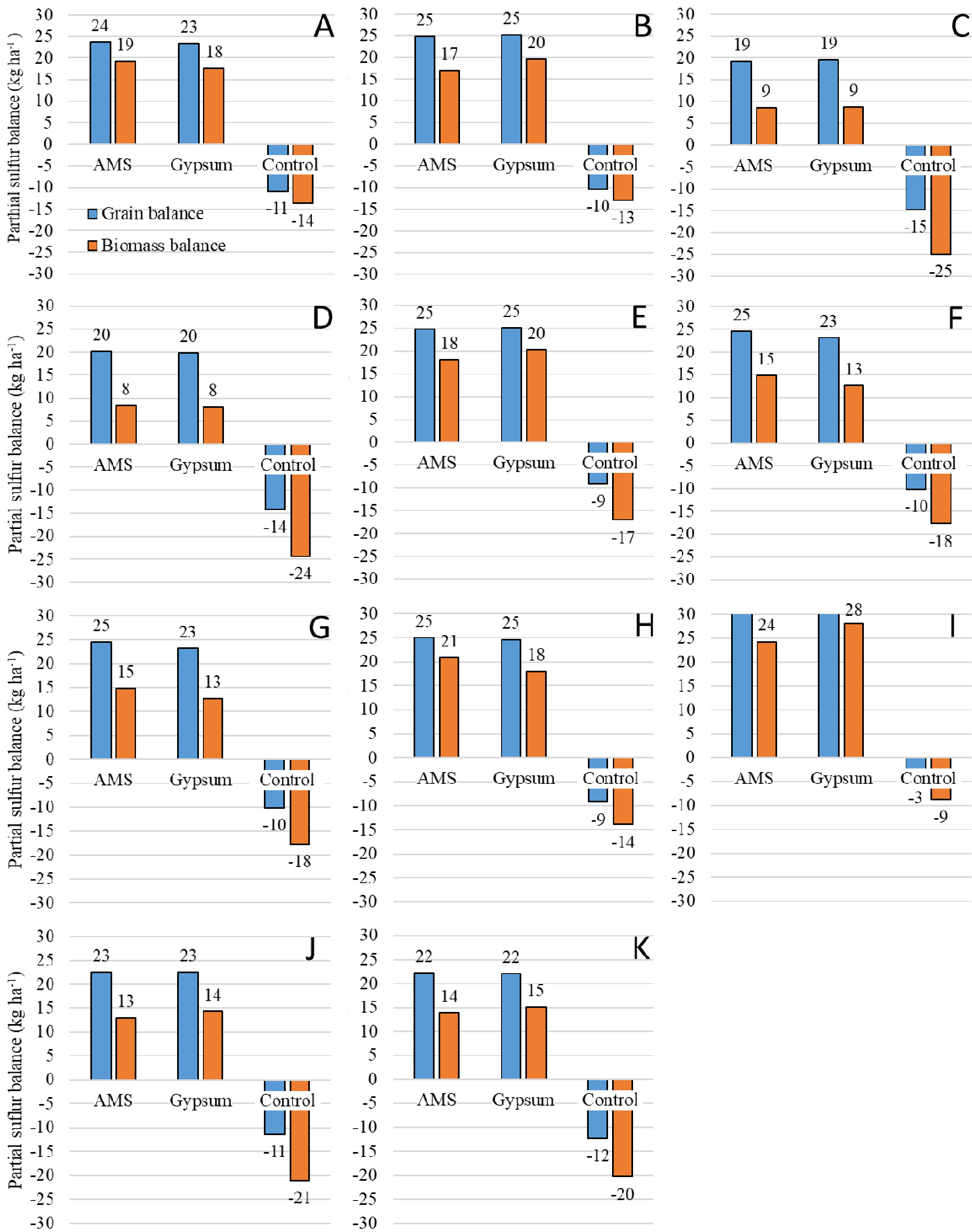

Figure 2.1. Partial S balances both grain and biomass for 11 trials as impacted by S application with S as ammonium sulfate (AMS), Gypsum versus a no-S Control. (2018 trials): A, 1. Wayne; B, 3. Orleans; C, 4. Genesee; D, 5. Livingston; E, 6. Livingston; F, 7. Ontario; G, 8. Wayne; H, 9. Livingston; (2019 trials): I, 10. Genesee; J, 11. Cayuga; K, 12. Livingston.

The soil S supply estimated from the control treatments, taking into account S uptake by the plant (biomass) and S removal with harvest (grain) versus S deposition only (partial balance) showed, across trials, that about 60% of the S taken up from the soil accumulated in the grain (Figure 2.2). This was consistent with the harvest index of 57% and slightly higher S content of grain compared to the stems and leaves.

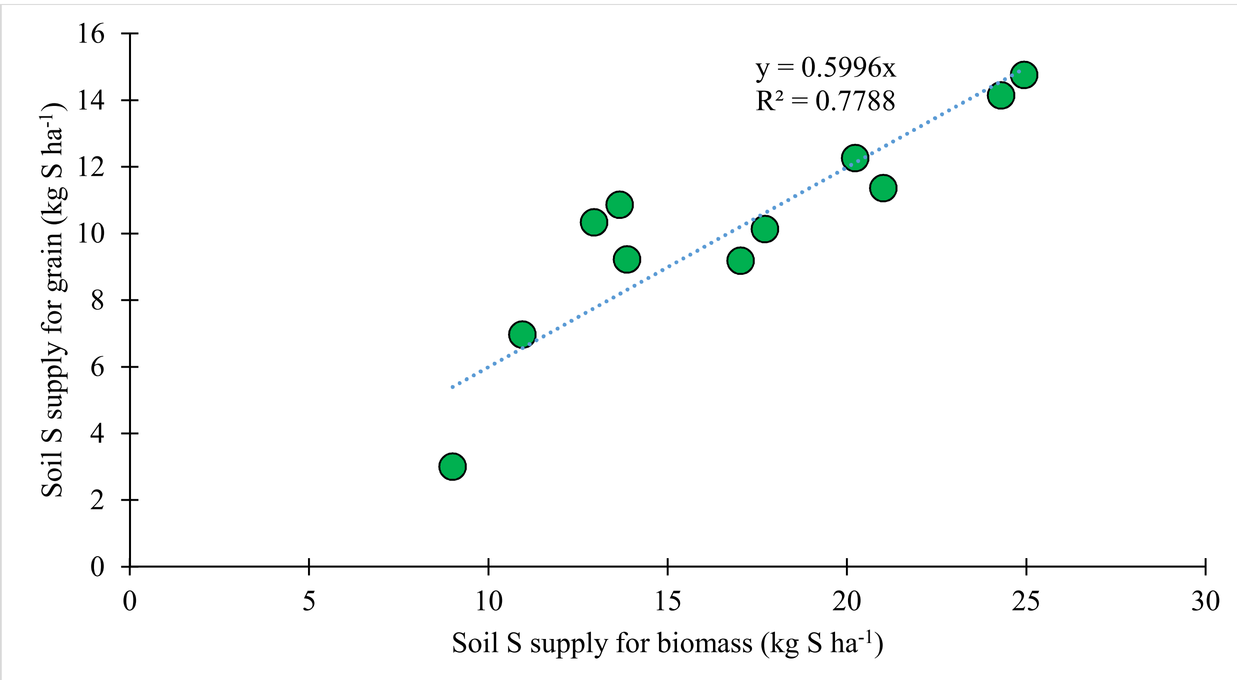

Figure 2.2: Soil S supply for biomass and for grain only based on the control plots of each trial in the study except trial 1 in 2017 and trial 5 in 2018.

Soil organic matter was not a good predictor of soil S supply (Figure 2.3). For example, the highest soil S contributions were determined for trials 5 and 6 which had SOM levels of 1.5 and 2.5 mg kg^{-1}, respectively, considerably lower than some of the other trials. When not all soil available S is taken up by the crop due to other limitations, control plot partial balances can underestimate the actual soil S supply. While SOM indicated minimum soil S supply, trial results suggest substantial soil S supply for all trials, sufficient for both yield and CP content. The negative partial balances for the control plots also indicate that S addition might be needed in future years as farming with negative nutrient balances is not sustainable.

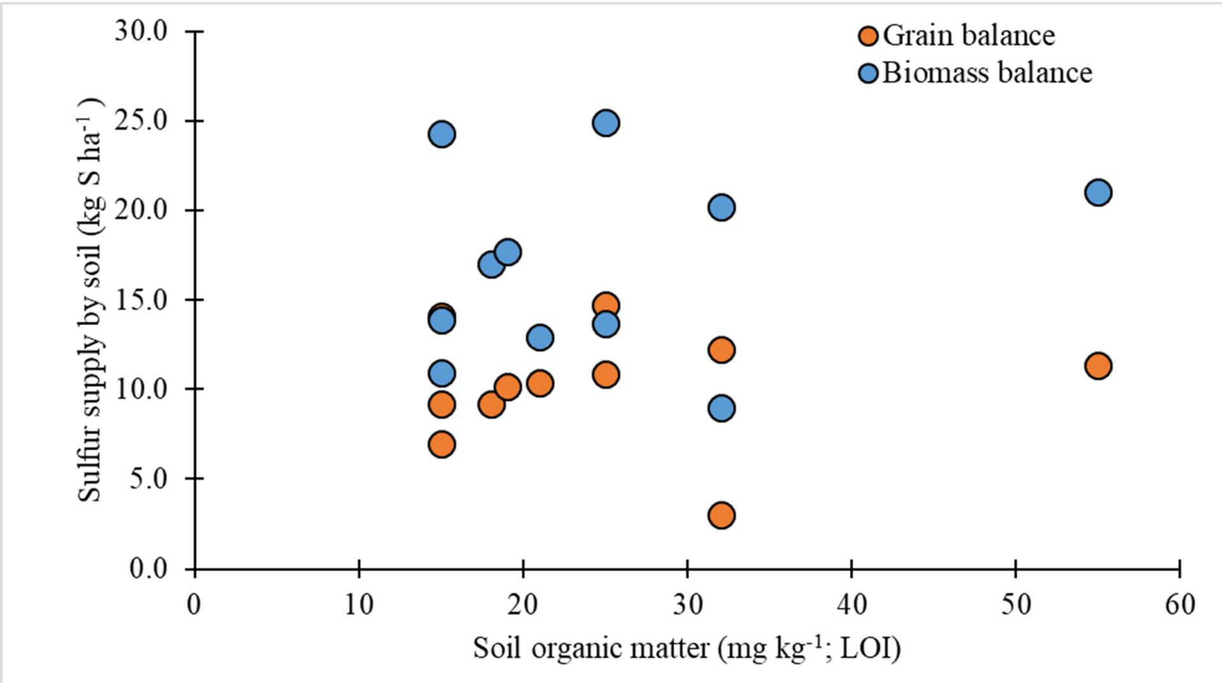

Figure 2.3: Soil S supply, measured as kg S ha^{-1} removed minus S deposition, for the control plots (no fertilizer S) of eleven trials conducted in 2018 and 2019.

Conclusion

The S concentrations and N:S ratios in mid-season tissue tests and whole plants at harvest suggested sufficient S available for optimal soybean yield, consistent with the lack of a yield response. Soil test data could not be calibrated due to the lack of a response in yield to S addition. Research is needed to calibrate currently used tests as critical values interpretations for the Mehlich-3 test based on Pennsylvania State University Agricultural Analytical Services Laboratory. The $CaCl_2$ test calibrated for alfalfa in New York suggest critical values might need to be lower than currently suggested for these tests. Use of current guidance based on soil testing could result in S recommendations for fields that do not need the extra S for crop response. It is possible that S addition changes the amino acid content of soybeans which could impact feed quality. Further research is needed on this topic. We conclude that S addition is unlikely to increase soybean yield under current farming conditions in New York, but that continued evaluation is needed given without S addition, a large portion of S taken up by the crop will come from S mineralized by the soil.

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