Final Report to Maryland Soybean Board
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Managing Sulfur Fertility to Enhance Soybean Yield and Protein Quality

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Abstract

Sulfur (S) is an essential macronutrient and a key component in the essential amino acids methionine and cysteine (METH+CYST) that are the building blocks of protein. The nutritional value of protein from soybeans, like that from most grain legumes, is limited by relatively low levels of METH+CYST. Sulfur deficiencies are becoming more widespread as soil reserves of S are depleted greater removals in high yielding crops in combination with much lower inadvertent S input. Sulfur deficiencies are most common on sandy soils that are low in organic matter and anion exchange capacity. Sulfur management has largely been ignored in soil fertility because (1) historically many fertilizers and pesticides contained S "impurities"; (2) until the late 1990s, sufficient S was supplied through atmospheric deposition of sulfur dioxide emissions from coal-fired power plants; and (3) until the inventions and widespread laboratory use of sophisticated and expensive instrumental methods (Inductively Coupled Plasma-Atomic Emission Spectroscopy, ICP-AES), the measurement of S in plant and soil samples was cumbersome and unreliable. In order to compare two common sulfate sources of sulfur and determine whether sulfur fertilization could enhance the yield and amino acid make-up of soybean protein, we conducted a series of eight field experiments. We also attempted to find a soil testing protocol that could predict S availability and identify fields where a soybean response to S application is likely. The experiments had a randomized split plot design with two levels (applied or not) of S sources factors (Gypsum, Epson salt) to give four treatments: 1) control (G0E0), 2) 560 kg/ha

gypsum (17% S) broadcast at planting (G1), 3) 86 kg/ha Epsom salt (13% S) as a foliar spray at soybean R1 growth stage (E1), and 4) the combination gypsum + Epsom (G1E1). Soybean yield, seed S concentration, seed S yield, and amino acid content were measured to determine the effects of S fertilization. In each of two years (2017 and 2018) this experiment was conducted using two types of soybean crops (full season and double crop) and two soil types (relatively coarse and fine) for a total of eight site-years. Soybean seed yield, seed S content, and S yield were significantly ($p<0.1$) increased with the S treatment in three out of the eight site-years. Sulfur-containing amino acid (METH+CYST) content of the seed was significantly increased by all three S application treatments ($p<0.1$). Results of this experiment show that applied S, on low available S soils, can produce significant yield increases (up to 35%) and stimulate dramatic increases (up to 90%) in the METH+CYST content the seed.

We also attempted to evaluate soil test methods for predicting where S application would be beneficial. Soil S levels were analyzed using four extraction protocols: (1) 0.01 Molar calcium chloride shaken with soil at a ratio of 5:1, (2) 500 ppm calcium phosphate in water shaken with soil at a ratio of 2.5:1, (3)500 ppm calcium phosphate in 2 Molar acetic acid shaken with soil at a ratio of 2.5:1, and (4) Mehlich-3 extracting solution shaken with soil at a ratio of 10:1 (referred to as CaCl2, CaHPO4, CaHPO4-HOAc, and Mehlich-3, respectively). For the four S soil test protocols, the measured soil S levels in the 0-10 cm A1 layer, in the subsoil below the A horizon to 30 cm, and the weighted average of all layers 0-30 cm were compared to the soybean crop responses to applied S at up to 23 sites (122 individual blocks). Relative soybean seed yield (kg soybean seed/ha) and relative soybean S yield (kg S in seeds/ ha) were the crop responses used to calibrate the soil tests. The calibration involved calculation of a critical value intended to demarcate fields so deficient in S that soybeans would likely respond positively to S

applications from fields sufficient in S such that applying additional S would not be expected to have an effect. When considering the weighted average of the full 0-30 cm sampled soil depth, the critical values were 5.5, 4.4, between 9.9 and 11.3, and between 16.2 mg S/kg soil, respectively, for the CaCl2, CaHPO4, CaHPO4-HOAc, and Mehlich-3 soil test protocols. Using just the upper 10cm of soils, the CaHPO4-HOAc soil test correctly identified 87.5% of the responsive soils as having extractable S below the critical level of 10.6 mg S/kg. The commonly used Mehlich-3 soil test was the second best of the four, correctly identifying 78% of the responsive fields as having extractable S below the critical level of 16.2 mg S/kg soil when using the upper 10 cm of soil. Including deeper soil layers in the sample did not improve the accuracy of these tests. More research needs to be done on a wider range of varieties, soils and environments and on testing methods to identify S-responsive soil.

Introduction

For several reasons, compared to N, P and K, little attention has been paid to managing S in most crops. Prior to the 1850s farmers relied heavily on organic amendments, legumes as well gypsum ($CaSO_4$) for soil fertility management. After the 1850s superphosphate became widely used to enhance P fertility, but this material also contains sufficient S as impurities to meet crop demand (Gilbert, 1951; Russel and Williams, 1977; Scherer, 2001). With the rise of industrialization in the early 1900s, in addition to impurities in chemical fertilizers popular at the time (mainly ammonium sulfate and superphosphate) (Eriksen, 2008), farm soils (especially in the Eastern United States) received large amounts of S as atmospheric deposition from the emissions of coal fired power plants. After the implementation of amendments to the Clean Air Act in 1990, S emissions from coal-fired power plants were drastically reduced (Ketterings et al., 2011; Klimont et al., 2013). The combination of 1) the reduction of S deposition due to the

successful regulation of S dioxide emissions, 2) use of chemical fertilizers with lower amounts of S impurities (e.g. diammonium phosphate and urea) and 3) movement away from animal-based amendments has led to increasing rates of S deficiency.

Sulfur deficiency may significantly impact crop yield and nutritional quality. It is not new information that S, along with nitrogen (N), phosphorous (P), potassium (K), magnesium (Mg), and calcium (Ca), is macronutrient essential for crop growth. Sulfur is generally taken up by soybeans in similar quantities to P (Bender et al., 2015). However, the need to actively manage S and research on how best apply this nutrient has only recently received much attention by farmers and researchers in humid regions. Although sulfur has been often ignored in soil fertility programs (Eriksen, 2008), it is a key component of the S containing amino acids (SCAAs) methionine and cysteine which are essential amino acids that often limit the nutritional quality of vegetable proteins. When the sum of these two amino acids (hereafter referred to as METH+CYST) is limiting, the health of humans and non-ruminant animals is affected because they cannot synthesize METH+CYST on their own and must receive them from a dietary source (Ruiz et al., 2005; Jez and Fukagawa, 2008). Previous research in Maryland has shown that S fertilization on deficient soils may not only improve yield but also protein quality of soybean through increased METH+CYST content in the seed (Weil and Notto, 2018).

Plant- available sulfur concentration in the soil is affected by atmospheric deposition, decomposing organic material, fertilizer inputs, S leaching, plant uptake, and microbial activity. Plants take up S as sulfate (SO_4^{2-}) which is relatively mobile in the soil and easily leached down the soil profile. Sulfur in the top layer (A horizon) of soil is generally mineralized from organic material, which includes both humus (stabilized soil organic matter) and recent crop residues left on or in the surface soil (Schoenau, 2008). The rate at which this mineralization occurs is

moderated by temperature, pH, moisture, aeration and the C:S ratio of the material (Weil and Brady, 2017). The plant-available SO_4^{2-}, including both SO_4^{2-} in the soil solution and adsorbed SO_4^{2-} , typically accounts for less than 5% of the total S in humid region soils. This (Scherer, 2009). Typically, soils that are low in organic matter and have a coarse texture are more likely to experience S deficiency due to the low mineralization and high leaching potential of SO_4^{2-} (Dick et al., 2008). Such soils are common on the sandy sediments of the Atlantic coastal plain of North America, including on the Eastern shore of Maryland.

Sulfur plays an important role in many plant physiological functions and growth processes including photosynthesis, protein synthesis, nitrogen fixation, and oil synthesis (Epstein and Bloom, 2005). Legumes, such as soybeans, have an especially high S demand because of the S required for N fixation. Although there is a general understanding of the essential role that S plays in the health of plants and in turn the nutritional health of humans and animals, there has been limited work done to evaluate the most effective rate, timing, and source of S to be used in agronomic practices.

Sulfur is most commonly applied to soils as gypsum ($CaSO_4 \bullet 2H_2O$). Soil-applied gypsum is a relatively low-cost and is widely available as a mined mineral and also as a very low-cost byproduct of many industrial processes. A commonly used form of gypsum is Flue-Gas Desulfurization gypsum (FDG) produced by the scrubbers used to remove SO_2 from coal fired power plant emissions (Miller and Sumner, 1997). Gypsum powder is a moderately soluble (~2.0–2.5 g/l at 25 °C) and can be applied at the time of planting and provide SO_4^{2-} for the plants to access during the entire the growing season. In Ohio Chen et al. (Chen et al., 2005) studied the effectiveness and potential environmental impacts of using FDG as a fertilizer for alfalfa and soybeans. They reported that FGD has good potential as a S fertilizer for both alfalfa and

soybeans (they observed 4-11% yield increases) and may provide other nutrient elements from impurities contained in the gypsum. In Brazil researchers (Caires et al., 2011) studying lime and gypsum applied to corn and soybean under no till systems found a significant effect of a gypsum on corn but not on soybean yield A study in India on the application of gypsum (at 0, 20, 40, and 60 kg S/ha) to soybeans growing on Vertisols found that up to 20 kg S/ha increased nodule production, leaf chlorophyll and yield, but above that level nodule production and leaf chlorophyll reached a plateau, while yield increased with up to 40 kg S/ha before plateauing (Ganeshamurthy and Sammi Reddy, 2000).

Research indicates that soybean S demand greatly increased at the beginning of seed production and pod filling (R1-3 growth stages) and S deficiency at this time could reduce yield and protein quality (Wang et al., 2008; Bender et al., 2015). A study done in Argentina compared ammonium sulfate and gypsum, both applied as a subsurface band at the time of planting, (Gutierrez Boem et al., 2007). and reported increased (6-14%) seed yield but no difference between the two sources of S.

Another potential source of S is Epsom salt (MgSO4), which is highly soluble (250 g/L 20°C) so it could be easily applied as a foliarly spray during the growing season. However, we were not able to find any research or recommendations in the literature for its use as a S fertilizer on soybean. We could also find no research or extension guidelines in the literature on the appropriate rate of Epsom application to avoid leaf burn while still supplying sufficient S to remediate any S deficiency. In previous work in Maryland a rate of 86 kg Epsom ha $^{-1}$ (which corresponds to 11 kg S ha $^{-1}$) was applied to soybeans without any damage to the leaves (Weil and Notto, 2018). However, more research is needed to determine appropriate timing and rate of both methods of applications.

In addition to improving yield, appropriate timing and rate of application of S may be important for the nutritional quality of soybeans. A preliminary survey of commercial soybean fields in Maryland found the content of S and METH+CYST to be highly variable.. While total S is a relatively easy measurement to take, measuring SCAA content is expensive and time consuming. Porter et al (1974) reported The total S content of grain legume seeds (dry beans(*Phaseolus vulgaris*, mung beans (*Vigna radiata*) and cowpeas(*Vigna unguiculate*)) was significantly correlated with the percent of protein present on the form of METH+CYST (Porter et al., 1974). That 1974 report used S determined by nitric acid-perchloric acid digestion followed by turbidimetric sulfate analysis. The current standard method for such total S determinations is ICP (citation). However, portable X-Ray fluorescence (XRF) is an emerging, rapid, non-destructive low-cost method for semi-quantitative determination of plant tissue elemental composition, including of elements as light as S and P if a vacuum is applied to the XRF detector head (Towett et al., 2016). Currently quantification of plant tissue S content by XRF is a lack of a calibration equations that relate XRF normalized photons to independently determined values of tissue S content.

Effective management of sulfur (S) fertility with S-containing amendments would be greatly aided by a reliable and accurate soil test for S. A reliable S soil test would provide values that correlate with plant uptake of S and identify fields where crops are likely to response to S application. Since S becomes available to plants mainly by the release of sulfate ions and soluble organic S compounds from decomposition of soil organic matter and by the desorption or dissolution of sulfate ions from iron and aluminum oxide coated clay surfaces, a reliable S soil test should dissolve an amount of S that is related to what could become available throughout the growing season from both of these sources in the soil (Ketterings et al., 2011).

Soil testing for S has lagged behind other essential macronutrients for several reasons. Until relatively recently farmers received agronomically sufficient S from impurities in common fertilizers and atmospheric deposition that S deficiency was a relatively uncommon phenomenon. However, after the passage of the 1990 amendments to the clean air act, that regulated SO_2 emissions, drastically decreased S atmospheric deposition, especially in the northeastern United States. Decreased atmospheric deposition coupled with higher yielding crop production and higher analysis fertilizers have led to increased S removal from soil without replenishment. Retention of plant available SO_4^{2-} relies on the anion exchange capacity of the soils which is greater in soils with higher clay content and iron/aluminum oxides (Ensminger, 1954; Reisenauer and Dickson, 1961; Metson, 1979). The sandy surface soils that are characteristic of the coastal plain region in the mid-Atlantic generally have low anion exchange capacity and are thought to be susceptible to S deficiency. However, SO_4^{2-} that leaches form the surface soil can be adsorbed onto subsoil clays and iron/aluminum oxides and serve as a significant source of plant available S throughout the growing season (Metson, 1979). Therefore, a soil test for S must take into account both S at the surface as well as subsoil S.

Most labs using Mehlich-3 soil test results report S levels as “plant available S” and give interpretations such as “low,” “medium,” or “high” which would suggest that critical levels had been determined by the soil test calibration studies. A study (Kowalenko et al., 2014) done in British Columbia compared the ability of 5 different extracting solutions to extract different fractions of soil S to identify a test that could provide accurate recommendations to farmers for a wide range of nutrients including S, which has not been extensively studied (Kowalenko et al., 2014). Although Mehlich 3 would be a convenient way to measure a wide range of elements and would simplify the soil test process since it is already widely used for other nutrients, more work

needs to be done to evaluate the relationship between extracted S and plant response to applied S. Mehlich-3 extracting solution (Mehlich, 1984) is composed of 0.015 *M* NH_4F + 0.2 *M* CH_3COOH + 0.013 *M* nitric acid (HNO_3) + 0.25 *M* ammonium nitrate (NH_4NO_3) + 0.001 *M* ethylenediaminetetraacetic acid (EDTA)+ 0.015*M* ammonium fluoride (NH_4F) + 0.5 *M* acetic acid (CH_3COOH) + 1.0 *M* ammonium acetate (CH_3COONH_4). Kowalenko et al. (Kowalenko et al., 2014) reported that soil extracting solutions such as $CaCl_2$ and $Ca(H_2PO_4)_2$ showed more promise than Mehlich-3, however they concluded that more research needs to be done before wide adoption of any of the tested methods

A New York Study done with alfalfa (Ketterings et al., 2011; Ketterings et al., 2012) found that 0.01 *M* $CaCl_2$ was the extract that responded most consistently to S additions, whereas the 1.0 mol L^{-1} NH_4OAc, 0.016 mol L^{-1} KH_2PO_4, 0.01 mol L^{-1} $Ca(H_2PO_4)_2$, Morgan, NaOAc, and Mehlich-3 extracting solutions did not respond consistently across the studied soils to the same S additions. The 0.01 *M* $CaCl_2$ extract also showed the most sensitivity to the different treatments of applied S which was determined to be beneficial because it indicates more sensitivity when identifying deficient soils (Ketterings et al., 2011).

The present study was undertaken to advance the S fertility management for soybean yield and quality by: 1) Evaluating the relationship between total S and METH+CYST contents of soybean seeds; 2) Producing a calibration equation to allow quantification of total S in plant tissue by XRF. 3) Conducting a series of field experiments on the mid-Atlantic coastal plain soils evaluate the potential for applied S to improve both yield and METH+CYST content of soybeans; 4) Comparing the efficacy of soil-applied gypsum and foliar-applied Epsom salt for S fertilization of soybeans. 5) evaluating four alternative S soil test protocols. The main hypotheses of the field experiments were (1) S application will increase yield of soybeans on

low- S soils, (2) S treatment will increase S content of soybeans, (3) S treatment will increase the concentration of METH+CYST in the seed, (4) The METH+CYST concentration will correlate with total S content of seed, (5) Foliar Epsom salt will have a greater impact on yield and S in the seed than soil applied gypsum.

Materials and Methods

Locations

Field trials to determine soybean response to S applications were carried out over two years, 2017 and 2018, on a total of 8 sites within the Central Maryland Research and Education Center (CMREC) in Beltsville, Maryland (within a 2 km radius of 39.012162, -76.833329) with a humid temperate climate averaging 14ºC and approximately 1000 mm y^{-1} of precipitation approximately evenly spread among 12 month (NOAA, 2020). Weather conditions for the two growing seasons of the study (May – November in 2017 and 2018) are illustrated in Figure 1. Weather conditions varied significantly between the two years. Figure 1 shows the weather from the CMREC research farm during the soybean growing season (May-November) during the two years this study took place. The graph is showing daily high temperature, daily low temperature, and daily cumulative rainfall compared to the historic average temperature and cumulative rainfall over a 30 year period from 1980 – 2010 from historic weather data from the BWI weather stations ("National Centers for Environmental Information"). The 2017 growing season was much drier (67 cm cumulative rainfall) than normal (97 cm) while 2018 (104 cm cumulative rainfall) was much wetter than

Each year four field experiments were conducted, two using double crop (DC) soybeans (planted after winter wheat harvest) and two using full season (FS) soybeans . For crop type the pair of sites were characterized by soils of contrasting textures, one formed from coarse sandy

sediments (Downer-Hammonton complexes referred to hereafter as “coarse”) and one formed from silty to clayey sediments (Russet-Christiana complexes referred to hereafter as “fine”). All eight fields were located within MLRA 149A which is the Northern Coastal plain with very deep parent material of fluviomarine deposits (Soil Survey Staff, 2020). Table 1 provides soil characteristics for each of the eight sites used in the study based on auger profile examinations at two locations on each site. Table 1 lists the agronomic practices use for each treatment at each site. Table 2 lists the management history for the eight sites, including crop rotation, fertilizer application, and mapped soil series confirmed by bucket auger samples that confirmed soil color using Munsell guide and texture by feel.

Experimental Design and Treatments

Experimental plots were laid out as a randomized split-plot trial with three to five replications for each site-year. The whole plot treatment was with or without 560 kg gypsum /ha broadcast at the time of planting, and the subplots were with or without 86 kg Epsom salt /ha applied as a foliar spray the R1 growth stage. Each year the experiments were carried out on four fields which included both soybean types, namely a relatively coarse and a relatively fine soil field with double crop soybean planted after wheat harvest and a relatively coarse and a relatively fine soil field with full season soybean fields planted after winter cover crop burn down. Soybeans were no-till planted in 37.5 cm rows in plots on average 15 m wide and 20 m long and farm-scale machinery was used for all planting, spraying and harvest operations.

Soil sampling

Soil samples were collected near the time of soybean planting, but before any S treatments were applied. Four 30 cm deep cores were collected from each control (no S added) plot using a 1.8 cm diameter push probe, and divided into three segments (0-10 cm, 10-20 cm or

the bottom of the A horizon if that boundary, and 20 cm or the bottom of A horizon to 30 cm). The length of each individual core segment was recorded and the four segments from each depth increment were composted with each replication. Thus the surface sample was always 10 cm deep, but the depth and thickness of the other two sampled layers varied with depth of the A horizon boundary, which was easily visible in these soils. After collection, soil was transported on ice back to the lab, fan-dried at room temperature for 24 – 48 hours, ground, and passed through a 2mm sieve before being stored for analysis.

Additional soil samples were collected in 2018-2019 in the manner just described from other fields where S was applied and soybean or other grain legume yield and seed S content responses were measured for the purpose of calibrating and evaluating different S soil test protocols.

Plant Sampling

Seed samples were collected by hand from each experimental unit. Seed samples were collected right before combine harvest by cutting 2 cm above the soil surface all the plants from three, 3-m long sections of row per plot. After harvesting, the plants were dried at 40° C for at least 48 hours. The seeds were then threshed from the plant and a subsample of seeds for each plot was collected for analysis. Seed yield and moisture content from all plots were measured by the a calibrated combine yield monitor and then normalized to 13% moisture content.

Seed S and Amino Acid Content

The total S content of the seed was determined by two methods. Subsamples ground to pass a 1 mm sieve were sent to a commercial lab for total S analysis using ICP (Waypoint Analytics, Richmond, VA). Sulfur content was also determined using XRF, as described below. and by by XRF (Bruker Tracer 3-SD) as described below.

All seeds were ground in a household coffee grinder (proctor silex, E160BYR) for 90 seconds for XRF analysis. Before XRF analysis samples were placed in an open top plastic XRF sample cup with a 4 micrometer prolene film bottom. Samples were lightly tamped down in plastic cup in order to minimize air space that could interfere with XRF beam. Enough sample was used to create a layer at least 3 mm thick (~ 5 g of sample). This thickness was determined earlier to provide an "infinite" absorption of x-rays. Samples were then scanned by the XRF instrument under a vacuum pressure <5 torr for 120 seconds at 15 kV, 25 microamps, and 200 pulse length with no filter. Spectra files were generated using SP1XRF software (Bruker Elemental, Kennewick, WA, United States). The spectrum of each analysis and counts per second (intensity in cps) were downloaded as .csv files which were then loaded, along with corresponding ICP values into the CloudCal software to generate calibration curves (Drake, 2018). The Lucas Tooth model built into CloudCal (Lucas-Tooth and Pyne, 1963; Drake, 2018) was used to normalize the XRF data taking into account non-linear inter-element effects to predict S content values. A liner regression was then used to define the relationship between the predicted S and the S measured independently by ICP. The calibration model used thus developed is shown in Figure 2.

A selection of 24 samples from two of the 2017 experiments (one DC and one FS) were sent for amino acid profile analysis (AAA) to the Molecular Structure Facility of the University of California, Davis, CA. After de-lipidization, the amino acids were determined with two separate analyses using a Hitachi L8900 Amino Acid Analyzer (Hitachi, USA, Santa Clara, CA) with post-column, ninhydrin derivatization. The first analysis run quantifies all the common amino acids except for cysteine (Cys), Methionine (MetI and Tryptophan (Trp). (Hitsuda et al., 2004, 2005)The second analysis run was performed on a separate oxidized aliquot of each

sample and detects cysteic acid (which is the combination of cysteine and cystine) and met-sulfone (oxidized/hydrolyzed stable form of methionine). Results for each amino acid were expressed as a percent of the extracted protein (g amino acid/100 g protein). The total protein content of the samples was calculated from total N content as determined on separate subsamples by by high-temperature combustion/gas chromatography (LECO, St. Joseph, MI) as recommended by Tabatabai and Bremner, 1991) and an N-to-protein conversion factor for soy protein of 5.71 (FAO, 2003) as:

g total protein / g seed = g N/g dry matter * 5.71 g soy protein/g N

and the content of the amino acid in the seed was calculated as:

g amino acid/ g seed = reported % amino acid / 100 * Crude Protein

The same 24 samples were also analyzed for total % S by ICP (Waypoint Analytical Labs, Richmond, Va).

After developing the calibration model, the model was applied to the remaining samples without any corresponding ICP values in order to determine S percent values for all samples. The S yield (kg/ha) was then calculated for each plot as

S yield (kg/ha) = (yield (kg/ha) * % S by XRF/100

The N/S ratio was also calculated for each of the 24 samples to identify S deficiency. A N/S ratio of greater than 18 has been used to identify S deficiency in soybean seeds (Hitsuda et al., 2004, Hallmark, 1992).

Soil Extraction procedures

Soil S levels were analyzed using four different extraction methods (table 1). The four extraction methods used were (1) 0.01 M $CaCl_2$, (2) 500 ppm $Ca(H_2PO_4)_2$ in water, (3)500 ppm

$Ca(H_2PO_4)_2$ in 2N HOAC, and (4) Mehlich-3. For the CaCl2 extraction 5 grams of soil were weighed into a 50 ml centrifuge tube, 25 ml of 0.01 M CaCl2 solution was pipetted into the centrifuge tube. Tubes were shaken for 30 min at 180 rpm, left to settle for 15 min and then filtered through Whatman no. 42 filter paper. For the 500 ppm $Ca(H_2PO_4)_2$ in water and 500 ppm $Ca(H_2PO_4)_2$ in 2N HOAc, 10 g of soil was measured into a 50 ml centrifuge tube, 25 ml of solution was pipetted into the 50 ml centrifuge tube. Tubes were then tightly capped and shaken at 180 rpm for 30 min, tubes were left to settle for 15 min and then filtered through Whatman no. 42 filter paper. For the Mehlich 3 extraction 2 g of soil was weighed into a 50 ml centrifuge tube, 20 ml of Mehlich 3 extracting solution was transferred with an autopipette into the 50 ml centrifuge tube, the tubes were tightly capped and shaken for 5 minutes at 180 rpm and then immediately filtered through Whatman no. 41 filter paper.

After extraction 10 – 15 ml of each extracted solution were transferred to a 15 ml centrifuge tube and then frozen. All the frozen samples along with 500 ml of the extracting solution used were then sent with freezer-packs in an insulated box to the Pennsylvania State University Agricultural Analytical Services Laboratory in University Park, Pennsylvania to be analyzed for total S content by ICP – AES (Table 1).

Data analysis

Effect of S treatments on response variables yield, seed S concentration, S yield, and seed METH+CYST (CYS + MET) concentration were determined by a split plot ANOVA in R (R Core Team, 2018), using the ‘car’, “nlme’ and ‘lsmeans’ packages. Gypsum application was the main plot factor and Epsom salt application was the subplot factor. Soil type (coarse or fine) was considered to be a fixed effect. Field, year, and block were considered random effects with block nested within a field. Unless otherwise indicated, a significance level of $\alpha = 0.05$ was used to

determine significant differences between treatments. An F-protected post hoc Tukey HSD test was conducted to determine significance levels between groups.

A linear regression analysis was done between %CYS+MET vs crude protein, CYS + MET vs %S, %S vs. crude protein in order to determine the correlation between seed METH+CYST content and total S. This was done in order to determine if total S (as determined by ICP or XRF) can be used as a predictor for METH+CYST content which in turn could be used as an indicator of overall protein quality.

Four categories of sites were identified for evaluations soil tests based on crop responses to S as determined with a split plot ANOVA performed in R, using the 'car', 'nlme' and 'lsmeans' packages. Crop responses to applied S (yield, seed S concentration, S yield) were determined using application of Epsom salt as a foliar spray and/or gypsum as a pre-plant soil amendment. Sulfur yield was defined as:

$$\text{S Yield } (\frac{\text{g S}}{\text{ha}}) = seed\ S\ content\ \frac{g\ S}{kg} * seed\ yield\ \frac{kg}{ha}$$

When both were used, Gypsum was a main plot factor and Epsom salt was a sub plot factor in split plot trials. Field and Block were considered random effects. Unless otherwise indicated, a significance level of $\alpha = 0.05$ was used to determine significant differences between treatments. An F-protected post hoc Tukey HSD test was conducted to determine significance levels between groups. Based on the results of the ANOVA fields were categorized into four groups: 1) non-responsive (NR), 2) significant yield response (YS), 3) significant seed S content response (SS), and 4) both a yield response and seed S content response (YSS).

The Cate-Nelson method of dividing bivariate data into responsive and unresponsive sites was used to determine the critical value of S that would predict the highest crop response for four response variables: relative yield, relative S yield, yield response, and S response in the whole 0-

30 cm soil profile as well as just the topsoil (0-10 cm) or subsoil (bottom of A or 20cm – 30 cm) (Cate and Nelson, 1971). This was done using the "rcompanion" package in R (R Core Team, 2018). The Cate Nelson analysis was followed by testing Pearson's Chi – squared to reject or accept the null hypothesis that crop response to increased S level was evenly distributed across all four quadrants. The soil test extraction most correlated with crop response was determined, as well as the ability to accurately identify site in the four categories responsiveness (NR,YS, SS, and YSS). Fields were grouped into four Cate-Nelson categories (I) Non- responsive and above critical level (II) Non-responsive and below critical level, (III) Responsive and below critical level, and (IV) Responsive and above critical level. The number of fields within each category was used to determine the percent of sites correctly identified by a soil test (100*(I fields + III Fields)/total fields) and the percent of responsive sites correctly identified (100*III Fields/ (III Fields + IV Fields).

The four response variables were calculated as follows:

$$Relative\ Yield = \frac{Yield\ of\ Control\ Plot}{Highest\ yield\ for\ that\ Crop\ x\ Year}$$

$$Relative\ S\ Yield$$

$$= \frac{\left(seed\ S\ content\ of\ control\ plot\ \left(\frac{g}{kg}\right) * Yield\ of\ control\ plot\ \left(\frac{kg}{ha}\right)\right) * 100}{Highest\ S\ yield\ for\ that\ Crop\ x\ Year}$$

$$\%\ Yield\ Response\ (Rep)$$

$$= \frac{Highest\ yielding\ S\ fertilized\ plot\ \frac{kg}{ha} - control\ plot\ yield\ \frac{kg}{ha}}{Control\ plot\ yield\ \frac{kg}{ha}} * 100$$

$$S\ Response = \frac{Highest\ S\ yield\ plot\ \frac{g\ S}{kg} - control\ plot\ S\ yield\ \frac{g\ S}{kg}}{Control\ plot\ \frac{g\ S}{kg}} * 100$$

Results

Yield and Seed %S Content

The main effects of S treatment on yield for 2017 and 2018 are summarized in Table 4 Table one shows the average yield by crop (DC or FS) for each of the four treatments. In the four double crop fields both the E and combined G+ E treatments were significantly different from the control with p values < 0.10 from the post hoc Tukey HSD test. In the four full season fields all treatments were significantly different from the control with p values <0.10 from the post hoc Tukey HSD test. Out of the eight fields used over the course of the two years, only three individual fields showed a significant response to applied S with a p value < 0.10. Overall in the four full-season soybean sites-years over the two years, yields for all S application treatments were significantly higher (2-6%) than the control yields with average yields ranging. In the four double crop soybean site-years both the E and GE treatments had significantly higher yields than the control, with E and GE averaging yields 17% and 15%, respectively, higher than the average control yields.

The main effects of S treatment on seed S (%) as measured by XRF are presented in table 4. Only one out of four full season fields showed a response to S treatment which was a negative response to E application at p<0.10. Three out of the four double crop fields showed significantly different responses from the control treatment at p<0.10. However, again one of the three fields showing a significantly different response was a negative response to E with significantly lower seed S% in both the E and GE treatments. Two out of the four had significantly higher yields in

both the E and G and one out of the four had significantly higher yields in the combined GE treatment. Averaged across four site years, none of the S treatments for full season or double crop soybeans affected seed S content significantly as compared to the no S control.

The main effects of S treatment on S yield are presented in Table 4. The S treatments did not significantly affect the full season soybean S yield (kg S/ha) at any of the four individual sites-year or when all four site-years were analyzed together. In contrast to the full season soybeans, S yields at three out of the four double crop soybean site-years exhibited significant responses to the S treatments. When the four double crop soybean site years were combined, the G treatment S yield was significantly greater than that of the control ($P<0.1$). The S yields at one of the four double crop site-years was greater than the control for all three treatments with S application, at one only the GE treatment gave a higher S yield and at one double crop soybean site-year both the E and GE treatments had S yields lower than the control.

During both 2017 and 2018 applied S had a greater effect on seed S content and yield at sites with the coarser textured soils. The yield was significantly ($p<0.05$) different from the control for all treatments with S applied (G, E, and GE) on the coarse textured soils but not on the silty soils. On the coarse soil sites, seed S content was significantly ($p<0.10$) higher in the G treatment than in the control. None of the treatments significantly affected seed S compared to the control at the fine soil sites.

Sulfur Containing Amino Acid Content

Figure 4 shows the linear relationship between crude protein and seed S % by ICP and %S by ICP vs % Cysteic Acid. Both show a positive linear relationship with and R^2 value of 0.65 and 0.64 respectively. The main effect of S treatment on seed SCAA content (METH+CYST /

total extracted protein) is summarized in Figure 5. All treatments had a significant impact on seed METH+CYST content, and the Epsom treatment was significantly higher than the gypsum treatment but the gypsum + Epsom treatment was not significantly different than either the Epsom or gypsum treatments alone.

Soil Test Evaluations

Table 7 lists the means of crop performance variables for each of the 23 sites used in the soil test protocol evaluations. The yield is the mean yield (kg/ha) of all the replications for that site-year. The relative yield is percent of the highest single replicate yield for that year-crop type combination. The use of relative yields and relative responses (as a percent of the highest single replicate response) allows the effects of S application to be isolated from the much greater year to year and full season to double variation. Based on the results of the ANOVA, fields were categorized into four groups: 1) non-responsive (NR), 2) significant yield response (YS), 3) significant seed S content response (SS), and 4) both a yield response and seed S content response (YSS). Fields in which the crop significantly responded to applied S in terms of yield, seed S content, or both yield and seed S content, were designated as *responsive fields*. Fields in which the crops exhibited no significant response to applied S were designated as *non-responsive*.

Table 8 presents the soil test S values by the four extraction protocols for the three soil horizons (depth segments) and the weighted mean of the complete 0-30 cm layer. Generally, the median values for the Mehlich-3 extraction were 3 or more times as large as the S values for the other three extracting protocols. For the 2017 soils the CaCl2 extracted much less S than the other solutions. The S soil test values using the four soil extraction protocols were not all closely

correlated with each other, especially in the B horizon where sorbed sulfate was likely an important component of the S present. Figure 6 shows how results from the four extraction protocols were related to each other. The CaCl2 extractant, a dilute neutral salt solution, correlated quite well with the much stronger Mehlich-3 extraction in the A1 horizons, suggesting that both extracted mainly water soluble sulfate (such as gypsum,) sulfate weakly held by organic matter or sulfate dissolved in the soil solution. The correlation was much weaker for the B horizon samples because much of the S in the subsoil is tightly sorbed sulfate ions on clay and metal oxide coatings from which the strong exchangers in Mehlich-3, but not the Cl^- ion could remove them. The S extracted by acidified calcium phosphate solution ($Ca(H_2PO_4)_2$ in 2N HOAC) was not correlated with the S extracted by the other three solutions.

Table 9 summarizes the abilities of four soil extractions to identify soils in which crops responded to S application during 2017-2019. Critical S soil test values above which crop response would not be expected, are indicated for each of the four extraction protocols as developed in relation to four different crop response variables. The critical soil test value was determined by Cate-Nelson (Cate and Nelson, 1971) analysis of data from 122 individual blocks within the 23 fields. Table 9 indicates the total number of fields and the number categorized in each Cate-Nelson quadrat, the percent of sites identified in the correct quadrate and the percent of S-responsive sites identified by the four soil test protocols. The results are presented for the 0-10 cm A1 horizon, the B horizon and entire 0-30 cm soil samples. Fields were grouped into four Cate-Nelson categories (I) Non- responsive and above critical level (II) Non-responsive and below critical level, (III) Responsive and below critical level, and (IV) Responsive and above critical level. The number of fields within each category was used to determine the % of sites

correctly identified by a soil test (100*(I fields + +III Fields)/total fields) and the % of responsive sites correctly identified (100*III Fields/ (III Fields + IV Fields).

The ANOVA identified 9 out of 23 fields in which crops showed a significant response (YS, SS, or YSS) to applied S. The Cate-Nelson analysis identified the critical levels for the four extractions (based on the weighted mean of the 0-30 cm sample) as 5.5, 4.4, between 9.9-11.3, and 16.2 mg S/kg for $CaCl_2$, $CaHPO_4$ (water), $CaHPO_4$ (HOAc), and Mehlich 3 respectively. The critical values were very similar whether determined using relative yield or relative S yield. The results of the Cate-Nelson analyses are shown in Figures 3-6. Only extractable S results for the A1, B, and weighted mean of all three soil layers (0-30cm) are shown. The A2 segment was considered a transition horizon that may have had some mixing between the surface and subsoils. It is not shown separately, but is included in the weighted average.

Sites were considered correctly identified by the Cate-Nelson analysis if non-responsive site had extractable S above the critical value (NR) and responsive sites (YS, SS, or YSS) has extractable S below the critical value (Table 6). In almost every case, data for the A1 horizon more accurately identified responsive soils that did data for the B horizon alone, and using the weighted mean for 0-30cm which included the B horizon rarely increased the accuracy of the soil tests. The $CaHPO_4$ (water) extractable S content for the A1 horizon was able to correctly identify 80% of sites based on relative yield, relative S yield, and yield response and identified 71.4% of the responsive sites. The $CaHPO_4$(water) extractable S content for the weighted mean correctly identified 70% of sites and 57.1% of sites that showed a significant response (based in only the 10 sites from 2017-208 for which $CaHPO_4$(water) data was available). The $CaHPO_4$ (HOAc) extractable S in the A1 horizon correctly predicted 68.2% of the total sites and 87.5% of the responsive sites based on both relative yield and relative S yield. The $CaHPO_4$ (HOAc)

extractable S for the 0-30 cm weighted mean correctly identified 59.1% of total sites and 75% of responsive sites. The $CaCl_2$ extractable S content for the A1 horizon accurately identified 73.9% of total sites but only 33.3% of responsive sites. The weighted mean for $CaCl_2$ correctly identified 43.5% and 66.7% of total and responsive sites, respectively. Mehlich-3 extractable S for the A1 horizon correctly identified 52.2% and 60.9% based on relative yield and relative S yield, respectively, and 77.8% of the responsive sites. In almost every case soil test extractable S was able to better categorize Cate-Nelson quadrant based relative yield and relative S yield than yield response or S response.

There were no significant responses to applied S by any of the soybeans grown in 2019 (DC or FS). Additionally, the $CaHPO_4$ (water) test was not completed on the 2019 samples. In order to confirm the comparison made when all 23 fields were included for the other three soil test protocols, we re-ran the comparisons using just the ten 2017-2018 fields for which all four extractions were performed. In this analysis (data not shown), $CaHPO_4$(HOAc) using just the A1 horizon accurately identified 100% of the 10 sites and 88.9% of the sites based on the weighted mean for 0-30 cm. For the 2017-2018 fields, Mehlich 3 accurately identified 80% of the sites using the A1 horizon soil and 50% and 70% of the sites for relative yield and relative S yield, respectively, for the 0-30cm weighted mean. The $CaCl_2$ extraction accurately identified 60% of sites for both the A1 and 0-30 cm weighted mean. In agreement with the results from all 23 fields, including the soil test values from the B horizon did not improve the percentage of responsive sites identified when only the 10 fields from 2017-2018 were analyzed.

Discussion

Differences in weather patterns undoubtedly affected soybean growth and response to S. In 2018 the weather was hot (>30^{o}C) and dry (no significant rain) for the week prior to and the

week after Epsom spraying while in 2017 it was cooler and wetter. These contrasting temperature and moisture conditions could have affected the likelihood of osmotic stress from the Epsom salt spray. These conditions also occurred during flowering when plants are starting to put more energy into seed production and the appropriate amounts of nutrients and water are critical to reach maximum yield potential. In addition, due to wet soil conditions in fall 2018, harvest occurred about a month later than in 2017.

The contrasting weather patterns most likely influenced the soybean yields in the two years. The full season soybean yields in 2018 on the site with coarse soils were significantly higher than on the site with finer soils, likely because the better drainage on coarse soils was beneficial during 2018 as this year was much wetter than 2017. However, the double crop yields in 2017 and 2018 were not significantly different between the soil types which likely has to do with the timing of the planting. Double crop soybeans are planted after the wheat harvest (or other cereal grain) in Maryland typically in late June or early July. In both years this corresponded with a period of low rainfall and high temperatures which may have impacted the overall yield if the plants were late to get started. A recent review (Hansel et al., 2019) reported that the main factors limiting yield potential of double crop soybeans were late planting date, water stress, low temperatures in fall, excessive crop residue at planting, soil nutrient deficiencies, early frost dates and factors that affect access by harvest equipment. These observations about yield impacts due to temperature and rainfall were consistent with the double crop soybean performance in this study.

Limited research has been done on the effect of timing of S application on the transport of S to seeds and allocation of S to amino acids within the seed. Nor is there much data on the effect of drought on soybean yield and protein content. Research done by Bender et al. (2015)

found that total nutrient uptake was significantly impacted by year when one year had above average temperatures and the other had below average temperatures during the growing season. Their data show that around 40 days after planting soybean S demand exponentially increased, but that the uptake of S was relatively evenly distributed across both the vegetative and reproductive (seed filling) growth stages (Bender et al., 2015). Our results show differing response to the different methods and timing of S applications in the yield, seed S content, and S yield. These results suggest that the underlying S uptake and transport mechanisms may be affected by S application timing and method of, by soil properties, and by weather conditions, all of which differed among the eight site-years.

In addition to weather and soil effects on plant uptake and of S, some plants may not be able to effectively transport S younger more photosynthetically active eaves and to the seed were storage proteins are synthesized(Sunarpi and Anderson, 1997; Paek et al., 2000; Naeve and Shibles, 2005). The younger leaves in the upper soybean canopy seem to be more effective than older leaves at assimilating SO_4 taken up by the plant into amino acids and other essential compounds (Naeve and Shibles, 2005). We chose to apply the Epsom as a foliar spray at the beginning of the plant reproductive stage (between R1-R3) because Epsom is highly soluble, most farmers are well equipped to apply foliar sprays, and that is the point at which plants are rapidly accumulating S and new leaves are still expanding. However, in agreement with prior research, our results show that although both the treatments with Epsom had more significant impact on overall and individual field yields, the treatments with Gypsum overall had higher seed S content. This may suggest that although the later treatment of sprayed Epsom was successful at meeting the plant S demand, the plants were not successfully able to transport that S into the seeds. Therefore, the findings that Naeve and Shibles (2005) report suggest that 56-

59% of the total plant S is accumulated in the vegetative stage and that the plant transports S to the seed if it has accumulated sufficient S during the vegetative stages. Additionally, only 10% of the seed S content came from the leaves, suggesting that solely applying a foliar application of S at the beginning of the reproductive stage may not give the plants enough time to transport sufficient S to the seed (Naeve and Shibles, 2005).

In addition to timing and source of applied S, soil type and S present in the soil also have an effect on S uptake and mobilization within the plant. It is expected that coarser textured fields would respond more significantly than finer textured fields to applied S. Sulfate ions are highly susceptible to leaching unless adsorbed onto the surfaces of clays and Fe or Al oxides. Sandier fields, which are low in clay and Fe or Al oxides than finer textured soils, have lower anion exchange capacity and thus are more susceptible to SO_4^{2-} leaching. The finer textured soils that characterized four of the sites in this study likely had sufficient S to meet crop needs stored in the soil organic matter and subsoil iron coated clay so crop growth at these sites may not have been limited by S before S application. This is likely the reason why the addition of S from gypsum or Epsom salt treatments failed to increase yield above that on the control plots. .

In contrast, soybean yields of on all of the relatively coarse fields were increased by S treatment, even though the seed S content was significantly improved only by the gypsum treatment and S yield was significantly impacted only by the combined gypsum and Epsom treatment. Several factors may explain why the S treatments increased the yield more frequently than they increased the seed S content.

Seeds from 24 plots in the 2017 experiments were analyzed for their content of the amino acids methionine and cysteine (METH+CYST). The results showed that all S application treatments increased the proportion of meth+cyst in the soybean seeds, with the greatest increase

from the Epsom salt treatments (Figure 7). This outcome supports our hypothesis that that soybean seed protein quality could be enhanced by S fertility management. Prior efforts to improve METH+CYST content of the soybean protein largely focused on breeding for improved METH+CYST content (Krishnan, 2008; Krishnan and Jez, 2018). Our results warrant further research into the potential for S fertility management for enhancement of soybean nutritional quality. Our study indicates that protein composition is not the same for all soybeans and for all growing conditions and suggests that fertility management, in addition to plant breeding, should be considered to maintain or improve the nutritional quality of soybeans for both humans and non-ruminant animas. Increases in seed METH+CYST content of the magnitudes observed in this study could have very large impacts on the economic value of soybeans as feed (McVey et al., 1995).

The strong correlation between the %S by XRF and total S measurements by ICP suggests that XRF is a reliable method to analyze plant tissue samples, including seeds, for S content. The XRF is an easy, rapid, non-destructive method that could easily be used by a grain elevator or wholesale purchaser of soybeans to measure nutrient concentration of incoming crops. Our results suggest that total S could be used as a proxy measurement for protein quality as it is strongly correlated with both crude protein and METH+CYST content. Further work needs to be done to confirm these relationships and create a calibration equation between total S and METH+CYST content that includes a wider range of plant genotypes and environments.

Conclusion

This study confirmed that application of S to soybeans is advisable on sandy soils. The rate and timing of Epsom salt foliar applications was effective, but further research needs to be done to be able to determine if lower rates, earlier timing, or other sources (such as potassium

sulfate or ammonium sulfate) might be more effective. The results are also inconclusive as to whether or not surface applied gypsum at the time of planting or foliar applied Epsom at the R1 is a more appropriate time to apply S. If the objectives were restricted just to yield, it would follow that Epsom provided a significant increase in yields in more site-years with or without gypsum than the gypsum alone treatment. However, when seed S content and S yield are included in the objectives, it becomes less clear because gypsum increased seed S content in more site years than Epsom salt alone.

Because the two years during which these experiments took place were characterized by contrasting growing season weather conditions, further studies should be carried out on a wider range of soil types and under different weather regimes. It is also possible that all the fields may have been S deficient but due to other stresses, such as drought, extreme rainfall, or other nutrient deficiencies, the plant did not respond to the S because those problems were more significant than the S deficiency. For example, if the plant is N deficient at any point, it will not respond to S fertilization, despite having an S deficiency, until the N demands are met because N is needed in larger amounts than S.

The highly significant linear relationship between seed S content measured by standard digestion and ICP analysis and seed S content determined by XRF scan suggest the potential for XRF to be deployed for rapid, inexpensive seed S analysis. Our amino acid data show that S nutrition of the plant affects protein quality in the seed. With further research to refine the technique for portable use, XRF has the potential to be applied to market differentiation that pays farmers for improved nutritional quality of the crop.

The exploration of sulfur soil test protocols was only partially successful. The CaCl2, CaHPO4(water) and the Mehlich-3 all proved to have some value in identifying soils on which

soybeans are likely to respond positively to applied S. However, all the protocols also mis-identified some fields as not needing S when the soybean did respond and some as needing sulfur when the soybean did not respond. The Mechlich-3 test was as good as any of the others and correctly identified about six or seven of the nine responsive soils in the study. Including soil from below the A horizon did not seem to improve the soil test accuracy.

The results of this study support continued research on S fertility management for soybeans, especially in regions with low soil organic matter and sandy textured soils. Due to current SO_2 emissions regulations and high analysis fertilizer use with low S impurities, S deficiency is likely to become an increasingly common in non S-fertilized soybeans in the mid-Atlantic and elsewhere. Continued research on a wide range of soil types and environments will be key to accurate S fertility recommendations.

Table 1. Agronomic practices and timing of operations at the eight study sites. No Insecticides or fungicides, other than seed treatment, were applied, all fields under no-till management for at least the past five years. All fields received four treatments: G1E0: Gypsum applied at a rate of 560 kg/ha broadcast at time of planting, G0E1: Epsom Salt applied at a rate of 86 kg/ha as a foliar spray between R1-R3, G1E1: combined gypsum and Epsom Salt, and G0E0: No treatment control. DC=Double Crop Soybean planted after cereal grain (usually wheat), FS=Full Season Soybean

Year	Field ID	Crop	Variety	Prior S Application (kg-S/ha)[1]	Crop Rotation	Herbicide Application	Plant Date	Gypsum Applied	Epsom Applied	Harvest Date
2017	5-43A	DC	TA3959R2S	2019-0 2018-20 2017-32 2016-20 2015-0	2017 Wheat DC Soybean 2016 FS Soybean 2015 Sorghum/FS Soybean 2014 Wheat DC Soybean 2013 FS Soybean 2012 Corn	glufosinate @ 0.85 L Ammonium Sulfate @1.3 kg glyphosate	7/11/17	4/11/17	8/31/17	10/31/17
2017	5-39B	DC	TA3959R2S	2019-20 2018-0 2017-20 2016-0 2015-20	2017 Wheat DC Bean 2016 FS Bean 2015 Wheat DC Bean, 2014 FS Bean 2013 Corn 2012 Wheat Barley DC Bean	glufosinate @ 0.85 L 1.3 kg Ammonium Sulfate, glyphosate	7/11/17	4/11/17	8/31/17	10/31/17
2017	5-43B	FS	Pioneer 40T84X	2019-32 2018-20 2017-150 as gypsum 2016-32 2015-20	2017 FS Bean, 2016 Corn 2015 Wheat DC Bean 2014 FS Beans 2013 Corn	0.95L glyphosate,0.13 Sulfentrazone 0.75 L pt. Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide, Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	6/10/17	4/12/17	8/10/17	10/18/17
2017	5-18O	FS	Pioneer 40T84X	2019-0/32 2018- 0/32 2017-0/32 2016-0/32 2015-0/32	2017 FS Bean 2016 Corn 2015 FS Bean 2014 Corn 2013 Wheat DC Beans 2012 FS Beans	0.95L glyphosate,0.13 Sulfentrazone 0.75 L pt. Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide, Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	6/10/17	4/12/17	8/26/17	10/26/17
2018	5-17C	DC	Asgrow 4135	2019-32 2018- 20	2018 Wheat DC Bean 2017 Corn	0.85L glufosinate , 0.17 L Clethodim, 1.3 kg Ammonium Sulfate,1.4L Glyphosate,	7/10/18	7/1/18	9/4/18	12/6/18

				2017-32+150 as gypsum 2016-20 2015-32	2016 Wheat DC Bean 2015 Corn 2014 Wheat DC Bean 2013 Corn					
2018	5-25A	DC	Asgrow 4135	2019-0/32 2018 - 0/32 2017-0/32 +150 as gypsum 2016-0/32 2015-0/32	2018 Wheat DC Bean 2017 FS Bean 2016 Corn 2015 Wheat DC Bean 2014 FS Bean 2013 Corn	0.85L glufosinate , 0.17 L Clethodim, 1.3 kg Ammonium Sulfate,1.4L Glyphosate,	7/10/18	7/1/18	9/4/2018	11/29/18
2018	5-39C	FS	Pioneer 31A22	2019-20 2018 – 0 2017-32 2016-32 2015-32	2018 FS Bean 2017 Corn 2016 Corn 2015 Corn 2014 Wheat DC Bean 2013 Corn	0.95L glyphosate, 0.13 Sulfentrazone 0.75 L pt. Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	6/18/18	5/25/18	8/5/18	10/9/18
2018	5-18E	FS	Pioneer 31A22	2019-0/32 2018 - 0/32 2017-0/32 2016-0/32 2015-0/32	2018 FS Bean 2017 Corn 2016 FS Bean 2015 Corn 2014 Wheat DC Bean 2013 FS Beans	0.95L glyphosate, 0.13 Sulfentrazone 0.75 L pt. Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	6/18/18	5/25/18	8/17/18	12/10/18

[1]All prior S treatments applies as 22-0-0-5S analysis fertilizer

Table 2. Soil characterization data for 2017 and 2018 fields at CMREC Beltsville research facility. Soil organic matter (SOM) determined by loss on ignition (LOI), pH measured in water, and Mehlich 3 extractable ,P,K, and S. Topsoil$_1$= 0-10 cm, Topsoil$_2$= 10-20 cm or bottom of A horizon, Subsoil = bottom of A or 20 cm – 30 cm.

Field ID	Surface Texture	Soil Series	Taxonomy	Soil type designation	Horizon	pH	SOM (%)	P (mg/kg)	K (mg/kg)	Est. CEC (meq/100g)
5-43A	Sandy Loam	Christiana	Fine, kaolinitic, mesic Aquic Hapludults	Fine	Topsoil$_1$	5.5	1.25	57.76	124.00	5.20
					Topsoil$_2$	5.5	0.55	55.19	59.10	3.90
					Subsoil	5.7	0.45	12.00	57.00	3.60
5-39B	Loamy Sand	Downer-Hammonton Complex	Coarse-loamy, siliceous, semiactive, mesic Typic Hapludults,	Coarse	Topsoil$_1$	5.6	0.60	71.39	73.56	5.00
					Topsoil$_2$	5.7	0.75	73.90	59.29	3.20
					Subsoil	6.0	0.20	23.67	42.79	2.90
5-43B	Sandy loam	Downer	Coarse-loamy, siliceous, semiactive, mesic Typic Hapludults	Coarse	Topsoil$_1$	5.9	1.40	60.41	99.51	4.60
					Topsoil$_2$	5.7	0.65	61.92	58.35	3.70
					Subsoil	5.6	0.30	38.36	42.73	3.10
5-18O	Sandy loam	Downer	Coarse-loamy, siliceous, semiactive, mesic Typic Hapludults	Coarse	Topsoil$_1$	6.5	2.50	199.39	89.82	4.60
					Topsoil$_2$	6.6	1.70	177.68	58.35	3.70
					Subsoil	5.5	0.85	2.90	42.73	3.10
5-17C	Sandy loam	Russett-Christiana	Fine-loamy, mixed, semiactive, mesic Aquic Hapludults	Fine	Topsoil$_1$	5.7	1.70	40.80	43.50	5.20
					Topsoil$_2$	5.7	1.70	45.20	31.40	3.90
			Fine, kaolinitic, mesic Aquic Hapludults		Subsoil	5.7	0.40	24.80	30.10	2.70
5-25A	Sandy Loam	Russett-Christiana	Fine-loamy, mixed, semiactive, mesic Aquic Hapludults	Fine	Topsoil$_1$	6.2	2.35	181.00	83.15	7.00
					Topsoil$_2$	6.1	1.15	112.60	35.50	5.90
			Fine, kaolinitic, mesic Aquic Hapludults		Subsoil	5.9	0.85	9.50	36.98	5.40
5-39C	Loamy Sand	Downer-Hammonton	Coarse-loamy, siliceous, semiactive, mesic Typic Hapludults,	Coarse	Topsoil$_1$	5.6	0.60	71.39	73.56	5.00
					Topsoil$_2$	5.7	0.75	73.90	59.29	3.20
					Subsoil	6.0	0.20	23.67	42.79	2.90
			Coarse-loamy, siliceous, semiactive, mesic Aquic Hapludults							
5-18E	Sandy Loam	Russett-Christiana	Fine-loamy, mixed, semiactive, mesic Aquic Hapludults Fine, kaolinitic, mesic Aquic Hapludults	Fine	Topsoil$_1$	6.4	3.35	225.93	80.38	14.50
					Topsoil$_2$	6.2	2.15	173.32	36.68	12.40
					Subsoil	6.5	1.45	67.78	30.97	8.80

Table 3. Analysis of Variance (ANOVA) for effects of S treatments on soybean yield (kg/ha), Seed S Content (mg/g), and S yield (kg-S/ha) of two types of soybean crops (full season and double crop) grown in two years (2017-2018) at CMREC Beltsville on two types of soils (coarse and fine textured) for a total of eight site years. Year, crop and soil were considered random effects, Epsom and Gypsum were fixed effects. Significant p values are in bold font.

		Yield	**Seed S Content**	**S-Yield**
Source of variation	**df**		**p-value**	
Block (Soil*Year)	32			
Gypsum	1	0.2904	**0.01438**	**0.0815**
Epsom	1	0.2833	**0.08329**	0.85202
Year	1	0.8178	**0.00664**	0.44465
Gypsum*Epsom	1	**0.0833**	0.87432	0.19625
Soil*Gypsum	1	**0.0137**	**0.05541**	**0.00727**
Soil*Epsom	1	0.1066	**0.08749**	**0.09429**
Soil*Year	1	**0.0000**	0.15017	**0.0000**
Gypsum*Year	1	0.868	0.12271	0.54631
Epsom*Year	1	0.9699	**0.01123**	0.25372
Crop*Year	1	**0.0571**	0.68958	0.14993
Crop*Epsom	1	0.253	0.60913	0.29667
Crop*Gypsum	1	0.6282	0.96423	0.68071
Soil*Gypsum*Epsom	1	0.3423	0.36778	0.33725
Soil*Gypsum*Year	1	0.7907	**0.01931**	0.21928
Soil*Epsom*Year	1	0.7776	0.67666	0.96897
Gypsum*Epsom*Year	1	0.9884	0.84141	0.92027
Crop*Gypsum*Epsom	1	0.9082	0.11369	0.44069
Soil*Gypsum*Epsom*Year	1	0.4835	0.85312	0.55763
Residuals	84			

*Table 4. Mean seed yields (top), seed S contents (middle), and S yields (bottom) for full season and double crop (planted after wheat harvest) soybean at Beltsville as affected by S treatment, soil type and year. *, ** denote statistically significant difference from average yield of the control by post hoc Tukey test at P <0.05 and <0.10. Means within a site-year followed by the same lower-case letter do not differ significantly at P <0.05. C= control; E = Epsom applied at a rate of 86 kg/ha as a foliar spray at first flower; G=Gypsum applied at a rate of 560 kg/ha at the time of planting;, and; GE = combination of gypsum and Epsom.*

Treatment	Fine Soil	Coarse Soil	Fine Soil	Coarse Soil	Crop Mean	Grand Mean
	2017		2018			
Soybean Yield						
---------- **kg/ha** ----------						
	Crop: Full Season Soybean					
	n=5	n=5	n=4	n-4		
C	3933a	2840b	2661a	4314a	3431b	2793b
E	3866a	3415a*	2846a	4407a	3634a**	3053a*
G	3892a	3422a*	2630a	4457a	3607a**	3011a*
GE	3725a	3328a**	2683a	4345a	3521a**	2974a*
	Crop: Double Crop Soybean					
	n=3	n=3	n=4	n-4		
C	1892a	2136b	2087b	1794a	1972b	
E	1914a	2560a**	2680b	2040a	2308a*	
G	1684a	2391ab	2667b	2140a	2247b	
GE	1684a	2659a**	2822a*	1870a	2271a**	
Soybean S Content						
---------- **%**----------						
	Crop: Full Season Soybean					
C	0.36a	0.3b	0.35a	0.34a	0.34a	0.34ab
E	0.35a	0.25a*	0.34a	0.36a	0.33a	0.34b
G	0.36a	0.33b	0.36a	0.33a	0.35b*	0.36a
GE	0.36a	0.32b	0.35a	0.35a	0.34a	0.35a
	Crop: Double Crop Soybean					
C	0.37a	0.31b	0.36a	0.34b	0.35a	
E	0.29b**	0.37a**	0.36a	0.36a**	0.35a	
G	0.37a	0.38a*	0.37a	0.36a*	0.37a	
GE	0.28b**	0.36ab	0.37a	0.37a*	0.35a	
Soybean S yield (kg/ha)						
---------- kg S / ha ----------						
	Crop: Full Season Soybean					
C	14.30a	8.43a	9.66a	14.81a	11.69a	8.84b
E	13.68a	8.34a	9.81a	15.37a	11.70a	9.397ab
G	13.95a	11.75a	9.25a	14.71a	12.37a	9.818a*
GE	13.28a	10.52a	9.39a	15.07a	11.87a	9.646ab
	Crop: Double Crop Soybean					
C	7.03a	6.74b	6.44a	7.18b	6.84b	
E	5.62ab*	9.57a*	7.34a	9.64b	8.11b	
G	6.23a	9.02a**	7.89a	9.72b	8.30a**	
GE	4.61b**	9.48a*	6.97a	10.32a**	8.22b	

Table 5. Summary of site cropping history and agronomic treatments for each field used for soil test evaluations in 2017, 2018, and 2019. Table includes Field ID, Crop, Variety, Sulfur treatments applied in field trial, Tillage History, S application to field prior to use in study, prior crop rotation, herbicide application during study, plant data, harvest date, Epsom Salt Spray date, Gypsum application date. DC= double crop Soybean planted after wheat harvest; FS = full season soybean;BB= Black Bean; E = Epsom applied at a rate of 86 kg/ha as a foliar spray at first flower; G=Gypsum applied at a rate of 560 kg/ha at the time of planting; C= control, and; GE = combination of gypsum and Epsom

Year	Field ID	Crop	Variety	Treatments	Tillage (past 5 years)	Prior S Application (kg-S/ha)[1]	Crop Rotation	Herbicide Application	Plant Date	Harvest Date	Epsom Applied	Gypsum Applied
2017	5-43A	DC	TA3959R2S	E, G, E+G, C	No-Till	2015-0, 2016-20, 2017-32, 2018- 20, 2019-0	2017 Wheat DC Soybean 2016 FS Soybean 2015 Sorghum/FS Soybean 2014 Wheat DC Soybean 2013 FS Soybean 2012 Corn	glufosinate @ 0.85 L Ammonium Sulfate @1.3 kg glyphosate	7/11/17	10/31/17	8/31/17	4/11/17
2017	5-39B	DC	TA3959R2S	E, G, E+G, C	No-Till	2015-20, 2016-0, 2017-20, 2018 - 0, 2019-20	2017 Wheat DC Bean 2016 FS Bean 2015 Wheat DC Bean, 2014 FS Bean 2013 Corn 2012 Wheat Barley DC Bean	glufosinate @ 0.85 L 1.3 kg Ammonium Sulfate, glyphosate	7/11/17	10/31/17	8/31/17	4/11/17
2017	5-43B	FS	Pioneer 40T84X	E, G, E+G, C	No-Till	2015-20, 2016-32, 2017-0+150 as gypsum 2018-20, 2019-32	2017 FS Bean, 2016 Corn 2015 Wheat DC Bean,2014 FS Beans 2013 Corn	0.95L glyphosate,0.13 Sulfentrazone 0.75 L pt. Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide, Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	6/10/17	10/18/17	8/10/17	4/12/17
2017	5-18O	FS	Pioneer 40T84X	E, G, E+G, C	No-Till	2015-0/32, 2016-0/32, 2017-0/32, 2018- 0/32, 2019-0/32	2017 FS Bean 2016 Corn 2015 FS Bean 2014 Corn 2013 Wheat DC Beans 2012 FS Beans	0.95L glyphosate,0.13 Sulfentrazone 0.75 L pt. Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide, Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	6/10/17	10/26/17	8/26/17	4/12/17
2018	5-17C	DC	Asgrow 4135	E, G,E+G,C	No-Till	2015-32, 2016-20, 2017-32, 150 as gypsum 2018- 20,	2018 Wheat DC Bean 2017 Corn 2016 Wheat DC Bean 2015 Corn 2014 Wheat DC Bean 2013 Corn	0.85L glufosinate , 0.17 L Clethodim, 1.3 kg Ammonium Sulfate,1.4L Glyphosate,	7/10/18	12/6/18	9/4/18	7/1/18

						2019-32						
2018	5-25A	DC	Asgrow 4135	E, G, E+G,C	No-Till	2015-0/32, 2016-0/32, 2017-0/32, 150 as gypsum 2018 - 0/32, 2019-0/32	2018 Wheat DC Bean 2017 FS Bean 2016 Corn 2015 Wheat DC Bean 2014 FS Bean 2013 Corn	0.85L glufosinate , 0.17 L Clethodim, 1.3 kg Ammonium Sulfate,1.4L Glyphosate,	7/10/18	11/29/18	9-4-2-18	7/1/18
2018	5-39C	FS	Pioneer 31A22	E, G, E+G, C	No-Till	2015-32, 2016-32, 2017-32, 2018 - 0, 2019-20	2018 FS Bean 2017 Corn 2016 Corn 2015 Corn 2014 Wheat DC Bean 2013 Corn	0.95L glyphosate, 0.13 Sulfentrazone 0.75 L pt. Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	6/18/18	10/9/18	8/5/18	5/25/18
2018	5-18E	FS	Pioneer 31A22	E, G, E+G, C	No-Till	2015-0/32, 2016-0/32, 2017-0/32, 2018 - 0/32, 2019-0/32	2018 FS Bean 2017 Corn 2016 FS Bean 2015 Corn 2014 Wheat DC Bean 2013 FS Beans	0.95L glyphosate, 0.13 Sulfentrazone 0.75 L pt. Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	6/18/18	12/10/18	8/17/18	5/25/18
2018	UMBB	BB	Midnight Black Turtle	E, G, E+G, C	2018[1]		2018 BB bean 2017 No Crop 2016 Soybeans 2015 Soybeans 2014 corn 2013 Soybeans	none	6/19/18	9/11/18	8/8/18	6/19/18
2018	5-39B	BB	Midnight Black Turtle	E, G, E+G, C	2018[1]	2015-20, 2016-0, 2017-20, 2018 - 0, 2019-20	2018 BBean 2017 Barley DC Bean 2016 Wheat DC Bean 2015 Corn 2014 Wheat DC Bean 2013 Corn	0.94 L Paraquat, 0.02 L halosulfuron methyl, 0.75 L Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide, 0.4 L Clethodim	5/30/18	9/11/18	8/8/19	6/1/19
2019	5-39A	DC	Asgrow 43X7	E,C	No-Till	2015-20, 2016-32, 2017-0, 2018 - 32, 2019-20	2019 Wheat DC Bean 2018 Wheat DC Bean 2017 FS Bean 2016 Corn 2015 Wheat DC Bean 2014 FS Bean	0.85 L glufosinate, 0.35 L Fluazifop-P-butyl, 1.3 kg Ammonium sulfate8-1-2018, 1.4 L glyphosate	7/14/19	11/4/19	8/26/19	NA

2019	5-7F	DC	Asgrow 43X7	E,C	No-Till	2015-NA, 2016-32 2017-0 2018 - 32 2019-NA	2019 Oats DC Bean 2018 Corn 2017 Wheat DC Bean 2016 Corn 2015 Wheat DC Bean 2014 Corn 2013 Wheat DC Bean	0.85 L glufosinate, 0.35 L Fluazifop-P-butyl, 1.3 kg Ammonium sulfate8-1-2018, 1.4 L glyphosate	7/15/19	11/6/19	8/26/19	NA
2019	5-43A	FS	Pioneer P29A25X	E,C	No-Till	2015-0, 2016-20, 2017-32 + 150 as gypsum 2018 - 20, 2019-0	2019 FS Bean 2018 Wheat DC bean 2017 Corn 2016 Wheat DC Soybean 2015 FS Bean 2014 Wheat DC Soybean	0.95L glyphosate, 0.13 Sulfentrazone 0.75 L Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	5/31/19	10/3/19	7/28/19	NA
2019	5-25C	FS	Pioneer P29A25X	E,C	No-Till		2019 FS Bean 2018 Wheat DC Bean 2017 Corn 2016 Corn 2015 Wheat DC Bean 2014 FS Bean	0.95L glyphosate, 0.13 Sulfentrazone 0.75 L pt. Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	6/4/19	10/3/19	7/28/19	NA
2019	5-40	FS	Pioneer P42A96X	E,C	No-Till		2019 FS Bean 2018 Corn 2017 Wheat DC Bean 2016 FS Bean 2015 Corn 2014 Wheat DC bean	0.95L glyphosate, 0.13 Sulfentrazone 0.75 L pt. Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	6/8/19	10/14/19	7/28/19	NA
2019	5-17A	FS	Pioneer P42A96X	E,C	No-Till	2015-32, 2016-32, 2017-20, 2018 - 32, 2019-0	2019 FS Bean 2018 Corn 2017 Wheat DC Bean 2016 Corn 2015 Wheat DC Bean 2014 FS Bean	0.95L glyphosate, 0.13 Sulfentrazone 0.75 L pt. Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid), 0.65 L glyphosate	6/8/19	10/11/19	7/28/19	NA
2019	5-39A	BB	Eclipse	E, G, E+G, C	2019[2]	2015-20, 2016-32, 2017-0, 2018 - 32, 2019-20	2019 Black Bean 2018 Wheat DC Bean 2017 FS Bean 2016 Corn 2015 Wheat DC Bean 2014 FS Bean	0.01 L Halosulfuron-Methyl, 0.56 L Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide	6/6/19	10/4/19	7/20/19	6/6/19

2019	5-7A	BB	Eclipse	E, G, E+G, C	2019[2]	2015-0, 2016-32, 2017-NA, 2018 - 32, 2019-NA	2019 Black Bean 2018 FS Bean 2017 Vegetables 2016 Vegetables 2015 Sweet Corn 2014 Vegetables	0.01 L Halosulfuron-Methyl, 0.56 L Metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide	6/6/19	10/19/19	7/20/19	6/6/19
2019	SK1	DC	Pioneer P41T65PR	E,C	No Till		corn, wheat/DC beans	0.95 L glyphosate	6/17/19	10/18/19		
2019	JL1	FS	Pioneer 33A24X	E,C	2016[2]		Beans, Corn, beans, corn, beans, corn etc.	0.95 L glyphosate	4/11/19	9/27/19		
2019	DS1	FS	HS44X80	E,C	2016, 2018[2]		2019 - DC Wheat, Beans 2018- Potatoes (rye covercrop) 2017- Watermelons (rye covercrop) 2016- Full season beans 2015- Milo 2014- DC wheat / beans		6/28/19	11/5/19	8/6/19	

[1]All prior S treatments applies as 22-0-0-5S analysis fertilizer, except gypsum where indicated.

[2]Surface Till, No Till prior five years

[3]Sludge Application with Conservation tillage, prior five years no till

[4]Moldboard plowed every three before 2016, Chisel plowed in 2018

Table 6 Soil characterization data for all fields used for S soil test evaluations in 2017, 2018, and 2019 seasons. Soil organic matter (SOM) determined by loss on ignition (LOI), pH measured in water, Mehlich 3 extractable P and K and estimated CEC. A1=0-10 cm, A2=10-20 cm or bottom of A horizon, B = bottom of A horizon or 20 cm – 30 cm, Mean = weighted average for the 0-30 cm sample based on the depth of the sample and the bulk density of a representative "Coarse" and "Fine" field at CMREC Beltsville.

Field	Horizon	Textural Class	Soil Series	Taxonomy	pH	SOM (%)	P (mg/kg)	K (mg/kg)	Est. CEC (meq/100g)
5-18O	A1		Downer	Coarse-loamy, siliceous, semiactive, mesic	6.5	2.50	199.39	89.82	4.60
	A2			Typic Hapludults	6.6	1.70	177.68	58.35	3.70
	B				5.5	0.85	2.90	42.73	3.10
	Mean				6.2	1.65	127.96	62.27	3.76
5-39B	A1		Downer-	Coarse-loamy, siliceous, semiactive, mesic Typic	5.6	0.60	71.39	73.56	5.00
	A2		Hammonton	Hapludults	5.7	0.75	73.90	59.29	3.20
	B		Complex	Coarse-loamy, siliceous, semiactive, mesic Aquic	6.0	0.20	23.67	42.79	2.90
	Mean			Hapludults	5.8	0.58	61.71	59.68	3.66
5-43A	A1		Christiana	Fine, kaolinitic, mesic Aquic Hapludults	5.5	1.25	57.76	124.00	5.20
	A2				5.5	0.55	55.19	59.10	3.90
	B				5.7	0.45	12.00	57.00	3.60
	Mean				5.6	0.72	43.49	76.84	4.18
5-43B	A1		Downer	Coarse-loamy, siliceous, semiactive, mesic Typic	5.9	1.40	60.41	99.51	4.60
	A2			Hapludults	5.7	0.65	61.92	58.35	3.70
	B				5.6	0.30	38.36	42.73	3.10
	Mean				5.7	0.79	55.72	66.75	3.82
5-17C	A1		Russett-Christiana	Fine-loamy, mixed, semiactive, mesic Aquic	5.7	1.70	40.80	43.50	5.20
	A2			Hapludults Fine, kaolinitic, mesic Aquic	5.7	1.70	45.20	31.40	3.90
	B			Hapludults	5.7	0.40	24.80	30.10	2.70
	Mean				5.7	1.19	35.91	34.24	3.79
5-18	A1		Russett-Christiana	Fine-loamy, mixed, semiactive, mesic Aquic	6.4	3.35	225.93	80.38	14.50
	A2			Hapludults Fine, kaolinitic, mesic Aquic	6.2	2.15	173.32	36.68	12.40
	B			Hapludults	6.5	1.45	67.78	30.97	8.80
	Mean				6.4	2.20	145.36	46.59	11.53
5-39B	A1		Downer-	Coarse-loamy, siliceous, semiactive, mesic Typic	5.6	0.60	71.39	73.56	5.00
	A2		Hammonton	Hapludults, Coarse-loamy, siliceous, semiactive,	5.7	0.75	73.90	59.29	3.20
	B			mesic Aquic Hapludults	6.0	0.20	23.67	42.79	2.90
	Mean				5.8	0.53	57.07	58.00	3.61
UMBB	A1		Annapolis-	Fine-loamy, glauconitic, mesic Typic Hapludults,	6.3	3.05	41.38	151.05	11.70
	A2		Donlonton	Fine-loamy, glauconitic, mesic Aquic Hapludults	6.3	1.90	23.97	75.01	9.80
	B				5.1	1.30	13.55	79.89	11.40
	Mean				5.9	2.03	25.49	98.25	10.96
5-25C	A1		Russett-Christiana		6.2	2.35	181.00	83.15	7.00
	A2				6.1	1.15	112.60	35.50	5.90

	B		Fine-loamy, mixed, semiactive, mesic Aquic	5.9	0.85	9.50	36.98	5.40
	Mean		Hapludults Fine, kaolinitic, mesic Aquic Hapludults	6.1	1.36	89.83	49.41	6.05
5-39A	A1	Downer	Coarse-loamy, siliceous, semiactive, mesic Typic	6.7	1.15	89.60	90.70	3.90
	A2		Hapludults	6.6	0.45	109.20	51.35	2.40
	B			6.8	0.20	45.10	53.08	1.70
	Mean			6.7	0.56	82.74	62.96	2.53
5-40	A1	Downer-Hammonton	Coarse-loamy, siliceous, semiactive, mesic Typic	6.2	1.50	73.97	28.08	4.26
	A2		Hapludults, Coarse-loamy, siliceous, semiactive,	5.8	0.70	103.33	21.19	3.32
	B		mesic Aquic Hapludults	5.9	0.18	76.17	23.80	2.67
	Mean			5.9	0.77	87.18	23.89	3.37
5-43A	A1	Christiana	Fine, kaolinitic, mesic Aquic Hapludults	5.8	1.55	67.50	41.86	5.20
	A2			5.8	0.75	67.90	33.68	3.70
	B			6.1	0.30	29.30	33.08	3.00
	Mean			5.9	0.83	55.32	35.78	3.92
5-39A	A1	Downer	Coarse-loamy, siliceous, semiactive, mesic Typic	6.0	1.30	57.30	94.03	5.10
	A2		Hapludults	5.8	0.70	79.40	66.73	4.00
	B			6.0	0.40	28.30	67.82	3.40
	Mean			5.9	0.77	56.49	74.75	4.12
5-7A	A1	Russett-Christiana	Fine-loamy, mixed, semiactive, mesic Aquic	5.8	1.60	33.60	108.95	5.70
	A2		Hapludults Fine, kaolinitic, mesic Aquic	5.9	1.10	18.40	73.69	4.60
	B		Hapludults	6.1	0.85	6.10	91.01	4.70
	Mean			5.9	1.15	18.30	89.84	4.94
5-7F	A1	Russett-Christiana	Fine-loamy, mixed, semiactive, mesic Aquic	6.2	2.55	20.30	67.29	6.20
	A2		Hapludults Fine, kaolinitic, mesic Aquic	6.4	1.30	8.50	35.83	4.70
	B		Hapludults	6.3	1.05	1.60	31.19	5.40
	Mean			6.3	1.55	8.94	42.69	5.37
DS1	A1	Hambrook-Woodstown	Fine-loamy, mixed, active, mesic Aquic	6.2	0.85	99.10	105.63	3.90
	A2		Hapludults	6.1	0.70	100.10	62.86	3.50
	B			6.2	0.40	52.70	63.55	2.60
	Mean			6.2	0.64	83.22	75.05	3.24
JL1	A1	Hambrook	Fine-loamy, siliceous, semiactive, mesic Typic	6.7	1.75	93.90	172.64	6.50
	A2		Hapludults	6.6	0.85	40.30	110.75	4.30
	B			6.6	0.60	12.00	86.54	3.60
	Mean			6.6	1.01	44.51	118.81	4.67
SK1	A1	Queponco	Fine-loamy, mixed, semiactive, mesic Typic	5.6	2.00	155.70	180.53	7.00
	A2		Hapludults	6.3	1.10	200.60	129.53	4.90
	B			5.9	1.00	43.30	143.25	5.30
	Mean			5.9	1.34	154.93	147.01	5.63

Table 7. Yield, Relative Yield, Relative Sulfur Yield, % Yield Response, and % S response for 23 fields grown throughout the 2017, 2018 and 2019 growing seasons. Values represent the mean value and standard error for the field. FS= Full season soybean, DC= Double crop soybean grown after a cereal grain, BB= Black bean

Field	Year	Crop	N	Yield (kg/ha)	Relative Yield (%)	Relative S Yield (%)	Yield Response (%)	S Response %
5-18O	2017	FS	12	3913±34.07	92.36±0.8	87.33±1.09	1.59±0.45	0.84±0.3
5-39B	2017	DC	24	1892±5.82	69.34±0.21	68.49±0.33	6.47±3.24	0.01±0
5-43A	2017	DC	24	2136±62.07	78.28±2.27	64.68±4.46	25.56±4.65	23.8±6.38
5-43B	2017	FS	12	3131±62.94	73.9±1.49	55.23±3.98	11.58±2.44	0±0
5-17C	2018	DC	30	2087±66.44	62.95±2	55.65±1.49	45.24±6.5	6.61±0.87
5-18[1]	2018	FS	12	2661±59.55	56.29±1.26	40.86±7.11	10.44±3.03	3.31±0.53
5-25A	2018	DC	12	1794±87.96	54.12±2.65	49.92±1.87	29.93±6.52	6.54±2.16
5-39B	2018	BB	18	1570±130.41	52.87±4.39	45.09±3.61	76.59±10.08	3.5±0.86
5-39C	2018	FS	12	4314±44.13	91.26±0.93	62.67±11.2	4.99±0.92	6.26±2.56
UMBB	2018	BB	9	1054±114.74	35.5±3.86	32.39±3.35	25.82±13.48	4.57±0.98
5-17Ca	2019	FS	12	2226±65.53	29.48±0.87	21.01±0.8	12.56±2.21	5.24±2.31
5-17Cb	2019	FS	12	2226±65.53	29.48±0.87	21.01±0.8	12.56±2.21	5.24±2.31
5-25C	2019	FS	24	3371±90.39	44.63±1.2	37.03±0.91	2.46±0.85	6.07±1.48
5-39[1]	2019	BB	24	2026±80.9	78.1±3.12	63.64±2.3	17.28±3.57	13.94±1.31
5-39A	2019	DC	9	1410±116.7	36.08±2.99	29.94±2.61	20.23±3.89	5.22±1.04
5-40	2019	FS	12	1510±122.63	20±1.62	13.33±1.11	17.83±7.13	7.63±1.16
5-40b	2019	FS	30	1510±122.63	20±1.62	13.33±1.11	17.83±7.13	7.63±1.16
5-43A	2019	FS	18	2146±69.69	28.42±0.92	19.8±0.63	5.54±1.83	19.95±0.81
5-7A	2019	BB	12	1813±90.84	69.88±3.5	58.45±2.8	24.85±4.67	16.52±2.96
5-7F	2019	DC	12	1798±34.64	45.98±0.89	38.93±1	6.64±1.32	8.34±1.62
DS1	2019	DC	12	3102±100.5	79.36±2.57	68.47±2.47	4.36±0.89	5.76±1.3
JL1	2019	FS	12	6706±141.38	88.79±1.87	65.65±1.3	1.05±0.36	2.91±0.97
SK1	2019	DC	12	3625±65.88	92.73±1.69	86.76±1.98	2.48±0.63	6.03±1.25

Table 8. Sulfur determined by ICP for four extractants (1) 0.01 M CaCl2, (2) Mehlich 3, (3) 500 ppm Ca(H2PO4)2 in water, and (4) 500 ppm Ca(H2PO4)2 in 2N HOAC for three horizons and the weighted average for the full 0-30cm soil sample. A1=0-10 cm, A2=10-20 cm or bottom of A horizon, B = bottom of A horizon or 20 cm – 30 cm, Mean = weighted average for the 0-30 cm sample based on the depth of the sample and the bulk density of a representative "Sandy" and "Silty" field at CMREC Beltsville. FS=Full season soybean, DC=Double crop soybean planted after winter cereal grain harvest (typically wheat), BB = Black bean.

Field ID	Year	Crop	Horizon	N	CaCl2	Mehlich-3	$Ca(H_2PO_4)_2$	$Ca(H_2PO_4)_2$-HOAc
					----------mg S/kg soil ----------			
5-18O	2017	FS	A1	10	1.115±0.082	19.0±0.82	10.667±0.82	11.47±1.19
			A2	10	0.539±0.07	20.8±1.5	10.376±1.09	11.9±2.04
			B	10	0.416±0.19	38.3±5.4	34.27±5.85	26.56±3.52
			Mean		0.66±0.048	25.7±1.4	17.655±1.972	16.52±1.19
5-39B	2017	DC	A1	3	0.687±0.084	14.04±0.49	2.561±0.533	2.41±0.27
			A2	3	0.347±0.052	13.2±0.86	2.834±0.449	1.37±0.02
			B	3	0.158±0.021	9.31±0.19	2.267±0.749	1.28±0.22
			Mean		0.4±0.041	12.52±0.45	2.64±0.493	1.66±0.1
5-43A	2017	DC	A1	3	0.668±0.072	12.54±0.33	3.252±0.532	2.73±0.57
			A2	3	0.468±0.062	10.26±0.68	2.232±0.149	1.96±0.05
			B	3	0.178±0.072	9.3±0.43	3.142±0.167	1.7±0.15
			Mean		0.443±0.023	10.64±0.37	2.788±0.105	1.81±0.4
5-43B	2017	FS	A1	10	0.615±0.059	11.68±0.9	4.849±0.547	3.21±0.29
			A2	10	0.319±0.035	10.58±0.67	3.88±0.408	2.38±0.24
			B	10	0.15±0.015	7.5±0.46	3.738±0.446	2.41±0.35
			Mean		0.37±0.033	10.19±0.56	4.167±0.377	2.54±0.28
5-18	2018	FS	A1	4	54.5±6.2	66.4±7.75	36.216±5.619	8.9±5.15
			A2	4	10.1±0.85	26.09±6.25	11.184±3.013	33.61±15.17
			B	4	10.7±1.1	34.24±8.71	33.535±10.23	42.49±7.94
			Mean		22.8±1.9	40.66±5.03	27.217±5.918	24.75±9.01
5-17C	2018	DC	A1	4	19.498±4.26	32.38±6.07	15.237±4.316	n.d.
			A2	4	9.753±3.426	25.73±1.9	18.491±5.934	n.d.
			B	4	12.377±1.096	25.96±2.22	16.704±3.709	n.d.
			Mean		13.491±1.483	27.66±3.07	16.882±3.928	
5-25A	2018	DC	A1	4	6.972±0.78	26.74±5.48	7.678±1.22	5.46±2.18
			A2	4	8.46±0.666	36.75±11.54	10.019±0.861	n.d.
			B	4	8.949±1.667	32.21±12.24	9.233±2.504	79.95±31.47
			Mean		8.242±0.948	32.12±8.63	9.047±1.186	17.49±10.77
5-39B	2018	BB	A1	4	3.944±0.52	13.89±1.02	4.323±0.546	2.19±0.52
			A2	4	2.028±0.207	13.21±1.36	2.84±0.65	2.51±2.16
			B	4	18.489±16.474	25.34±16.78	18.056±15.037	37.85±34.71
			Mean		8.658±5.875	18.05±6.46	8.929±5.218	8.12±6.66
5-39C	2018	FS	A1	4	100.493±28.453	88.12±21.02	119.879±36.963	55.26±51.85
			A2	4	2.867±0.618	14.38±2.71	4.137±0.96	5.38±1.06
			B	4	2.137±0.233	9.83±0.94	3.882±0.471	22.45±9.09
			Mean		29.622±8.082	33.01±5.96	36.098±10.585	21.69±13.75
UMBB*	2018	BB	A1	4	3.642±0.251	14.2±0.82	3.484±0.606	4.98±0.45
			A2	4	1.731±0.113	12.77±1.14	2.685±0.797	4.27±0.39
			B	4	1.06±0.046	12.86±0.85	3.412±0.481	3.49±1.05
			Mean		2.064±0.094	13.31±0.48	3.117±0.31	4.15±0.43

5-17Ca	2019	FS	A1	8	1.468±0.233	12.89±1.02	n.d	9.78±2.08
			A2	8	2.216±0.916	11.6±0.51	n.d	9.79±2.21
			B	8	1.366±0.535	11.46±0.74	n.d	7.8±2.36
			Mean		1.721±0.533	11.98±0.61	n.d	8.75±1.37
5-17Cb	2019	FS	A1	8	2.076±0.38	13.44±0.94	n.d	9.9±2.96
			A2	8	0.918±0.088	13.32±0.92	n.d	14.77±3.17
			B	8	0.962±0.195	11.9±1.11	n.d	17.69±3.16
			Mean		1.27±0.184	12.85±0.69	n.d	13.59±2.36
5-25C	2019	FS	A1	4	2.648±0.288	11.77±1.19	n.d	20.23±5.78
			A2	4	2.742±0.548	11.6±0.64	n.d	15.47±5.83
			B	4	6.08±1.374	13.05±3.99	n.d	21.09±4.76
			Mean		4.009±0.709	11.97±1.86	n.d	19.25±1.93
5-39A	2019	BB	A1	6	4.484±1.606	13.4±1.31	n.d	17.75±2.84
			A2	6	2.457±0.258	10.49±0.55	n.d	19.82±1.05
			B	6	5.943±3.463	8.18±0.29	n.d	18.43±2.17
			Mean		1.493±0.146	10.42±0.43	n.d	18.73±1.56
5-39A	2019	DC	A1	4	1.592±0.431	16.23±0.78	n.d	3.16±0.36
			A2	4	1.4±0.079	12.48±1.02	n.d	2.61±0.11
			B	4	3.786±1.147	12.49±1.09	n.d	2.83±0.47
			Mean		1.727±0.121	13.6±0.33	n.d	2.8±0.13
5-40a	2019	FS	A1	8	1.304±0.187	14.57±0.71	n.d	3.26±0.3
			A2	8	0.74±0.073	14.89±0.62	n.d	2.92±0.18
			B	8	0.563±0.041	11.35±0.64	n.d	3.4±0.34
			Mean		0.843±0.061	13.84±0.59	n.d	2.97±0.22
5-40b	2019	FS	A1	8	2.11±0.287	14.03±0.95	n.d	2.44±0.15
			A2	8	0.94±0.106	12.39±0.73	n.d	2.21±0.19
			B	8	0.671±0.046	9.72±0.66	n.d	2.12±0.19
			Mean		1.192±0.05	12.14±0.56	n.d	2.26±0.16
5-43A	2019	FS	A1	4	2.151±0.15	13.93±0.58	n.d	17.62±4.2
			A2	4	1.385±0.081	11.97±0.6	n.d	11.41±5.35
			B	4	1.169±0.209	8.83±0.52	n.d	11.46±5.23
			Mean		1.511±0.139	11.59±0.62	n.d	13.38±2.27
5-7A	2019	BB	A1	6	1.337±0.09	13.56±1.28	n.d	1.93±0.11
			A2	6	1.128±0.06	10.39±0.8	n.d	2.06±0.13
			B	6	0.913±0.078	10.76±1.03	n.d	1.95±0.17
			Mean		1.117±0.037	11.44±0.6	n.d	1.98±0.11
5-7F	2019	DC	A1	4	5.587±0.863	21.15±1.03	n.d	7.01±1.07
			A2	3	3.413±0.254	15.5±0.82	n.d	8.27±1.93
			B	4	8.271±1.297	31.57±4.55	n.d	13.23±3
			Mean		5.994±0.811	23.86±2.85	n.d	10.03±1.88
DS1	2019	DC	A1	4	4.737±0.602	9.02±3.06	n.d	5.1±1.93
			A2	4	5.696±0.269	11.93±0.47	n.d	5.79±2.85
			B	4	4.784±0.6	8.84±0.83	n.d	5.43±3.02
			Mean		5.129±0.123	10.08±0.75	n.d	5.69±1.44
JL1	2019	FS	A1	4	2.824±0.096	19.67±0.38	n.d	3.93±0.3
			A2	4	1.731±0.1	15.78±1.69	n.d	2.56±0.05

			B	4	1.928±0.254	14.03±1.82	n.d	2.89±0.37
			Mean		2.127±0.069	16.25±0.38	n.d	3.09±0.15
SK1	2019	DC	A1	4	6.094±0.467	18.1±1.29	n.d	25.17±1.44
			A2	4	7.694±0.701	16.88±0.79	n.d	21.86±2.58
			B	4	7.146±0.77	18.72±1.53	n.d	23.47±1
			Mean		7.074±0.318	17.49±0.88	n.d	23.08±1.2

Table 9. Summary of the abilities of four soil extractions to predict response to S application on 23 fields sampled during 2017-2019. Critical values, total number of fields and number categorized in each Cate-Nelson quadrat with respect to critical value, percent of sites correctly identified and percent of responsive sites identified by four soil test protocols tested on 0-10 cm A1 horizon, B horizon and entire 0-30 cm soil samples. The four soil extractions used were (1) 0.01 M CaCl2, (2) 500 ppm $Ca(H_2PO_4)_2$ in water, (3) 500 ppm $Ca(H_2PO_4)_2$ in 2N HOAC, and (4) Mehlich 3. The critical x value was determined by Cate-Nelson analysis of data from 122 individual blocks within the 23 fields. Fields that had a significant response to applied S in terms of yield, seed S content, or both yield and seed S content were designated as responsive fields. Fields were grouped into four categories (I) Non- responsive and above critical level (II) Non-responsive and below critical level, (III) Responsive and below critical level, and (IV) Responsive and above critical level. The number of fields within each category was used to determine the % of sites correctly identified by a soil test (100(I fields + +III Fields)/total fields) and the % of responsive sites correctly identified (100*III Fields/ (III Fields + IV Fields). A1= 0-10 cm, B = Bottom of A or 20 cm to 30 cm, mean=Weighted average of 0-30 cm based on the average bulk density of similar soil types. Bold font highlights 70 or greater correct identification of Cate-Nelson quadrate or of responsiveness.*

	Critical Value	N	No Response Above Critical Level	No Response Below Critical level	Response below Critical Level	Response Above Critical Level	Correctly Identified by Soil test	Responsive Sites Identified
	mg S/ kg soil	Number of fields					%	%
Relative Yield								
CaCl2								
A1	0.7	23	14	0	3	6	**73.9**	33.3
B	1.5	23	7	7	5	4	52.2	55.6
Mean	5.5	23	4	10	6	3	43.5	66.7
$Ca(H_2PO_4)_2$								
A1	5.3	10	3	0	5	2	**80.0**	**71.4**
B	3.2	10	3	0	2	5	50.0	28.6
Mean	4.4	10	3	0	4	3	**70.0**	57.1
$Ca(H_2PO_4)_2$ -HOAc								
A1	8.3	22	8	6	7	1	68.2	**87.5**
B	11.1	22	8	6	5	3	59.1	62.5
Mean	11.3	22	7	7	6	2	59.1	**75.0**
Mehlich-3								
A1	18.1	23	5	9	7	2	52.2	77.8
B	17.3	23	4	10	6	3	43.5	66.7
Mean	16.2	23	6	8	6	3	52.2	66.7
Relative S Yield								
$CaCl_2$								
A1	0.8	23	14	0	3	6	**73.9**	33.3
B	0.4	23	14	0	3	6	**73.9**	33.3
Mean	5.5	23	4	10	6	3	43.5	66.7
$Ca(H_2PO_4)_2$								
A1	5.3	10	3	0	5	2	**80.0**	**71.4**
B	9.7	10	2	1	5	2	**70.0**	**71.4**
Mean	4.4	10	3	0	4	3	**70.0**	57.1
$Ca(H_2PO_4)_2$ - HOAc								
A1	8.3	22	8	6	7	1	68.2	**87.5**
B	7.1	22	9	5	5	3	63.6	62.5

Mean	9.9	22	8	6	6	2	63.6	**75.0**
Mehlich-3								
A1	14.6	23	7	7	7	2	60.9	**77.8**
B	17.3	23	4	10	6	3	43.5	66.7
Mean	16.2	23	6	8	6	3	52.2	66.7
Yield Response (%)								
$CaCl_2$								
A1	4.2	23	5	9	6	3	47.8	66.7
B	6.2	23	3	11	6	3	39.1	66.7
Mean	8.4	23	2	12	7	2	39.1	**77.8**
$Ca(H_2PO_4)_2$								
A1	5.3	10	3	0	5	2	**80.0**	**71.4**
B	4.5	10	2	1	4	3	60.0	57.1
Mean	3.2	10	3	0	3	4	60.0	42.9
$Ca(H_2PO_4)_2$ -HOAc								
A1	2.8	22	13	1	4	4	**77.3**	50.0
B	2.7	22	13	1	4	4	**77.3**	50.0
Mean	3.1	22	10	4	4	4	63.6	50.0
Mehlich-3								
A1	13.1	23	11	3	2	7	56.5	22.2
B	8.6	23	14	0	2	7	69.6	22.2
Mean	14.0	23	6	8	6	3	52.2	66.7
S Response (%)								
$CaCl_2$								
A1	0.8	23	14	0	3	6	**73.9**	33.3
B	0.4	23	14	0	3	6	**73.9**	33.3
Mean	1.0	23	12	2	3	6	65.2	33.3
$Ca(H_2PO_4)_2$								
A1	2.4	10	3	0	0	7	30.0	0.0
B	3.3	10	3	0	2	5	50.0	28.6
Mean	8.0	10	3	0	4	3	**70.0**	57.1
$Ca(H_2PO_4)_2$ - HOAc								
A1	3.8	22	11	3	5	3	**72.7**	62.5
B	2.5	22	13	1	4	4	**77.3**	50.0
Mean	2.4	22	13	1	3	5	**72.7**	37.5
Mehlich-3								
A1	13.0	23	11	3	2	7	56.5	22.2
B	10.3	23	10	4	4	5	60.9	44.4
Mean	10.3	23	13	1	1	8	60.9	11.1

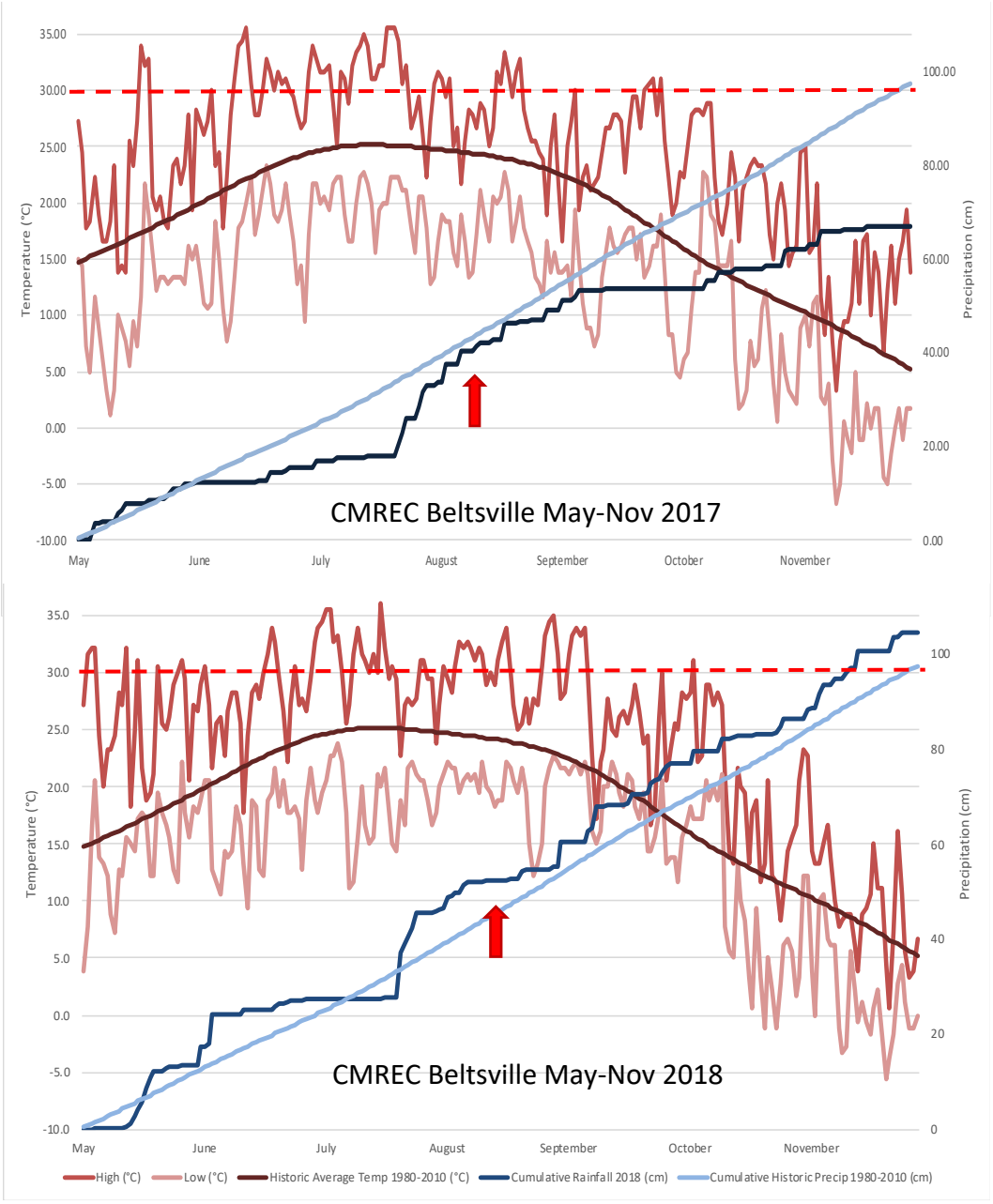

Figure 1 Daily high temperature (°C), daily low temperature (°C), and daily precipitation (cm) at Beltsville for 2017 (upper) and 2018 (lower) and the 1980-2010 average temperature(°C) and average precipitation (cm) at Baltimore (BWI NOAA weather station). The red arrow indicates when Epsom salt was sprayed. The dashed red horizontal line indicates high temperature stress above 30°C.

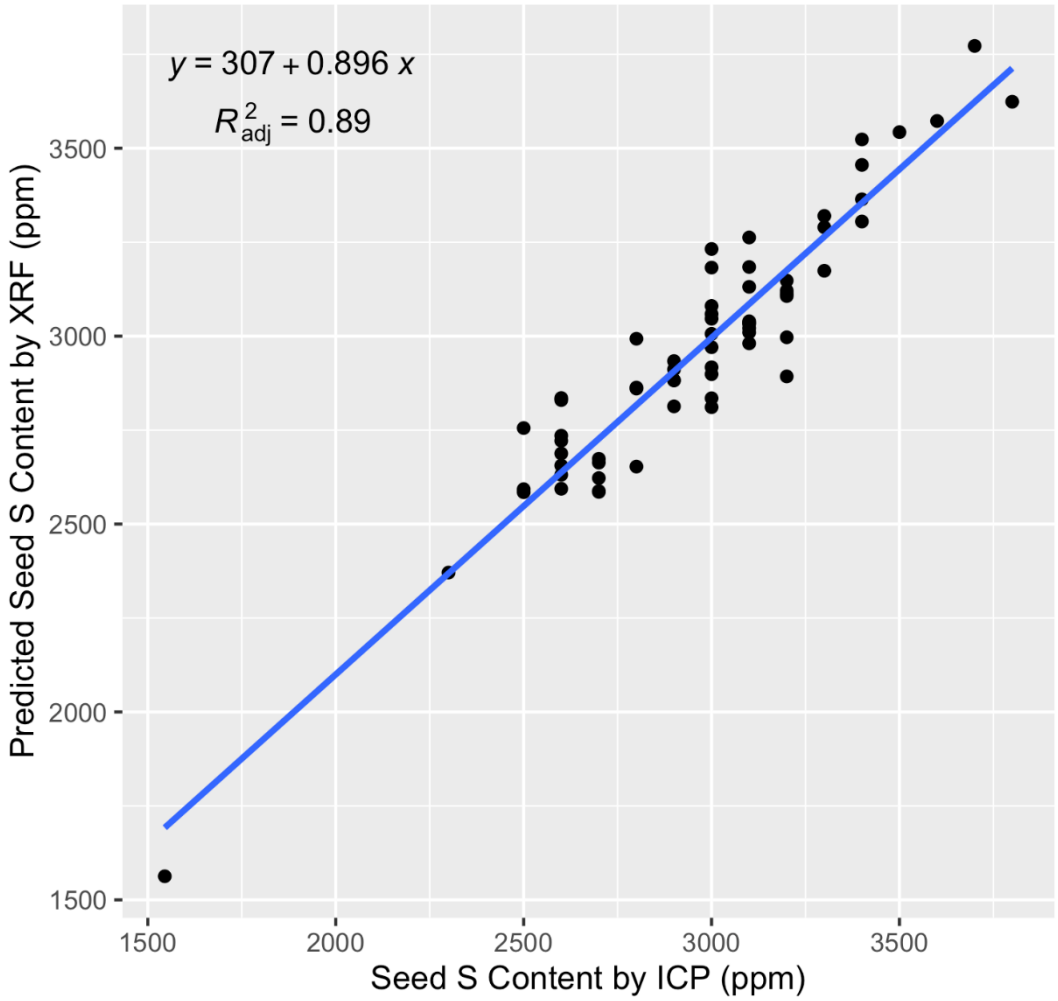

Figure 2 *Calibration curve developed using spectral normalized net photon values from XRF spectra and S concentration values obtained by independent ICP analysis for a set of 88 plant tissue samples. Calibration was smoothed using the Lucas Tooth mode (Lucas-Tooth and Pyne, 1963)) and CloudCal software (from xrf.guru.com)*

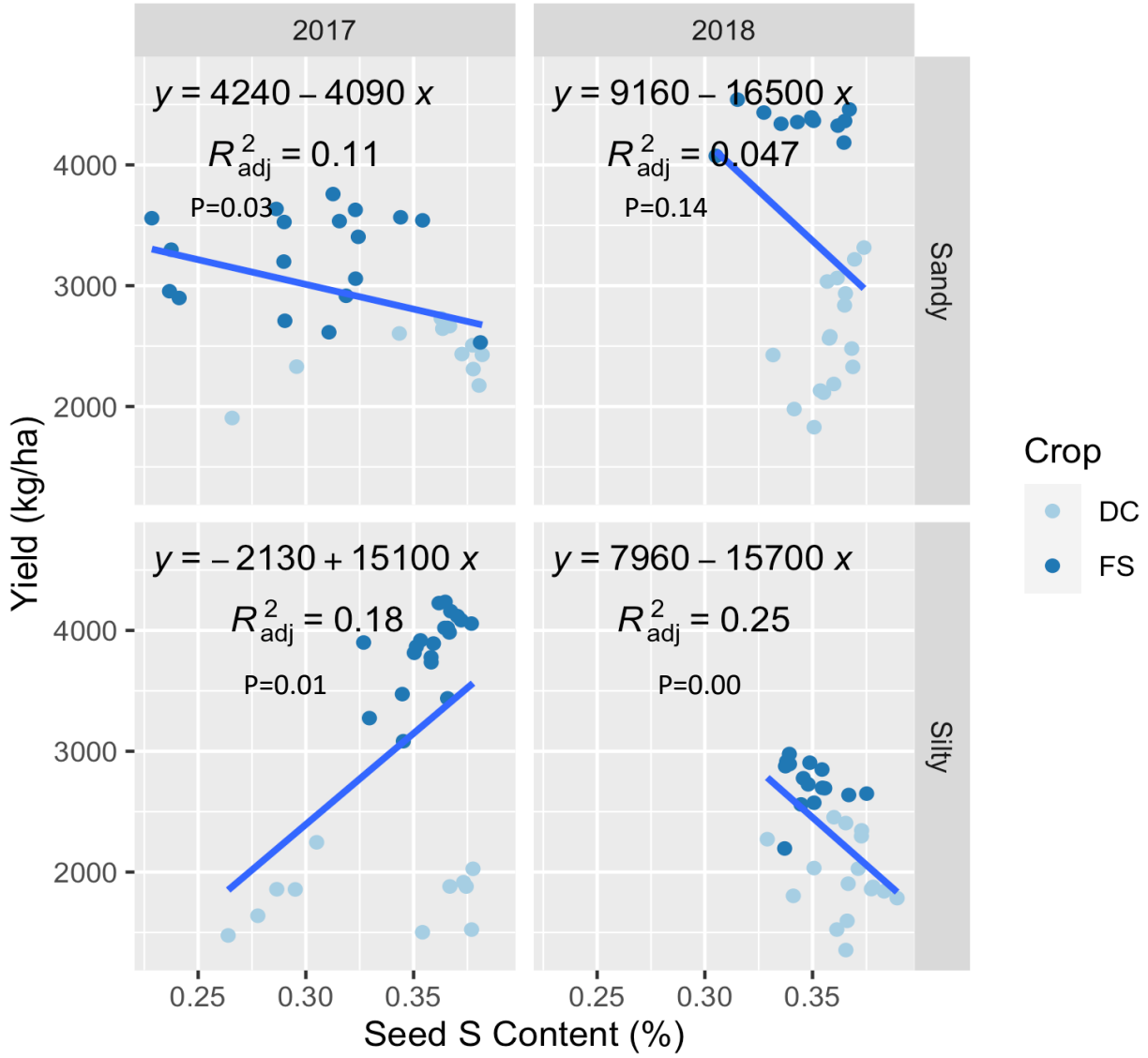

Figure 3 Linear relationship between Seed S Content and soybean yield (kg/ha) for full season (FS) and double crop (DC) soybeans grown at eight sites near Beltsville, MD with relatively coarse or fine textured soils in 2017 and 2018.

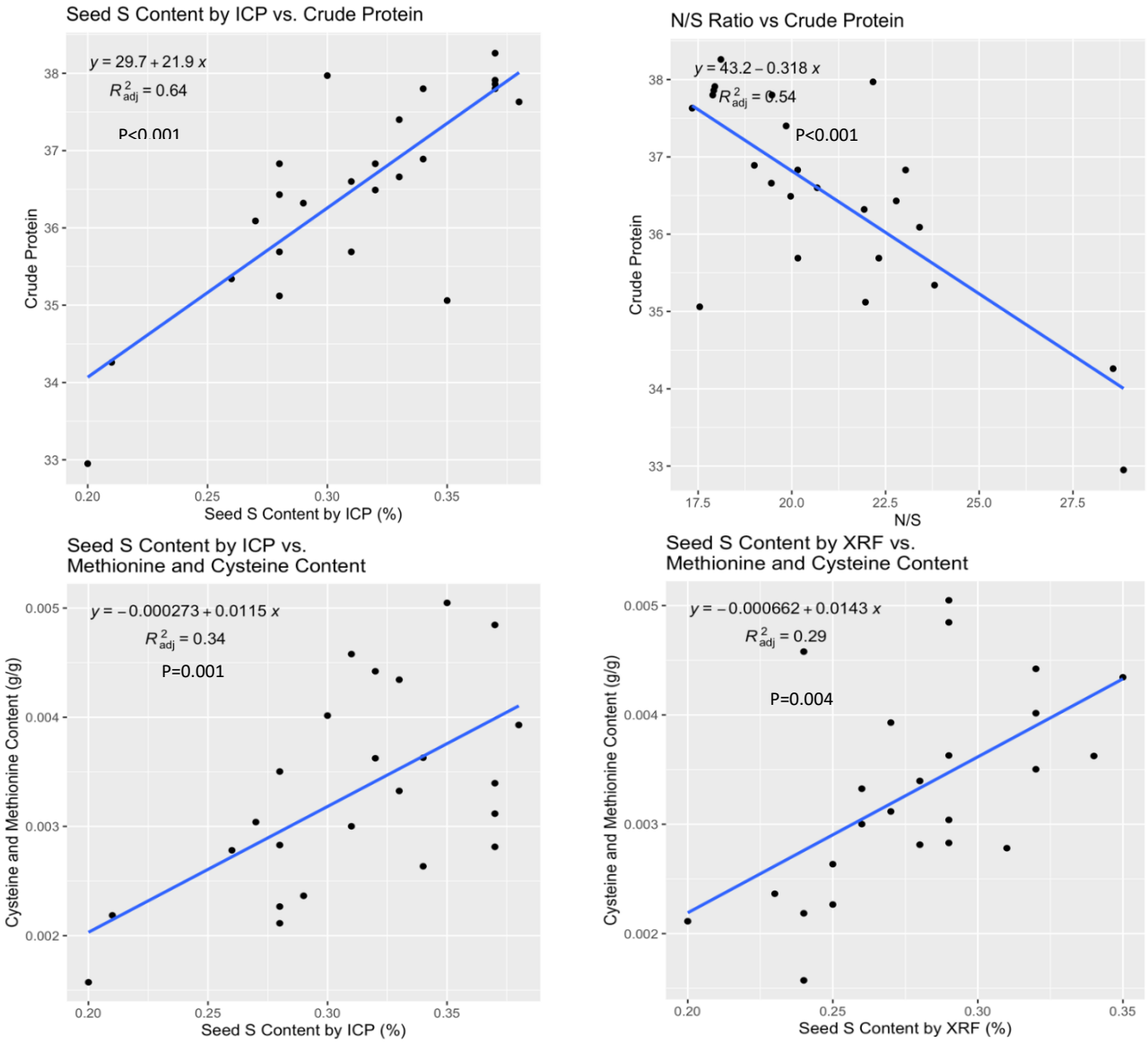

*Figure 4 Relationship between seed S content or seed N/S ratio and crude protein and methione+cysteine in the seed. Crude Protein calculated as total N % (by high temperature combustion) * 5.71 (top left). Data are for a total of 32 samples, 12 from a DC and a FS sites in 2017, 4 each from a DC and a FS site in 2018. All samples were analyzed for amino acid content (expressed as a % of total protein) by HPLC and for S in the seed by ICP. that the linear regression analyses show that seed S content is positively related with both Crude Protein and cysteine + methionine content of the seed. The relationship between N/S ratio and crude protein shows a negative relationship indicating as S deficiency increased, crude protein decreased.*

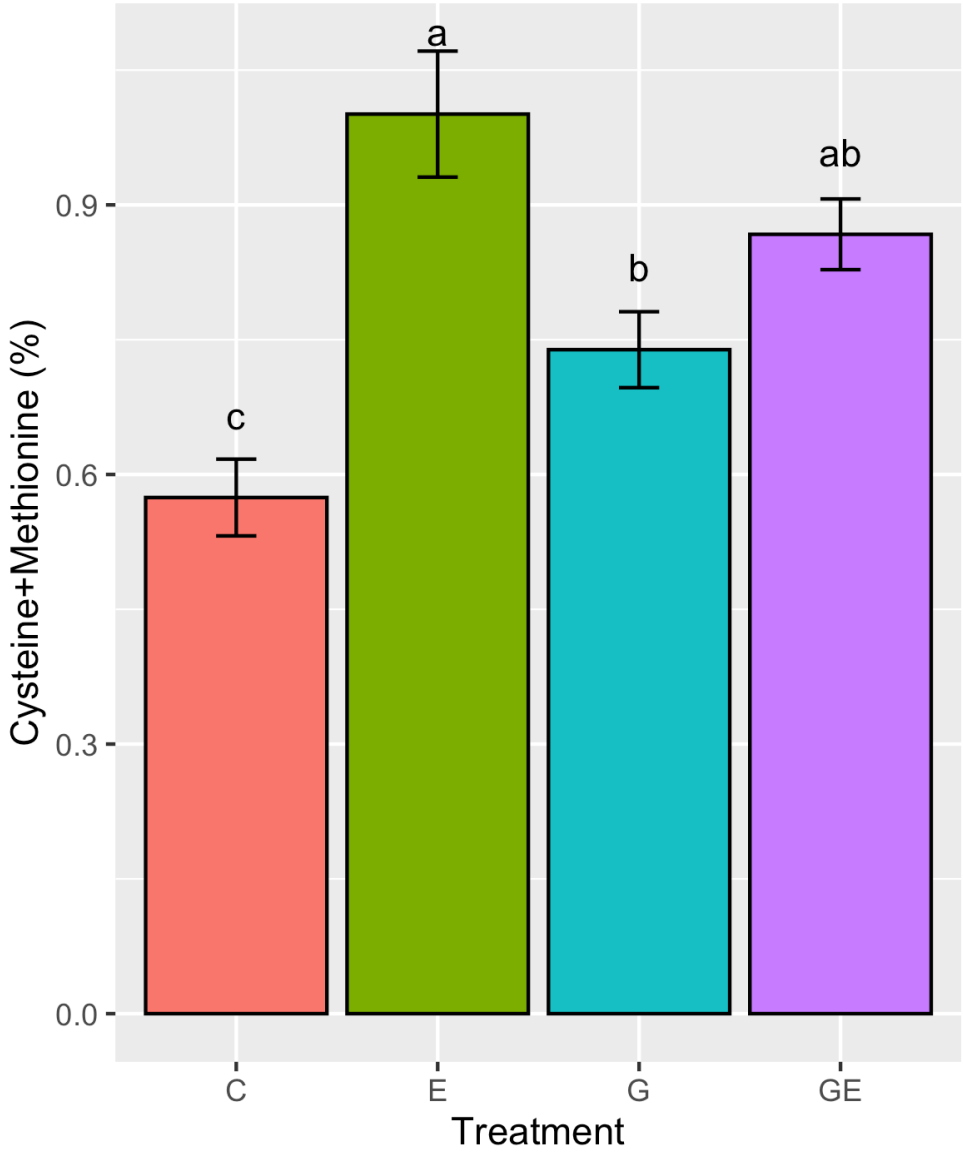

Figure 5 The effect of S treatment on the Cysteine and Methionine concentration as a percent of extracted protein in soybean seeds. Means of 3 replications from each of two sites at CMREC Beltsville in 2017 (24 samples in all) one site with full season and one with double crop soybeans . G0E0=No S control; G0E1 = Epsom applied at a rate of 86 kg/ha as a foliar spray at first flower; G1E0=Gypsum applied at a rate of 560 kg/ha at the time of planting; and; G1E1 = combination of gypsum and Epsom. Means with the same lower-case letter are not significantly different at $p<0.05$ level by post hoc Tukey HSD test.

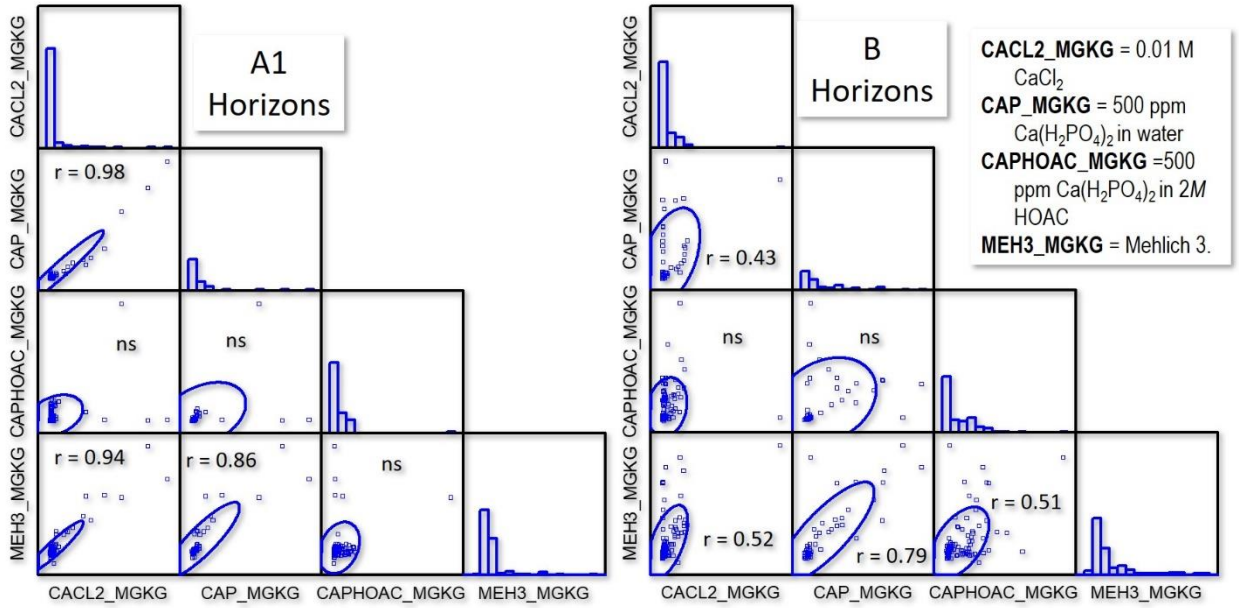

Figure 6. Correlation matrices for soil S extracted by four soil test protocols in the A1 (0-10cm) soil samples and in the B horizon (bottom of A to 30 cm) soil samples from 2017, 2018 and 2019 study fields. CaCl2, a dilute neutral salt correlated quite well with the much stronger Mehlich-3 extraction in the A1 horizons, suggesting that both extracted mainly water soluble sulfate (such as gypsum,) sulfate weakly held by organic matter or sulfate dissolved in the soil solution. The correlation was much weaker for the B horizon samples because much of the S in the subsoil is tightly sorbed sulfate ions on clay and metal oxide coatings from which the strong exchangers in Mehlich-3, but not the Cl^- ion could remove them. Extractable S by Mehlich-3 and $Ca(H_2PO_4)_2$ in water were moderately well correlated for both the A1 and B horizon soils. Results from $Ca(H_2PO_4)_2)_2$ in 2N HOAC were not correlated with results from the other three extraction protocols.

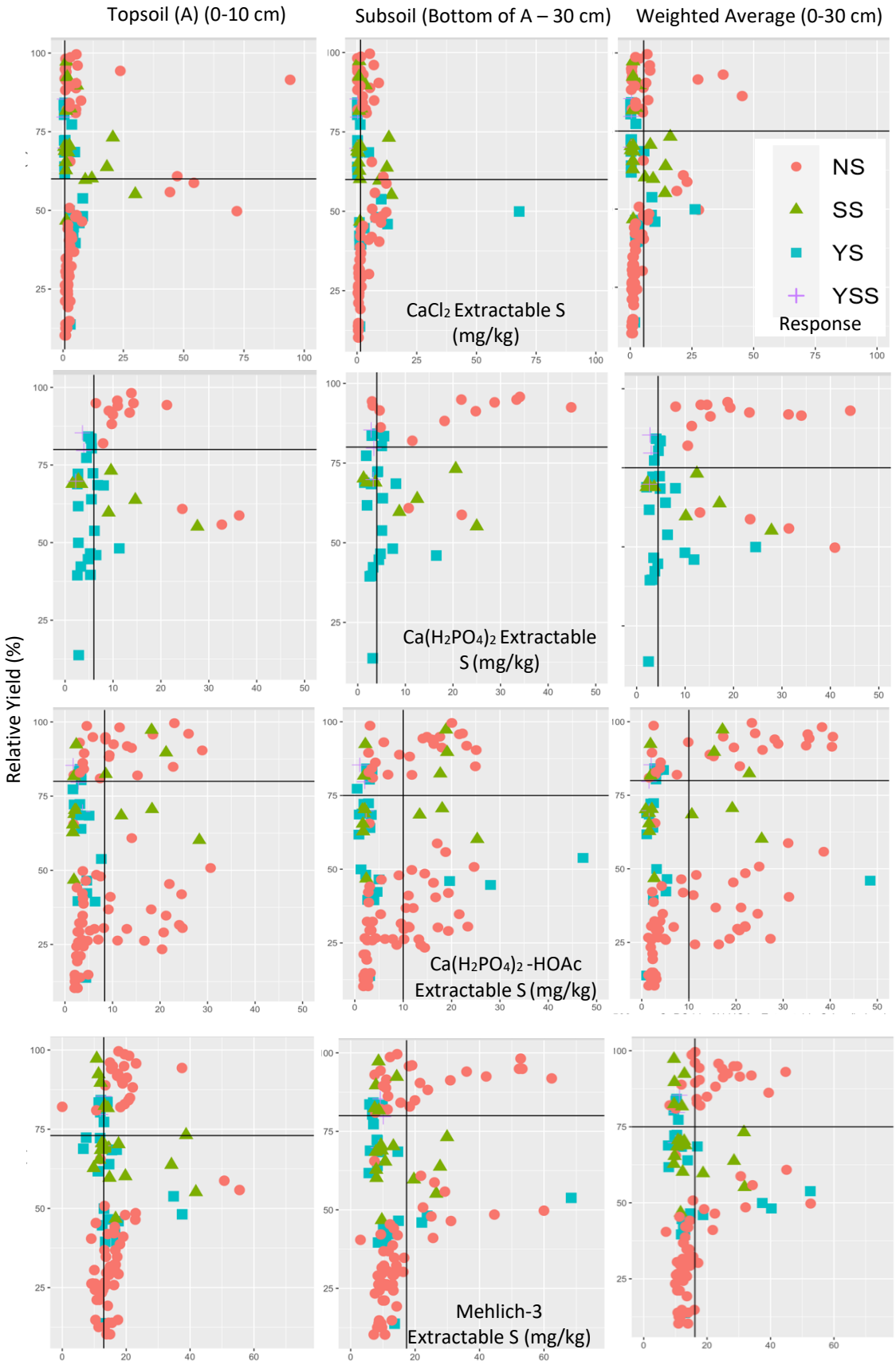

Figure 7 Cate Nelson graphs identifying critical level for extractable S and Relative Yield (%) Calculated as the yield of the no S treatment divided by the highest yielding plot for that crop x year for four different soil extractants (1) 0.01 M CaCl2, (2) Mehlich 3, (3) 500 ppm $Ca(H_2PO_4)_2$ in water, and (4) 500 ppm $Ca(H_2PO_4)_2$ in 2N HOAC for 0-10 cm and subsoil (bottom of A or 20 cm-30cm) horizons and the weighted average for the full 0-30cm soil sample. NS=Fields that did not have a significant yield or S response, SS=Fields with a significant S response, YS=Fields with significant yield response, and YSS = fields with significant yield and S response based on ANOVA.

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