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Nitrogen Sources and Rates Affect Soybean Seed Composition in Mississippi

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Abstract: Soybean (*Glycine max* L.) seed is a major source of protein, oil, carbohydrates and other nutrients that are important for human and animal nutrition. Producers have considered applying nitrogen (N) fertilizer to soybean crop to maximize seed yield; however, its effect on seed composition is not well understood. The objective of this two-year (2015 and 2016) study was to evaluate the effects of N fertilizer sources and application rates (45, 90, 135 and 179 kg N ha^{-1}) on soybean seed composition on two soil textures (clay and silt-loam) in Mississippi. The three fertilizer sources included in this study were urea with N-(n butyl) thiophosphoric triamide (Urea+NBPT), polymer-coated urea (PCU), and ammonium sulfate (AMS). Nitrogen application at 179 kg ha^{-1} on clay soil reduced seed protein by 1.05% compared to unfertilized soybeans in 2016. However, N application at 179 kg ha^{-1} increased oil content by 0.7% on clay soil compared to the unfertilized soybeans only in 2016. Nitrogen applications reduced stachyose content on both soil textures in 2015. The fatty acids showed variable response to N applications. Since, seed quality is not a trait from which growers receive an economic incentive, they are unlikely to adopt this practice for standard soybean production.

Keywords: protein; oil; fatty acids; sugars; fiber; starch

1. Introduction

Soybean fulfills its nitrogen (N) requirement mainly through symbiotic biological nitrogen fixation (BNF) or by N uptake from soil. On average, the BNF satisfies only 50–60% of soybean N demand [1] which is enough to achieve only 80–90% of soybean yield possible with N fertilization [2–4]. Any gap between the soybean N demand and N supply by BNF needs to be supplied by N uptake from other sources [5]. In an effort to provide soybeans with adequate N for maximum seed yield, producers have considered applying N fertilizer to their soybean crop. Previous studies have reported inconsistent responses of soybean seed yield to N fertilizer applications [6–18]. For instance, in the Southern US, no effect was reported in Texas [7] and Alabama [6]. However, Ray et al. (2006) reported an increase in soybean seed yield by 7.7% and 15.5% for irrigated and non-irrigated environments, respectively, due to a large amount of N applications in Mississippi [17]. McCoy (2016) reported an increase in soybean seed yield of 4% and 8% on sandy/silt loam and clay soil, respectively, due to N applications in Mississippi [19]. Soybean yield response to N applications depends upon soil properties (soil temperature, pH, moisture, soil nitrate content at planting) [8,13,20], irrigation [13] and cultivars [8]. Previous studies on N application effects in soybeans have examined seed yields, biomass production,

nodulation or other plant growth parameters in the southern US [8,21,22]. However, the impact of N applications on soybean seed composition is not well documented.

Soybean seed is a major source of protein, oil, carbohydrates and other nutrients that are important for humans and animals. Soybean is composed of approximately 40% protein, 20% oil and 33% carbohydrates [23]. The protein and oil concentration determines the economic value of soybean seed [24]. The oil portion of the soybean seed mainly consists of saturated fatty acids (such as palmitic and stearic acid), and unsaturated fatty acids (such as oleic, linoleic, and linolenic acid) [25]. Lower palmitic acid, linoleic acid and linolenic acid, and higher oleic acid content is desirable for enhancing soybean oil shelf life and flavor stability [26]. Linoleic and linolenic acids are essential fatty acids that mammals cannot synthesize and thus, need to be supplied through their diets [27]. Soybean also contains both structural (cell wall polysaccharides such as cellulose, hemicellulose, pectins) and nonstructural carbohydrates (starch, mono-, di-, and oligosaccharides). On average, soybean seed consists of 9–12% of soluble carbohydrates such as sucrose, raffinose, and stachyose, which contributes to seed quality. Low levels of raffinose and stachyose are preferred in soybean seeds for better seed quality as these two carbohydrates cause diarrhea in non-ruminant animals. Higher sucrose levels in soybean seed improve taste and flavor of food products. Starch is a storage polysaccharide that accumulates in developing soybean seeds until 30–40 days after flowering and then, its concentration declines rapidly to <1% at maturity [27]. Cell wall polysaccharides along with lignin, enzyme-resistant starch and oligosaccharides constitute a non-digestible part of food/feed known as dietary fiber [27]. Dietary fiber is measured using different methods including crude fiber analysis, enzymatic-gravimetric dietary fiber analysis, acid detergent fiber (ADF) or neutral detergent fiber (NDF) methods.

Protein and fatty acid synthesis occurs at different rates and times during seed development and can be affected by N availability during seed development and maturation period [28]. Some previous research from the southern US addresses the effect of N fertilizer on soybean protein and oil content [16,22]. Among those that did, N fertilization did not affect soybean seed protein and oil content in Alabama [8,22]. For studies conducted in Florida and Arkansas, Purcell and King (1996) reported a decrease in seed protein and increase in oil concentration due to N applications at 112 kg ha^{-1} rate at V6, R2 or V6 and R2 stages [29]. In Mississippi, the impact of N application on soybean seed composition was only reported by Ray et al. (2006) [16] and Bellaloui et al. (2011) [30]. Ray et al. (2006) reported reduced protein concentration and increased oil concentration in both irrigated and non-irrigated environments resulting from very large applications of N (averaged over application rates of 290, 310 and 360 kg N ha^{-1}) on clay soil [16]. Bellaloui et al. (2011) found that N application at 112 kg ha^{-1} in irrigated and non-irrigated soybeans in Mississippi affected protein, oil, and fatty acid composition including palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid [30].

While these previous studies have begun to address the effect of N fertilizer on soybean seed composition they either only investigated a single N rate [30] or agronomically unrealistic N rates [16]. As more producers in the mid-southern US consider N fertilization of soybeans, information on the effect of multiple N rates and multiple N fertilizer sources on total soybean seed composition will be required. Therefore, the objective of this study was to evaluate the effect of N rate and N source on soybean seed composition including protein, oil, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, fiber, starch, ash, ADF, and NDF.

2. Materials and Methods

Field studies were conducted at the Mississippi State University Delta Research and Extension Center in Stoneville, MS (33°25′ N, 90°54′ W) in 2015 and 2016 on two soil textures commonly used for commercial soybean production in the Mississippi Delta. In 2015, the field experiment was conducted on a Dubbs silt-loam (fine-silty, mixed, active, thermic Typic Hapludalfs) and a Tunica clay (clayey over loamy, smectitic over mixed, superactive, nonacid, thermic Vertic Epiaquepts). The study was conducted only on a Tunica clay in 2016.

The soybean variety 'Pioneer 49T80' was seeded into conventionally tilled plots at 333,585 seeds ha^{-1} on 16 April for silt-loam soil and 30 April for clay soil in 2015. In 2016, soybean variety 'Asgrow 4632' was planted at 333,585 seeds ha^{-1} on 7 May. Each plot consisted of four rows of soybean spaced 100 cm apart. Pest management and irrigation scheduling were performed according to Mississippi State University Extension recommendations.

The experimental design was a randomized complete block arrangement with four replications. Treatments consisted of a factorial arrangement of three N fertilizer sources (N source), and five N fertilizer application rates (N rate). Nitrogen sources used in this study were: ammonium sulfate (AMS; 21-0-0-24S), polymer-coated urea (PCU; 44-0-0) (Environmentally Smart Nitrogen, Agrium Inc., Calgary, AB, Canada), and Urea+NBPT [N-(n butyl) thiophosphoric triamide] (46-0-0). The NBPT product used was Agrotain (Koch Agronomic Services, Wichita, KS, USA) which is a urease inhibitor that limits N loss from urea via a reduction in ammonia volatilization. All N sources were broadcast by hand to assigned plots at N rates of 0, 45, 90, 135, and 179 kg N ha^{-1} at the R1 growth stage of soybean. The initial soil characterization information is provided in Table 1 and details about soil sampling and analysis is provided in McCoy (2016) [19].

Table 1. Selected soil chemical properties of research fields used for studies in Stoneville, MS in 2014 and 2015.

Year	Soil Texture	Soil Description	Soil pH	O.M. [†]	CEC [†]	Mehlich-3 Extractable Nutrients						
						P	K	Ca	Mg	Na	S	Zn
			(1:2)	%	Meq 100 g^{-1}	mg kg^{-1}						
2015	Silt loam	Dubbs Silt Loam	6.3	0.7	11.1	29	263	1171	254	13	10	3.5
2015	Clay	Tunica Clay	6.8	1.7	29.7	105	421	4163	991	29	18	7.0
2016	Clay	Tunica Clay	7.7	2.7	38.7	44	266	5445	1305	84	177	5.0

[†] O.M., organic matter; CEC, cation exchange capacity.

Soybean was harvested from the middle two rows of each plot using a small-plot combine (Kincaid Equipment, Haven, KS, USA). A single 0.5 kg seed sample was collected from each plot for analysis of seed quality parameters including protein, oil, fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid), fiber, starch, ash, ADF, NDF and sugars (sucrose, raffinose, and stachyose). Soybean seeds were analyzed for sugars only in 2015.

A 25 g soybean seed sub-sample was ground using the laboratory Mill 3600 (Perten, Springfield, IL, USA) and the protein, oil, fatty acids, sugars, acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined by near-infrared reflectance using the AD 7200 array feed analyzer (Perten, Springfield, IL, USA) [31,32]. Perten's Thermo Galactic PLS IQ software was used by the University of Minnesota for developing calibrations and a calibration curve was updated regularly according to Association of Official Analytical Chemists (AOAC) methods [30]. Seed protein, oil, fiber, starch, ash, ADF, NDF, and sugar concentrations were determined based on a seed dry matter, whereas fatty acids concentrations were determined based on total oil concentration.

Data were analyzed using the PROC MIXED procedure in SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Due to differences in soil texture and soybean variety, each site-year was analyzed separately. Nitrogen source and N rate were considered fixed effects while replication was considered a random effect. Least square means were calculated and mean separation ($p < 0.1$) was produced using PDMIX800 in SAS (Statistical Analysis System), a macro for converting mean separation output to letter groupings [33].

3. Results

3.1. Soybean Seed Composition under Silt-Loam Soil

Soybean seed protein, oil, palmitic acid, oleic acid, stearic acid, linolenic acid, and sugars including raffinose, and sucrose concentrations (Table 2) were not affected by the N fertilization on the silt-loam soil in 2015. The mean concentrations for these seed composition parameters are provided in Tables 2 and 3.

Table 2. Soybean seed protein, oil, fatty acids, starch, fiber, and ash as affected by the N application rates in 2015 and 2016.

Soil Texture	Year	N Rates	Protein	Oil	Fatty Acids					Fiber	Starch	Ash	ADF	NDF
					Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid					
		kg N ha^{-1}						g kg^{-1}						
Silt loam	2015	0	382.6a [†]	236.2a	108.0a	41.9a	316.4a	474.2d	61.8a	63.2a	82.1a	58.8a	120.2b	173.0a
		45	396.3a	233.9a	105.1a	40.5a	299.4a	491.0ab	62.0a	59.6bc	66.2b	59.1a	126.1a	170.5a
		90	385.6a	235.0a	106.4a	41.5a	303.7a	486.9bc	62.4a	62.0ab	75.3ab	59.2a	123.2ab	170.4a
		135	398.4a	234.3a	103.2a	39.9a	298.4a	497.8a	62.3a	58.2c	68.6b	59.6a	120.4b	165.0b
		179	385.0a	233.7a	105.9a	41.6a	306.7a	477.7cd	61.5a	62.0ab	74.4ab	59.6a	124.2ab	170.9a
Clay	2015	0	391.7c	219.6a	115.5a	42.5a	283.6a	482.3a	75.1a	62.7a	77.0ab	57.9a	132.3a	172.8a
		45	394.1abc	219.6a	113.9a	41.4a	289.6a	480.1a	73.2ab	61.8a	75.2ab	58.0a	131.1a	167.8a
		90	401.8a	217.4a	112.9a	41.7a	292.6a	479.4a	71.2b	62.0a	68.2c	58.2a	135.1a	170.5a
		135	398.9ab	217.2a	114.2a	41.8a	288.1a	481.4a	70.9b	61.9a	72.1bc	57.9a	135.1a	169.6a
		179	394.1bc	218.8a	114.2a	42.3a	294.9a	474.7a	74.5a	61.6a	79.4a	58.6a	133.1a	169.7a
Clay	2016	0	402.9ab	214.9b	111.2a	44.0bc	238.4a	514.0a	73.8a	58.1a	61.8a	54.9c	145.1a	165.9a
		45	403.4a	213.2c	112.5a	45.4a	239.0a	506.5a	74.6a	59.1a	61.8a	55.4a	145.5a	166.6a
		90	400.6bc	215.5ab	111.8a	43.5c	236.3a	513.3a	73.3a	59.0a	63.6a	55.1bc	144.8a	166.4a
		135	401.8ab	215.2ab	111.2a	45.1ab	234.4a	511.4a	74.0a	58.5a	64.1a	55.3ab	144.0a	166.9a
		179	398.7c	216.3a	111.0a	44.7ab	230.8a	514.0a	74.7a	59.2a	64.7a	55.2abc	145.8a	166.1a

[†] Same letters within a column for each soil type indicates no significant differences between means at $p < 0.1$. ADF, acid detergent fiber; NDF, neutral detergent fiber.

Table 3. Soybean seed sugars including raffinose, stachyose and sucrose as affected by the N application rates in 2015.

Soil Texture	N Rate	Raffinose	Stachyose	Sucrose
	kg N ha^{-1}		g kg^{-1}	
Silt loam	0	7.1a [†]	27.4a	20.9a
	45	6.7a	24.3bc	18.2a
	90	7.0a	26.4ab	21.6a
	135	7.1a	24.0bc	19.1a
	179	7.4a	22.1c	23.3a
Clay	0	7.2a	28.0a	34.0a
	45	7.1a	25.7ab	31.8a
	90	6.9a	23.6b	29.1a
	135	6.8a	23.4b	29.7a
	179	6.9a	24.0b	30.7a

[†] Same letters within a column for each soil type indicates no significant differences between means at $p < 0.1$.

Nitrogen application rates affected linoleic acid, fiber starch, ADF, NDF and stachyose sugar concentrations on the silt-loam soil in 2015 (Tables 2 and 3). The N application up to a rate of 135 kg ha^{-1} increased linoleic acid concentration compared to unfertilized soybeans by 2.7 to 5% (Table 2). However, N application at 179 kg ha^{-1} had a linoleic concentration similar to that from unfertilized soybeans. Increasing N application rate from 90 to 135 kg ha^{-1} increased linoleic acid concentration by 2.2%.

Nitrogen application at 90 and 179 kg ha^{-1} rate caused no change in fiber and starch content of soybean seed compared to unfertilized soybean. However, the N application at 45 and 135 kg ha^{-1} rates reduced fiber concentration by 5.8% and 8%, respectively, compared to unfertilized soybeans. Similar to fiber concentration, N application at 45 and 135 kg N ha^{-1} rate reduced starch concentration compared to unfertilized soybean by 19% and 16.5%, respectively. Nitrogen application at 45 kg N ha^{-1} resulted in 5% higher ADF content than the unfertilized soybeans. Any further increase in N application rate did not result in any increase in ADF compared to unfertilized soybeans. The NDF concentration in soybean seed was significantly lower with 135 kg N ha^{-1} compared to other N application rates and unfertilized soybean.

Application of N at 135 and 179 kg N ha^{-1} reduced stachyose sugar content in soybean seed compared to unfertilized soybean (Table 3). Seed oil and stachyose sugar content was affected by N source on the silt-loam soil in 2015 (Table 4). Using AMS as the N source resulted in a 2% increase of seed oil concentration compared to PCU and Urea+NBPT. Seed stachyose concentration was 15% and 10% higher when AMS was used as the N source compared to PCU and Urea+NBPT, respectively.

Table 4. Soybean seed oil and stachyose sugar concentration as affected by the N sources on silt-loam soil in 2015.

N Source	Oil	Stachyose
	g kg^{-1}	g kg^{-1}
AMS	237.7a [†]	26.9a
PCU	233.1b	23.5b
Urea+NBPT	233.1b	24.3b

[†] Same letters within a column indicates no significant differences between means at $p < 0.1$. Urea+NBPT, [N-(n butyl) thiophosphoric triamide]; PCU polymer-coated urea, AMS ammonium sulfate.

3.2. Soybean Seed Composition under Clay Soil

3.2.1. Seed Composition in 2015

In 2015, the N application rates affected only protein, linolenic acid, starch, and stachyose sugar concentration on clay soil (Table 2). Application of 90 and 135 kg N ha^{-1} increased protein concentration by 2.6% and 1.8% compared to the unfertilized soybean. However, protein concentration was not increased significantly compared to unfertilized soybean when N was applied at either 45 or 179 kg ha^{-1}. No differences were found for protein concentration between N rates of 45, 90 and 135 kg N ha^{-1} as well as between 135 and 179 kg N ha^{-1} on clay soil in 2015. No clear trends were obtained for the protein concentration on clay soil in 2015 due to N applications.

Application of 45 kg N ha^{-1} did not cause any change in linolenic acid concentration on clay soil in 2015 compared to unfertilized soybean (Table 2). Further increasing N rate to 90 and 135 kg N ha^{-1} reduced linolenic acid concentration by 5.2% and 5.5% compared to unfertilized soybeans.

Starch concentration with 90 and 135 kg N ha^{-1} was 14% and 9% lower compared to N application at 179 kg ha^{-1}. Application of 45 kg N ha^{-1} did not cause any change in stachyose concentration compared to unfertilized soybeans on clay soil in 2015 (Table 3). However, N application at 90, 135 and 179 kg N ha^{-1} reduced stachyose sugar by 14% to 16% compared to unfertilized soybean.

3.2.2. Seed Composition in 2016

Nitrogen fertilization in soybeans on clay soil in 2016 did not affect palmitic acid, oleic acid, linolenic acid, fiber, starch, ADF, NDF, raffinose, stachyose and sucrose (Table 2).

In 2016, N application at 179 kg ha^{-1} on clay soil reduced seed protein concentration by 1.05% compared to unfertilized soybeans (Table 2). The protein concentration from unfertilized soybeans was similar to N application rates of 45, 90 and 135 kg ha^{-1}. The seed oil concentration was increased by 0.7% and 1.4% due to N application at 179 kg ha^{-1} compared to unfertilized soybeans and N application rate of 45 kg ha^{-1} (Table 2). No differences were found between N application rates of 90, 135 and 179 kg N ha^{-1} for seed oil concentration on clay soil in 2016. Stearic acid concentration was 3.2% and 4.2% higher with N application at 45 kg ha^{-1} than unfertilized soybeans and 90 kg N ha^{-1}, respectively.

When AMS was used as N source, the linoleic acid concentration was 10–15.5 g kg^{-1} less for N application rate of 135 kg ha^{-1} compared to unfertilized soybeans and N application at 90 and 179 kg ha^{-1} (Table 5). Linoleic acid was reduced by 13–14 g kg^{-1} due to N application at 45 kg ha^{-1} than for 135 and 179 kg N ha^{-1} using PCU as N source. Urea+NBPT application at 45 kg ha^{-1} had 11.6 g kg^{-1} less linoleic acid concentration than unfertilized soybeans.

Table 5. Soybean seed linoleic acid and ash concentration as affected by the N source and N rate for study conducted on clay soil in 2016.

N Source	N Application Rate	Linoleic Acid	Ash
	kg N ha^{-1}	g kg^{-1}	
AMS	0	514.0ab [†]	54.9de
	45	512.2abc	54.9de
	90	519.5a	54.7e
	135	504.0c	55.6ab
	179	518.8a	55.1bcde
PCU	0	514.0ab	54.9de
	45	504.8bc	56.0a
	90	510.0abc	55.5ab
	135	519.0a	54.9de
	179	517.8a	55.2bcde

Table 5. *Cont.*

N Source	N Application Rate	Linoleic Acid	Ash
	kg N ha^{-1}	g kg^{-1}	
Urea+NBPT	0	514.0ab	54.9de
	45	502.4c	55.4bcd
	90	510.4abc	55.0cde
	135	511.3abc	55.5abc
	179	505.3bc	55.3bcde

[†] Same letters within a column indicates no significant differences between means at $p < 0.1$. AMS, ammonium sulfate; PCU, polymer coated urea; NBPT, [N-(n butyl) thiophosphoric triamide].

4. Discussion

For studies conducted in Mississippi, soybean seed composition showed inconsistent responses to N fertilization between soil textures and years, probably due to differences in environmental conditions during the growing season [34–36], soil properties [35], soybean varieties, and/or crop management practices such as planting dates, tillage, fertility, and plant density [37]. Climatic conditions such as lower temperatures and higher precipitation amounts during seed filling may reduce protein and oil concentration, whereas high air temperatures and moderate rainfall at the seed filling period can result in higher protein concentration in soybean seeds [35,38]. In general, N sources performed similarly and did not show differences for most of the seed composition parameters measured in our study, in agreement with findings from other studies [15]. McCoy (2016) reported that belowground biomass, root length, root area, root diameter and nodule numbers were significantly lower for clay soil compared to silt-loam soil, which possibly affected biological nitrogen fixation and consequently, soybean response to N fertilizer applications [19].

The decrease in protein concentration with N application at 179 kg ha^{-1} was only observed on clay soil in one out of two years of study. Increase in oil concentrations due to N applications occurred on clay soil in 2016, whereas no changes occur in oil concentration in 2015. Nitrogen fertilization did not change seed protein concentration on silt-loam soil in 2015. Previous studies have shown that protein and oil concentrations were negatively correlated resulting in the opposite trend for soybean seed protein and oil concentration in response to N applications in our study on clay soil [16]. In agreement with our results, multiple studies have also reported inconsistent responses of soybean seed protein and oil content to N applications [10,15,16,39–42]. In Arkansas, 112 kg N ha^{-1} when applied at the R2 growth stage in soybeans caused no changes in seed protein and oil concentration compared to unfertilized soybeans on silt loam soil [40]. Wesley et al. (1998) found an increase in protein and oil content by 1% and 0.3–0.5% because of N applications at R3-R4 stage at rates of 22 and 44 kg ha^{-1} using multiple N sources at some of the locations in Kansas, however, N applications showed inconsistent and overall no significant effects on seed protein and oil content, when combined over multiple locations [10]. A study in Minnesota showed N application at 84 kg ha^{-1} increased protein content by 0.4 g kg^{-1} and showed no change in oil content when averaged over N application timing, methods and sources [12]. Particularly in Mississippi State, the protein concentration of irrigated soybeans decreased by 2.7% and oil concentration was increased by 2.2% when a large amount of N was applied at 290, 310 and 360 kg ha^{-1} at planting on the Sharkey clay soil [16]. Another study in the Mississippi Delta region found that N application at 112 kg ha^{-1} at V3-V4 growth stage reduced protein content and increased oil content only in one out of two years of study on silty clay loam soil [30].

Palmitic acid, oleic acid, raffinose and sucrose sugar showed no response to N fertilization in the two years of this study. Stearic acid, and linoleic acid concentrations showed a variable response to N fertilization in the two years of this study. Similarly, stearic acid, oleic acid, and linoleic acid concentrations, averaged over two years, were increased by 0.85, 7.5, and 16.1 g kg^{-1} when 112 kg N ha^{-1} was applied at the V3-V4 growth stage of soybean on silty clay loam soil in Mississippi [30]. However, the same study reported that linolenic acid concentration was reduced by

6.2 g kg^{-1} in the first year and increased in another year by 10.8 g kg^{-1} due to N applications [30]. No changes in fatty acids content due to N applications was observed by Ham et al. (1975) and Bennett and Krishnan (2005) [35,39]. Ham et al. (1975) found that fatty acids including palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid did not change with pre-plant N application at 224 kg ha^{-1} in Minnesota [39].

Linolenic acid, fiber, starch, ash, NDF and ADF concentrations showed variable response to N application rates in all study years and soil textures. Similarly, Howell and Collins (1957) reported that linoleic and linolenic acid levels were inversely correlated with temperature and little or not affected by N, P, K and S nutrition, the addition of manure/plant residues [43]. Seed fiber content, ADF and NDF were affected by N rate only on silt loam soil. Barker and Sawyer (2005) found that fiber concentration in soybean seeds remained unchanged when N was applied at 45 and 90 kg ha^{-1} during reproductive stages [15].

5. Conclusions

In this study, the impacts of nitrogenous fertilizer application were examined with regards to its impact on seed composition. Ammonium sulfate (AMS; 21-0-0-24S), polymer-coated urea (PCU; 44-0-0), and Urea+NBPT [N-(n butyl) thiophosphoric triamide] (46-0-0) were applied at rates of 0, 45, 90, 135, and 179 kg N ha^{-1} at the R1 growth stage of soybean. Although there was an increase of oil in one out of two years of study on clay soil at the rate of 179 kg ha^{-1} regardless of its source, inconsistent responses of some seed composition components including protein, fiber, sugars and fatty acids were also found. The responses of seed composition constituents were different, depending on soil type, the rate of application, and location. The response of seed composition due to the nitrogenous application has also shown to be inconsistent in previous studies. Further, economic analysis for our study showed that N application is not an economically feasible option for increasing soybean yield as net returns with N application at any rate was lower than the unfertilized soybeans for both soil types (unpublished data) [44]. Since there is no economic incentive to growers for seed oil or protein at the grain elevator, it is unlikely that N fertilizer will be adopted strictly for increasing seed composition constituents as yield is the main determinant factor for profit.

Author Contributions: J.M. Orlowski, B.R. Golden and J.M. McCoy designed the experiments and collected data; G. Kaur and J.M. McCoy analyzed the data; N. Bellaloui contributed analysis tools; all authors were involved with manuscript preparation and editing.

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