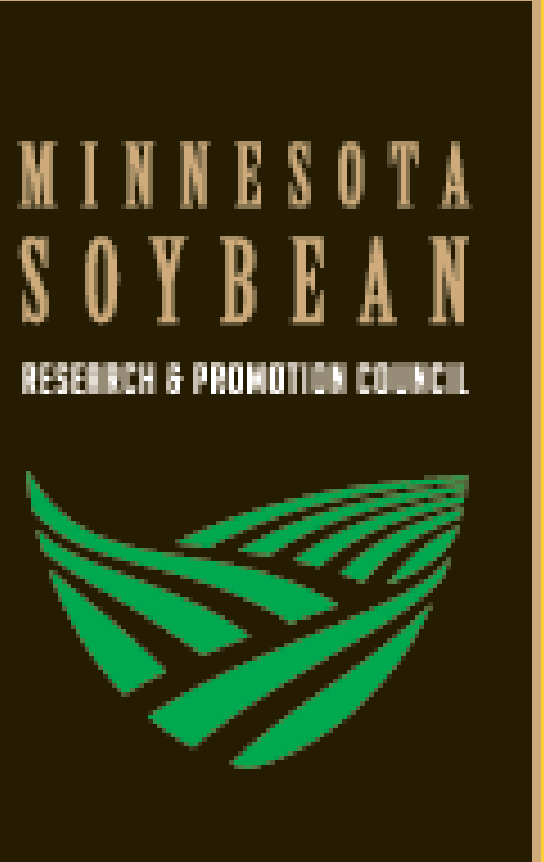# Changing the Source-Sink Ratio in Soybean Affects the Amount and Quality of Seed Protein

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Abstract

The soybean [*Glycine max* (L.) Merr.] crop of the Northern and Western Corn Belt has historically had lower total protein than other United States growing regions. However, it is not well documented if changes in total protein coincide with changes in protein quality, or the relative abundance of certain amino acids. The importance of amino acid balance in protein is documented by discrepancies between crude protein value and the actual feed value of soybeans. Additionally, this change in protein quality may cause error when using proximate measures such as combustion nitrogen to estimate total amino acids. Field studies were conducted in 2013 and 2014 in southern Minnesota to produce seed with variable protein quantity to investigate these questions. Treatments included 40% depodding, 80% depodding, 80% shade, 66% defoliation and removal of non-harvest plants. Plots were harvested at maturity and protein analysis was conduction by two methods. Amino acids were measured by cation-exchange chromatography coupled with post-column ninhydrin derivatization. Crude protein was estimated by multiplying combustion nitrogen by the conventional 6.25 nitrogen-to-protein conversion factor. Comparison of the two protein measures revealed overestimation of the amino acids by crude protein (y = 0.88*x + 3.93). A correction was made by calculating the actual percent nitrogen in the total amino acids from chromatography and using this unique nitrogen-to-protein conversion factor for each sample. This improved the approximation of crude protein to total amino acids (y = 0.92*x + 2.47). Changing percent nitrogen in the soybean protein accounts for this correction and is a result of changes in relative abundance of amino acids including nitrogen-rich amino acids like arginine and lysine.

Materials and Methods

Objectives:
1) Create a wide range of protein quantities within environments and genotypes.
2) Examine quality relationships within the protein fraction.

Experimental Design: RCBD, Four repetitions
Main Plots: Seven source-sink treatments including control
Split Plots: Varieties P92Y32, P92Y22
Location: St. Paul, MN (2013 and 2014) and Waseca, MN (2013)

40% Pod Removal

Left: Example of 80% shade at R5, a source limiting treatment.
Right: Plant diagram explains moderate (40%) pod removal at R5, a sink limiting treatment (Proulx and Naeve, 2009).

Treatment	Crude Protein (dm basis)	
80% shade starting at R5	43.59	A
severe depodding at R5	42.37	A B
moderate depodding at R5	41.74	B
remove all but 1m center harvest row at R5	40.14	C
no treatment	39.24	C
66% defoliation at R6	37.61	D
66% defoliation at R5	37.55	D

Table 1. Treatments induced a 15% range in protein values as dry matter percentage of seed. Font colors indicate graph legend.

Results: Protein Nitrogen

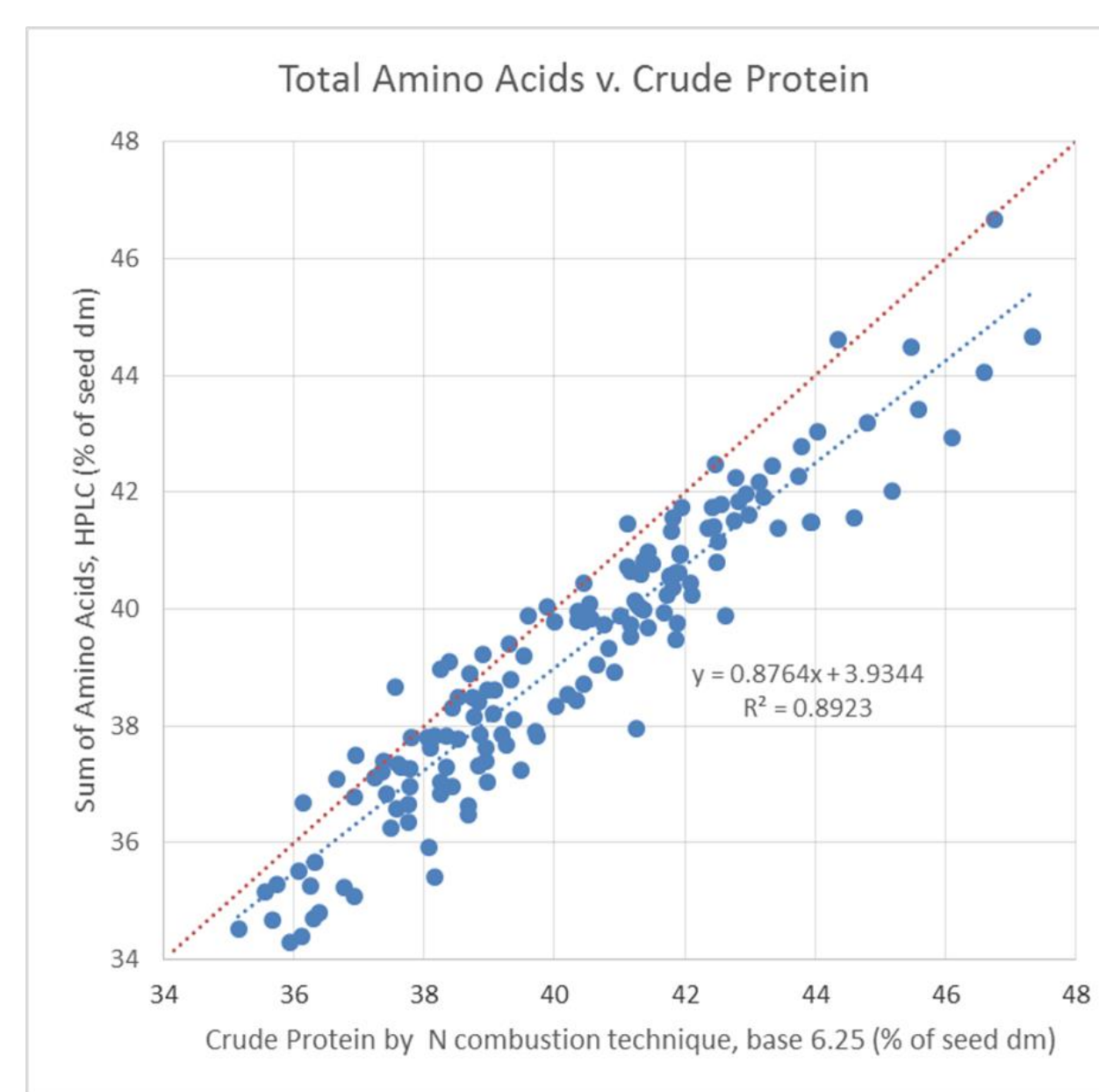| Amino Acid	%N by weight
Tyrosine	8.59
Phenylalanine	9.52
Methionine	10.69
Glutamic Acid	10.85
Aspartic Acid	12.17
Isoleucine	12.39
Leucine	12.39
Hydroxyproline	12.39
Taurine	13.08
Cysteine	13.59
Threonine	13.86
Valine	14.14
Proline	14.43
Tryptophan	15.05
Serine	16.09
Hydroxylysine	19.44
Alanine	19.72
Lysine	21.88
Glycine	24.56
Ornithine	24.56
Histidine	30.66
Arginine	35.90

Figure 1. Relationship between crude protein and total amino acids. Crude protein value overestimates total amino acids and the slope of 0.88 indicates that this is greater at higher protein levels.

Table 2. The percent nitrogen by weight of amino acids as measured by HPLC. Percent nitrogen in the protein $= \sum (n * \%N)...(n^i * \%N^i)$, where n is each AA fractional abundance
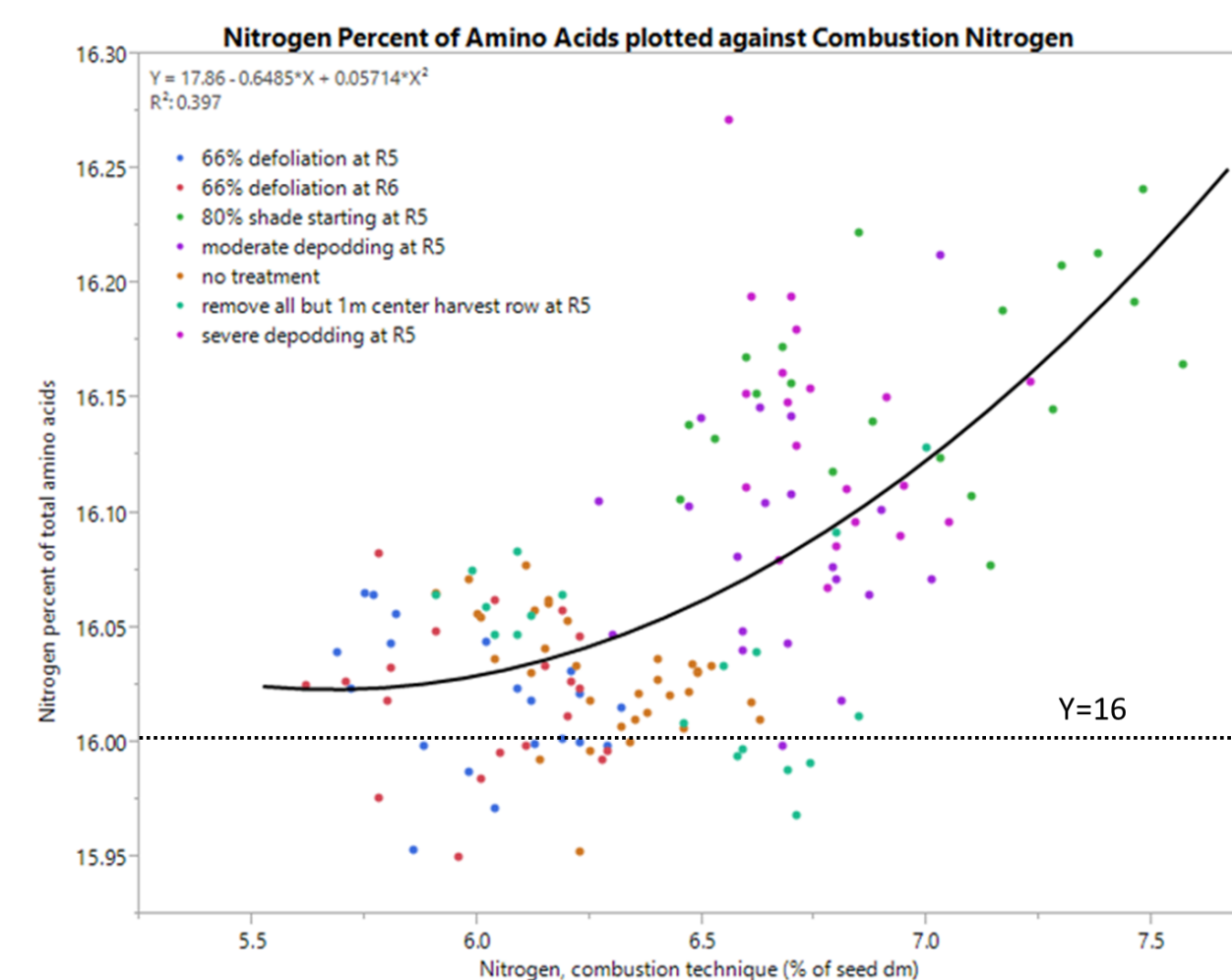

Figure 3. Percent nitrogen calculated from amino acid composition increases versus combustion nitrogen.
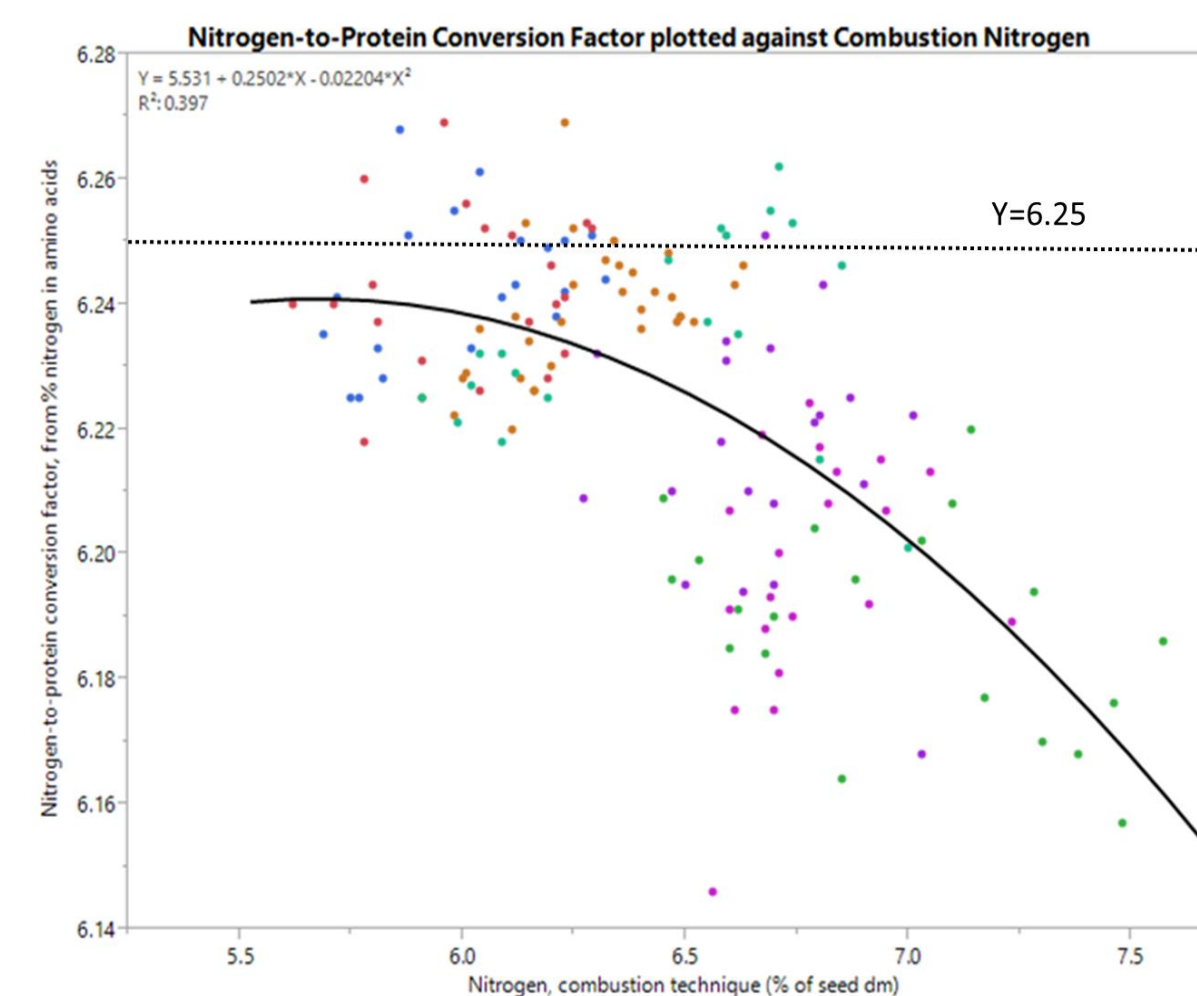
Figure 4. Nitrogen-to-protein ratio decreases versus combustion nitrogen.
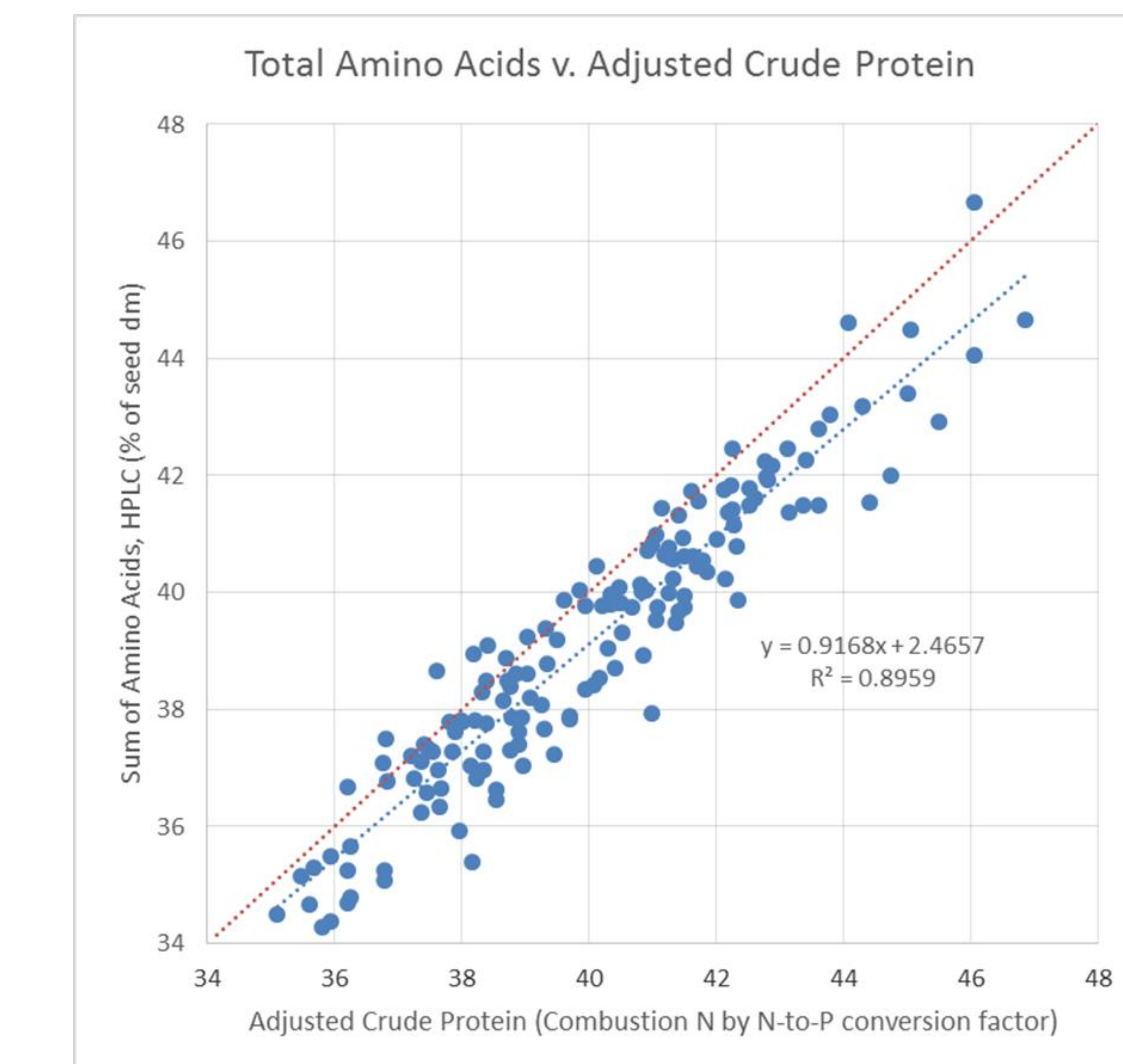

Figure 5. Sum of amino acids plotted against adjusted crude protein (combustion nitrogen multiplied by unique nitrogen-to-protein ratio in Figure 4). Improved the overestimation of total amino acids slightly (m = .92 > m = .88).
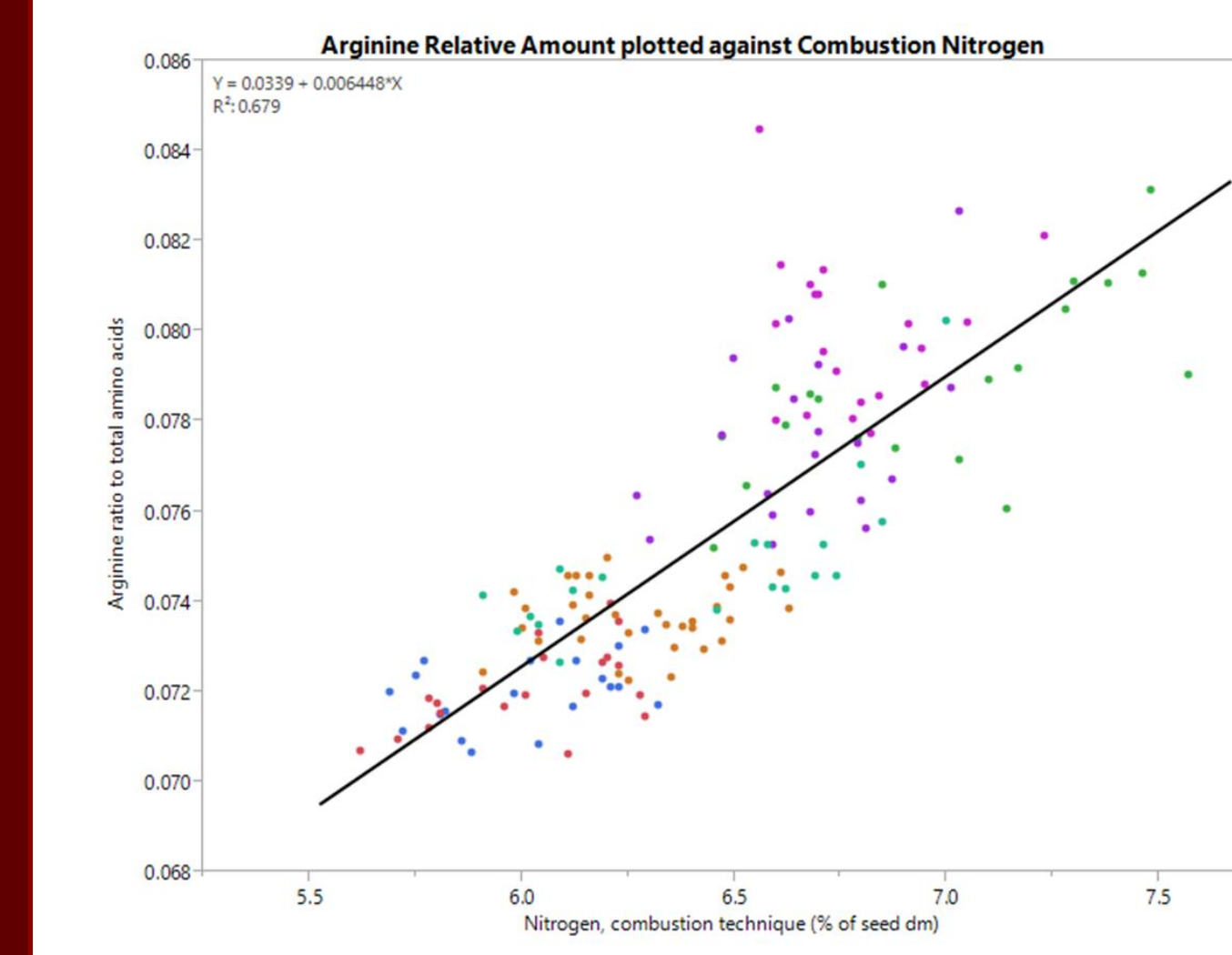
Results: Protein Quality

Figure 6. Arginine relative amount increases when plotted against combustion nitrogen. Arginine, the most nitrogen rich amino acid (Table 2), accounts for a large percent of variability in percent nitrogen in the protein.
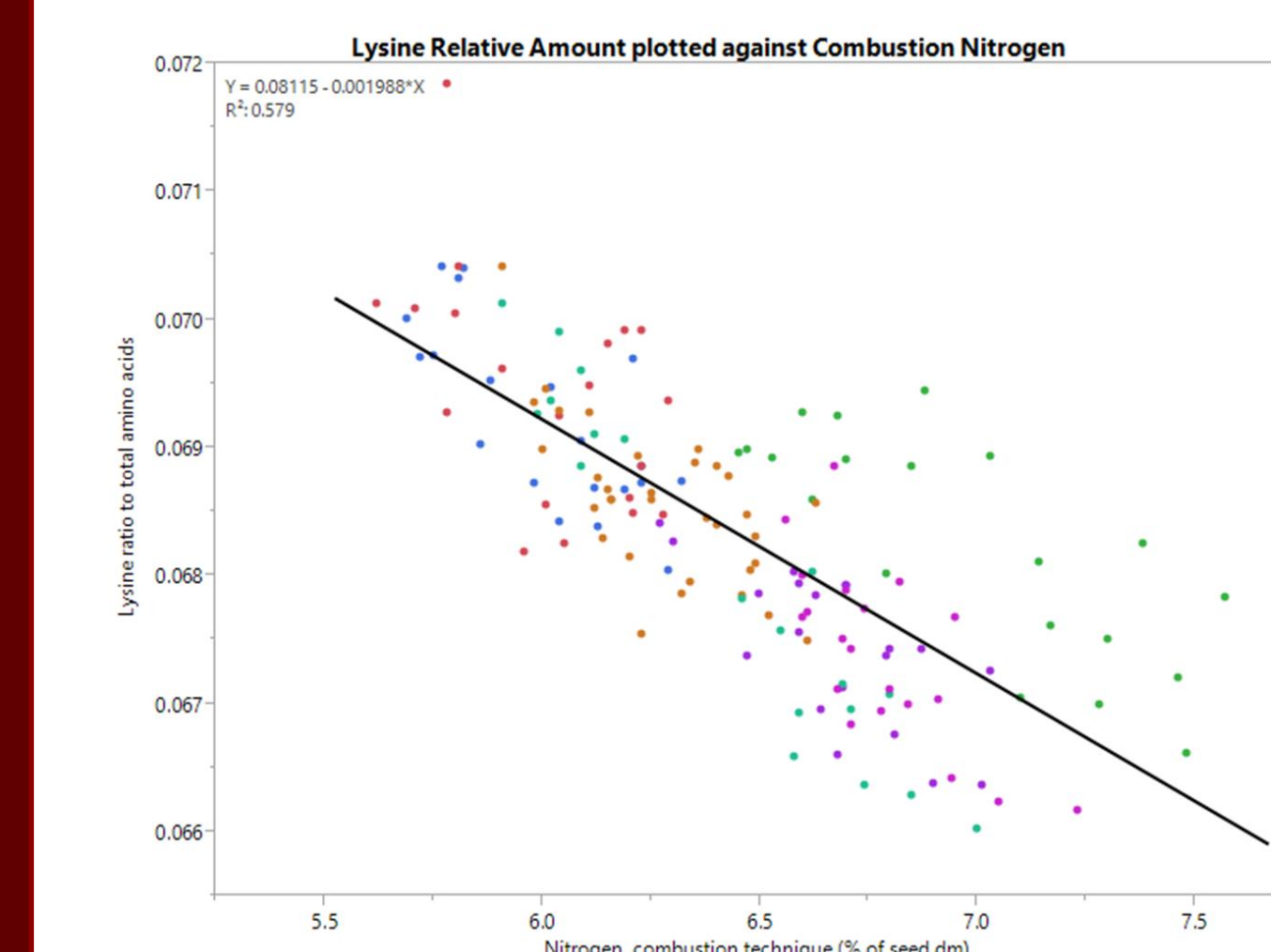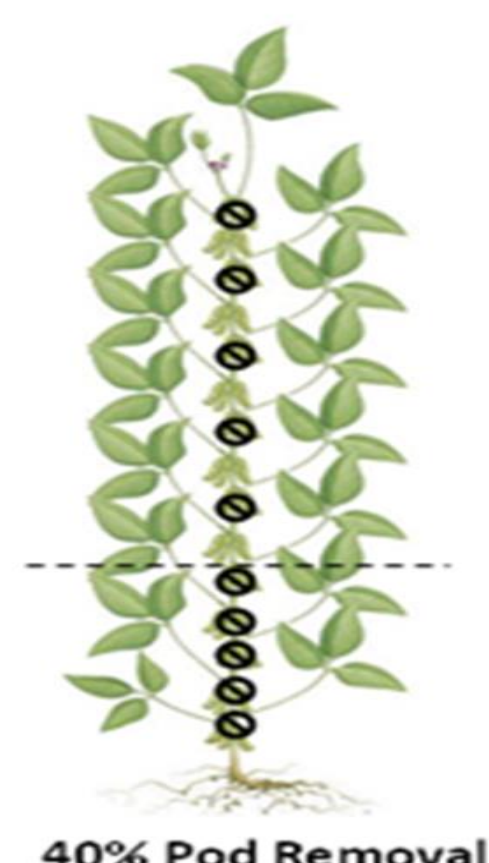
Figure 7. Lysine relative amount decreases when plotted against combustion nitrogen. Lysine, also high in nitrogen, partially offsets nitrogen enrichment from Arginine.

Conclusions

- Crude protein from combustion (6.25 base) over predicts the actual amino acids in soybean seed.

- Percent nitrogen in the protein (and therefore the nitrogen-to-protein conversion factor) varies in relation to total seed protein. Using an adjusted nitrogen-to-protein conversion factor ($y = -0.022 x^2 + 0.250 x + 5.531$, where x = combustion nitrogen and y = adjusted nitrogen to protein conversion factor) may slightly improve crude protein estimation.

- The abundance of nitrogen rich amino acids like arginine varies with combustion nitrogen. However, protein to nitrogen ratio changes relatively little. This may be due to other amino acids offsetting arginine.

Acknowledgements

References

Proulx, R. A., & Naeve, S. L. (2009). Pod removal, shade, and defoliation effects on soybean yield, protein, and oil. *Agronomy Journal, 101*(4), 971-978.