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Summary

Over time, plant breeding efforts for improving soybean [*Glycine max* (L.) Merr.] yield were prioritized and effects on seed nutritional quality were overlooked, decreasing protein concentration. This research aims to explore the effect of nitrogen (N) and sulfur (S) fertilization on soybean seed yield, seed protein and sulfur amino acids concentration. In 2018, ten field trials were conducted across the main US soybean producing region. The treatments were fertilization at 1) planting (NSP); during 2) vegetative growth (NSV); and 3) reproductive growth (NSR) and 4) unfertilized (Control). Nitrogen fertilization was applied at the rate of 40 lb/a utilizing urea ammonium nitrate (UAN), and S at 9 lb/a via ammonium sulfate (AMS). A meta-analysis was performed to consider small variations among experimental designs. A summary of the effect sizes did not show effects for seed yield. However, fertilization at planting (NSP) increased seed protein by 1% more than the control across all sites. Overall, sulfur amino acid concentration increased by 1.5% relative to the control, but the most consistent benefit came from fertilization during the reproductive growth (NSR), increasing sulfur amino acids by 1.9%. Although N and S fertilization did not affect seed yields, applying N and S in different stages of the crop growth can increase protein concentration and improve protein composition, providing the opportunity to open new US soybean markets.

Introduction

Soybean [*Glycine max* (L.) Merr.] demands a great amount of nitrogen (N) during the seed filling period compared to other legumes and cereals. The plant N assimilation from the soil supply and biological nitrogen fixation (BNF) frequently does not match the requirements for a high yielding crop. This gap between assimilation and requirement forces the plant to prematurely remobilize N from other organs and consequently establish a “self-destruction” status, hampering the synthesis of highly energetic compounds in the seed, such as proteins and amino acids (Sinclair and de Wit, 1975). Over the last decades, plant breeding efforts overlooked changes in seed quality (defined here as nutritional composition) and concentrated on increasing soybean yields. The latter was achieved, increasing production and profitability, but the former was diminished, creating a concern for the global industry and producers. This study aims to

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explore the effect of N and S fertilization on seed yield, seed protein, and concentration of sulfur amino acids—such as cysteine and methionine. We hypothesized that N and S fertilization as a management practice can help offset the reduction in protein levels and protein quality (amino acids concentration), especially when adopted during the seed filling period.

Procedures

Sites and Measurements

This research project was conducted across seven states of the main soybean producing region in the United States (KS, MN, AR, IL, IA, SD, and IN), investigating management practices with potential effects on soybean nutritional quality. However, experimental designs and treatments are slightly different across locations, requiring a preliminary selection of studies to perform the analysis in this report. Selection of trials, from the 2018 season, was done considering the presence of the following treatments in at least one variety × planting date combination (defined as the study): 1) fertilization at planting (NSP); during 2) vegetative growth (NSV); 3) reproductive growth (NSR); and 4) an unfertilized (Control). A description of the 10 selected studies and their soil properties before planting is presented in Table 1. Regarding fertilizers and nutrient rates, ammonium sulfate (AMS) was applied to provide 9 lb/a of S-SO₄, and urea ammonium nitrate (UAN) to provide 40 lb/a of N. At harvest, seed yield was recorded and seed samples were analyzed in terms of protein and sulfur amino acids concentration with the near infrared (NIR) method (Pazdernik et al., 1997).

Statistical Analysis

A meta-analysis was adopted considering the different experimental procedures and designs across locations. The response ratio effect sizes, in logarithmic scale, of each treatment relative to the control, were estimated according to Borenstein et al. (2009). First, the effect sizes were calculated per study and associated to the within-study variability. The between-study variability was also estimated in order to assign specific weights to each study (random effect model). Finally, the summary of the effect sizes was calculated for each of the treatments and variables. The I² parameter, percentage of between-study variance over the total variance, was calculated for each model and could be associated with specific conditions of each study (e.g. weather and soil), beside the random error. The R software (R Core Team, 2019) was used to perform calculations, analysis, and figures.

Results

Responses on Seed Yield and Quality

The summary of effect sizes shows no yield response from N and S fertilization applied at any time of the soybean season (Figure 1). Seed protein concentration across sites was increased by 1% more than the control only by the fertilization at planting (Figure 2). The sulfur amino acids were always enhanced after N and S application, increasing 1.7%, 1.5% and 1.9% when applied at NSP, NSV, and NSR, respectively—all relative to the control. In addition, for sulfur amino acids, the summary of effect sizes for the late fertilization (NSR) was the most precisely estimated, with smaller 95% confidence intervals (CI). Overall, the magnitude of changes in protein and amino acids was rela-

tively small, around 1–2% over the control, which represents less than 1% of changes on the basis of concentration by dry weight.

Final Considerations and Next Steps

Much of the between-study variance is not explained by the current meta-analysis model. A future step for fine-tuning this model could be to consider the input of weather and soil variables to improve the estimation of the summary effect size. In addition, more studies from the literature or from different field locations should be explored, minimizing the weight of specific sites on the final results, and even allowing statistical comparison of fertilization timings during the season.

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Table 1. Description of the ten studies relative to planting date, maturity group (MG), and soil properties (pH, clay, and soil organic matter)

State	Study	Planting date (2018)	MG [†]	pH	Clay (%)	SOM [‡] (%)
IN	IN1	05-11	3.4	6.4	25	3.4
	IN2	06-05	3.4	6.4	25	3.4
	IN3	05-24	2.4	6.2	20	3.7
	IN4	05-24	3.4	6.2	20	3.7
SD	SD1	05-15	1.1	6.1	30	4.7
	SD2	05-15	2.4	6.1	30	4.7
	SD3	06-04	1.1	6.1	30	4.7
	SD4	06-04	2.4	6.1	30	4.7
	SD5	05-17	1.1	6.6	35	3.4
	SD6	05-17	2.4	6.6	35	3.4

[†] Relative maturity group. [‡] Soil organic matter (loss-on-ignition).

Studies were located in Indiana (IN) and South Dakota (SD), with study codes representing single combinations of planting dates and MG.

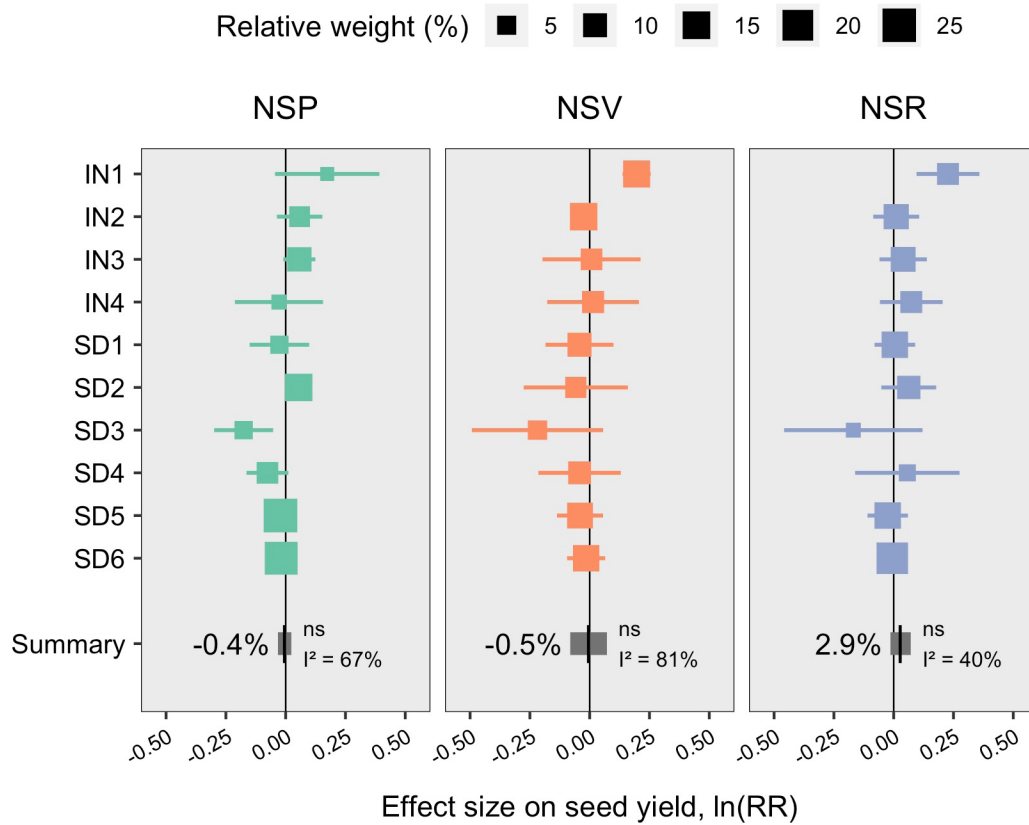


Figure 1. Treatment effect sizes for soybean seed yield across studies. Squares are located on the log of the response ratios (RR), or effect sizes. Size of the squares represent the weight of the study on the final summary, and horizontal bars represent the 95% confidence intervals (CI). The width of the gray bar on the effects summary determines whether the treatment had a positive, negative, or no effect (ns) on seed yield (95% CI). Percentages (left of the summary) indicate the final RR, and the I^2 represents the between-study variability. Nitrogen (N) and sulfur (S) application at planting is presented in the left panel (NSP), during the vegetative growth in the center (NSV), and during the reproductive growth in the right panel (NSR).

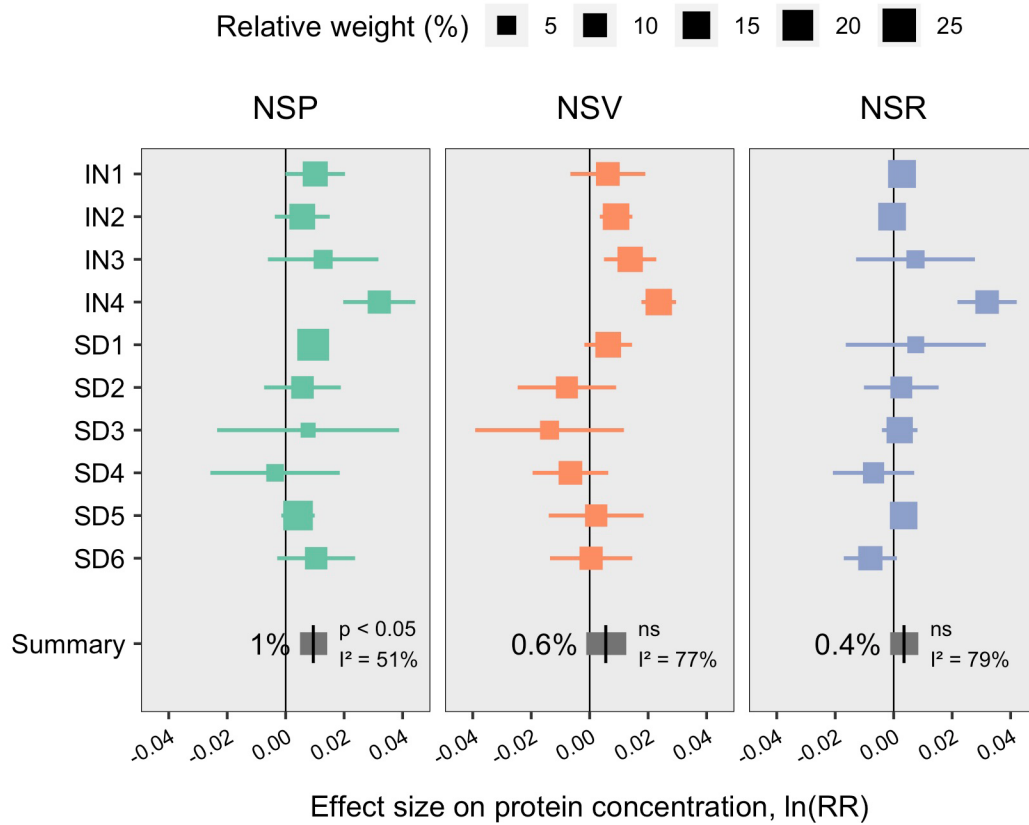


Figure 2. Treatment effect sizes for seed protein concentration across studies. Squares are located on the log of the response ratios (RR), or effect sizes. Size of the squares represent the weight of the study on the final summary, and horizontal bars represent the 95% confidence intervals (CI). The width of the gray bar on the effects summary determines whether the treatment had a positive, negative, or no effect (ns) on protein (95% CI). Percentages (left of the summary) indicate the RR, and the I^2 represents the between-study variability. Nitrogen (N) and sulfur (S) application at planting is shown in the left panel (NSP), during the vegetative growth in the center (NSV), and reproductive growth in the right (NSR).

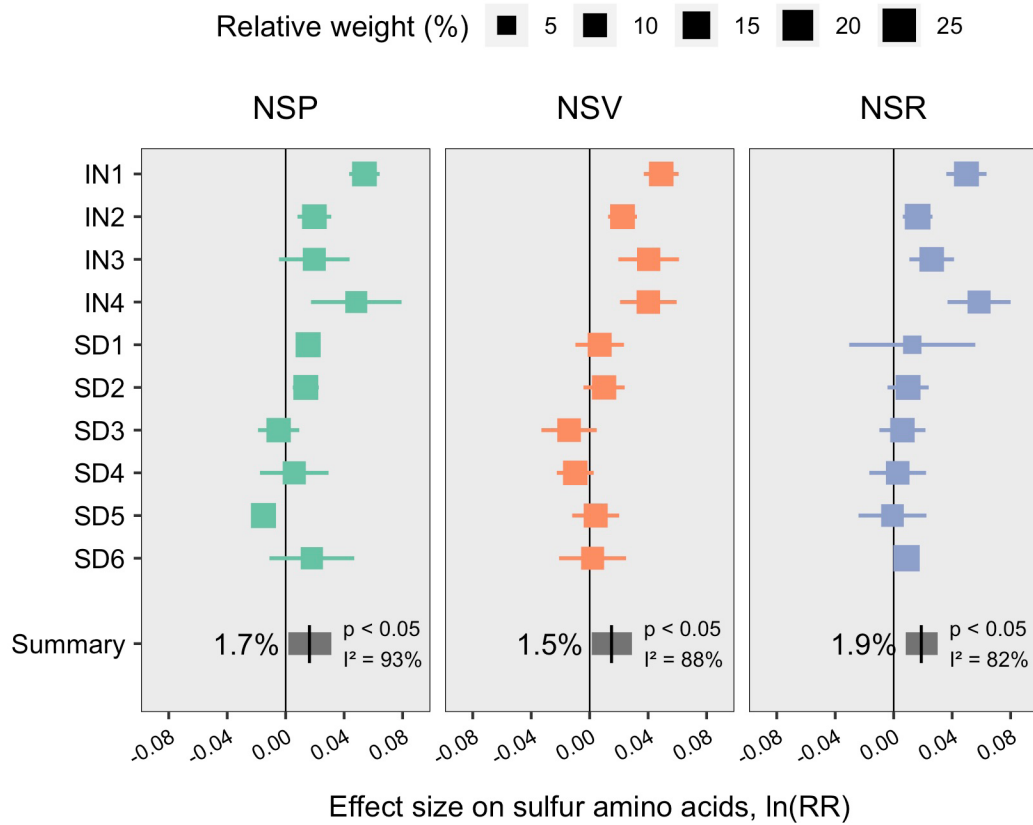


Figure 3. Treatment effect sizes for sulfur amino acids concentration across studies. Squares are located on the log of the response ratios (RR), or effect sizes. Size of the squares represent the weight of the study on the final summary, and horizontal bars represent the 95% confidence intervals (CI). The width of the gray bar on the effects summary determines whether the treatment had a positive, negative, or no effect (ns) on sulfur amino acids (95% CI). Percentages (left of the summary) indicate the summary RR, and the I^2 represents the between-study variability. Nitrogen (N) and sulfur (S) application at planting is presented in the left panel (NSP), during the vegetative growth in the center (NSV), and N and S applied during the reproductive growth is presented in the right panel (NSR).