SOYBEAN RESPONSE TO NITROGEN AND SULFUR FERTILIZATION

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agricultural and Applied Sciences

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

December 2016

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Soybean [*Glycine max* (L.) Merrill.] yields in North Dakota have not yet reached their genetic potential. Applying fertilizers may increase yields. This study was conducted to understand the impact of nitrogen (N) and sulfur (S) fertilization on soybean plant density, vigor, greenness, height, yield, test weight, protein and oil content, nodulation, vegetation, and root growth. Two varieties were planted in experiments across ten environments during 2015-2016. Yield differed between environments (up to 77%). Varieties responded differently to N and S fertilizer. Nitrogen (56 kg N ha^{-1}) in the form of broadcast incorporated urea increased vigor (13%) and yield (3.6%) but decreased plant density (5.7%) and nodulation (from 31.8 to 23.7 nodules $plant^{-1}$). Nitrogen application of 56 kg N ha^{-1} increased yield 118 kg ha^{-1} when averaged over all environments. Sulfur, in the form of broadcast incorporated gypsum, decreased protein concentration and increased early season nodule size.

ACKNOWLEDGEMENTS

I cannot express enough thanks to my graduate school advisor, Dr. Hans Kandel, for continually challenging me and pushing me to the limits of my potential. The encouragement and guidance throughout the research project and coursework was of paramount importance. I also extend sincere gratitude to my graduate committee members, Dr. Joel Ransom, Dr. Thomas DeSutter, and Dr. Ted Helms for their assistance in experimental implementation and design as well as reading this thesis and offering critical review to ensure it is of the highest quality. I am forever indebted to Dr. Jawahar Jyoti and Dr. Grant Mehring for their time, patience, and assistance with statistical analysis.

This project could never have been completed without the efforts of research technician Chad Deplazes, whose work ethic, dedication, and desire to see others succeed is second to none. Thank you also to research technician Darrin Eisinger for the energy and effort in the final stages of my research project. I am also singularly grateful to my colleagues, friends, and fellow graduate students ("Joel's Army") who were all instrumental in data collection and trial implementation and have the very brightest of futures ahead of them: Calli Feland, Nicholas Schimek, Tracy Hillenbrand, Missy Geiszler, Elizabeth Lovering, Matthew Rellaford, and J Stanley. I am also thankful for the contributions of student research assistants Garret Lungren, Sukhwinder Bali Kautish, Manish Sawant, Jake Riske, and Tanner Zetocha. To my parents and family: I thank you for the love and support for me to pursue graduate education. In addition, credit is owed to Andrew Hoiberg of Calcium Products Inc., Adam Spelhaug of Peterson Farms Seed, and Proseed's Keith Peltier for providing materials necessary to conduct this study. Finally, thank you to the North Dakota Soybean Council for funding this research project.

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LITERATURE REVIEW

Soybean as a Crop

Soybean [*Glycine max* (L.) Merrill.] varieties have been adapted to be grown in North Dakota and now play a key role in crop rotations. Agricultural producers in the Midwest, as well as throughout the US, utilize soybean as a dominant crop in seasonal crop rotations. Its agronomic properties and seed qualities, as well as end use, have also made soybean an important crop across the globe. Soybean yields have increased over time due to the breeding of new varieties, improved application of fertilizer and pesticides, and better management practices (Ash et al., 2006). The annual rate of soybean yield increase in the US averages 31 kg ha^{-1} (Specht et al., 1999).

The growth habit of soybean is separated into two main growth stages: vegetative and reproductive growth, within each main stage exists a number of individual stages (Fehr et al., 1971). The growth stage is partly dependent on heat units (Kandel and Akyüz, 2012) but is also dependent upon photoperiod, because soybean grown in the northern regions of the US is a short-day, photosensitive crop. The reproductive phase of the plant's life cycle does not begin until the critical length of nighttime occurs. Depending on the planting date, the plant has a varying number of nodes on the main stem, which can form branches. This gives the soybean plant an ability to compensate for short adverse conditions during the growing season.

In North Dakota, soybean is typically planted in mid-May when soil temperatures approach the minimum germination temperature of 9°C. Following germination, the soybean plant emerges by the active hypocotyl mechanism, which brings the growing point aboveground. This type of emergence makes the soybean very sensitive to late spring frost and other damage. Therefore, date of planting is critical. In North Dakota, soybean is typically harvested in late September or early October. The official test weight of a soybean crop is 772.3 kg m^{-3} and each seed contains roughly 20% oil and 40% protein by dry weight. As reported by the USDA, the average North Dakota soybean yield in 2012, 2013, 2014, and 2015 was 2318, 2049, 2318, and 2184 kg ha^{-1}, respectively (USDA, 2015b; USDA, 2016). Improving fertilization practices could increase North Dakota's average soybean yield.

Soybean Nodulation and Nitrogen Fixation

One of the main reasons soybean is such an appealing crop is the limited need for N fertilization in the US. Presently, no N application is recommended in North Dakota, except as a rescue treatment (Franzen, 2013). Soybean is capable of obtaining most of its N supply due to a symbiotic relationship with *Bradyrhizobium japonicum* bacteria in the soil. The soybean plant and bacteria in the soil undergo a chemical recognition process to initiate nodulation. The plant secretes flavonoid chemical compounds into the surrounding soil, while the bacteria release a chemical called "Nod factor" into the soil (Franzen, 2013). The chemical recognition of the Nod factor by the plant triggers increased cell division in the root cortex (primary nodule meristem); this process is termed "root hair curling." The bacteria then attach to the root hair and secondary cell division begins in the pericycle (secondary nodule meristem). The bacteria form an infection thread, which penetrates the root cell. The primary and secondary nodule meristems undergo a fusion and the cells begin to elongate and differentiate as a nodule. A connection is made from the nodule to the plants vascular tissue and the bacteria become functional "bacteroids." The bacteroids, which are capable of N fixation, provide N to the plant in exchange for carbohydrates (Taiz et al., 2015). This process is termed biological N fixation (BNF). It is recommended to inoculate soybean seed with an appropriate *B. japonicum* strain to encourage BNF when seed will be planted where there is no history of soybean production or where it has not been planted for 3 to 5 yr. Inoculation is also recommended when planting into soils with low pH (<6) and sandy-textured soils.

Nodules containing *Bradyrhizobium japonicum* bacteria are actively fixing N by the V2 stage of soybean growth. However, several researchers have expressed concern as to whether or not BNF is capable of meeting increased N demands of newly developed soybean varieties with higher yield potential (Yamada, 2000; Vitti and Trevisan, 2000; Lamond and Wesley, 2001; Cooper, 2003). Soybean plants accumulate approximately 30% of total N demand by R3 (Ritchie et al., 1982). Zotarelli (2000) reported that higher rates of BNF occur after flowering. More precisely, maximum BNF occurs between R3 and R5, but there is a reduction in BNF between the R5 and R7 growth stage (Zapata et al., 1987), which could limit yield potential during seed filling. The majority of N is demanded during the late reproductive stages, presumably during a time of reduced BNF. Zapata et al. (1987) concluded that BNF accounts for approximately 50% of the soybean's annual N demand. Harper (1974) concluded that a combination of N fixation and inorganic N is required to obtain optimum soybean yields. In order to fulfill N requirements, the soybean plant must either remobilize N from plant source regions to the seed, reducing overall photosynthetic capacity, or take N from the soil.

Fertilizer rates were determined after reviewing prior research pertaining to nodulation after N fertilization. Hungria et al. (2006) reported significant decreases in nodule number, root dry weight, and yield after various applications of N. Rates ranged from 30 kg N ha^{-1} up to 300 kg N ha^{-1} applied at various timings and timing combinations. Shoot dry weight was not affected. The study was conducted in Brazil with very different soil types and varieties than are available in the US. In a separate study, Nishi and Hungria (1996) reported reduced BNF in soybean and no economical yield benefits after application of up to 400 kg N ha^{-1}. Salvagiotti et al. (2009) reported an increased soybean yield following N fertilization of 180 kg ha^{-1} with no impact on nodulation. However, source and placement were important factors in that study because slow-release N sources were placed below the immediate root zone.

Sulfur seems to have a substantial impact on the nodulation of legumes. Varin et al. (2009) reported that S fertilization strongly influenced nodulation of white clover (*Trifolium repens* L.) grown in a hydroponic environment. Specifically, S fertilization significantly increased average nodule number (up to 4000 nodules plant^{-1}) and average nodule mass (150%) of white clover compared to white clover grown under S deficient conditions. In addition, nodule mass and density were highly correlated with the amount of N_2 derived from BNF. Furthermore, S had a specific effect on BNF, as N fixed per root area increased with S fertilizer. The percentage of N derived from BNF also increased with S fertilization, indicating that BNF is more dependent on S nutrition than N uptake (Varin et al., 2009). Gaw and Soong (1942) reported that S in the form of $CaSO_4$ significantly increased dry mass (10%) and nodule number (45%) of garden pea (*Pisum sativum* L.) while ammonium sulfate (AMS) depressed nodulation.

Nitrogen Fertilization

Nitrogen is an essential plant nutrient, as it is an important component of chlorophyll. It is also a key part of amino acids, the building blocks of protein molecules, and DNA, without which there would be no life on earth (Raven et al., 2005). The earth's atmosphere is approximately 78% N in the form of nitrogen gas (N_2) (Fields, 2004). Nitrogen in this form, however, is not available for plant use. Soybean requires a large amount of N because of the high protein concentration of the seed, approximately 35-40% (Saito et al., 2014).

Nitrogen application to soybean has significantly increased yields in ND, but the revenue increase did not outweigh the cost of fertilizer (H. Kandel, personal communication, 2015). Salvagiotti et al. (2009) reported that N fertilization may satisfy the additional N required to attain maximum yield of soybean when soil and BNF provide an inadequate N supply. Soybean acquires only between 25% and 75% of its required N by fixation (Deibert et al., 1979). This matches a range of 50-60% of average total N demand obtained through BNF reported by Salvagiotti et al. (2009). Kaiser and Lamb (2012) also reported that certain environmental conditions limit the ability of soybean nodules to supply adequate N late in the growing season, indicating that N fertilizer may be beneficial.

High N levels in the soil, specifically nitrate (NO_3^-), are associated with high expression of iron deficiency chlorosis (IDC) in soybean (Franzen, 2013; Kaiser and Lamb, 2012). Chlorosis is known to cause plant stress and presents itself as interveinal yellowing of leaves. Iron deficiency chlorosis can cause substantial yield loss in soybean (Hansen et al., 2003), especially in the poorly drained, calcareous, high bicarbonate soils of the upper Great Plains (Bloom et al., 2011). In the soils of eastern ND, iron (Fe) is plentiful but not readily available to soybean plants because of its form in the soil. Iron in soil is either in the ferrous (Fe^{2+}) or ferric (Fe^{3+}) ionic state, but only grasses can obtain Fe^{3+} from the soil by use of phytosiderphores and chelating agents (Charlson and Shoemaker, 2006). The soybean plant must take Fe from the soil in the Fe^{2+} form. To do so, the plant must acidify the surrounding soil by releasing protons (H^+) and reductants to facilitate the reduction of Fe^{3+} to Fe^{2+}, which the plant can then utilize (Jolley et al., 1996). When NO_3^- is taken into the plant it must be converted quickly into ammonium (NH_4^+). This process requires the use of protons (H^+) and makes it more difficult for the soybean plant to acidify the surrounding soil. Therefore, less Fe is converted to the form available for plant uptake. Special consideration should be given to N fertilization rate and N levels in the soil to avoid the aggravation of IDC and ensuing yield losses.

High soil NO_3^--N concentrations also decrease N fixation (Laysell and Moloney, 1994). Nitrogen as incorporated AMS and broadcast incorporated urea at planting may not increase soybean yield in certain environments and certain soil conditions (Beard and Hoover, 1971; Welch et al., 1973; Bahrati et al., 1986; Touchon and Rickerl, 1986). Heatherly et al. (2003) concluded that the application of 35 kg N ha^{-1} in the form of granular ammonium nitrate applied before V2 stage did not increase yield and decreased net returns by up to \$50 ha^{-1}. However, low rates of N at seeding have increased soybean yield and nodulation in some regions (Ying et al., 1992; Starling et al., 1998; Bhangoo and Albritton, 1976), possibly due to low N fixation at the beginning of the season. Zapata et al. (1987) stated that the main source of N during vegetative development is the absorption of NO_3^--N from the soil, which can be from soil mineralization or application of fertilizer.

Timing may be an important factor when considering N fertilizer application to soybean. Applying N at reproductive stages may increase yield by supplying N at a vital time when N supply may be limited. Barker and Sawyer (2005) concluded that fertilizing soybean at the beginning of R3 with 45 and 90 kg N ha^{-1} in the form of broadcast urea or band placement polymer-coated urea in Iowa only increased yield 49 kg ha^{-1} over the control (0 kg N ha^{-1}), which was not significant. Kinugasa et al. (2012) also reported that N fertilization after flowering significantly increased soybean seed number $plant^{-1}$ (100%).

Research conducted at NDSU in 2014 showed a significant yield increase in soybean treated with N and S fertilizer (Kandel, personal communication, 2015). Additionally, in a study conducted from 2000-2002 at South Dakota State University, Osborne and Riedell (2006) reported that a band application of ammonium nitrate or urea at planting at a rate of 16 kg N ha^{-1} increased soybean yield 6% in 2 years of the 3-yr study. In addition, Kaiser and Lamb (2012) reported that N application of 56 to 84 kg N ha^{-1} could be beneficial to soybean in some fields in the Red River Valley. Starling et al. (1998) reported an average soybean yield increase of 0.15

Mg ha^{-1} when treated with 50 kg N ha^{-1} compared to the control (0 kg N ha^{-1}). Dry matter also increased with N while average nodule number per root decreased. Osbourne and Riedell (2006) reported increased biomass and yield with increasing N rates from 0 to 8, 16, and 24 kg N ha^{-1}. However, the researchers reported that environmental conditions during the season and the time of planting greatly influence the results of fertilization. Ying et al. (1992) reported that N fertilizer rates of 25 and 50 kg N ha^{-1} did not significantly influence yield compared to the control of 0 kg N ha^{-1}, but did significantly decrease nodulation and increase above-ground dry matter. These results were obtained from various regions and environments. Kaiser and Lamb (2012) reported increased average biomass after application of 84 kg N ha^{-1} with no yield impact in MN.

Salvagiotti et al. (2008) reported that a 4031 kg ha^{-1} soybean crop removes approximately 325 kg N ha^{-1} from the field in stover and grain, of which 235 kg N ha^{-1} is removed from the field in the grain. The remainder is assumed to be recycled back into the soil with the breakdown of crop residue. Soil processes must also be considered when applying N fertilizers, including leaching and denitrification. These two processes of N depletion in the soil are especially important when considering tile drainage as a water management practice. In a study focused on tile drainage practices, Drury et al. (2009) reported that soybean yield was significantly higher (13.2%) when fertilized with 50 kg N ha^{-1} across 3 different drainage systems (unrestricted tile drainage, controlled drainage, and controlled drainage with subirrigation). In general, soil N depletion will continue as yields increase and more N is removed in grain.

Sulfur Fertilization

Sulfur is one of the 16 essential elements for plant growth and is a component of amino acids needed for protein synthesis (Jan et al., 2002). Following the enactment of the Clean Air Act in 1963, more soils show S deficiencies possibly relating to lower sulfur dioxide emissions.

This is supported by trends reported by the National Atmospheric Deposition Program (2015) of a nearly 50% reduction in sulfate (SO_4^{2-}) concentration and deposition since 1987 in North Dakota although precipitation has remained relatively constant. David et al. (2016) reported that atmospheric deposition of S in the Great Lakes states of the upper Midwest is 25% of its peak amount. Sulfur is becoming deficient in soils due to the introduction of high yielding varieties, the use of high grade S free fertilizers, and the reduced emission of S from industrial processes (Scheerer, 2009). Soil S levels have decreased as S removal and crop yields have increased and deposition of SO_4-S via rainfall, fertilizer, and pesticides has decreased (Ferguson et al., 2000; Dick et al., 2008). Sulfur deficiencies not only reduce yield, but also decrease the feed value of soybean (Sexton et al., 1997). Unfortunately, it is difficult to measure soil S levels because of highly variable soil test results (Franzen, 2013, Kaiser and Lamb, 2012). Soils typically at risk for S deficiency include coarse-textured soils, soils low in organic matter, soils experiencing large amounts of rainfall in the fall or spring, and soils located on higher landscape positions (Chen et al., 2005).

Recent studies in Minnesota indicate that fertilizing soybean with S increases plant growth but not yield (Kaiser and Lamb, 2012). However, trials conducted in 2011 and 2012 across various soil types in Minnesota reported average soybean yield increase of approximately 134 kg ha^{-1} across S fertilizer rates of 0 to 56 kg S ha^{-1} (Kruger et al., 2014). The application of gypsum in Ohio in 2000, at rates of 16 kg S ha^{-1} and 67 kg S ha^{-1}, increased soybean yield by 4.8% and 11.6%, respectively (Chen et al., 2005). Ganeshamurthy and Reddy (2000) reported that the application of gypsum alone up to a rate of 40 kg S ha^{-1} 30 d after planting increased the yield of soybean nearly 40%, and yield continued to increase with the additional application of farm yard manure which contains N. However, manure contains many other essential plant nutrients, and the study was conducted in India, which has a much different climate than North Dakota. A study conducted in Pakistan reported that soybean plant height, seed yield, and dry matter increased 14, 20, and 26%, respectively, when compared to the control after treatment of 30 kg S ha^{-1} in the form of gypsum (Hussain et al., 2011). Additionally, N uptake and BNF of inoculated soybean increased 50% and 72%, respectively, for the treatment of gypsum at 30 kg S ha^{-1} compared to the control (Hussain et al., 2011).

Sulfur also seems to have an effect on the root system of soybean. Zhao et al. (2008) reported that S supply as elemental sulfur can promote the growth of the soybean root and enhance the plants ability to absorb nutrients. When fertilized in pots with 60 mg S kg^{-1} soil, the average number of nodules plant^{-1} increased by 36%, the plant dry weight increased by 76%, and the seed yield increased by 12.8% compared to the control (0 mg S kg^{-1} soil) (Zhao et al., 2008). The study was conducted in pots in the greenhouse and field plot results were not reported.

Bonde (2013) showed that S concentration in corn (*Zea mays* L.) increased with in-season ammonium sulfate fertilizer applications in Minnesota, which indicates the plant's ability to uptake S throughout the season. Similarly, Thurgood (2014) highlighted a small S concentration increase in soybean petiole at R1 following a split S application, although the increase was not statistically significant. The treatments were applied prior to spring tillage and again shortly after planting.

In a series of Iowa field trials conducted by Sawyer et al. (2011) between 2006 and 2008, corn yield increased by an average of 816 kg ha^{-1} across 45 environments with different soil textures after a broadcast application of gypsum shortly after planting. Rates of S used were 0, 11, 22, and 44 kg ha^{-1}. The average yields increased on fine-textured and coarse-textured soil groups by 941 and 1757 kg ha^{-1}, respectively. This research supports the accepted theory that course-textured soils are typically more at risk for S deficiency due to leaching potential and, therefore, more responsive on average than fine-textured soils to S fertilization. Research by Sawyer et al. (2011) also supports the viability of gypsum as a spring-applied broadcast fertilizer.

Halley and Deibert (1996) conducted an experiment in Rock Lake, ND to determine the impact of landscape position on S deficiency, and canola (*Brassica napus* L.) response to S fertilization at different rates and in different forms. The study showed that a Buse soil (fine-loamy, mixed, superactive, frigid Typic Calciudolls) on a hilltop landscape position would have consistently lower yields than a Barnes soil (fine-loamy, mixed, superactive, frigid Calcic Hapludolls) on a slope landscape position and that both would yield lower than a Svea soil (fine-loamy, mixed, superactive, frigid Pachic Hapludolls) on a footslope landscape position. Ammonium sulfate fertilization at rates of 22 and 44 kg S ha^{-1} increased yields significantly on each soil and landscape position. The added S also improved yield so the average yield for each S rate was similar regardless of landscape position.

DeSutter et al. (2011) reported about a 2008 and 2009 study conducted near Langdon, ND that the application of S in the form of flue gas desulfurization gypsum and N in the form of urea at rates of 33.6 kg S ha^{-1} and 30 kg N ha^{-1} in canola significantly increased yield (~50%) over N applied at a rate of 30 kg N ha^{-1}. This apparent relationship between N and S has been analyzed in other rapeseed (*Brassica napus* L.) species as well (Kaur et al. 2011), and it suggests that the availability of S impacts N uptake and assimilation and, ultimately, seed yield. This potential "synergism" between N and S has been noted in previous studies conducted in soybean as well (H. Kandel, personal communication, 2015; De Wit, 1992).

Ammonium sulfate is a commonly used S fertilizer source. However, AMS also contains N in the form of ammonium and is far more soluble (754 g L^{-1}) than gypsum (2.05 g L^{-1})

(Gutierrez Boem et al., 2007). Gutierrez Boem et al. (2007) reported that the fertilizer efficiency of AMS and gypsum was not different despite the substantially different water solubility. The lack of difference in efficiency was attributed to a volume of water in the soil large enough to negate any difference due to solubility. A larger response to AMS compared to gypsum could be attributed directly to N fertilization. According to the "law of the optimum," N supply could increase S uptake or utilization efficiency when both N and S are deficient (De Wit, 1992). Furthermore, both N and S have been shown to have positive effects on nodule number, root length, and leghemoglobin concentration in soybean (Sharma and Sharma, 2014).

It is noteworthy that soybean is deficient in the S-containing amino acids methionine and cysteine (Sharma and Sharma, 2014) considering the S-N relationship and the nutrient mobility in plants. Bellaloui et al. (2011) reported that S applied alone at a rate of 44.8 kg ha^{-1} or applied with 112 kg N ha^{-1} consistently increased seed protein and oleic acid concentrations but decreased oil and linoleic acid concentration compared with the control. According to these sources, utilizing common N and S fertilizers could influence soybean protein and quality parameters. However, it has also been reported that soybean seed protein requires a high mobilization of stored vegetative N (Sinclair and DeWitt, 1975; Shibles and Sundberg, 1998) and S (Anderson and Fitzgerald, 2001).

Gypsum application over time has provided agronomic benefits for some crops in some soils, but the economic benefits to farmers require further analysis (Smith et al., 2009). In addition, gypsum has a relatively low solubility (2.05 g L^{-1}), so form and application timing are both important considerations. The low water solubility of gypsum but high leaching potential of sulfate in select soils should especially be taken into consideration when using gypsum fertilizer with tile drainage for water management. Tile drainage can decrease the water content in the soil profile, reduce the amount of water available to solubilize gypsum, and transport sulfate to groundwater and watersheds.

Tile Drainage

There are many reasons for the installation of tile drainage in agricultural fields. Tile drainage can improve soil health, structure, and aeration and, therefore, enhance microbial activity (Gardner et al. 1994). In addition, it promotes higher spring soil temperatures, earlier planting and germination (Liefers and Rothwell, 1987), and also increases trafficability (Chieng et al., 1986). The use of heavy equipment on moist soils can result in soil compaction, a structure-damaging process (Wind, 1976). Excess moisture in the soil profile can limit oxygen (O_2) in soil pores. Plants in this anaerobic condition exhibit stress and, if the anaerobic conditions persist, can die. The initiation of stress-induced senescence may only take 2 or 3 d.

The growth stage of the plant when the soil becomes waterlogged also is important. Early in the growing season, a waterlogged soil may prevent germination, while a waterlogged soil may not have as drastic an effect on plant growth later in the season. The growth stage and length of time the soil is saturated both play an important role in plant stress response. Tile drainage can be an important water management tool, especially in eastern North Dakota and northwestern Minnesota where soils typically have a high water holding capacity and water table close to the soil surface.

Tile drainage is also an important factor in nutrient management. Waterlogged soils release increased amounts of nitrous oxide (N_2O), a particularly damaging greenhouse gas (McFarlane et al., 1992). The conversion of NO_3^--N to N gases such as N_2 and N_2O by soil bacteria (termed denitrification) represents an important N loss process in agricultural production, as well as a detriment to the environment and agricultural sustainability. Another important N loss process with regards to moisture is leaching. Leaching occurs when NO_3^- (a readily available form of N for plants) moves downward through the profile with water. The soil water may eventually reach the ground water and possibly fresh water bodies such as rivers and streams. This can be especially detrimental to the environment, because excess nitrate (NO_3^-) in fresh water may lead to hypoxic conditions.

Hypoxia is a condition of low oxygen, which damages aquatic life. It is especially prevalent in the Gulf of Mexico where many tributaries flow from agricultural areas in North America. Kladivko et al. (2004) reported that NO_3^- leaching was significantly influenced by tile drainage design, N inputs, crop rotation, and management practices. In a separate 4-y study focused on different tile drainage systems, crop rotations, and N fertilizer rates on a Brookston clay loam soil in Ontario, Drury et al. (2009) reported that all measurements revealed N concentrations which exceeded the long-term aquatic life limit of 4.7 mg N L^{-1}, regardless of system, crop, or fertilizer rate. This indicates that current practices in the Mississippi watershed could potentially be hazardous to aquatic species and the environment. Crop rotation, tile drainage system design, management of the water table, and future fertilization practices will be important to mitigate NO_3^- losses in agricultural areas and reduce the severity of hypoxia.

Subsurface tile drainage has also proved to decrease soil salinity (Ghumman et al., 2011) as soluble salts leach from the profile in gravitational water when the water table is lowered. This benefit would be further enhanced by the addition of gypsum to sodic soils; soils affected by high concentrations sodium (Na^+). High Na^+ concentrations on soil exchange sites can lead to soil processes such as slaking, swelling, clay dispersion, crusting, clogging of soil pores, and harmful soil structural changes, which are all very undesirable for agricultural production (DeSutter and Cihacek, 2009). As gypsum is added to soil and solubilized, calcium (Ca^{2+}) replaces Na^+ on exchange sites and the Na^+ is leached from the root zone of the soil profile, leaving SO_4^- available for plant uptake. This process can take decades, but long-term gypsum application along with subsurface tile drainage may be utilized to remediate sodic soils.

Increased demand for soybean and potential for higher yielding varieties supports the need for further research into soybean production and management practices, including fertility management. The emphasis of this research is focused on understanding the relationship between N and S and the impact of the two nutrients on soybean yield and plant and seed qualities. The potential yield and quality benefits associated with N and S fertilization have been noticed in several experiments in soybean and other crops (DeSutter et al., 2011; Naeve and Shibles, 2005; Thurgood, 2014), although the benefits to soybean in North Dakota are undetermined. Therefore, the objectives of this study were to determine the effect of N and S fertilization on soybean plant density, vigor, greenness and height, seed yield, test weight, protein and oil content, nodulation, vegetation, and root growth. Results will enhance the understanding of best management practices with regards to soybean fertilization in North Dakota. Specifically, the study will provide research-based results to support future economic and environmentally conscious fertilizer decisions to improve the sustainability of soybean production in North Dakota. This research will be used to determine if practical, farm-level fertilization decisions can improve yield or other quality characteristics of soybean varieties grown in North Dakota.

MATERIALS AND METHODS

Experimental Design and Treatments

Experiments were conducted in five environments in 2015 and were repeated in 2016. All experiments were conducted in North Dakota. For all environments the experiment was designed as a randomized complete block (RCBD) with a factorial arrangement of varieties, N, and S. There were four replications at Fargo and three replications for the other environments. Per environment each replication consisted of 18 experimental units containing one of two soybean varieties to which a fertilizer treatment was applied. The experimental unit was four rows wide with 0.354 m row spacing. The experimental unit size was 1.52 x 7.62 m. The main location was North Dakota State University's (NDSU) NW22 experiment station (46.932N, -96.859W) located near Fargo. This site had two separate environments; it included controlled tile drainage (CTD) (with 7.6 m tile spacing) and naturally drained (NAD) ground.

The soil at the NW22 location is a complex of Fargo (Fine, smectitic, frigid Typic Epiaquerts) and Ryan (Fine, smectitic, frigid Typic Natraquerts) silty clay (USDA, 2015a) (Table 1). Both are naturally poorly or very poorly drained and slowly permeable. The soil without tile drainage has a relatively low crop productivity index rating (67) and is not considered prime farmland (USDA, 2015a). The soil's natural fertility is somewhat limited, and the nutrient leaching is rated as very limited. The parent material of the soil is clayey glaciolacustrine deposits (USDA, 2015a). Soil samples were taken prior to the fertilizer application and analyzed at the NDSU Soil Testing Laboratory (Table 2). The previous crop at NW22 was wheat [*Triticum aestivum* (L.) emend. Thell.] in 2015 and corn in 2016. Measurements were taken throughout the growing season with a Solinst Model 101 Water Level Meter (Solinst, Georgetown, ON, Canada) to determine the depth of the water table at NW22. Precipitation accumulated between dates of measurement in 2015 and 2016 was added and is shown in Figure 1 and Figure 2, respectively. In the CTD environment, a control structure was used to control drainage and maintain the water table at a depth of approximately 105 cm.

In 2015, the experiment was replicated in Richland, Ransom, and Sargent counties. Sites were located near Mooreton, Lisbon, and Gwinner, respectively. In 2016, the experiment was replicated in Ransom, Sargent, and Steele counties. Sites were located near Lisbon, Gwinner, and Hope, respectively.

The soil at the Richland County site (46.261N, -96.816W) is a mix between a Fargo silty clay loam (Fine, smectitic, frigid Typic Epiaquerts) and a Fargo-Enloe complex (Fine, smectitic, frigid Typic Epiaquerts) (Table 1). The natural fertility of the soil is rated as somewhat limited, while the nutrient leaching is very limited. The very limited amount of leaching is attributed to a high water table and high clay content that provides sites for nutrients to adhere to. The soil has a high crop productivity index rating (85) and is considered to be prime farmland. The parent material of the soil is clayey glaciolacustrine deposits (USDA, 2015a). The previous crop at the 2015 Richland County site was sugarbeet (*Beta vulgaris* L.).

The soil at the Sargent County site (46.212N, -97.654W) is a Hamerly-Tonka complex (Fine-loamy, mixed, superactive, frigid Aeric Calciaquolls) (Table 1). The natural soil fertility and nutrient leaching are both classified as very limited. The soil has only a moderate crop productivity index rating (64) but is considered to be prime farmland if properly drained because it also is classified as poorly drained. The soil parent material is fine-loamy till (USDA, 2015a). The previous crop at the Sargent County location was corn both years.

The soil at the Ransom County site (46.443N, -97.834W) is a Barnes-Svea loam soil (Fine-loamy, mixed, superactive, frigid Calcic Hapludolls) (Table 1). The soil's natural fertility is not limited and the nutrient leaching potential is somewhat limited. The soil has a crop productivity index rating of 85 and is considered to be prime farmland. The natural drainage class of the soil is well drained and the parent material is fine-loamy till (USDA, 2015a). Corn was the previous crop at the Ransom County site both years.

The soil at the Steele County site (47.440N, -97.653W) is a Brantford loam soil (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, frigid Typic Hapludolls) (Table 1). The soil's natural fertility and nutrient leaching potential are somewhat limited. The soil is well drained and the parent material is shaly glaciofluvial deposits. The soil's crop productivity rating is 48 and is not considered prime farmland (USDA, 2015a). The previous crop at the Steele County site was conventional soybean. All soils in the experiment were considered low response soils due to high clay concentration and low leaching potential.

Table 1. Soil series, taxonomy, and previous crop at Fargo, Ransom, Richland, Sargent, and Steele Counties, ND, in 2015 and 2016.

Location	Soil Series[†]	Soil Taxonomy[†]	Prev. Crop[†]	PI[†]
Fargo	Fargo-Ryan	Fine, smectitic, frigid Typic Epiaquerts	Wheat/Corn	67
		Fine, smectitic, frigid Typic Natraquerts		
Ransom	Barnes-Svea	Fine-loamy, mixed, superactive, frigid Calcic Hapludolls	Corn	85
		Fine-loamy, mixed, superactive, frigid Pachic Hapludolls		
Richland	Fargo-Enloe	Fine, smectitic, frigid Typic Epiaquerts	Sugarbeet	85
		Fine, smectitic, frigid Argiaquic Argialbolls		
Sargent	Hamerly-Tonka	Fine-loamy, mixed, superactive, frigid Aeric Calciaquolls	Corn	64
		Fine, smectitic, frigid Argiaquic Argialbolls		
Steele	Brantford	Fine-loamy over sandy or sandy-skeletal, mixed, superactive, frigid Typic Hapludolls	Soybean	48

[†]Soil data obtained from (USDA, 2015a). Prev. Crop = Previous Crop, PI = Crop Productivity Index.

Table 2. Soil test results at all environments in 2015 and 2016.

Year	Location	Depth	NO_3-N	P	K	SO_4-S	pH	OM†
		cm	kg ha^{-1}	mg kg^{-1}		kg ha^{-1}		%
2015	Ransom	0-30	16	6	180	511	7.4	3.2
		30-61	9	1	90	794	8.1	1.8
	Richland	0-30	9	3	240	25	7.8	4
		30-61	7	1	250	96	8.1	3.1
	Sargent	0-30	4	6	180	34	7.5	4.3
		30-61	4	6	215	316	7.7	3.1
	NW22NAD†	0-30	17	12	425	‡	8.1	4.7
		30-61	3	5	440	‡	8.2	2.9
	NW22CTD†	0-30	19	14	428	‡	8	4.8
		30-61	3	4	329	‡	8.3	3
2016	Ransom	0-30	40	11	146	20	7	4
		30-61	38	4	114	43	7.9	1.8
	Sargent	0-30	53	12	174	258	7.6	4.6
		30-61	138	9	168	652	7.5	3.3
	Steele	0-30	29	12	126	13	6.8	2.9
		30-61	27	6	102	20	7.8	1.6
	NW22NAD†	0-15	33	14	634	‡	7.7	5.6
		15-61	39	6	311	‡	8.1	3.7
	NW22CTD†	0-15	29	17	544	‡	7.6	5.9
		15-61	46	8	288	‡	7.9	4

†OM = Organic matter. NAD = naturally drained, CTD = controlled tile drained.
‡Value for this soil test unavailable.

Two soybean varieties were used: Peterson Farm Seed 15R07 (PFS 15R07) and Proseed 30-80 (PS 30-80). The two soybean varieties are similar in maturity rating, but significantly different in IDC scores determined in 2014 variety trials (Helms, 2014) and different in plant architecture (Table 3). Consideration was given to IDC during variety selection due to a known interaction between high soil NO_3^- levels and an increased probability of IDC (Franzen, 2013). Increased levels of IDC are considered to decrease photosynthesis and, therefore, overall yield. Both varieties are glyphosate-resistant and rated as resistant to soybean cyst nematode (SCN).

Table 3. Soybean varieties used and descriptive features.

Variety	Company	Maturity	IDC†	SCN‡	Canopy	Height
15R07	Peterson Seed	0.7	1.8	R‡	Bushy	Medium
30-80	Proseed	0.8	2.4	R	Medium	Medium Tall

† IDC = iron deficiency chlorosis. IDC scored on 1-5 scale (1=green, 5=yellow).
‡SCN = soybean cyst nematode. R=resistant.

Both varieties' seeds were treated with ApronMaxx RTA fungicide (Mefenoxam [(R)-2-[(2,6-dimethylphenyl) methoxyacetylamino]propionic acid methyl ester] and Fludioxonil [4-(2,2-Difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile]) (Syngenta Crop Protection, LLC, Basel, Switzerland) prior to planting at a rate of 3.26 mL kg^{-1} of seed in order to limit early disease pressure. This seed treatment provides protection against damping-off and seed rots due to *Pythium sylvaticum*, *Phytophthora sojae*, *Rhizoctonia solani*, and several *Fusarium* species. In addition, the seed treatment suppresses seed borne *Sclerotinia sclerotiorum* and *Phomopsis longicolla*. Both varieties were also inoculated with Vault SP (*Bradyrhizobium japonicum*) inoculum (BASF, Ludwigshafen, Germany) at a rate of 1.8 g kg^{-1} the day of planting to encourage nodulation.

A ragdoll germination test was conducted using a moist paper towel at room temperature to find a germination percentage. Planting rates were adjusted based on the germination test to achieve the targeted plant density of 370 500 plants ha^{-1}. A Mettler Toledo XS6001S scale (Mettler-Toledo, LLC, Columbus, OH) was used to weigh out the amounts of soybean seed required for each plot. The seed for each plot was packaged in envelopes prior to planting.

Soil samples were taken at each site prior to fertilizer application (Table 2). Nitrogen was applied in the form of urea (46-0-0) and S was applied in the form of gypsum (0-0-0-18). Gypsum was chosen as an S source instead of AMS because AMS also contains N, and it was essential to the experimental design to isolate S as a fertilizer treatment. The gypsum used was

SuperCal SO4; pelletized agricultural gypsum obtained from Calcium Products, Inc. (Calcium Products, Inc., Ames, IA) with a guaranteed chemical analysis of 17% S. Treatments were applied by hand the day of planting to simulate broadcast application and incorporated with an S-tine cultivator to a depth of approximately 2.5 to 3.5 cm. In 2016, the Fargo and Ransom County environments were fertilized 1 and 3 d before planting, respectively, due to weather conditions.

There were 9 fertilizer treatments that included all combinations of 3 N rates and 3 S rates. Rates were selected based on those used by previous researchers. Fertilizer treatments are summarized in Table 4.

Table 4. Fertilizer treatments used in the factorial study.

Fertilizer	kg N ha^{-1}	kg S ha^{-1}
Check	0	0
Urea	28	0
Urea	56	0
Urea+gypsum	28	11.2
Urea+gypsum	28	22.4
Urea+gypsum	56	11.2
Urea+gypsum	56	22.4
Gypsum	0	11.2
Gypsum	0	22.4

The plots were planted with a Hege 1000 no-till planter (Hege Company, Waldenberg, Germany). Seeds were sown to a depth of approximately 0.03 m. Plant density was determined shortly after soybean emergence (VE) by counting all plants in 0.914 m of both of the inner 2 rows of each plot.

Weeds were controlled using Roundup WeatherMAX (a.i. 48.8% glyphosate, N-(phosphonomethyl) glycine, in the form of its potassium salt) (Monsanto Co., St. Louis, MO). The herbicide was applied using TeeJet 8001 XR nozzles at a rate of 1.6 L ha^{-1} in 94 L ha^{-1} water and a spray pressure of 200 kPa. SelectMax (12.6% (E)-2-[1-[[(3-chloro-2-

propenyl)oxy]imino]propyl]- 5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) (Valent U.S.A. Corporation, Walnut Creek, CA) was applied at a rate of 0.8 L ha^{-1} along with the Roundup WeatherMAX at all sites that produced corn the previous season. Along with chemical application, weeds were removed from plots by hand and alleys were kept weed free by rototilling.

Disease and insect pressure was monitored throughout the season and foliar insecticide was applied as needed. Cobalt Advanced (a.i. 28.12% chlorpyfiros: O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate and 1.44% *Lambda*-cyhalothrin) (Dow AgroSciences, Indianapolis, IN) was applied in 2015 to control soybean aphid (*Aphis glycines* Matsumura) pressure at NW22 and the Sargent and Ransom County locations. The insecticide was applied at a rate of 1.9 L ha^{-1} at all locations. Mustang Maxx (a.i. 9.15% S-Cyano(3-phenoxyphenyl)methyl (+/-)-cis/trans-3-(2,2-dichloethenyl)-2,2-dimethylcyclopropanecarboxylate) (FMC Corporation, Philadelphia, PA) was applied at NW22 in 2016 at a rate of 1.75 L ha^{-1} to suppress grasshopper (Orthoptera: Acrididae) pressure.

Each plot was scored visually for vigor and greenness at the V4 growth stage (Fehr et al., 1971). Vigor was scored on a scale of 1-9 (9 being most vigorous), and greenness was scored visually on a scale of 1-5 (1 being most green). Vigor was also measured by using a handheld GreenSeeker crop sensing system (Trimble, Sunnyvale, CA) at R4. The sensor emits bursts of red and infrared light and measures the amount of each type of light that is reflected back to the instrument and displays the percent of the target area containing green vegetative tissue. The instrument displays the measurement in terms of a normalized difference vegetation index (NDVI) ranging from 0.00 to 0.99 with 0.99 being the most vigorous (99% target area containing green vegetative tissue). Although the GreenSeeker detects green vegetation, the NDVI score is mostly a measure of vigor and crop canopy and does not necessarily represent greenness. Plant height was measured before harvest at R8 by averaging the height from the soil level to the top node of 5 random soybean plants in each plot.

The plots were harvested using a Wintersteiger Classic plot combine (Wintersteiger Ag, Ried, Austria) after physiological maturity at proper harvest moisture content. The samples were cleaned using a Clipper seed cleaner (Ferrell-Ross, Bluffton, IN) and the whole plot samples were then weighed on a Mettler Toledo XS6001S scale (Mettler-Toledo, LLC, Columbus, OH) to determine yield. Oil and protein content was obtained using a Perten Instruments DA 7250 NIR analyzer (Perten Instruments, Inc., Springfield, IL), and moisture and test weight were determined using a GAC 2100 moisture tester (DICKEY-John Corp., Minneapolis, MN). Observations were corrected to 13% moisture content. The dates of field observations, measurements, and applications are presented in Table 5.

Table 5. Dates of important measurements and field operations at Fargo, Ransom, Richland, Sargent, and Steele County, ND, in 2015 and 2016.

Measurement/Operation	Fargo	Ransom	Richland	Sargent	Steele
	---------------------------------Date---------------------------------				
			2015		
Soil test/fertilize/plant	4 May	5 May	5 May	5 May	-
Fertilize observation plots	5 June	4 June	4 June	4 June	-
First herbicide application	10 June	12 June	12 June	12 June	-
Second herbicide application	7 July	7 July	7 July	7 July	-
Insecticide	24 July	27 Aug	-	27 Aug	-
Stand count	11 June	15 June	15 June	15 June	-
Vigor/greenness score	23 June	24 June	24 June	24 June	-
First root dig	1 July	2 July	2 July	2 July	-
Second root dig	29 July	28 July	28 July	28 July	-
GreenSeeker	4 Aug	5 Aug	5 Aug	5 Aug	-
Height measurement	11 Sep	15 Sep	10 Sep	15 Sep	-
Harvest	22 Sep	24 Sep	24 Sep	24 Sep	-
			2016		
Soil test/fertilize	5 May	9 May	-	9 May	16 May
Plant	6 May	12 May	-	9 May	16 May
Fertilize observation plots	1 June	2 June	-	2 June	3 June
First herbicide application	10 June	2 June	-	2 June	10 June
Second herbicide application	21 July	20 July	-	20 July	18 July
Insecticide	7 Aug	-	-	-	-
Stand count	13 June	16 June	-	16 June	14 June
Vigor score	23 June	16 June	-	22 June	22 June
Greenness score	13 June	16 June	-	22 June	14 June
First root dig	7/8 July†	29 June	-	29 June	30 June
Second root dig	1 Aug	29 July	-	29 July	28 July
GreenSeeker	15 July	14 July	-	14 July	15 July
Height measurement	14 Sep	12 Sep	-	12 Sep	13 Sep
Harvest	27 Sep	28 Sep	-	28 Sep	29 Sep

†Reps 3 and 4 were done one day later due to rain.

Weather data for the NW22 location was collected from the North Dakota Agricultural Weather Network (NDAWN) station in Fargo (46.897N, -96.812W). A WatchDog 2000 series weather station (Spectrum Technologies, Aurora, IL) was also placed at the NW22 location to collect weather data including precipitation and soil temperature. Water table levels at NW22 were measured throughout the growing season with a water table level meter (Model 101, Solinst, Georgetown, ON, Canada). Weather data for the Richland, Sargent, Ransom, and Steele County environments was collected from the NDAWN weather station located near Wahpeton (46.355N, -96.666W), Oakes (46.074N, -98.093W), Lisbon (46.445N, -97.721W), and Finley (47.526N, -97.847W), respectively.

Statistical analysis was conducted using standard procedures according to Carmer et al. (1989) for a randomized complete block design with a three-factor factorial arrangement. All dependent variables were analyzed using a general linear model (PROC GLM) on SAS 9.3 (SAS Institute Inc., Cary, NC). Variety and N and S fertilizer treatments were considered fixed variables, and environment was considered a random variable. The data was analyzed for each environment in 2015 and 2016 separately. After confirming homogeneity of variance according to Bartlett's Chi-Square test, data was then combined and analyzed over all environments in the study. Treatment means were separated using Fisher's protected least significant difference (LSD) at the 95% level of confidence (α=0.05). Different LSDs were calculated for each individual environment and the combined analysis. To avoid a Type I error, LSDs were only calculated if there was significance at the 95% level of confidence. The ANOVA table (Table 6) shows degrees of freedom (df) for each source of variation in the factorial study for the CTD environment and the NAD environment, each with four replications. Other environments were similar except there were three replications per environment. It is important to note that df for some sources of variation (SOV) are different for various environments because NW22 environments had four reps while other environments had three reps.

Table 6. ANOVA of factorial study at Fargo, ND, NW22 environments.

SOV†	df‡ equation	Df
Rep§	(r-1)	3
Variety(VT)	(VT-1)	1
Nitrogen (N)	(N-1)	2
Sulfur (S)	(S-1)	2
VTxN	(VT-1)(N-1)	2
VTxS	(VT-1)(S-1)	2
NxS	(N-1)(S-1)	4
VTxNxS	(VT-1)(N-1)(S-1)	4
Error	(r-1)[(VT)(N)(S)-1)]	51
Total	r(VT)(N)(S)-1	71

†SOV=Source of variation.
‡df=Degrees of freedom.
§3 replications at environments outside NW22.

Nodulation Study

This experiment was conducted at all environments previously described. There were four replications at Fargo in the NW22CTD and NW22NAD environment. Each county environment had two replications for a total of 14 replications across five environments in 2015 and duplicated in 2016. At each environment, 12 observational mini plots were included in each rep: two varieties x six fertilizer treatments. These plots were included for the purpose of destructive sampling and collecting data on nodule number and size, as well as above ground plant and root biomass. This sampling was done to determine the effect of N and S fertilization on plant roots and nodules. No yield data was obtained from observational plots. Each whole plot (1.52 x 7.62 m) for this experiment was planted with one variety and split into three subplots. Each subplot received different rates of N and S. The subplots were made by dividing each whole plot equally into three subplots (1.52 x 1.83 m) with 1.07 m of each end of the whole plot as a border.

Treatments were randomized and applied to observational plots shortly after VE by using a hoe to create a small furrow approximately 2.5 to 3.5 cm deep next to the plant row and applying fertilizer to the furrow by hand and then covering the furrow with soil. Plants from 1 m of one of the inner 2 rows in each observational portion were removed from the plot using a shovel. The number of plants taken from each subplot varied between eight and twenty-two depending on the plant density of the randomly selected area. The roots of each plant were then rinsed in a water bucket to remove soil particles and the nodules on each plant were counted and rated for size. The nodule size on each plant was rated in percentages of small (<1 mm), medium (1-4 mm), and large (>4 mm). Size ratings and nodule counts were averaged over observed plants and expressed as weighted averages. Plant samples from plots were collected in cloth bags and dried for 10 d at approximately 50 ^{o}C. After drying, the samples were weighed. The roots were then removed from the samples, and the samples were reweighed without roots to determine the average plant mass, shoot mass, and root mass per plant within each treatment. This process was done at a late vegetative stage (approximately V4) and again at the R4 stage. The treatments applied to each variety are summarized in Table 7.

Table 7. Fertilizer treatments applied to observational plots.

Fertilizer	kg N ha^{-1}	kg S ha^{-1}
Check	0	0
Urea	140	0
Urea	280	0
Urea+gypsum	140	112
Urea+gypsum	280	112
Gypsum	0	112

Statistical analysis was conducted using standard procedures according to Carmer et al. (1989) for a randomized complete block design with a two-factor factorial arrangement within a split plot, with variety being the main plot. All dependent variables were analyzed with a mixed model (PROC MIXED) on SAS 9.3 (SAS Institute Inc., Cary, NC). Variety and fertilizer treatment were considered fixed variables, and environment was considered a random variable. The data were analyzed for each environment in 2015 and 2016 separately. After confirming homogeneity of variance according to Bartlett's Chi-Square test, data was then combined and analyzed over all environments and years of the study. Treatment means were separated using Fisher's protected least significant difference (LSD) at the 95% level of confidence (α=0.05). Different LSDs were calculated for each individual environment and the combined analysis. To avoid a Type I error, LSDs were only calculated if the treatment F-test was significant at the 95% level of confidence. The ANOVA table below (Table 8) shows degrees of freedom for each source of variation in the nodulation study at NW22.

Table 8. ANOVA of nodulation study conducted at Fargo, ND.

SOV†	df‡ equation	df‡
Rep	(r-1)	3§
Variety (VT)	(VT-1)	1
Error (a)	(VT-1)(r-1)	3§
Nitrogen (N)	(N-1)	2
VTxN	(VT-1)(N-1)	2
Sulfur (S)	(S-1)	1
VTxS	(VT-1)(S-1)	1
NxS	(N-1)(S-1)	2
VTxNxS	(VT-1)(N-1)(S-1)	2
Residual Error	(VT)(N)(r-1)(S-1)	18
Total		35

†SOV=Source of variation.
‡df = degrees of freedom.
§1 degree of freedom and 2 replications at locations outside NW22.

RESULTS AND DISCUSSION

Weather Data

In 2015, the environments at NW22 experienced above normal amounts of rain in May immediately after planting (Table 9). Precipitation in 2015 was lower than the normal (30-year average period from 1981-2010) in June, August, and September and average in July. Air temperature was normal in each month besides September, which was warmer than normal. The 2016 growing season was much more similar to normal with the exception of unusually high rainfall in July (Table 9). Weather events also impacted the environments at NW22 in 2016. Severe thunderstorms occurred on 4 July, 7 July, and 27 August that included quarter sized hail, golf ball sized hail, and quarter sized hail, respectively. The soybean plants were damaged uniformly throughout the experimental area.

Table 9. Air temp and precipitation for each month during the growing season in 2015 and 2016, Fargo, ND.

	Max Air Temp			Min Air Temp			Precipitation		
Month	2015	2016	Norm.†	2015	2016	Norm.†	2015	2016	Norm.†
	------°C------			------°C------			------mm------		
May	19	22	21	7	9	7	200	33	71
June	26	26	25	14	14	13	64	69	99
July	28	28	28	17	17	16	71	132	71
Aug	27	27	27	14	15	14	54	48	65
Sept	25	22	22	12	11	8	41	80	65
Total							430	362	371

†Norm. represents a 30-yr average from 1981-2010. Data obtained from North Dakota Agricultural Weather Network.

In Ransom County in 2015, early season precipitation was above normal in May following planting and lower than normal in July, August, and September (Table 10). Average air temperatures in 2015 were similar to normal for each month aside from September, which was slightly above normal. There was also very little rainfall in September in 2015. Conditions in 2016 were similar to normal except very low precipitation in June and very high precipitation late in the growing season in August (Table 10). The Ransom County site also experienced a severe thunderstorm event with quarter-sized hail 7 September 2016. The soybean plants were uniformly damaged because the storm duration and intensity were consistent over the experimental area.

Table 10. Air temp and precipitation for each month during the growing season in 2015 and 2016, Ransom County, ND.

	Max Air Temp			Min Air Temp			Precipitation		
Month	2015	2016	Norm.†	2015	2016	Norm.†	2015	2016	Norm.†
	------°C------			------°C------			------mm------		
May	19	23	21	6	8	7	154	67	75
June	26	27	26	14	14	13	91	39	80
July	29	29	29	16	16	16	35	81	80
Aug	28	28	28	13	14	14	43	128	54
Sept	26	23	22	11	10	8	7	44	65
Total							330	359	354

†Norm. represents a 30-yr average from 1981-2010. Data obtained from North Dakota Agricultural Weather Network.

Total precipitation in 2015 was similar to normal in Richland County (Table 11). Total rainfall was much higher than normal in May, however, and much lower than normal in August and September. Monthly air temperatures were near normal, except for lower average air temperature in May and above average temperature in September.

Table 11. Air temp and precipitation for each month in the 2015 growing season, Richland County, ND.

	Max Air Temp		Min Air Temp		Precipitation	
Month	2015	Norm.†	2015	Norm.†	2015	Norm.†
	------°C------		------°C------		------mm------	
May	19	22	6	8	153	81
June	26	26	13	13	62	83
July	28	29	15	16	70	81
Aug	27	28	13	14	26	62
Sept	26	22	11	9	11	74
Total					322	381

†Norm. represents a 30-yr average from 1981-2010. Data obtained from North Dakota Agricultural Weather Network.

Precipitation in 2015 near the Sargent County location was substantially higher in May and lower than normal in the subsequent four months, especially in July (Table 12). Average air temperature was normal throughout the growing season, although the average monthly air temperature in September was higher than normal. The 2016 growing season was dry early in the season, in May and June, but above average precipitation was experienced late in the growing season in July and August (Table 12). The Sargent County site also experienced a severe thunderstorm with quarter-sized hail 7 September 2016. The soybean plants were uniformly damaged throughout the experimental area.

Table 12. Air temp and precipitation for each month during the growing season in 2015 and 2016, Sargent County, ND.

	Max Air Temp			Min Air Temp			Precipitation		
Month	2015	2016	Norm.†	2015	2016	Norm.†	2015	2016	Norm.†
	------ºC------			------ºC------			------mm------		
May	18	22	21	6	7	6	152	51	75
June	26	28	25	13	14	12	81	66	96
July	29	29	28	14	16	14	20	140	82
Aug	27	28	28	13	15	13	33	129	60
Sept	25	24	22	9	11	7	20	26	64
Total							306	412	377

†Norm. represents a 30-yr average from 1981-2010. Data obtained from North Dakota Agricultural Weather Network.

Temperatures were near normal in the Steele County environment in 2016 (Table 13). Total precipitation in the first two months of the growing season was also similar to normal. However, rainfall was higher than normal in July and September.

The highest yielding environments in the study were Ransom County and Steele County in 2016 with average yields of 4488 kg ha^{-1} and 4152 kg ha^{-1}, respectively.

Table 13. Air temp and precipitation for each month in the 2016 growing season, Steele County, ND.

	Max Air Temp		Min Air Temp		Precipitation	
Month	2016	Norm.†	2016	Norm.†	2016	Norm.†
	------°C------		------°C------		------mm------	
May	21	19	7	6	70	68
June	25	23	12	13	93	95
July	26	26	15	15	112	79
Aug	26	26	13	13	54	69
Sept	21	20	9	10	73	46
Total					402	357

†Norm. represents a 30-yr average from 1981-2010. Data obtained from North Dakota Agricultural Weather Network.

Water Table

The water table level at NW22 was different between years and was influenced by rainfall (Figures 1 and 2). As indicated in Figure 1, tile drainage practice had an impact on water table depth at times of high precipitation in 2015. The depth of the water table was significantly different ($p \leq 0.05$) between the naturally drained (NAD) and controlled tile drained (CTD) environments for all measurement dates in 2015 from 21 May to 6 July (Figure 1). In 2016, however, water table depth was relatively consistent between tile drainage practices. Both NAD and CTD environments responded similarly to precipitation throughout both growing seasons by fluctuating in response to rainfall events. However, the water table in the NAD environment was influenced more by precipitation events than the water table in the CTD environment. The water table depth fluctuated more in response to precipitation in the NAD environment and remained at a more consistent level in the CTD environment.

This trend was more apparent in 2015 than in 2016 for two main reasons. First, drainage was taking place at the beginning of the 2015 growing season due to large amounts of early precipitation. In addition, early spring rains in 2015 allowed the water table to remain at a shallow depth in both environments. The water table in 2015 was higher in general compared to

2016, possibly due to a very dry winter preceding the 2016 growing season. Additionally, the preceding dry winter and hot, dry growing conditions caused large cracks (approximately 7-9 cm) to form and open at the soil surface in 2016. These macropores allowed for preferential flow to occur after precipitation as rainfall quickly flowed into and deeper into the soil profile via the macropores. Figure 2 shows that the water table level of the 2016 growing season never rose to even the lowest water table depth of the 2015 growing season. The mean soybean yield over both years for the NAD environment and CTD environment was 2909 kg ha^{-1} and 2949 kg ha^{-1}, respectively.

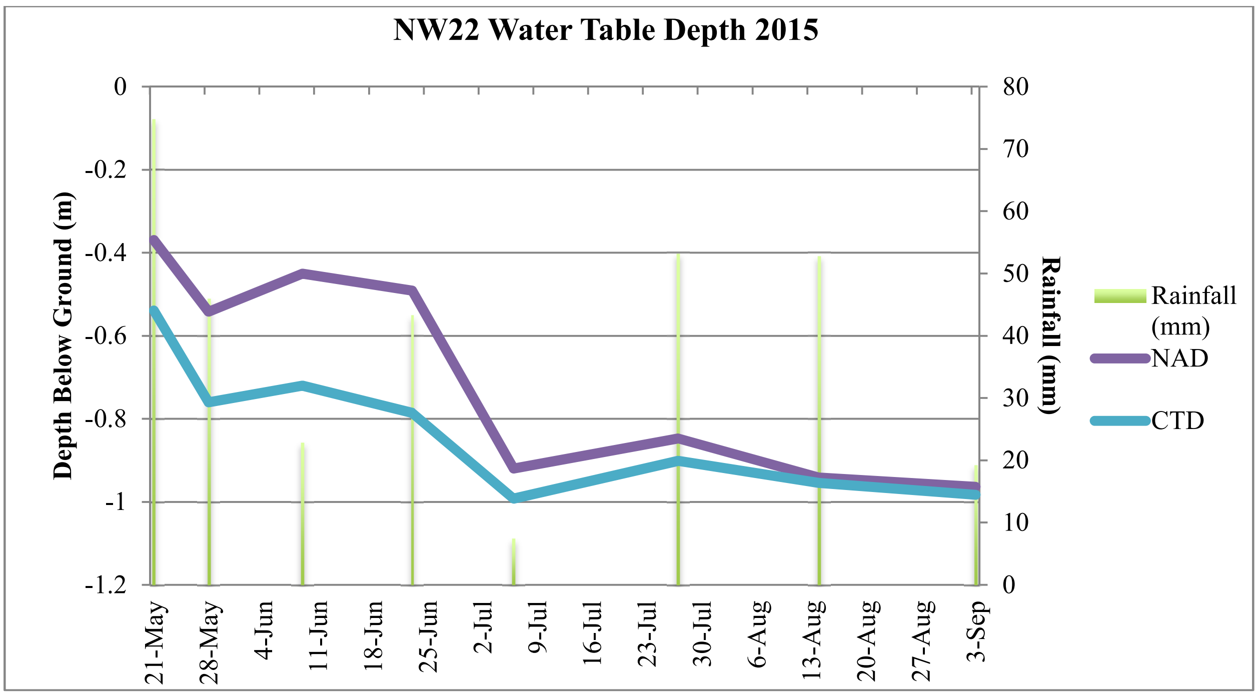

Figure 1. NW22 water table level and precipitation throughout the 2015 growing season in both the NAD (naturally drained) and CTD (controlled tile drained) environments.
†Water table depth was significantly different ($p \leq 0.05$) between NAD and CTD environments on each measurement date from 21 May 2015 to 27 July 2015.

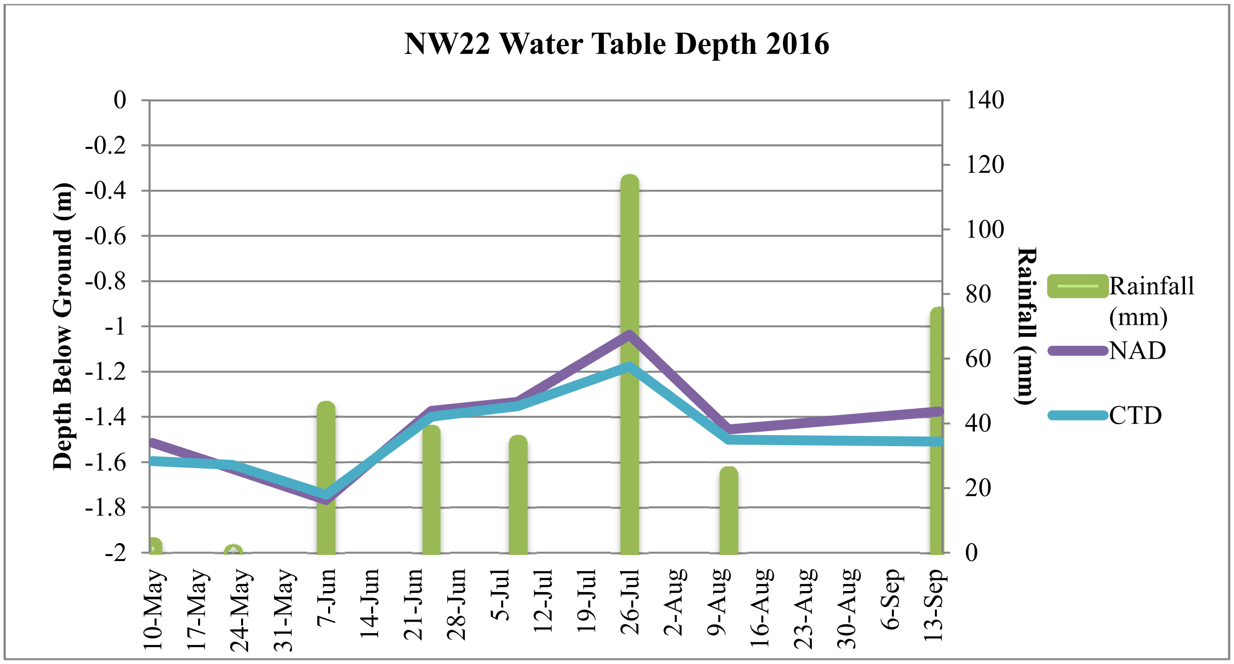

Figure 2. NW22 water table level and precipitation throughout the 2016 growing season in both the NAD (naturally drained) and CTD (controlled tile drained) environments.
†Water table depth was not significantly ($p \leq 0.05$) different between environments at any measurement dates in 2016.

Factorial Study

Residual mean squares for each environment were homogenous according to Bartlett's Chi-square and, therefore, environments were combined for analysis. Levels of significance for variety, treatment, and various interactions are presented in Table 14.

Table 14. Levels of significance for the ANOVA of agronomic traits for ten environments in 2015 and 2016.

SOV[†]	df	Density	Vigor	G	GS	Height	Yield	TW	Protein	Oil
Env [Environment]	9									
Rep(Env)	24									
VT [Variety]	1	ns	***	ns	ns	ns	ns	**	ns	ns
Env * VT	9	ns	ns	ns	ns	ns	ns	ns	ns	ns
N [Nitrogen]	2	**	*	*	**	**	**	ns	ns	ns
Env * N	18	ns	ns	*	ns	ns	*	ns	ns	ns
VT * N	2	ns	ns	ns	ns	ns	ns	ns	ns	ns
S [Sulfur]	2	ns	ns	ns	ns	ns	ns	ns	*	ns
Env * S	18	ns	ns	ns	ns	ns	ns	ns	ns	ns
VT * S	2	*	ns	ns	ns	ns	ns	ns	ns	ns
N * S	4	ns	ns	**	ns	ns	ns	ns	ns	ns
VT * N * S	4	ns	ns	ns	ns	ns	ns	ns	ns	ns
Residual Error	408									

ns, *, **, *** = not significant, significant at (p≤0.05), (p≤0.01), and (p≤0.001), respectively.
[†]SOV = source of variation, df = degrees of freedom, G = visual greenness score, GS = GreenSeeker NDVI value, TW = test weight.

Variety

Vigor and test weight were significantly different between varieties (Table 15). The variety PS 30-80 was more vigorous than PFS 15R07 in all environments except for the 2016 NW22 CTD environment, in which the vigor scores between varieties were similar. This is attributed mostly to lower than normal precipitation in May before the date of vigor scoring (23 June). The lower than normal precipitation caused dry conditions, especially in the CTD environment. The dry conditions in the environment limited overall plant growth and significant vigor difference between varieties was not observed. Across all environments, the average vigor of PS 30-80 and PFS 15R07 was 6.0 and 5.1, respectively. The observed differences in vigor and test weight between varieties are expected due to genotypic differences between varieties (Helms, 2014).

Nitrogen

Nitrogen significantly influenced a number of dependent variables measured in the study including plant density, vigor, greenness score, GreenSeeker NDVI value, height and yield (Table 15).

Table 15. Nitrogen effect on plant density, vigor, visual greenness score, GreenSeeker value, plant height, and yield across all environments and both years of the study.

Rate	Density	Vigor†	G	GS	Height	Yield
kg N ha^{-1}	plants ha^{-1}	(1-9)	(1-5)	(0-0.99)	cm	kg ha^{-1}
0	491000a	5.2a	2.2a	0.842a	73.0a	3260a
28	487000a	5.6ab	1.9ab	0.848a	74.4 b	3320 b
56	465000 b	5.9 b	1.8 b	0.852 b	75.0 b	3380 b

†Vigor was scored visually on a scale of 1-9 (9 = most vigorous), G = greenness scored visually on 1-5 scale (1 = most green), GS = GreenSeeker value (NDVI measured on scale of 0 – 0.99 with 0.99 being most vigorous).
‡Means in a column followed by the same letter are not significantly different at ($p \leq 0.05$), tested according to Carmer et al. (1989).

Plant density significantly decreased as N rate increased to 56 kg N ha^{-1}. This may be attributed to ammonia volatilization toxicity of the urea fertilizer damaging seedlings early in the season. Urea is quickly hydrolysed to ammonia in the soil by urease enzyme, and the accumulation of ammonia can cause toxicity to germination and seedling growth (Bremner and Krogmeier, 1988; Buresh, 1987; Haden et al., 2011). Ammonia volatilization that inhibited seed germination and reduced plant density following urea application has been seen in wheat (Wan et al., 2016), corn (Creamer and Fox, 1980; Ouyang et al., 1998), and other crops (Bennett and Adams, 1970). These studies focused on understanding the effect of ammonia on germination were conducted in laboratory settings using solutions containing ammonia. Most toxicity is associated with in-furrow application of urea or application methods that involve close proximity to or direct contact with the seed. Broadcasted urea could cause toxicity and reduced establishment, especially when broadcast at one time instead of split applications and when environmental conditions are conducive including excess soil moisture. In addition, the use of slow-release N fertilizers or N fertilizers treated with urease inhibitors could mitigate damage due to toxicity. However, in this study high precipitation in many environments shortly after planting (Tables 9-13) may have caused the N fertilizer to solubilize and damage germinating seeds taking in water with toxic ammonia concentration. Specifically, the NW22 NAD and Sargent County environments in 2015 experienced significantly reduced stands following N application (Table 16). Both environments experienced unusually high precipitation after planting in May (Tables 9 and 12), which supports the possibility that plant density was reduced as the result of ammonia volatilization toxicity after urea application. This trend was not significant in any environment in 2016 (Table 16). The results of this study support the importance of appropriate fertilizer type, placement and timing of fertilizer application.

Table 16. Nitrogen effect on average plant density at each environment in 2015 and 2016.

Rate	Year	NW22 NAD	NW22 CTD	Ransom	Richland	Sargent	Steele
kg N ha^{-1}	2015	--	--	plants ha^{-1}	--	--	
0		513000a†	471000a	457000a	442000a	437000a	-
28		470000ab	481000a	466000a	443000a	407000ab	-
56		459000 b	457000a	420000a	413000a	379000 b	-
	2016						
0		555000a	532000a	481000a	-	473000a	551000a
28		534000a	544000a	500000a	-	495000a	534000a
56		551000a	517000a	468000a	-	472000a	511000a

†Means in a column, within a year, followed by the same letter are not significantly different at ($p \leq 0.05$).

The highest rate of 56 kg N fertilizer ha^{-1} significantly increased vigor, visual greenness score, GreenSeeker value, height, and yield over the control. Vigor increased as N rate increased from 0 to 56 kg N ha^{-1}. However, vigor was not different between the rates of 0 and 28 kg N ha^{-1} or 28 and 56 kg N ha^{-1} (Table 15). Sij et al. (1979) concluded that a low rate of urea fertilizer banded at planting can increase early season soybean vigor when unfavorable environmental conditions delay BNF. Vigor was significantly different between N rates in several environments but not every environment (Table 17) because environmental conditions (Tables 9-13) and soil N levels (Table 2) were highly variable between environments.

Table 17. Nitrogen effect on visual vigor score at each environment in 2015 and 2016.

Rate	Year	NW22 NAD	NW22 CTD	Ransom	Richland	Sargent	Steele
kg N ha^{-1}	2015	---------------------------------------(1-9)---					
0		5.2 b†	5.9 b	4.5 b	6.3a	6.0a	-
28		5.5ab	6.3ab	6.1a	5.4a	5.1a	-
56		6.1a	6.6a	6.4a	5.3a	5.4a	-
	2016						
0		4.7 b	5.0a	3.6 b	-	4.6a	6.2a
28		5.6a	5.3a	5.3a	-	5.4a	6.4a
56		6.0a	5.7a	6.1a	-	5.1a	6.1a

†Means in a column, within a year, followed by the same letter are not significantly different at ($p \leq 0.05$). Vigor scored visually on 1-9 scale with 9 being most vigorous.

The GreenSeeker value (NDVI) was also different between the highest rate of N and the lower rate of N fertilizer of 28 kg N ha^{-1} (Table 15). The increase in NDVI after 56 kg N ha^{-1} supports a similar trend of increased vigor score over the control following 56 kg N ha^{-1} application (Table 15). Although not significant in any individual environment in 2015, GreenSeeker score was significantly different between N rates in 2016 at the NW22 NAD and NW22 CTD environments (Table 18).

Table 18. Nitrogen effect on NDVI score at each environment in 2015 and 2016.

Rate	Year	NW22 NAD	NW22 CTD	Ransom	Richland	Sargent	Steele
kg N ha^{-1}	2015	--(0-0.99)--					
0		0.88a†	0.87a	0.86a	0.83a	0.85a	-
28		0.88a	0.87a	0.86a	0.83a	0.86a	-
56		0.88a	0.87a	0.86a	0.83a	0.86a	-
	2016						
0		0.79 c	0.79 b	0.83a	-	0.88a	0.87a
28		0.81 b	0.80 b	0.82a	-	0.88a	0.87a
56		0.82a	0.81a	0.82a	-	0.88a	0.87a

†Means in a column, within a year, followed by the same letter are not significantly different at ($p \leq 0.05$). NDVI score is a measure of NDVI obtained by a handheld GreenSeeker on a scale of 0 - 0.99 with 0.99 being most vigorous.

Visual greenness score improved with added N early in the growing season, which was unanticipated. Greenness scores by N rate for each environment in 2015 and 2016 are presented in Table 19. Added N was expected to aggravate IDC, causing plants to yellow. However, no IDC was observed and added N actually made plants greener in general due to increased overall photosynthesis. This theory is supported by a strong relationship between N per unit leaf area and photosynthesis (Sinclair, 2004).

Table 19. Nitrogen effect on visual greenness score at each environment in 2015 and 2016.

Rate	Year	NW22 NAD	NW22 CTD	Ransom	Richland	Sargent	Steele
kg N ha^{-1}	2015	----------	----------	----------(1-5)	----------	----------	----------
0		2.9 b†	2.8 c	3.0 b	2.0 b	2.4a	-
28		2.2a	2.3 b	2.1a	2.5ab	2.5a	-
56		2.0a	1.9a	1.9a	2.6a	2.5a	-
	2016						
0		1.6a	1.5a	2.0 c	-	1.7a	1.8 b
28		1.5a	1.5a	1.6 b	-	1.6a	1.4a
56		1.6a	1.4a	1.3a	-	1.6a	1.3a

†Means in a column, within a year, followed by the same letter are not significantly different at ($p \leq 0.05$). Greenness scored on scale of 1-5 with 1 being most green.

Visual greenness was significantly different between N rates in several environments in 2015 (Table 19) including NW22 NAD and NW22 CTD. Significant differences in visual greenness were not observed between N rates at NW22 NAD or NW22 CTD in 2016. Greenness scores were better (lower) in general in 2016 than in 2015 (Table 19) and favorable growing conditions and high N mineralization in 2016 may have improved the visual greenness in control plots so differences between N rates were less apparent. Additionally, NDVI score responded opposite of the trend seen in visual greenness. The NDVI score was not significantly different between N rates in any environment in 2015, but was significantly different between N rates in the NW22 NAD and NW22 CTD environments in 2016. In 2016, the average NDVI score increased as N rate increased at both environments (Table 18). In addition, GreenSeeker NDVI scores were lower at NW22 NAD and NW22 CTD in 2016 than in 2015 in general. Hail damage that reduced crop canopy in the environments at Fargo in early July before GreenSeeker measurement may explain why NDVI scores were generally lower in 2016 than 2015. More research is needed to determine why visual greenness and NDVI scores responded oppositely between years in the NW22 NAD and NW22 CTD environments.

Average plant height was 1.4 cm and 2 cm greater than the control when treated with 28 and 56 kg N ha^{-1}, respectively (Table 15). Nitrogen significantly influenced average plant height in the NW22 NAD environment in both years of the study and the Ransom County environment in 2015, but did not significantly impact plant height in any of the other individual environments (Table 20). The mean plant heights for N rates in each environment in 2015 and 2016 are presented in Table 20. A relationship was expected between height and yield in this study. Height and yield both increased with N fertilizer, but the relationship between height and yield was weak (r^2=0.2702).

Table 20. Nitrogen effect on plant height at each environment in 2015 and 2016.

Rate	Year	NW22 NAD	NW22 CTD	Ransom	Richland	Sargent	Steele
kg N ha^{-1}	2015	----------------------	----------------------	cm	----------------------	----------------------	----------------------
0		69.7 b†	68.9a	83.9 b	67.6a	84.5a	-
28		73.0ab	68.2a	86.7ab	67.8a	85.8a	-
56		73.8a	68.5a	89.6a	67.0a	87.3a	-
	2016						
0		53.0 b	52.4a	91.7a	-	71.5a	87.2a
28		55.0ab	55.1a	92.1a	-	71.3a	88.9a
56		56.4a	54.5a	92.5a	-	71.9a	88.6a

†Means in a column followed by the same letter are not significantly different at ($p \leq 0.05$).

Application of 28 and 56 kg N ha^{-1} increased yield 66 and 118 kg ha^{-1}, respectively, compared to no N application (Table 15). It is possible that the yield differences are not as big as expected due to inefficient N fertilization methods, N mineralization, environmental conditions, and inefficient production practices such as plant competition and inefficient plant density. Salvagiotti et al. (2008) and Beutow (2015) indicated that placement of slow-release N, such as a polymer-coated urea, below the immediate root zone or N application during reproductive stages could achieve a greater yield response in high-yielding environments as opposed to broadcast application by maximizing the efficiency of BNF and fertilizer N combination. In addition, adverse environmental conditions during the growing season such as dry conditions and hail in some environments may have reduced overall yield potential, obscuring the effect of fertilizer. For example, average yield across both environments at NW22 in 2015 and 2016 was 3155 and 2708 kg ha^{-1}, respectively, which goes against the general trend in this study and in North Dakota of higher yields in 2016 than in 2015. Average yields for N rates in each environment in 2015 and 2016 are presented in Table 21.

Table 21. Nitrogen effect on yield at each environment in 2015 and 2016.

Rate	Year	NW22 NAD	NW22 CTD	Ransom	Richland	Sargent	Steele
kg N ha^{-1}	2015			-----kg ha^{-1}-----			
0		3076 b†	3108a	2930a	3226a	2584a	-
28		3129ab	3156a	2903a	3304a	2452a	-
56		3252a	3206a	2891a	3257a	2562a	-
	2016						
0		2571 b	2619 b	4376 b	-	4003a	4092a
28		2646ab	2786a	4504a	-	4114a	4245a
56		2785a	2843a	4587a	-	4243a	4137a

†Means in a column, within an environment and year, followed by the same letter are not significantly different at ($p \leq 0.05$).

Sulfur

Although there were variable SO_4-S levels in the soil of each environment (Table 2), there was no significant interaction between environment and S in the combined analysis (Table 14). The soils in this study were not selected for an anticipated response to S. Protein concentration was significantly different between S fertilizer rates. As kg S ha^{-1} increased, mean protein concentration actually decreased. Average seed protein concentration for the treatment of 0, 11.2, and 22.4 kg S ha^{-1} was 337.3, 336.5, and 335.7 g kg^{-1}, respectively. The result of this study disagrees with those by Bellaloui et al. (2011) who reported that seed protein consistently increased in field trials when fertilized with S or N and S. All protein concentration means were lower than the anticipated range of 35-40%. However, Naeve et al. (2011) noted that protein levels in Minnesota are generally lower than those reported in southern U.S. states. The observed relationship between S and protein is noteworthy because S is one of the elements in amino acids, part of the structure of proteins. In addition, an inverse relationship between protein and yield has been previously reported in soybean (Burton, 1985; Hartwig and Hinson, 1972; Sebern and Lambert, 1984; Wehrmann et al., 1987) and other crops such as wheat (Pleijel et al., 1999). The decrease in protein concentration in this study with increasing S rate could potentially be related to an increase in yield. However, the average yields of 0, 11.2 and 22.4 kg S ha^{-1} were 3310, 3330, and 3310 kg ha^{-1}, respectively, and were not significantly different. Additionally, S alone did not significantly influence yield in any individual environment in the study.

Breeding methods have already been aimed at increasing the sulfur-containing amino acids methionine and cysteine in soybean to improve the soybean's nutritional value (Krishnan, 2005). Kaiser and Kim (2013) reported that S fertilizer significantly increased protein concentration at two of four sites in Minnesota. However, they concluded that due to the inconsistent results S fertilizer may not be beneficial on all soil types. Conversely, the observed trend in this study illustrates that fertilizer S actually decreased mean protein concentration. Samples in this study were not analyzed to determine which specific amino acids were affected. The results of this study may be different because dry fertilizer was used and Kaiser and Kim (2013) utilized liquid starter fertilizer forms of N and S. Even though agronomic effectiveness of fertilizers is related to their solubility, all sulfate fertilizers are assumed to have similar agronomic effects (Pederson et al., 1998). Similar responses to S fertilizer were observed when different sulfate fertilizers were compared in wheat (Mitchell and Mullins, 1990), rapeseed (Gupta et al., 1997), and cotton (*Gossypium* L.) (Mullins, 1998). Additionally, Krishnan et al. (2005) concluded that there is an inverse relationship between overall protein concentration and S-containing amino acid concentration in soybean.

Further research is required to determine if S fertilizer can be used to increase protein levels in low protein varieties, environments where S is limiting, or potentially under environmental stresses that inhibit adequate S uptake or protein formation during key parts of the growing season. The results of this study indicate that S fertilizer does not increase protein concentration, but actually reduces it. The observed differences in protein concentration in this study, although statistically significant, may not be meaningful on a practical level because of the small range of values.

Variety x Sulfur

The interaction of variety x S significantly influenced plant density. However, the relationship is sporadic and may not be a true interaction (Figure 3). The plant density of PFS 15R07 increased as S rate increased from 0 to 11.2 kg S ha^{-1}, but then decreased as S rate increased from 11.2 to 22.4 kg S ha^{-1}. PS 30-80 responded in the opposite manner. The plant density of PS 30-80 decreased as S rate increased from 0 to 11.2 kg S ha^{-1}, but then increased as S rate increased from 11.2 to 22.4 kg S ha^{-1}. This relationship offers more evidence that varietal genotypic differences are very important with regards to management decisions, including varietal selection and fertilization techniques. More research is required to better understand how varieties respond to S and what factors lead to differential responses.

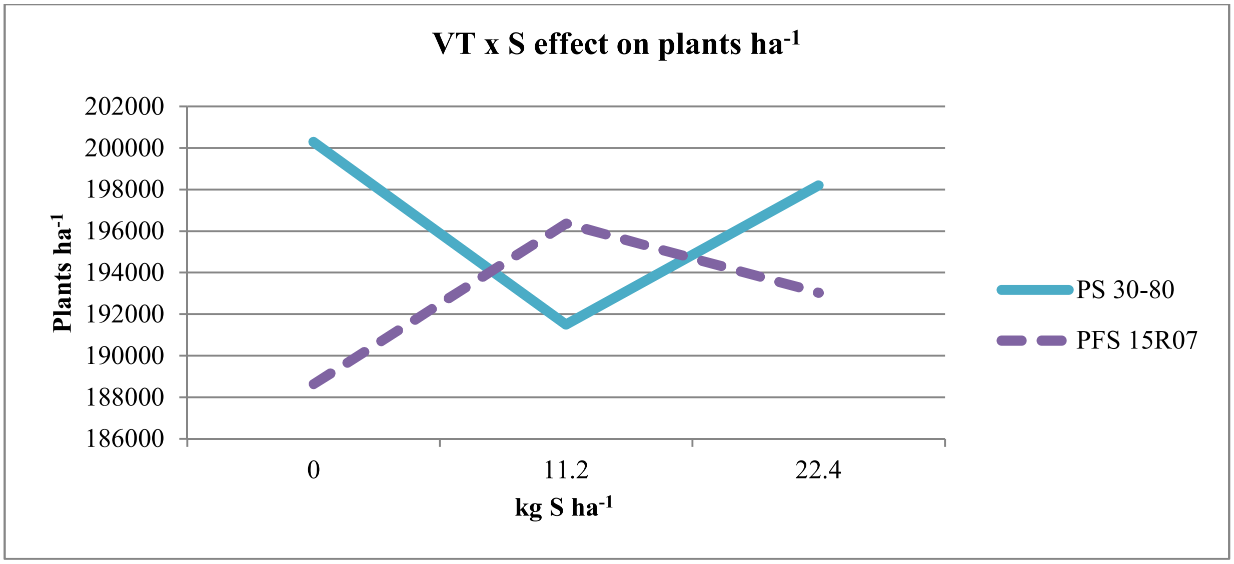

Figure 3. Variety x sulfur effect on plant density when analyzed across all environments in both years of the study.

Nitrogen x Sulfur

The interaction of N x S significantly influenced visual greenness score. Visual greenness was scored on a scale of 1-5 with 1 being the most green and 5 being yellow. The plants which received 28 or 56 kg N ha^{-1} were greener compared with the control (0 kg N ha^{-1}) (Figure 4). The relationship makes sense biologically, because both N and S are important components in photosynthesis. However, the limitations of the research include that greenness was scored visually and is inherently subject to bias. Additionally, the observed trend shown in Figure 4 was not supported by NDVI data obtained from the handheld GreenSeeker. The greenness was scored visually earlier in the growing season than NDVI scores were measured by the GreenSeeker (Table 5) and the GreenSeeker NDVI is a measure of aboveground plant biomass. However, the observed visual differences in greenness could have changed throughout the growing season, which would explain why the NDVI scores did not support the visual greenness scores shown in Figure 4.

Understanding the relationship between N and S and the influence of the two nutrients on yield was a main objective of this study and a yield response to N x S was anticipated. However, no significant yield response to N and S was recorded in any environment in the study. The mean yields by N x S across all environments are presented in Table 22.

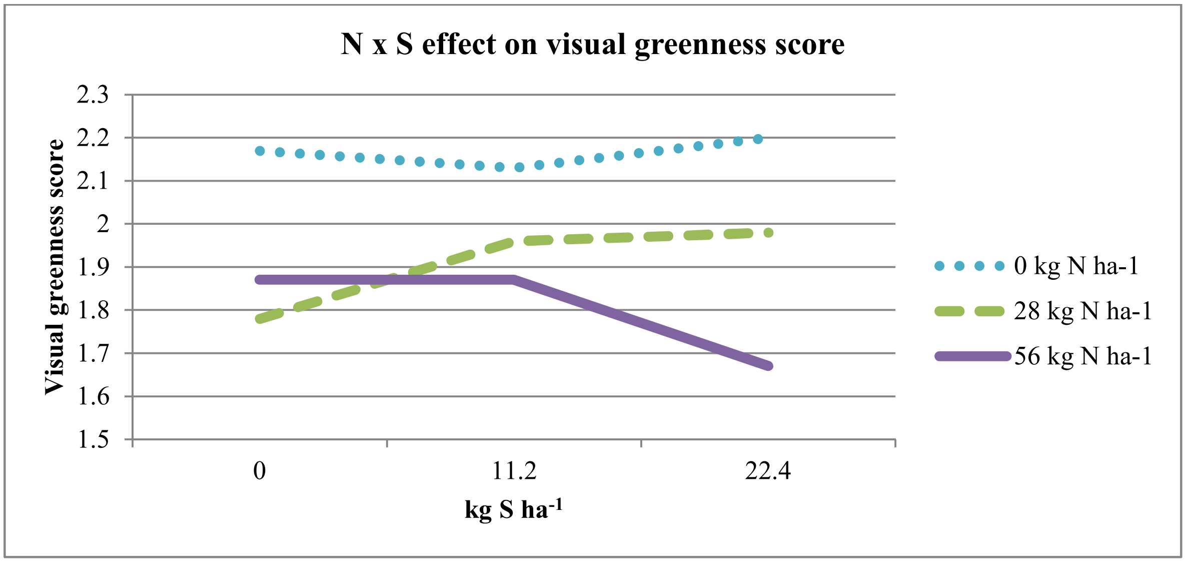

Figure 4. Nitrogen x sulfur effect on mean visual greenness score when analyzed across all environments in both years of the study.

Table 22. Nitrogen x sulfur effect on average yield across all environments.

Fertilizer rate		Yield
kg N ha^{-1}	kg S ha^{-1}	--kg ha^{-1}--
0	0	3225
0	11.2	3252
0	22.4	3299
28	0	3326
28	11.2	3360
28	22.4	3292
56	0	3393
56	11.2	3380
56	22.4	3353
LSD (0.05)		ns

Nodulation Study

Residual mean squares for each environment were homogenous according to Bartlett's Chi-square test and, therefore, environments were combined for analysis. Levels of significance for variety, treatment, and various interactions for the first (V4) and second (R4) nodulation experiments are presented in Table 23 and Table 24, respectively.

Table 23. Significance levels for the ANOVA for root characteristics of the first (V4) nodulation experiment combined across all environments.

SOV†	df	AN	Sm	M	L	PM	SM	RM
Env [Environment]	9							
Rep(Env)	18							
VT [Variety]	1	***	ns	ns	ns	ns	ns	*
VT * Env	9							
N [Nitrogen]	2	***	***	ns	***	ns	ns	ns
VT * N	2	ns	ns	ns	ns	ns	ns	ns
S [Sulfur]	1	ns	ns	ns	ns	ns	ns	ns
VT * S	1	*	ns	ns	ns	ns	ns	ns
N * S	2	ns	ns	ns	ns	ns	ns	ns
VT * N * S	2	ns	ns	ns	ns	ns	ns	ns
Residual Error	180							

ns, *, **, *** = not significant, significant at (p≤0.05), (p≤0.01), and (p≤0.001), respectively, tested according to Carmer et al. (1989).
†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass per pant, RM = average root mass per plant.

Environment played a key role in the magnitude of plant response to a number of treatments. For example, average nodule number per plant was highly variable between environments at the V4 and R4 growth stage (Figure 5) regardless of treatment. In addition, the percent increase in average nodule number between V4 and R4 was different between environments. In 2016, the average nodule number per plant increased 5% and 73% in the Ransom County and Steele County environment, respectively. Possible explanations for differential environmental response include numerous factors such soil type and previous crop

(Table 1), weather conditions (Tables 9-13), and level of soil N at the start of the experiment (Table 2). In addition, the soil in the 2016 Ransom County environment was extremely dry and compacted at the R4 growth stage, which caused root damage during sample collection. Although environments varied in response magnitude to many treatments, general trends followed a similar pattern for most treatments in all environments. Deviations from general trends will be discussed in more detail in the following sections pertaining to particular independent variables.

Table 24. Significance levels for the ANOVA for root characteristics of the second (R4) nodulation experiment combined across all environments.

SOV†	df	AN	Sm	M	L	PM	SM	RM
Env [Environment]	9							
Rep(Env)	18							
VT [Variety]	1	ns	ns	*	*	ns	ns	ns
VT * Env	9							
N [Nitrogen]	2	***	***	ns	***	ns	ns	ns
VT * N	2	ns	ns	*	ns	*	*	*
S [Sulfur]	1	ns	ns	*	ns	ns	ns	ns
VT * S	1	ns	*	ns	*	ns	ns	ns
N * S	2	ns	ns	ns	ns	ns	ns	ns
VT * N * S	2	ns	ns	ns	ns	ns	ns	ns
Residual Error	180							

ns, *, **, *** = not significant, significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).
†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass per plant, RM = average root mass per plant.

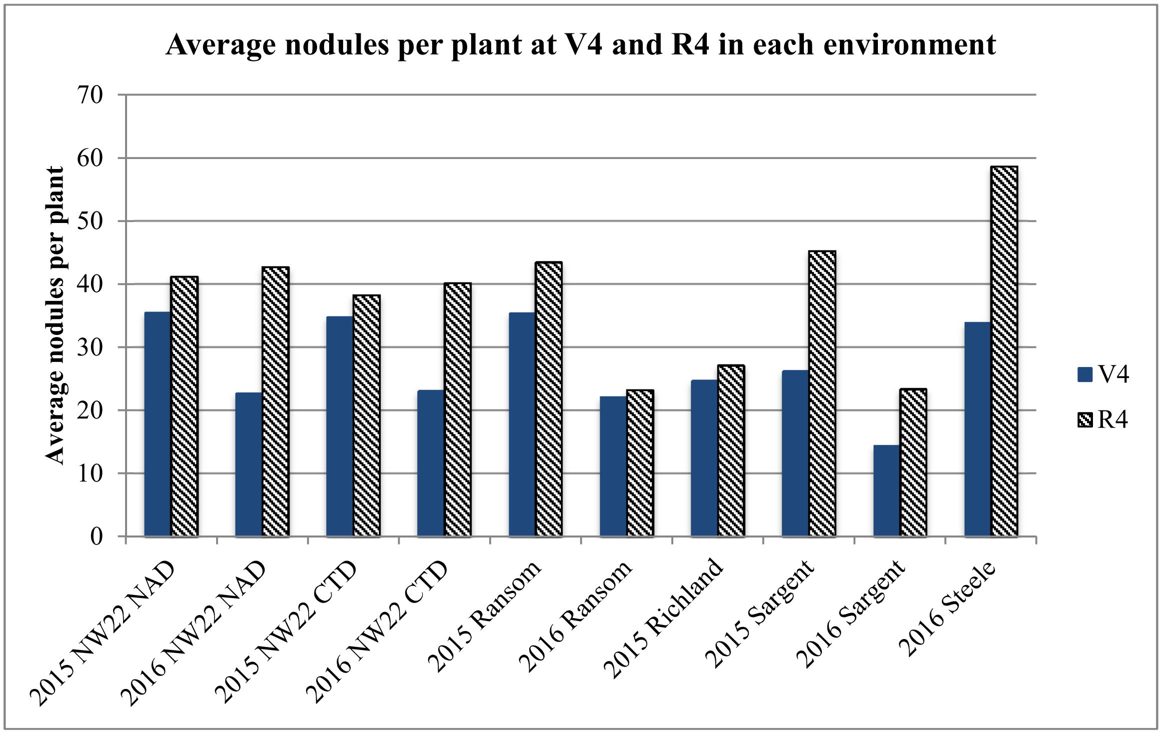

Figure 5. Average nodules per plant at both V4 and R4 in each environment.

Variety

Average nodule number per plant was significantly different at the V4 stage between varieties. However, by the R4 stage variety had no significant impact on average nodule number per plant. This indicates that the two varieties evened out in average nodule number per plant during the growing season (Table 25). A significant difference in root mass between varieties was also observed at the V4 growth stage, but evened out by the R4 growth stage (Table 25).

Table 25. Variety effect on average nodule number and root mass per plant across all environments at V4 and R4.

	AN†		RM	
Variety	V4	R4	V4	R4
	--#--	--#--	-----------g-----------	
PS 30-80	30.3a‡	39.8a	0.49a	1.68a
PFS 15R07	24.4 b	36.9a	0.47 b	1.74a

†AN = average nodule number, RM = average root mass per plant.
‡Means in a column followed by the same letter are not significantly different at ($p \leq 0.05$).

As noted in Table 25, the average nodule number per plant appeared to be related to average root mass at the V4 stage. As root mass increased, average nodule number per plant also increased. The larger root mass of PS 30-80 at V4 allowed for more symbiotic relationships to be formed with *Bradyrhizobium japonicum* possibly due to an increased total root surface area. However, analysis of all V4 data to determine if a relationship existed between root mass and average nodule number provided an r^2=0.0055 value (n=416, $p \leq 0.05$), which does not indicate a strong relationship. This increase in average number of nodules per plant at the V4 stage may help explain the difference in average nodule size and overall nodulation between varieties at the R4 stage (Table 26).

Table 26 shows varietal differences in average percent small, medium, and large nodules at the R4 stage. PFS 15R07 had an average of 5.2% more large nodules per plant than PS 30-80 at the R4 stage. As aforementioned, PFS 15R07 had significantly fewer nodules on average than PS 30-80 at the V4 growth stage. This helps explain the difference in average nodule size at the R4 stage because the PFS 15R07 variety had significantly fewer nodules per plant to sustain and allocate resources to. The result was possibly greater average carbohydrate supply to each nodule of the PFS 15R07 plant root resulting in larger nodules.

Table 26. Variety effect on percent small, medium, and large nodules per plant across all environments at V4 and R4.

Variety	Sm†		M		L	
	V4	R4	V4	R4	V4	R4
	----------------	----------------	----------------	%	----------------	----------------
PS 30-80	29.7a‡	27.6a	54.3a	60.6a	19.5a	11.8a
PFS 15R07	28.0a	26.2a	49.6a	57.1 b	22.6a	17.0 b

†Sm = average small (<1 mm) nodules per plant, M = average medium (1-4 mm) nodules per plant, L = average large (>4 mm) nodules per plant.
‡Means in a column followed by the same letter are not significantly different at ($p \leq 0.05$).

Nitrogen

Nitrogen strongly influenced nodulation at the V4 and R4 stage, particularly in regards to average number of nodules per plant and average percent of small and large nodules per plant. As the N rate increased at both growth stages, average nodule number per plant decreased and average nodule size decreased (Table 27). The average nodule number per plant was significantly lower than the control at either N rate at both stages (Table 27). The average number of nodules per plant was not significantly different between the rates of 140 kg N ha^{-1} and 280 kg N ha^{-1} at either stage, however. As N rate increased, the average percent of small nodules per plant increased at both stages and the average percent of large nodules decreased.

Table 27. Nitrogen effect on average nodules per plant, and percent small, medium, and large nodules per plant across all environments at V4 and R4.

Rate	AN†		Sm		M		L	
	V4	R4	V4	R4	V4	R4	V4	R4
kg N ha^{-1}	-#-	-#-	----------------	----------------	----%	----------------	----------------	----------------
0	31.8a‡	44.3a	19.8 b	22.0 c	47.9a	56.7a	32.4a	21.4a
140	26.5 b	37.3 b	30.5a	26.8 b	56.9a	59.9a	17.9 b	13.7 b
280	23.7 b	33.5 b	36.3a	32.0a	51.1a	60.0a	12.8 b	8.0 c

†AN = average nodule number, Sm = average number of small (<1 mm) nodules per plant, M = average medium (1-4 mm) nodules per plant, L = average number of large (>4 mm) nodules per plant.
‡Means in a column followed by the same letter are not significantly different at ($p \leq 0.05$).

Results agree with those by Streeter (1988) as well as Gibson and Harper (1985) and Laysell and Moloney (1994) who indicated a strong inhibition of nodulation and N fixation activity under high nitrate conditions. Furthermore, the results of this study confirm those reported by Hungria et al. (2006) and Mendes et al. (2003) who conclude that N fertilization at rates of 30 to 400 kg N ha^{-1} decreased nodulation and the contribution of BNF. However, Mendes et al. (2003) reported that average nodule number was 50% lower for plants treated with 40 kg N ha^{-1} compared to the control 15 d after emergence, but these significant differences in average nodule number per plant had disappeared by the R1 stage. The significant difference between average nodule number at R4 reported in this study could be attributed to the higher rates of N fertilizer used.

Results also agree with those concluded by Salvagiotti et al. (2008) who showed a negative N fixation response as N fertilizer was added to the soil surface or incorporated in the topmost layers. Specifically, nitrate has been shown to decrease nodule number, nodule mass, and N fixation activity, and accelerate nodule senescence. Results of this study show the same trend of decreased average nodule number and decreased average nodule mass. Saito et al. (2014) concluded that rapid inhibition of nodule activity was attributed to a decrease in transport of photosynthate to nodules from the shoot. In addition, Ohyama et al. (2011) reported that soybean nodule growth completely stopped after 1 d of application of 0.005 mol L^{-1} NO_3^- solution. Many hypotheses have been proposed as the cause of nodulation inhibition by nitrate including carbohydrate-deprivation in nodules (Streeter, 1988), feedback inhibition by a product of nitrate metabolism (Neo and Layzell, 1997; Bacanamwo and Harper; 1997), and decreased oxygen diffusion into nodules (Gordon et al., 2002; Schuller et al., 1988).

Nitrogen was expected to impact the average root mass and shoot mass of the soybean plant. Nitrogen did not significantly influence plant mass, shoot mass, or root mass in this study at either growth stage when data was combined and analyzed over all environments (Table 28).

Table 28. Average plant mass, shoot mass, and root mass at each N rate at both V4 and R4 combined across all environments.

Rate	PM†		SM		RM	
	V4	R4	V4	R4	V4	R4
kg N ha^{-1}	----------	----------	----------	g ----------	----------	----------
0	2.10a‡	11.01a	1.68a	9.28a	0.48a	1.72a
28	2.28a	11.54a	1.78a	9.80a	0.50a	1.74a
56	2.34a	11.52a	1.86a	9.86a	0.48a	1.67a

†PM = average plant mass, SM = average shoot mass per plant, RM = average root mass.
‡Means in a column followed by the same letter are not significantly different at ($p \leq 0.05$).

Nitrogen had no impact on root mass in any of the individual environments. Plant mass and shoot mass were responsive to N treatment at V4 in two environments in 2015 and one environment in 2016 in this study. In 2015, N influenced V4 plant mass in the NW22 CTD environment and the Sargent County environment (Table 29). Shoot mass was also influenced by N treatment at V4 in 2015 in the Sargent County environment (Table 29). In 2016, plant mass and shoot mass were significantly different at R4 between N treatments in the Sargent County environment (Table 29). Plant mass and shoot mass were significantly different between N rates at V4 when analyzed over all environments in 2015 but not different when analyzed over all environments in 2016 (Table 30).

Table 29. Nitrogen effect on average plant mass and shoot mass at V4 in the NW22 CTD and Sargent County environment in 2015 and average plant mass and shoot mass at R4 in the Sargent County, ND, environment in 2016.

Rate	V4				R4	
	NW22 CTD		Sargent			
	2015				2016	
	PM†	SM	PM	SM	PM	SM
kg N ha^{-1}	--	------	---	g ---	------	--
0	1.32 b‡	16.05a	1.64 b	1.32 b	9.86 b	8.27 b
28	1.47ab	18.05a	1.86 b	1.44 b	10.02 b	8.26 b
56	1.64a	20.34a	2.36a	1.96a	12.20a	10.39a

†PM = average plant mass, SM = average shoot mass per plant.
‡Means in a column, within a year, followed by the same letter are not significantly different at ($p \leq 0.05$).

Table 30. Nitrogen effect on average plant mass and shoot mass at V4 and R4 across all environments in 2015 and 2016.

Rate	Year	V4		R4	
		PM†	SM	PM	SM
kg N ha^{-1}	2015	-------------------------	------ g	------	-------------------------
0		1.63 b‡	1.26 b	11.65a	9.93a
28		1.79ab	1.40ab	11.57a	9.89a
56		1.94a	1.56a	11.88a	10.18a
	2016				
0		2.67a	2.09a	9.39a	7.78a
28		2.71a	2.10a	10.48a	8.81a
56		2.66a	2.09a	10.04a	8.55a

†PM = average plant mass, SM = average shoot mass per plant.
‡Means in a column, within a year, followed by the same letter are not significantly different at ($p \leq 0.05$), tested according to Carmer et al. (1989).

The results shown in Table 30 could possibly be due to variable soil test N levels between years. Soil NO_3^--N levels were generally higher in 2016 than in 2015 according to soil test results (Table 2). Conditions during the 2016 growing season were particularly conducive to N mineralization, potentially obscuring plant response to additional N fertilization in environments in 2016 (Franzen, 2016, personal communication). The significant difference in plant mass and shoot mass between N rates at V4 in 2015 was not present at R4 (Table 30), indicating that N had a greater impact on plant growth at the beginning of the growing season. Plant growth had evened out between N treatments by the R4 stage. This result shows that N fertilizer can influence early season plant growth in some environments. Results also agree with those by Sij et al. (1979), who concluded that a small amount of N fertilizer can increase early season soybean vigor when unfavorable environmental conditions delay BNF.

Sulfur

At the V4 growth stage, S had no significant effect on any of the measured dependent variables. However, at the R4 growth stage S significantly impacted nodule size (Table 31). These results supported those reported by Varin et al. (2009) who indicated S significantly increased nodule size of white clover. The results of this study show that S at a rate of 112 kg S ha^{-1} decreased the average percent of medium sized nodules per plant at R4 compared to the control (Table 31). However, the average percent of small and large sized nodules per plant did not change in response to S. More research is necessary to determine the impact of S on soybean nodule size.

Table 31. Sulfur effect on average nodules per plant, and average percent small, medium, and large nodules per plant across all environments at V4 and R4.

Rate	AN†		Sm		M		L	
	V4	R4	V4	R4	V4	R4	V4	R4
kg S ha^{-1}			-------------	-------------	-------------	-------%------	-------------	-------------
0	27.0a‡	38.3a	29.3a	28.3a	51.2a	57.2 b	19.6a	14.6a
112	27.7a	38.3a	28.4a	25.5a	52.8a	60.5a	22.5a	14.1a

†AN = average nodules per plant, Sm = average small (<1 mm) nodules per plant, M = average medium (1-4 mm) nodules per plant, L = average large (>4 mm) nodules per plant.
‡Means in a column followed by the same letter are not significantly different at (p≤0.05).

Variety x Nitrogen

Analysis showed a significant VT x N interaction at the R4 stage for average percent medium nodules per plant, average plant mass, and average shoot mass per plant (Figures 6-9). The average number of medium nodules per plant tended to increase for PFS 15R07 as N level increased. The average percent medium nodules per plant for PS 30-80 increased as N rate increased from 0 to 140 kg N ha^{-1}, but then decreased as N rate increased from 140 kg N ha^{-1} to 280 kg N ha^{-1} (Figure 6). The trend reflects the influence of N on nodulation. As N rate increased, nodule size became smaller on average. However, there was a difference in percent medium and large nodules between varieties at R4 (Table 26), indicating that the varieties had different nodule sizes in general, regardless of treatment. PFS 15R07 had greater average plant mass, shoot mass, and root mass than PS 30-80 when no N was applied. When 140 kg N ha^{-1} was applied the plant mass (Figure 7), shoot mass (Figure 8), and root mass (Figure 9) of PS 30-80 increased and was greater than PFS 15R07. However, the average plant mass, shoot mass, and root mass of PFS 15R07 increased when 280 kg N ha^{-1} was applied and average plant mass, shoot mass, and root mas of PS 30-80 decreased. This interaction indicates that the two varieties responded differently to N fertilizer treatment. The difference in varietal response to N was not anticipated. The results seem to indicate that a general recommendation cannot be made for all soybean varieties because varieties respond differently with regards to nodule size, plant mass, and shoot mass. Future research should investigate in more detail the response differences to fertilizer application for various soybean genotypes.

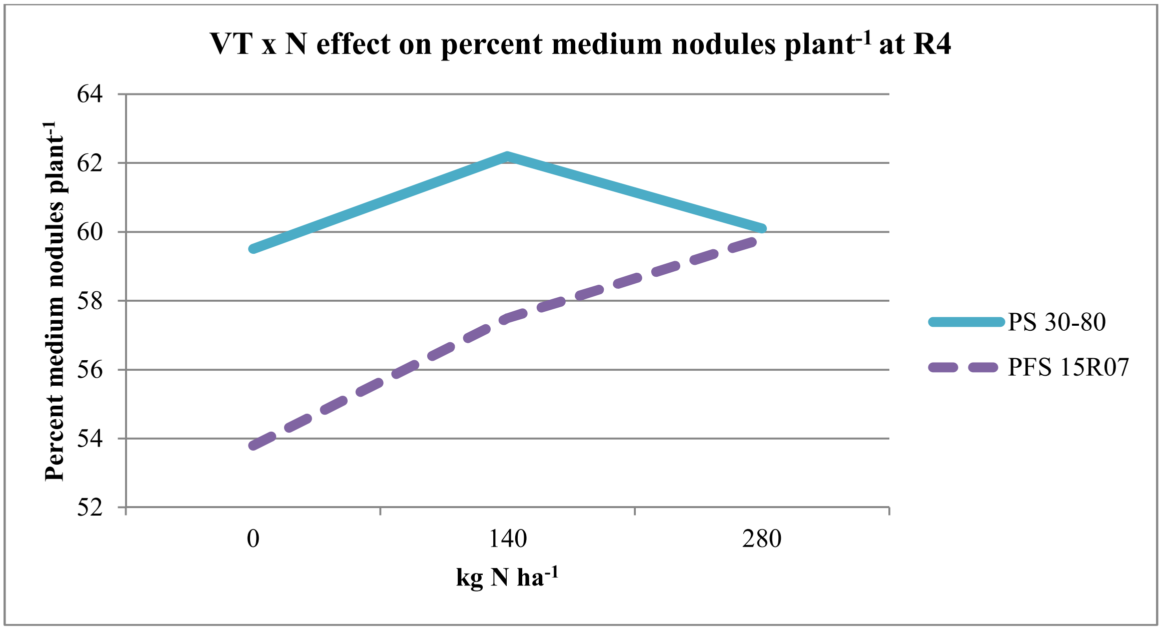

Figure 6. Variety x nitrogen effect on average percent medium nodules per plant at R4.

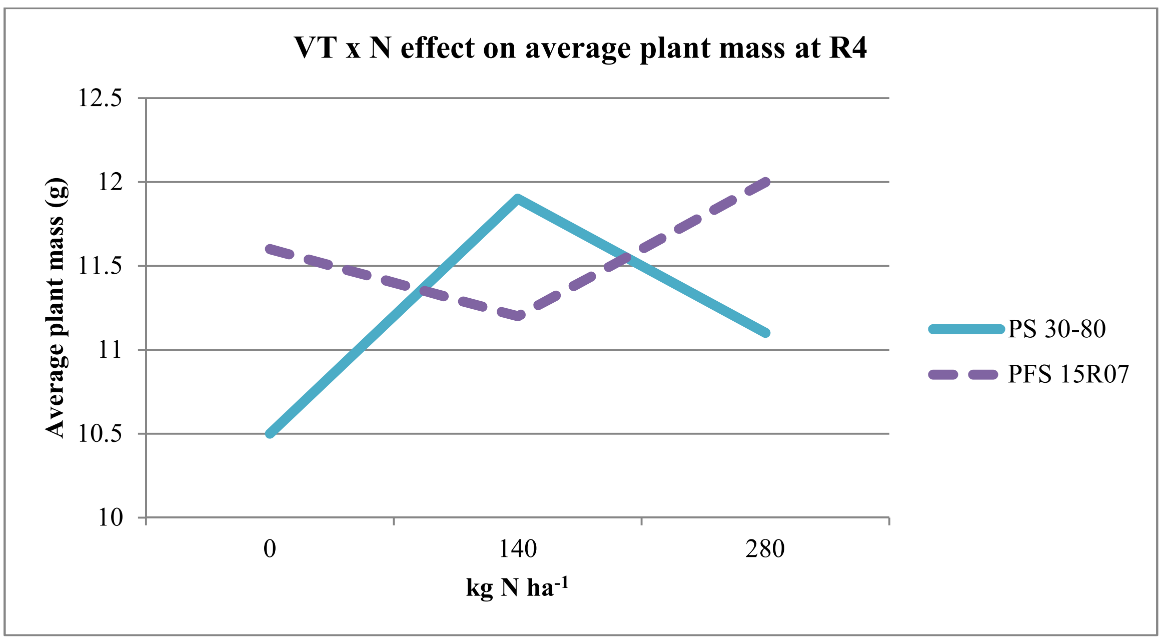

Figure 7. Variety x nitrogen effect on average plant mass at R4.

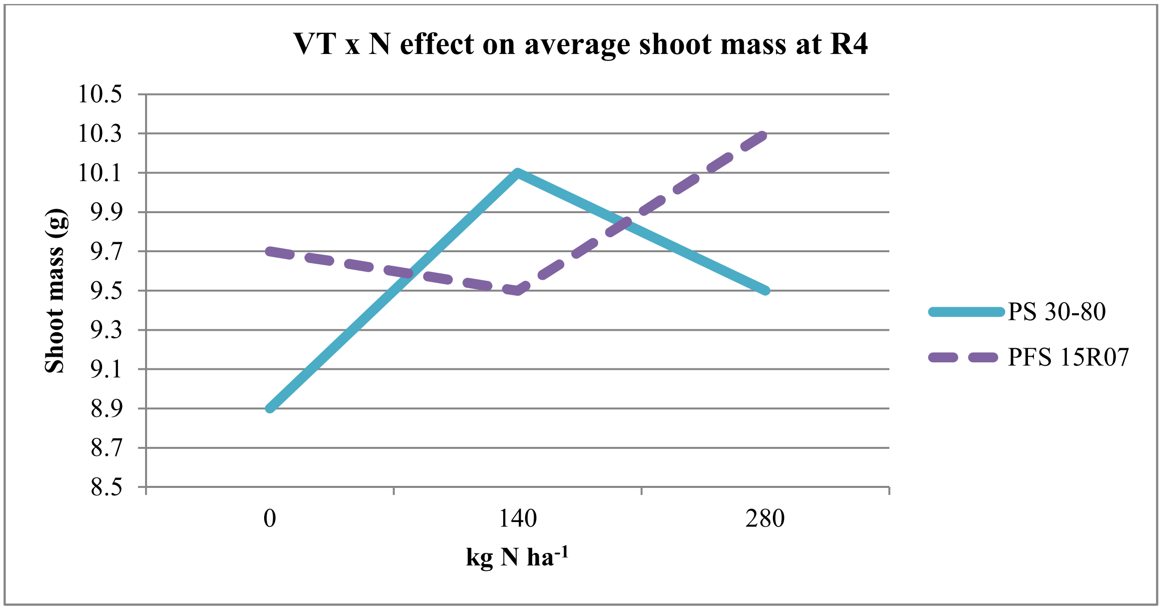

Figure 8. Variety x nitrogen effect on average shoot mass per plant at R4.

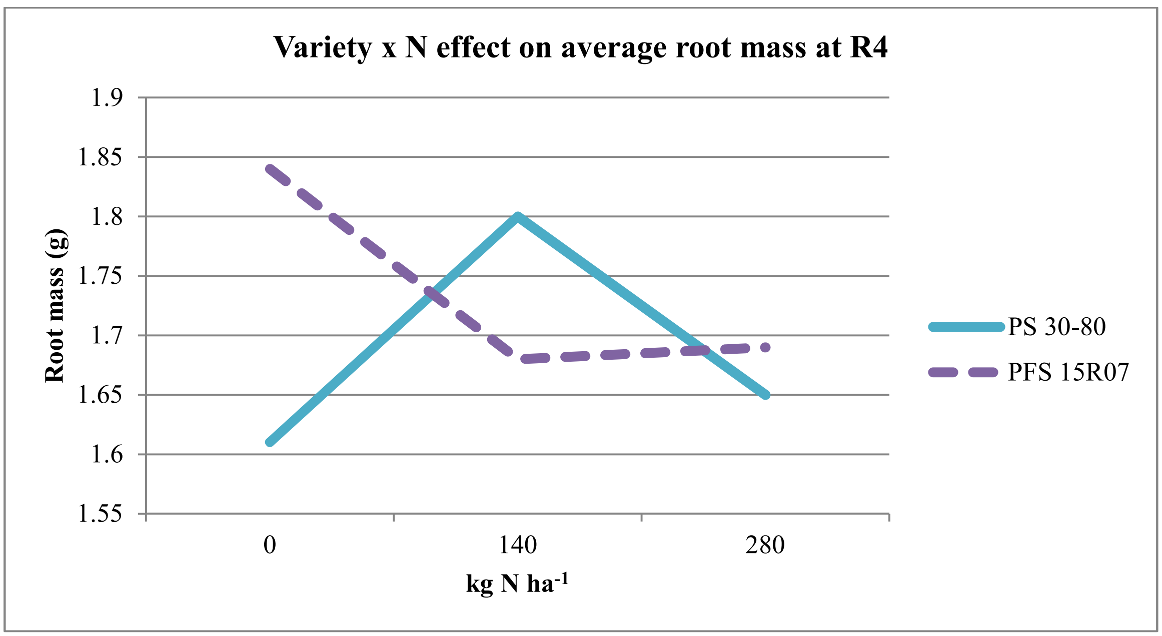

Figure 9. Variety x nitrogen effect on average root mass per plant at R4.

Variety x Sulfur

Analysis showed a VT x S interaction at the V4 growth stage on the average number of nodules per plant (Figure 10). The significant difference may be the result of a difference in magnitude between varieties and not a true interaction. As indicated in Figure 10, significant differences in nodule number between varieties existed at V4 regardless of S treatment. The significant difference between varieties but not S rates indicates that this was a difference in magnitude and not a crossover interaction.

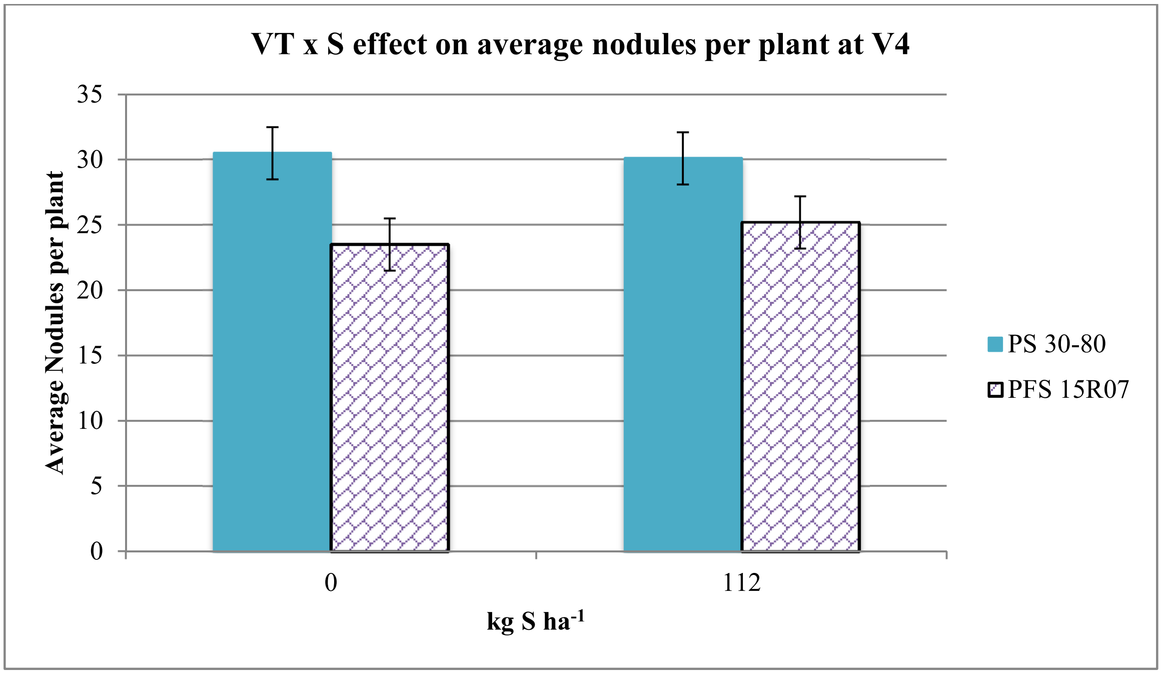

Figure 10. Variety x sulfur effect on average nodules per plant at V4.
†Nodule number is significantly different ($p \leq 0.05$) between varieties at both S rates. Nodule number for individual varieties are not significantly different ($p \leq 0.05$) between S rates.

There was also a VT x S interaction at the R4 stage for average percent small nodules per plant and average percent large nodules per plant (Figures 11 and 13), but not for percent medium nodules per plant at R4 (Figure 12). PS 30-80 tended to have lower percent small nodules and higher percent large nodules as S rate increased from 0 to 112 kg S ha^{-1}. Conversely, PFS 15R07 tended to have higher percent small nodules and lower percent large nodules as S rate increased from 0 kg to 112 kg S ha^{-1}. Figure 13 illustrates how completely differently the varieties responded to S with regards to nodule size. Further research is needed to determine what genotypic differences caused the plants to respond differently to S treatment.

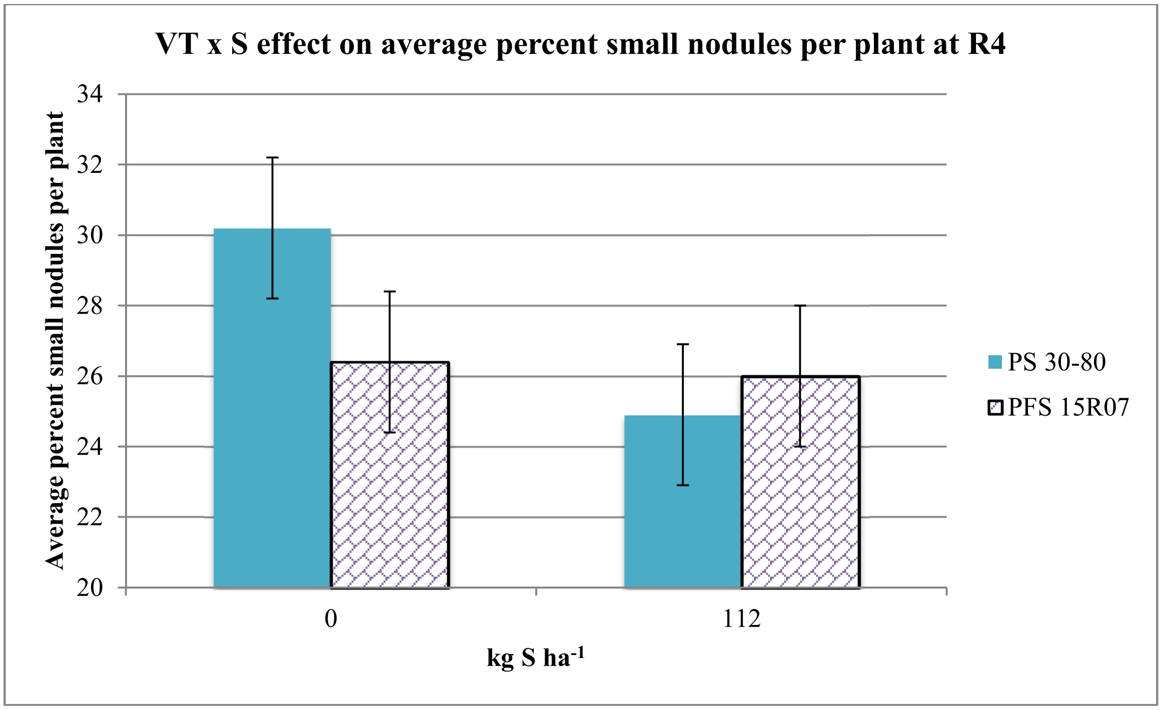

Figure 11. Variety x sulfur effect on average percent small nodules per plant at R4.
†Small nodule percent was significantly different ($p \leq 0.05$) between S rates for PS 30-80, but not between varieties at either S rate.

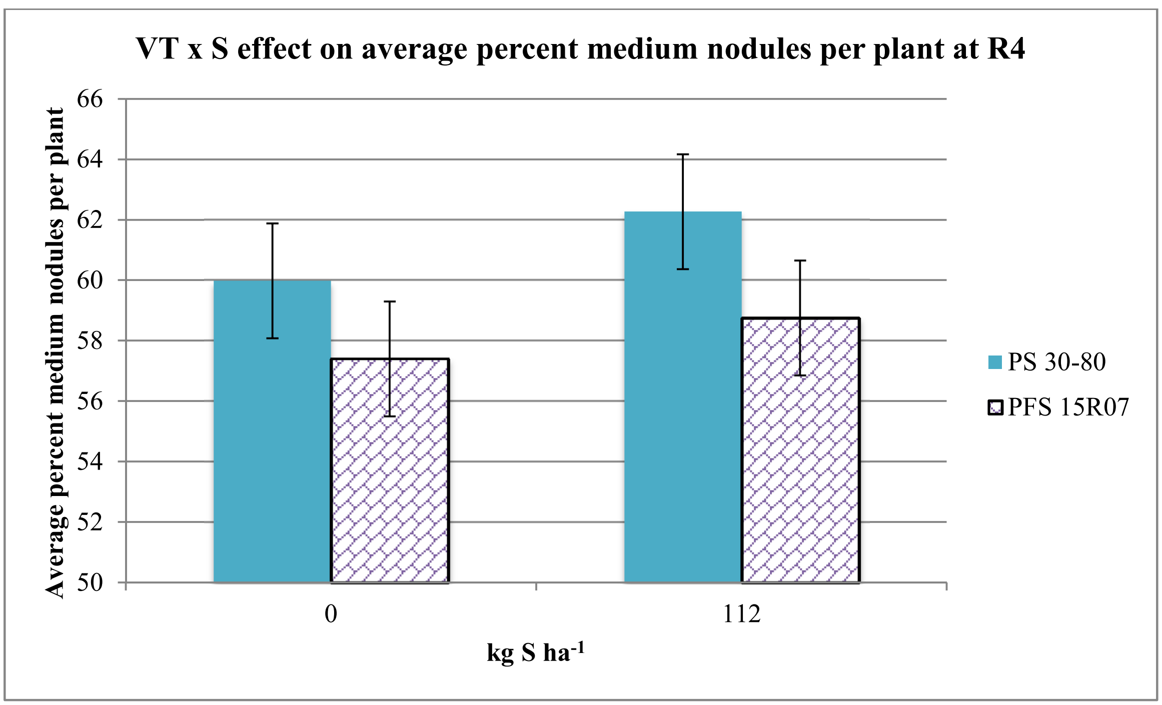

Figure 12. Variety x sulfur effect on average percent medium nodules per plant at R4.
†Medium nodule percent is not significantly different ($p \leq 0.05$) between varieties or S rates.

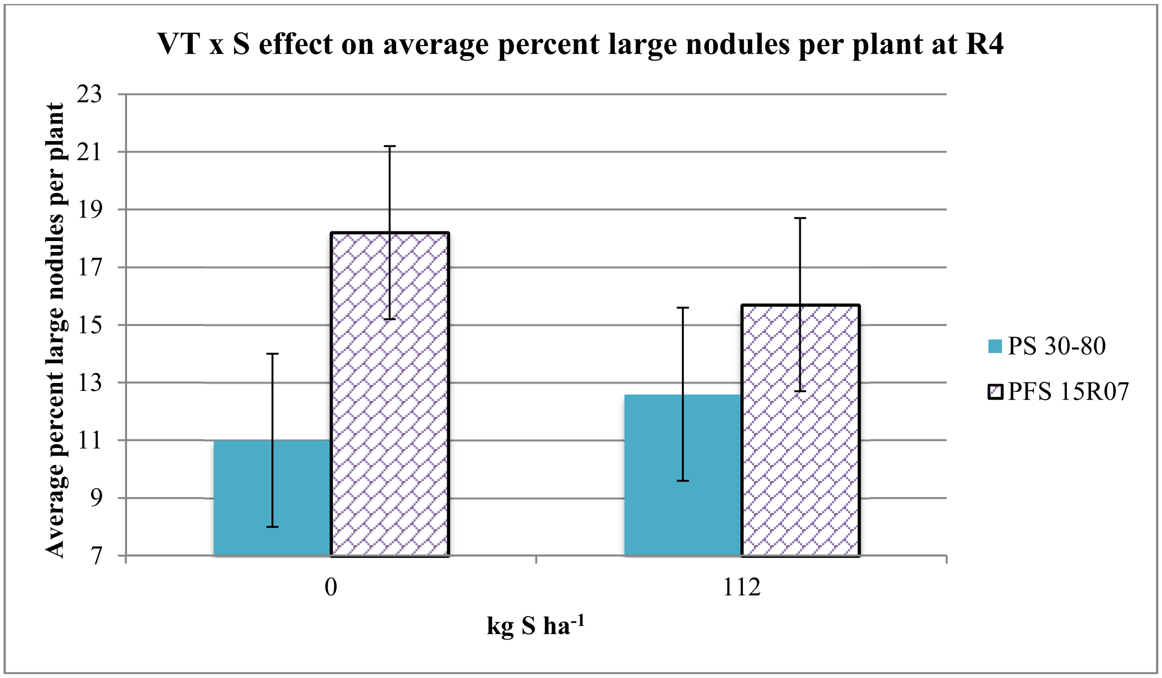

Figure 13. Variety x sulfur effect on average percent large nodules per plant at R4.
†Large nodule percent is significantly different ($p \leq 0.05$) between varieties at 0 kg S ha-1.
Large nodule percent is not significantly different ($p \leq 0.05$) between varieties at 112 kg S ha^{-1}.
Large nodule percent for each variety is not significantly different ($p \leq 0.05$) between S rates.

CONCLUSION

The results of this study indicate that N application significantly increased vigor, visual greenness, NDVI score, and height. Nitrogen also increased yield, but reduced plant stand and nodulation. Treatments of 28 kg N ha^{-1} and 56 kg N ha^{-1} increased yield 66 kg ha^{-1} and 118 kg ha^{-1}, respectively, compared to the control. The rate of 56 kg N ha^{-1} also significantly reduced plant stand compared to the lower N rate (28 kg N ha^{-1}) and the control (0 kg N ha^{-1}). Although N increased yield 5%, fertilization may not be economical when considering the cost of N fertilizer and application cost. At current urea prices as of 18 November 2016, 56 kg N ha^{-1} would cost approximately \$43.10 ha^{-1}. The yield increase of 118 kg ha^{-1} at current commodity prices as of 18 November 2016 represents an increased profit of \$63.06 ha^{-1}. A total net gain of \$19.96 ha^{-1} was observed in this study for the application of 56 kg N ha^{-1}. However, the average yield in this study was approximately 42% higher than the state average yield in North Dakota (USDA, 2016). A 5% yield increase over the state average may not increase profits enough to make N application economical. Commodity prices, fertilizer prices, and yield level are all factors that must be considered when determining if N application is economical. Future research should focus not only on fertilization practices, but also general production practices such as maintaining optimum plant densities following fertilization.

Sulfur did not significantly influence yield. The average yield for 0, 11.2, and 22.4 kg S ha^{-1} were 3314, 3331, and 3314 kg ha^{-1}, respectively, and were not significantly different. However, environments with S-deficient soils were not specifically selected for this study. The county environments represent random producer conditions. Future S fertilization research should focus on environments where a response to S would be more likely, such as coarse-textured soils or soils low in organic matter. Sulfur significantly impacted seed protein concentration. Protein concentration at 0, 11.2, and 22.4 kg S ha^{-1} was 33.73%, 33.65%, and

33.57%, respectively. Although statistically significant, this response to S fertilizer may not be relevant on a practical level because of the miniscule differences between protein concentrations (0.16%). Future research is necessary to understand the relationship between yield, protein concentration, and S-amino acid concentration in new high-yielding soybean varieties.

Varieties responded differently between environments and treatments, indicating the importance of appropriate varietal selection and environmental placement. Environment had a strong influence on plant yield, regardless of treatment. For example, average yield differed between environments by up to 77%. Vigor and test weight were different between varieties. PS 30-80 was more vigorous in general compared to PFS 15R07. However, the NDVI score obtained from the handheld GreenSeeker did not support this trend of greater vigor for PS 30-80.

The interaction of N and S did not significantly influence any of the measured dependent variables in this study aside from visual greenness score. Control plots (0 kg N ha^{-1}) were less visually green than plots that received 28 or 56 kg N ha^{-1}. A yield response to N and S fertilizer was expected, but not observed across all environments or in any individual environment in this study. Although in this thesis an alpha level of 0.05 is used, it is noteworthy that yield was significantly different for N x S at the 90% level of confidence (Pr>F = 0.0781) when analyzed across all environments. As previously discussed, yield increased in general as N rate increased. However, S influenced yield differently at the different N rates. For example, yield increased as S rate increased at the level of 0 kg N ha^{-1}. Conversely, yield decreased as S rate increased at the 50 kg N ha^{-1} rate. Future research should focus on the N x S interaction in more detail.

Unfortunately, no general N or S fertilizer recommendation can be made for soybean due to the highly variable responses between environments and varieties in this study. This study did highlight the importance of varietal selection and fertilizer placement. In addition, atypical environmental conditions leading to high N mineralization may have obscured results of this study indicated by the variable responses between years.

With regards to nodulation, varietal differences existed between average nodule number per plant at the V4 stage. PS 30-80 averaged 30.3 nodules per plant while PFS 15R07 averaged 24.4 nodules per plant. Root mass also differed between varieties at the R4 stage. The average root mass of PS 30-80 and PFS 15R07 was 0.49 g per root and 0.47 g per root, respectively, at R4. A relationship was expected between average nodule number and root mass. Overall there was no direct relationship between average nodules per plant and average root mass per plant (r^2=0.0055).

Nitrogen impacted a number of plant characteristics related to nodulation including average nodules per plant and nodule size. As N rate increased, the average nodules per plant decreased significantly. The average nodules per plant at 0, 140, and 280 kg N ha^{-1} were 31.8, 26.5, and 23.7, respectively, at V4. At R4, the average nodules per plant at 0, 140, and 280 kg N ha^{-1} were 44.3, 37.3, and 33.5, respectively. The average number of nodules per plant was significantly lower than the control at both growth stages when plants were treated with 140 and 280 kg N ha^{-1}, but the average number of nodules per plant was not different between the 140 and 280 kg N ha^{-1} treatments at either stage. Nitrogen also significantly influenced the average percent small and large nodules per plant at both stages. The general trend indicated that average nodule size became smaller as N application rate increased. At R4, the interaction of VT and N influenced the average percent medium nodules per plant, average plant mass, and average shoot mass. Varieties responded very differently to N rate with regards to plant mass and shoot mass. The interaction of VT and N for average percent medium nodules per plant indicates that average percent medium nodules per plant of PFS 15R07 increased as N rate increased, while PS 30-80 initially increased but then decreased. However, the average percent medium nodules per plant of PFS 15R07 appeared to respond more to N than PS 30-80.

Sulfur influenced nodule size at the R4 stage. However, the relationship remains unclear and more research is necessary to establish how S impacts nodule size. Additionally, the interaction of VT and S influenced several variables, including average nodules number at the V4 stage. The relationship indicates that the significance was due to a difference in magnitude for average nodules between varieties and was not a true interaction. In general, PS 30-80 appeared to be more responsive to S treatment with regards to nodulation, which resulted in numerous VT x S interactions for various measured dependent variables. The varieties responded completely differently to S application with regards to average percent large nodules per plant. The average percent large nodules per plant increased over the control for PS 30-80 when 112 kg S ha^{-1} was applied. Conversely, the average large nodules per plant decreased compared to the control for PFS 15R07 when 112 kg S ha^{-1} was applied. More research is needed to determine how S influences different soybean varieties in North Dakota.

Nodulation and plant vegetation was more effected by fertilizer in early vegetative growth than reproductive growth, indicating that plant growth throughout the season made up for early differences between treatments and control. Soil types varied by environment and likely impacted the results of the study, especially the nodulation study. Compaction and varying soil moisture levels made accurate sample collection very difficult.

The results of this study indicate that N and S fertilizer can influence soybean seed yield and several growth characteristics in North Dakota. Nitrogen fertilizer was a more dominant factor for impacting soybean yield and nodulation than S fertilizer. This study also showed that soybean varieties respond differently to fertilizer treatment. Additionally, environments played a key role in response to treatment but experimental sites in this study were not specifically selected for N and S research. Further important considerations are fertilizer form and application method. A main goal of this study was to understand the interaction of N and S on soybean yield, quality, and growth. Although not significant at 95% confidence, a relationship exists between N and S that should be investigated in more detail. Future research should focus on understanding genotypic variations in response to N and S fertilizer, soil-specific response to N and S application, and different forms of N and S fertilizer at various application timings to maximize the sustainability of soybean production practices in North Dakota.

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APPENDIX

Table A1. Mean squares and significance levels for the ANOVA for agronomic traits, NW22 naturally drained, 2015.

Source	DF	Density	Vigor	Greenness	GreenSeeker	Height	Yield	TW	Protein	Oil
Rep	3	1873808964	47.57***	1.3**	0.0075***	439.6***	108.5***	0.43	0.85**	0.59***
VT [Variety]	1	3442128979	22.67***	0.2	0.0000	0.2	9.6	8.62**	0.70*	3.19***
N [Nitrogen]	2	3264106373*	5.03***	6.2***	0.0003	111.6*	43.1*	0.11	0.1	0.30*
VT * N	2	781470846	1.47*	0.3	0.0002	5.5	9.8	0.01	0.12	0.07
S [Sulfur]	2	495343872	2.02**	0.1	0.0000	0.1	4.7	0.48	1.10**	0.15
VT * S	2	736292904	0.82	0.0	0.0005*	56.1	24.1	0.69	0.11	0.07
N * S	4	956804290	0.77	0.1	0.0002	29.1	5.6	0.54	0.19	0.02
VT * N * S	4	510403188	1.20*	0.2	0.0000	66.3*	7.7	0.67	0.14	0.04
Residual Error	51	904571248	0.36	0.2	0.0001	24.4	8.8	0.78	0.17	0.06

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively.

Table A2. Mean squares and significance levels for the ANOVA for agronomic traits, NW22 controlled tile drained, 2015.

Source	DF	Density	Vigor	Greenness	GreenSeeker	Height	Yield	TW	Protein	Oil
Rep	3	1688794533	3.4***	1.3***	0.0061***	571.8***	84.3***	0.29	1.22***	0.07
VT [Variety]	1	3442128981	15.1***	0.5	0.0001	0.0	3.4	0.00	0.08	1.64***
N [Nitrogen]	2	558808127	2.8**	5.4***	0.0003	3.1	12.8	0.41	0.21	0.10
VT * N	2	197384583	0.1	0.0	0.0001	8.9	2.4	0.59	0.01	0.00
S [Sulfur]	2	1189685827	0.9	0.6	0.0001	7.3	8.9	0.32	0.05	0.07
VT * S	2	860532244	0.9	0.1	0.0001	8.6	1.7	0.77	0.02	0.03
N * S	4	652391008	0.5	0.3	0.0001	14.2	7.2	1.07	0.08	0.03
VT * N * S	4	650777510	0.3	0.1	0.0000	16.6	8.4	0.42	0.07	0.10
Residual Error	51	904065052	0.5	0.2	0.0001	15.4	5.8	0.87	0.14	0.04

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively.

Table A3. Mean squares and significance levels for the ANOVA for agronomic traits, Ransom County, ND, 2015.

Source	DF	Density	Vigor	Greenness	GreenSeeker	Height	Yield	TW	Protein	Oil
Rep	2	659024279	0.5	0.1	0.0007*	40.5	10	0.31	0.08	0.18
VT [Variety]	1	1721781598	52.0***	0.8	0.0001	40.4	2.8	2.8***	0.5	0.02
N [Nitrogen]	2	1751900229	19.5***	6.5***	0.0002	149.2***	1.5	0.09	0.21	0.35*
VT * N	2	2414510062	1.5	0.4	0.0002	17.8	6.1	0.04	0.3	0.22
S [Sulfur]	2	1463621927	0.1	0.1	0.0001	8.4	7.6	0.3	0.08	0.06
VT * S	2	951605240	1.4	0.9	0.0005	4.3	2.7	0	0.06	0.15
N * S	4	1557204810	1	0.3	0.0000	25.3	30.9	0.14	0.36	0.18
VT * N * S	4	1086063403	0.7	0.3	0.0003	21.1	16	0.23	0.11	0.21
Residual Error	34	1009817716	1.3	0.3	0.0002	15.6	13.7	0.17	0.17	0.09

*, **, *** = significant at (p≤0.05), (p≤0.01), and (p≤0.001), respectively.

Table A4. Mean squares and significance levels for the ANOVA for agronomic traits, Richland County, ND, 2015.

Source	DF	Density	Vigor	Greenness	GreenSeeker	Height	Yield	TW	Protein	Oil
Rep	2	60237257	0.6	0.1	0.0012*	28.3	41.6	3.98***	0.48	0.00
VT [Variety]	1	1388325357	5.4	0.1	0.0000	27.6	4.6	0.01	0.42	0.14
N [Nitrogen]	2	854078252	5.0	1.7*	0.0001	3.5	6.3	0.01	0.13	0.14
VT * N	2	1356055394	3.2	0.1	0.0001	2.0	9.4	0.15	0.12	0.22
S [Sulfur]	2	587313256	2.4	0.8	0.0001	2.4	6.1	0.12	0.03	0.04
VT * S	2	895670644	0.6	1.0	0.0001	2.4	18.9	0.00	0.23	0.10
N * S	4	1944802873	2.4	0.6	0.0002	7.5	15.6	0.23	0.24	0.20
VT * N * S	4	856946695	10.3**	0.4	0.0001	17.1	6.9	0.21	0.13	0.07
Residual Error	34	1262198326	2.1	0.5	0.0003	10.6	12.7	0.23	0.21	0.11

*, **, *** = significant at (p≤0.05), (p≤0.01), and (p≤0.001), respectively.

Table A5. Mean squares and significance levels for the ANOVA for agronomic traits, Sargent County, ND, 2015.

Source	DF	Density	Vigor	Greenness	GreenSeeker	Height	Yield	TW	Protein	Oil
Rep	2	1574774011	6.4	0.2	0.0001	26.6	2.1	0.727*	0.56	0.19
VT [Variety]	1	6453992	20.2*	2.9	0.0000	6.2	34.3	4.392***	0.18	1.12*
N [Nitrogen]	2	2415944276*	3.9	0.1	0.0002	34.5	19.9	0.24	0.15	0.02
VT * N	2	174257780	0.3	0.3	0.0002	24.0	2.3	0.05	0.72	0.09
S [Sulfur]	2	135533828	3.9	0.9	0.0001	15.9	1.2	0.16	0.07	0.19
VT * S	2	699182449	2.4	0.4	0.0004	4.6	3.7	0.07	0.44	0.06
N * S	4	1770545093*	7.2	0.6	0.0006	71.3	6.6	0.37	0.66	0.14
VT * N * S	4	654004507	1.5	1.2	0.0004	67.3	5.0	0.00	1.69	0.04
Residual Error	34	552006126	3.4	0.9	0.0003	50.1	9.4	0.14	0.78	0.19

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively.

Table A6. Mean squares and significance levels for the ANOVA for agronomic traits, combined across environments in 2015†.

Source	DF	Density	Vigor	G	GS	Height	Yield	TW	Protein	Oil
Env [Environment]	4	8553268679***	6.2**	0.2	0.0192***	5091***	1067***	37.4***	73.7***	12.17***
Rep(Env)	12	1272990132	14***	0.7*	0.0037***	268***	57***	1.0*	0.8	0.23**
VT [Variety]	1	7434998598*	102.8**	1.5	0.0001	16	25	9.3	0.1	3.48
N [Nitrogen]	2	6398844071**	4.1	5.7	0.0003	136	16	0.0	0.4	0.12
VT * N	2	89369859	2.1	0.2	0.0000	16	1	0.2	0.1	0.18
S [Sulfur]	2	235759940	3.2	0.5	0.0001	2	8	0.4	0.8	0.34**
VT * S	2	1027350007	0.7	0.4	0.0003	25	5	0.2	0.1	0.03
N * S	4	2324433048	3.0	1.0*	0.0003	33	28	0.6	0.4	0.09
VT * N * S	4	650956787	2.1	0.5	0.0002	7	6	0.1	0.8	0.12
Residual Error	204	922829436	1.4	0.4	0.0002	23	10	0.5	0.6	0.09

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).
†Environments included in analysis: NW22 naturally drained, NW22 controlled tile drained, Ransom, Richland, and Sargent County. G = visual greenness score, GS = GreenSeeker value, TW = test weight.

Table A7. Mean squares and significance levels for the ANOVA for agronomic traits, NW22 naturally drained, 2016.

Source	DF	Density	Vigor	Greenness	GreenSeeker	Height	Yield	TW	Protein	Oil
Rep	3	356220913	3.4	3.1***	0.0111***	115***	162***	0.12	0.24	0.36
VT [Variety]	1	194140228	4.3	1.2*	0.0002	26	124**	2.72**	0.95*	0.45
N [Nitrogen]	2	498579759	11.5**	0.0	0.0051***	71*	63*	0.89	0.71*	0.41
VT * N	2	397482976	0.9	0.1	0.0005	40	59*	0.35	0.45	0.12
S [Sulfur]	2	1110091930	0.5	0.5	0.0001	3	3	0.11	0.25	0.00
VT * S	2	2635349750*	2.5	0.0	0.0003	14	30	0.55	0.17	0.27
N * S	4	529217663	1.7	0.4	0.0011	28	8	0.41	0.07	0.07
VT * N * S	4	631415399	1.8	0.1	0.0002	43	7	0.2	0.19	0.04
Residual Error	51	728657397	1.5	0.2	0.0005	17	13	0.38	0.19	0.13

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively.

Table A8. Mean squares and significance levels for the ANOVA for agronomic traits, NW22 controlled tile drained, 2016.

Source	DF	Density	Vigor	Greenness	GreenSeeker	Height	Yield	TW	Protein	Oil
Rep	3	3084300997*	8.5**	0.8**	0.0026***	75**	345***	0.27	4.05***	0.09
VT [Variety]	1	137691481	0	0.2	0.0023*	3	91***	5.84***	0.48	0.16
N [Nitrogen]	2	713719846	3.6	0.1	0.0030**	49	72***	0.05	0.14	0.27
VT * N	2	603979316	5.6*	0.2	0.0003	12	16	0.03	0.27	0
S [Sulfur]	2	1117085631	4.3	0.1	0.0003	25	10	0.37	0.24	0.04
VT * S	2	1052536008	3.4	0.1	0.0008	5	7	0.77	0.13	0.03
N * S	4	420048698	0.8	0.4	0.0006	1	8	0.99*	0.39*	0.12
VT * N * S	4	758874972	1.7	0.0	0.0001	3	6	0.19	0.09	0.13
Residual Error	51	1070264323	1.5	0.2	0.0004	17	7	0.36	0.15	0.1

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively.

Table A9. Mean squares and significance levels for the ANOVA for agronomic traits, Ransom County, ND, 2016.

Source	DF	Density	Vigor	Greenness	GreenSeeker	Height	Yield	TW	Protein	Oil
Rep	3	263179444	9.4**	0.2*	0.0088*	19*	4	0.94	0.7	0.29
VT [Variety]	1	25815967	33.4***	0.0	0.0001	0	14	1.85*	0.15	0
N [Nitrogen]	2	745077503	29.6***	1.9***	0.0002	3	45*	0.33	0.05	0.01
VT * N	2	1202593812	3.9	0.0	0.0015	5	7	0.63	0.3	0.05
S [Sulfur]	2	344930007	0.2	0.1	0.0017	3	4	0.53	0.18	0.03
VT * S	2	273218987	0.5	0.1	0.0004	4	9	0.5	0.21	0.04
N * S	4	91072996	0.5	0.0	0.0042	6	8	0.37	0.27	0.09
VT * N * S	4	359272212	1.5	0.1	0.0031	6	13	0.53	0.06	0.07
Residual Error	34	628399453	1.6	0.0	0.0019	5	10	0.34	0.29	0.11

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively.

Table A10. Mean squares and significance levels for the ANOVA for agronomic traits, Sargent County, ND, 2016.

Source	DF	Density	Vigor	Greenness	GreenSeeker	Height	Yield	TW	Protein	Oil
Rep	3	482618773	23.9**	2.1***	0.0002	81	40	0.37	0.24	0.02
VT [Variety]	1	35131947	3.3	0.5	0.0000	33	59	2.99**	1.75**	0.51*
N [Nitrogen]	2	504136178	2.8	0.0	0.0000	2	57	0.07	0.45	0.03
VT * N	2	1295801117	0.2	0.2	0.0001	0	17	0.28	0.28	0.13
S [Sulfur]	2	22232244	0.3	0.1	0.0000	45	51	0.41	0.07	0.04
VT * S	2	603100662	1.9	0.0	0.0000	32	32	0.28	0.02	0.07
N * S	4	347077684	1.5	0.1	0.0001	13	6	0.08	0.28	0.04
VT * N * S	4	372901866	2	0.1	0.0000	5	10	0.15	0.36	0.1
Residual Error	34	845560362	3.1	0.2	0.0001	44	40	0.29	0.17	0.09

*, **, *** = significant at (p≤0.05), (p≤0.01), and (p≤0.001), respectively.

Table A11. Mean squares and significance levels for the ANOVA for agronomic traits, Steele County, ND, 2016.

Source	DF	Density	Vigor	Greenness	GreenSeeker	Height	Yield	TW	Protein	Oil
Rep	3	4462623241**	7.6**	0.0	0.0001	121**	37	0.07	10.37***	0.03
VT [Variety]	1	1264992080	8.0*	0.6**	0.0000	78*	3	2.20***	0.11	0.06
N [Nitrogen]	2	1196862330	0.3	1.3***	0.0001	16	25	0.06	2.17*	0.30*
VT * N	2	105422114	0.8	0.1	0.0001	2	3	0.07	0.13	0.07
S [Sulfur]	2	99683126	0.2	0.0	0.0000	17	5	0.07	0.96	0.07
VT * S	2	1370412120	0.5	0.1	0.0000	8	5	0.05	0.31	0.09
N * S	4	342778398	0.7	0.1	0.0002	2	6	0.16	0.54	0.02
VT * N * S	4	314097282	0.7	0.1	0.0000	2	15	0.03	0.68	0.1
Residual Error	34	627386632	1.1	0.1	0.0002	18	17	0.09	0.45	0.08

*, **, *** = significant at ($p≤0.05$), ($p≤0.01$), and ($p≤0.001$), respectively.

Table A12. Mean squares and significance levels for the ANOVA for agronomic traits, combined across environments in 2016†.

SOV†	DF	Density	Vigor	G	GS	Height	Yield	TW	Protein	Oil
Env [Environment]	4	9159349684***	14.2	0.4*	0.0842***	19886***	10368***	11.79***	10.06***	15.33***
Rep(Env)	12	1728200721*	9.8***	1.4***	0.0049***	84***	140***	3.28***	2.96***	0.17
VT [Variety]	1	298087265	32.9	0.3	0.0002	18	32	8.55*	0.08	0.01
N [Nitrogen]	2	1429761662	28.4*	1.8	0.0025	77*	201**	1.11	0.24	0.03
VT * N	2	1473422318	3.7	0.2	0.0005	13	42	0.17	0.17	0.04
S [Sulfur]	2	607875074	0.8	0.1	0.0006	65*	6	1.2	0.4	0.01
VT * S	2	2978840773*	2.8	0.2	0.0001	9	19	2.61	0.27	0.08
N * S	4	634932909	0.5	0.2	0.0008	0	2	1.03	0.18	0.06
VT * N * S	4	452756817	2.1	0	0.0009	8	19	0.2	0.15	0.15
Residual Error	204	799954838	1.7	0.1	0.0006	20	16	1.04	0.24	0.1

*, **, *** = significant at ($p≤0.05$), ($p≤0.01$), and ($p≤0.001$), respectively, tested according to Carmer et al. (1989).
†Environments included in analysis: NW22 naturally drained, NW22 controlled tile drained, Ransom, Sargent, and Steele County. G = visual greenness score, GS = GreenSeeker value, TW = test weight.

Table A13. Mean squares and significance levels for the ANOVA for agronomic traits, combined across all environments in 2015 and 2016.

SOV[†]	DF	Density	Vigor	G	GS	Height	Yield	TW	Protein	Oil
Env [Environment]	9	20233849352**	11.2***	11.9**	0.0607***	11575***	6050***	23.29*	43.970***	60.10***
Rep(Env)	24	1500593439*	11.9***	1.0***	0.0043***	179***	99***	2.15***	1.878***	0.20**
VT [Variety]	1	2377861609	126.0***	1.6	0.000804	34	0	17.81**	0.001	1.53
Env * VT	9	979421914	5.0	0.6	0.000280	20	28	1.20	0.690	0.60
N [Nitrogen]	2	6656845289**	25.3*	6.9*	0.0048**	208**	154**	0.47	0.420	0.13
Env * N	18	684597215	6.3	1.7*	0.000565	25	39*	0.33	0.499	0.20
VT * N	2	1102741834	4.1	0.0	0.0006	20	17	0.25	0.080	0.12
S [Sulfur]	2	148288393	3.6	0.1	0.0002	22	4	0.53	1.173*	0.16
Env * S	18	712357758	1.2	0.4	0.000203	13	11	0.46	0.258	0.06
VT * S	2	3466328957*	0.4	0.5	0.0002	12	20	1.80	0.089	0.04
N * S	4	1208466276	1.2	0.9**	0.0001	15	22	0.71	0.311	0.09
Env * N * S	36	851189055	1.8	0.2	0.000423	20	9	0.66	0.343	0.09
VT * N * S	4	864576933	1.2	0.3	0.0001	8	9	0.23	0.656	0.08
Residual Error	408	1200406844	1.5	0.3	0.0004	21	13	0.77	0.426	0.10

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).
[†]Environments included in analysis: NW22 naturally drained, NW22 controlled tile drained, Ransom, Sargent, and Steele County. G = visual greenness score, GS = GreenSeeker value, TW = test weight.

Table A14. Mean squares and significance levels for the ANOVA for root characteristics, first nodulation experiment, NW22 naturally drained, 2015.

SOV†	DF	AN	Sm	M	L	PM	SM	RM
Rep	3	570.16**	1181.96***	216.23*	1365.59***	0.18	0.17	0.00
VT [Variety]	1	475.24*	442.86	11.46	311.86	0.00	0.00	0.00
Error (a)	3	66.71	83.91	39.79	122.41	0.23	0.20	0.00
N [Nitrogen]	2	1548.92***	4692.13***	84.26	4555.58***	0.03	0.06	0.01
VT * N	2	12.35	191.39	168.15	193.70	0.01	0.00	0.00
S [Sulfur]	1	336.84	188.08	5.00	254.44	0.01	0.04	0.01
VT * S	1	10.81	213.37	58.42	48.50	0.05	0.07	0.00
N * S	2	17.99	324.29	107.51	58.45	0.08	0.06	0.01
VT * N * S	2	57.32	84.64	192.80*	350.29	0.00	0.00	0.00
Residual Error	18	98.14	113.47	56.06	98.51*	0.13	0.10	0.01

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A15. Mean squares and significance levels for the ANOVA for root characteristics, first nodulation experiment, NW22 controlled tile drained, 2015.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Rep	3	207.58*	311.64	221.01*	1002.57***	0.09	0.06	0.01
VT [Variety]	1	644.65**	266.96	462.08*	1431.49***	0.00	0.00	0.00
Error (a)	3	54.53	243.41	120.17	159.31	0.20	0.13	0.01
N [Nitrogen]	2	581.28***	3894.52***	150.87	5541.33***	0.42*	0.29	0.01
VT * N	2	55.03	69.72	600.18***	499.77**	0.15	0.09	0.01
S [Sulfur]	1	430.06	338.23	37.99	149.51	0.27	0.14	0.02
VT * S	1	12.44	24.36	64.99	9.77	0.18	0.18	0.00
N * S	2	26.30	52.24	89.03	103.97	0.49	0.42	0.00
VT * N * S	2	72.28	3.32	57.67	34.33	0.02	0.02	0.00
Residual Error	18	57.91	141.91	66.55	93.51	0.13	0.09	0.01

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A16. Mean squares and significance levels for the ANOVA for root characteristics, first nodulation experiment, Ransom County, ND, 2015.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	104.07	226.18*	1522.16***	2921.86***	0.74*	0.60**	0.01
VT [Variety]	1	242.05*	37.57	7.43	11.59	0.04	0.01	0.01
Error (a)	1	1.65	49.89	28.26	153.24	0.15	0.15	0.00
N [Nitrogen]	2	44.21	44.28	474.05**	800.84**	0.42	0.42	0.00
VT * N	2	27.95	18.53	6.51	23.99	0.38*	0.35*	0.00
S [Sulfur]	1	126.36	72.82	285.64	646.91	0.57*	0.32	0.04
VT * S	1	80.79	147.93*	0.05	153.51	0.10	0.07	0.00
N * S	2	317.18*	35.51	116.36	278.52	0.13	0.04	0.03
VT * N * S	2	31.14	23.71	19.45	0.97	0.34	0.24	0.01
Residual Error	6	41.81	29.75	39.14	94.98	0.09	0.06	0.01

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A17. Mean squares and significance levels for the ANOVA for root characteristics, first nodulation experiment, Richland County, ND, 2015.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	248.77*	1068.18**	30.28	1458.16**	0.26	0.11	0.03
VT [Variety]	1	144.77*	398.98	130.64	73.01	0.02	0.03	0.00
Error (a)	1	3.19	3.37	0.27	5.57	0.09	0.09	0.00
N [Nitrogen]	2	79.13	453.00*	158.08	1118.70**	0.28	0.24	0.01
VT * N	2	36.61	135.71	99.95	33.57	0.25	0.31	0.01
S [Sulfur]	1	43.70	154.28**	1.55	186.72	0.32	0.41	0.01
VT * S	1	132.43*	0.98	287.78	255.19	0.09	0.05	0.00
N * S	2	4.02	25.60	43.75	44.18	0.05	0.04	0.00
VT * N * S	2	3.24	140.48	93.16	119.61	0.09	0.05	0.01
Residual Error	6	26.33	87.47	64.24	121.09	0.25	0.19	0.01

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A18. Mean squares and significance levels for the ANOVA for root characteristics, first nodulation experiment, Sargent County, ND, 2015.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	67.04	196.65*	64.67	486.86*	10.91***	8.78***	0.11***
VT [Variety]	1	41.30	5.85	84.53	134.86	0.02	0.03	0.01
Error (a)	1	3.50	20.05	1.70	33.41	0.00	0.01	0.00
N [Nitrogen]	2	195.91*	1162.37***	259.29**	1857.91***	0.81*	0.93*	0.01
VT * N	2	8.70	10.46	5.42	30.15	0.16	0.11	0.00
S [Sulfur]	1	1.67	548.87***	1.81	613.63*	0.31	1.59	0.01
VT * S	1	82.29	20.99	2.14	36.53	0.09	0.73	0.06**
N * S	2	20.03	93.23	2.36	75.97	0.14*	0.08*	0.01
VT * N * S	2	21.69	22.27	23.10	52.31	0.21	0.19	0.00
Residual Error	6	27.68	27.09	25.03	69.72	0.26	0.21	0.00

*, **, *** = significant at (*p*≤0.05), (*p*≤0.01), and (*p*≤0.001), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A19. Mean squares and significance levels for the ANOVA for first nodulation experiment (V4) root characteristics combined across all environments in 2015.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Env (Environment)	4	817.66**	2937.45***	386.93***	3935.28***	14.15***	10.71***	0.26
Rep(Env)	9	305.90**	663.54**	325.43***	1330.15***	1.41***	1.13***	0.02**
VT [Variety]	1	1193.55**	7.63	333.49	442.04	0.37	0.26	0.01
VT * Env	4	24.46	257.64	88.41	272.6	0.2	0.16	0.01
N [Nitrogen]	2	1320.48**	5593.55**	645.84	9998.83***	1.33**	1.46**	0.01
VT * N	2	36	26.88	86.88	97.3	0.31	0.30	0.00
S [Sulfur]	1	18.06	1125.07**	69.82	1755.44**	0.63	0.77*	0.01
VT * S	1	153.12	0.94	221.41	251.14	0.64	0.47	0.01
N * S	2	118.54	116.06	217.77*	85.35	0.13	0.10	0.01
VT * N * S	2	16.27	102.96	42.38	41.99	0.34	0.25	0.01
Residual Error	90	64.8	101.16	55.14	93.81	0.14	0.11	0.01

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A20. Mean squares and significance levels for the ANOVA for root characteristics, second nodulation experiment, NW22 naturally drained, 2015.

SOV†	DF	AN	Sm	M	L	PM	SM	RM
Rep	3	358.27**	358.68*	287.14	798.41**	19.31	13.08	0.62*
VT [Variety]	1	4.85	171.70	1087.62**	2123.60***	14.60	10.59	0.32
Error (a)	3	50.25	123.25	115.81	374.23	4.10	2.80	0.13
N [Nitrogen]	2	459.80**	772.27***	107.41	691.27*	2.77	1.91	0.11
VT * N	2	125.39	41.92	66.79	6.74	9.43	8.42	0.04
S [Sulfur]	1	21.05	3.98	33.00	59.91	14.41	12.10	0.10
VT * S	1	55.77	9.51	36.76	83.67	0.97	0.17	0.33
N * S	2	38.66	40.48	9.20	39.15	9.17	7.51	0.10
VT * N * S	2	79.20	4.37	96.45	141.67	0.52	0.78	0.03
Residual Error	18	64.80	82.60	105.09	144.53	9.51	7.35	0.17

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A21. Mean squares and significance levels for the ANOVA for root characteristics, second nodulation experiment, NW22 controlled tile drained, 2015.

SOV†	DF	AN	Sm	M	L	PM	SM	RM
Rep	3	93.86	98.86	126.26	35.52	5.37	4.67	0.09
VT [Variety]	1	516.24**	8.03	444.81*	333.29*	3.29	1.54	0.33
Error (a)	3	104.13	87.10	36.73	32.05	22.29	16.62	0.42
N [Nitrogen]	2	260.00*	1372.92***	1.69	1325.99**	2.48	1.88	0.04
VT * N	2	29.09	9.78	96.97	67.09	7.04	4.14	0.38
S [Sulfur]	1	141.80	1397.60	415.95	288.64	6.33	6.59	0.00
VT * S	1	93.49	96.31	109.74	0.44	0.00	0.00	0.00
N * S	2	32.74	197.14	293.12*	79.51	19.57	15.09	0.31
VT * N * S	2	67.45	34.40	41.12	143.42	7.45	4.92	0.27
Residual Error	18	54.70	98.14	78.87	65.54	8.25	6.08	0.19

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A22. Mean squares and significance levels for the ANOVA for root characteristics, second nodulation experiment, Ransom County, ND, 2015.

SOV†	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	352.92*	38.67	146.83	34.80	5.64	3.99	0.14
VT [Variety]	1	87.47	0.73	7.38	3.46	0.07	0.12	0.01
Error (a)	1	19.82	120.21	112.21	464.70	7.48	6.67	0.02
N [Nitrogen]	2	130.87	48.07	76.19	209.55	3.19	2.00	0.14
VT * N	2	47.87	9.14	14.79	2.13	6.32	5.65	0.05
S [Sulfur]	1	19.20	83.43	4.78	128.14	0.09	0.18	0.01
VT * S	1	243.04*	2.89	74.00	47.65	0.78	1.23	0.05
N * S	2	116.61	26.69	41.34	121.46	2.99	2.67	0.03
VT * N * S	2	7.80	15.43	0.09	13.52	1.37	0.74	0.10
Residual Error	6	47.91	48.18	61.39	109.28	5.92	4.56	0.10

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A23. Mean squares and significance levels for the ANOVA for root characteristics, second nodulation experiment, Richland County, ND, 2015.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	49.29	49.75	568.87*	282.17	57.09	49.24	0.29
VT [Variety]	1	30.51**	46.23	330.39	623.80	117.56*	86.26*	2.42**
Error (a)	1	0.00	84.63	31.60	12.81	12.64	7.83	0.57
N [Nitrogen]	2	202.03	245.36	519.21*	1450.69**	8.34	7.60	0.22
VT * N	2	0.02	44.38	57.09	54.82	11.66	10.83	0.04
S [Sulfur]	1	210.74	145.26	636.30	1389.60	1.48	1.04	0.04
VT * S	1	12.87	342.57	67.34	713.69	18.74	14.61	0.26
N * S	2	4.58	28.67	208.48	355.56	54.95	43.74	0.67
VT * N * S	2	72.52	69.08	86.69	310.30	18.31	14.95	0.17
Residual Error	6	92.32	81.53	88.06	183.09	20.76	16.87	0.25

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A24. Mean squares and significance levels for the ANOVA for root characteristics, second nodulation experiment, Sargent County, ND, 2015.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	822.22**	80.39	460.98***	926.38**	7.12	6.12	0.04
VT [Variety]	1	51.68	128.31	541.49**	1196.96	5.71	3.64	0.23
Error (a)	1	6.17	27.06	0.09	30.33	32.47	25.22	0.46
N [Nitrogen]	2	6.88	69.71	399.35*	429.95*	9.24	6.26	0.35
VT * N	2	45.68	21.57	194.37	322.87	2.75	3.12	0.05
S [Sulfur]	1	73.00	17.22	273.30	153.31	0.21	0.19	0.00
VT * S	1	19.42	10.32	228.69	141.86	27.30	23.53	0.14
N * S	2	12.33	55.27	58.81	62.01	4.39	4.22	0.01
VT * N * S	2	138.12	41.86	19.33	106.54	4.20	2.33	0.28
Residual Error	6	46.07	30.84	19.29	54.27	15.51	12.22	0.23

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A25. Mean squares for the ANOVA for root characteristics of the second nodulation experiment (R4) combined across all environments in 2015.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Env (Environment)	4	1263.75***	882.93***	529.16***	1476.33***	173.53***	140.05***	1.87***
Rep(Env)	9	286.75***	171.27	268.54**	416.13***	15.99	12.51	0.29
VT [Variety]	1	17.33	151.63	1772.83**	2961.42*	49.92	36.5	1.05
VT * Env	4	151.72	52.65	83.23	240.54	26.39	18.84	0.67
N [Nitrogen]	2	615.39**	1094.91*	608.02	3333.77***	2.87	2.85	0.00
VT * N	2	116.62*	8.21	333.68**	238.54	13.54	11.98	0.09
S [Sulfur]	1	382.33*	29.21	737.18	472.90	10.39*	9.48*	0.02
VT * S	1	66.65	129.01	207.52	663.78	1.53	1.45	0.00
N * S	2	39.02	105.42	19.67	56.23	27.68	22.37	0.28
VT * N * S	2	85.23	19.01	9.68	48.10	15.49	11.11	0.38
Residual Error	90	61.15	76.31	81.39	100.21	10.21	7.95	0.17

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A26. Mean squares and significance levels for the ANOVA for root characteristics, first nodulation experiment, NW22 naturally drained, 2016.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Rep	3	309.1***	455.9***	157.3*	493.2***	1.18	1.11	0.01
VT [Variety]	1	313.8***	256.9*	332.3*	4.84	0.49	0.44	0.00
Error (a)	3	19.80	25.95	2.76	11.79	0.01	0.01	0.03
N [Nitrogen]	2	3.77	13.48	224.5*	347.7**	0.02	0.04	0.00
VT * N	2	32.71	79.84	291.4**	215.1*	0.64	0.42	0.03
S [Sulfur]	1	1.88	6.40	176.00	115.28	0.25	0.16	0.01
VT * S	1	2.90	25.74	42.67	2.13	0.07	0.09	0.00
N * S	2	18.08	43.15	108.76	134.06	1.14*	0.84*	0.02
VT * N * S	2	6.25	1.47	108.24	90.36	0.46	0.40	0.00
Residual Error	18	17.61	50.86	45.31	55.80	0.73	0.51	0.03

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A27. Mean squares and significance levels for the ANOVA for root characteristics, first nodulation experiment, NW22 controlled tile drained, 2016.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Rep	3	129.7***	54.13	403***	292.6**	3.5**	2**	0.2***
VT [Variety]	1	213***	198.97	77.73	500.0	0.57	0.60	0.00
Error (a)	3	5.64	87.18	68.31	128.65	1.11	0.73	0.04
N [Nitrogen]	2	79.8**	149.64	429.9**	816.0*	1.20	0.81	0.05
VT * N	2	7.59	91.19	3.66	71.01	0.29	0.18	0.01
S [Sulfur]	1	20.35	45.13	105.90	206.5*	0.22	0.09	0.03
VT * S	1	0.66	0.05	47.08	50.25	1.73	1.21	0.05
N * S	2	34.74	300.2*	141.67	38.04	1.10	0.58	0.08
VT * N * S	2	0.88	42.41	62.59	2.18	0.75	0.57	0.03
Residual Error	18	10.56	60.00	57.15	58.45	0.66	0.44	0.03

*, **, *** = significant at (p≤0.05), (p≤0.01), and (p≤0.001), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A28. Mean squares and significance levels for the ANOVA for root characteristics, first nodulation experiment, Ransom County, ND, 2016.

SOV†	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	5.21	21.32	11177.92	158.38	0.43	0.41	0.00
VT [Variety]	1	346.10	2.30	9888.43	2.83	1.04	0.63	0.05*
Error (a)	1	104.14	45.24	10674.00	1.37	0.21	0.13	0.01
N [Nitrogen]	2	84.19	773.6*	11490.34	228.4	1.50	1.28	0.01
VT * N	2	64.86	173.62	8118.12	28.98	0.24	0.21	0.00
S [Sulfur]	1	17.38	218.68	9984.51	238.3	0.59	0.52	0.00
VT * S	1	53.17	2.56	11167.98	23.09	0.06	0.06	0.00
N * S	2	18.67	73.75	9995.73	36.99	0.55	0.41	0.01
VT * N * S	2	18.71	10.30	10780.58	99.68	0.17	0.15	0.00
Residual Error	6	57.47	117.68	9763.35	42.87	0.23	0.17	0.01

*, **, *** = significant at ($p\leq0.05$), ($p\leq0.01$), and ($p\leq0.001$), respectively, tested according to Carmer et al. (1989).

†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A29. Mean squares and significance levels for the ANOVA for root characteristics, first nodulation experiment, Sargent County, ND, 2016.

SOV†	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	100.15	731.7	342.33	73.06	10.01***	6.9***	0.298***
VT [Variety]	1	209.6*	2.99	2.64	11.26	0.10	0.05	0.01
Error (a)	1	6.69	266.06	108.58	34.71	0.01	0.01	0.00
N [Nitrogen]	2	3.50	191.95	195.19	5.01	0.21	0.15	0.01
VT * N	2	2.78	27.59	7.66	31.92	0.00	0.00	0.00
S [Sulfur]	1	75.19	910.2	601.5	31.86	1.80	1.18	0.06
VT * S	1	49.77	1478.8	936.9	61.58	0.08	0.08	0.00
N * S	2	22.57	28.71	37.53	16.80	0.36	0.26	0.01
VT * N * S	2	29.10	270.74	221.65	10.06	0.23	0.15	0.01
Residual Error	6	29.80	150.22	99.58	18.09	0.14	0.09	0.01

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A30. Mean squares and significance levels for the ANOVA for root characteristics, first nodulation experiment, Steele County, ND, 2016.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	39.05	41.99	0.02	49.12*	0.18	0.09	0.02
VT [Variety]	1	395.6*	4.17	7.67	19.55	0.17	0.21	0.00
Error (a)	1	57.52	254.98	305.19	3.57	0.22	0.08	0.03
N [Nitrogen]	2	891.7***	1469.36**	89.85	1283.27*	0.56	0.38	0.02
VT * N	2	22.32	31.77	4.43	23.38	0.24	0.10	0.03
S [Sulfur]	1	90.15	47.09	110.38	10.60	0.03	0.00	0.02
VT * S	1	0.31	692.76	358.65	48.9	0.03	0.02	0.00
N * S	2	24.41	142.80	86.29	29.4	0.13	0.05	0.02
VT * N * S	2	133.69	176.49	346.34	25.2	0.13	0.05	0.02
Residual Error	6	55.05	86.89	96.00	6.16	0.13	0.08	0.01

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A31. Mean squares for the ANOVA for root characteristics of the first nodulation experiment combined across all environments in 2016.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Env (Environment)	4	1156.47***	1774.49***	1629.57	361.82	6.12	4.17***	0.31***
Rep(Env)	9	162.32***	258.33**	1466.78	290.23*	2.74	1.85***	0.11***
VT [Variety]	1	1483.96**	182.74	1992.79	130.88	0.06	0.21	0.04
VT * Env	4	19.79	30.21	2181.05	81.91	0.62	0.64	0.01
N [Nitrogen]	2	433.84	1842.09*	3391.79	1785.00**	0.16	0.10	0.01
VT * N	2	16.91	47.9	1664.12	88.17	0.61	0.39	0.04
S [Sulfur]	1	0.24	484.22	1120	50.1	0.34	0.36	0.00
VT * S	1	57.3	17.27	2488.52	1.46	0.23	0.18	0.00
N * S	2	1.38	35.99	2346.67	35.52	1.33*	0.81*	0.07*
VT * N * S	2	39.25	153.41	2800.67	14.07	0.53	0.37	0.02
Residual Error	90	20.09	70.74	1129.68	45.54	0.52	0.36	0.02

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A32. Mean squares and significance levels for the ANOVA for root characteristics, second nodulation experiment, NW22 naturally drained, 2016.

SOV†	DF	AN	Sm	M	L	PM	SM	RM
Rep	3	111.70	374.547**	460.649***	138.52**	4.74	2.80	0.27
VT [Variety]	1	168.47	0.27	0.55	1.59	3.35	2.25	0.11
Error (a)	3	0.40	36.91	3.60	63.57	5.11	2.14	0.64
N [Nitrogen]	2	1293.46***	80.23	174.70	484.99**	0.76	0.24	0.23
VT * N	2	2.29	61.00	17.31	13.85	4.18	3.23	0.06
S [Sulfur]	1	175.19	95.65	40.01	11.93	7.29	5.52	0.12
VT * S	1	198.38	79.90	0.09	85.46	6.71	4.80	0.16
N * S	2	3.30	134.97	25.96	48.65	0.78	0.96	0.03
VT * N * S	2	188.26	45.48	59.56	0.98	4.81	3.26	0.15
Residual Error	18	63.63	62.55	61.10	21.66	5.68	3.98	0.19

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A33. Mean squares and significance levels for the ANOVA for root characteristics, second nodulation experiment, NW22 controlled tile drained, 2016.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Rep	3	169.10	359.84**	328.179***	218.04***	4.42	3.41	0.09
VT [Variety]	1	202.60	13.23	105.51	193.446**	20.685*	13.64*	0.49*
Error (a)	3	136.30	88.19	30.44	27.02	1.11	0.86	0.02
N [Nitrogen]	2	1296.43***	224.32	89.89	384.27***	0.13	0.03	0.27
VT * N	2	23.54	8.27	3.32	4.86	0.27	0.17	0.02
S [Sulfur]	1	151.86	174.75	167.91	0.07	0.93	0.94	0.00
VT * S	1	10.17	96.3	202.2	0.00	1.12	0.53	0.11
N * S	2	7.67	106.63	23.60	48.08	10.96	5.47	1.01*
VT * N * S	2	79.27	4.92	57.24	61.33	10.75	7.02	0.40
Residual Error	18	79.11	60.02	45.71	17.86	3.63	2.50	0.15

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A34. Mean squares and significance levels for the ANOVA for root characteristics, second nodulation experiment, Ransom County, ND, 2016.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	0.37	0.70	70.75	85.56	7.49	3.13	0.93
VT [Variety]	1	103.30	7.11	25.05	5.47	23.59	20.37*	0.12
Error (a)	1	159.49	58.47	0.33	50.03	0.35	0.03	0.17
N [Nitrogen]	2	50.00	284.84*	10.34	376.62	18.02	14.86	0.23
VT * N	2	14.26	15.95	8.28	1.80	6.76	4.81*	0.28
S [Sulfur]	1	42.77	798.20*	281.12	131.92	0.07	0.05	0.00
VT * S	1	115.64	3.11	0.57	6.34	0.92	0.32	0.15
N * S	2	60.06	87.98	73.94	16.84	4.95*	4.66*	0.01
VT * N * S	2	233.58	168.86	106.15	134.88	3.62	3.74	0.09
Residual Error	6	82.47	36.88	44.30	77.92	6.21	4.04	0.43

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A35. Mean squares and significance levels for the ANOVA for root characteristics, second nodulation experiment, Sargent County, ND, 2016.

SOV†	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	1240.13***	4.53	0.43	0.17	15.02	10.29	0.45
VT [Variety]	1	34.58	0.00	1.49	12.66	2.44	0.41	0.85
Error (a)	1	92.89	229.91	98.24	1.13	0.17	0.61	0.13
N [Nitrogen]	2	17.93	1.96	2.46	60.64	13.69*	12.07*	0.11
VT * N	2	20.79	62.52	121.52	5.40	9.71	6.22	0.39
S [Sulfur]	1	245.26	39.13	2.70	91.10	5.64	2.04	0.89
VT * S	1	26.16	226.35	100.51	0.07	1.68	1.15	0.05
N * S	2	57.84	47.35	15.97	175.01	20.84	15.06	0.60
VT * N * S	2	14.68	276.29	214.19	6.52	0.33	0.13	0.06
Residual Error	6	56.49	140.08	76.08	68.41	7.32	5.89	0.13

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A36. Mean squares and significance levels for the ANOVA for root characteristics, second nodulation experiment, Steele County, ND, 2016.

SOV†	DF	AN	Sm	M	L	PM	SM	RM
Rep	1	2.53	89.19	28.32	194.64*	0.05	0.00	0.08
VT [Variety]	1	387.64	151.56*	87.33	4.63	8.15	5.97	0.17
Error (a)	1	190.20	121.29	105.55	2.42	3.34	2.84	0.02
N [Nitrogen]	2	1283.37*	1069.99**	141.36	745.45	39.65	31.22	0.64
VT * N	2	10.66	38.95	16.36	25.46	27.20	16.45	1.34
S [Sulfur]	1	650.47	2.17	0.12	0.34	27.67	19.82	0.65
VT * S	1	1.50	26.11*	17.90	4.50	9.25	7.95	0.05
N * S	2	151.45	90.51	85.73	10.22	26.31	19.12	0.69
VT * N * S	2	3.97	39.27	87.65	11.06	65.16	41.90	2.61
Residual Error	6	236.29	31.67	27.92	35.85	7.54	4.59	0.43

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A37. Mean squares for the ANOVA for root characteristics of the second nodulation experiment combined across all environments in 2016.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Env (Environment)	4	5293.04***	71.21	405.55	126.99	88.36	59.87	2.79
Rep(Env)	9	231.72	256.15*	273.99**	150.01**	5.56*	3.56	0.28
VT [Variety]	1	709.56**	35.49	2.16	86.07	3.27	2.15	0.12
VT * Env	4	27.37	33.98	51.62	21.87	14.58	10.72	0.49
N [Nitrogen]	2	2371.24**	1169.98*	125.54	1430.12**	24.75	22.67	0.37
VT * N	2	2.92	6.81	47.49	14.09	32.48*	20.66*	1.35*
S [Sulfur]	1	310.03	662.4	185.26	140.45	5.2	4.79	0.01
VT * S	1	119.62	246.8	61.8	26.57	0.17	0.09	0.01
N * S	2	155.02	41.27	102.63	46.78	2.38	1.76	0.50
VT * N * S	2	205.17	12.73	90.96	67.12	7.08	4.53	0.30
Residual Error	90	87.82	63.08	53.65	34.25	5.86	3.77	0.23

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A38. Mean squares for the ANOVA for root characteristics of the first nodulation experiment combined across all environments.

SOV[†]	DF	AN	Sm	M	L	PM	SM	RM
Env [Environment]	9	1626.51**	4164.09**	1531.19	5652.14**	15.46***	10.37***	0.61***
Rep(Env)	18	234.18***	460.82**	895.38	810.19***	2.07***	1.49***	0.07***
VT [Variety]	1	2673.98***	135.66	2007.68	521.24	0.06	0	0.05*
VT * Env	9	19.81	129.66	1034.97	167.47	0.41	0.35	0.01
N [Nitrogen]	2	1613.66***	6859.32***	2507.59	10014***	1.06	0.96	0.02
VT * N	2	4.54	69.38	927.98	170.26	0.66	0.54	0.03
S [Sulfur]	1	4.94	38.57	296.61	523.56	0.02	0.04	0
VT * S	1	198.22*	5.08	2182.48	106.15	0.04	0.03	0
N * S	2	47.06	11.75	760.32	52.05	0.59	0.33	0.05
VT * N * S	2	19.55	126.28	1099.98	6.77	0.03	0.04	0
Residual Error	180	44.94	85.15	592.33	68.06	0.33	0.23	0.01

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

[†]SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A39. Mean squares for the ANOVA for root characteristics of the second nodulation experiment combined across all environments.

SOV†	DF	AN	Sm	M	L	PM	SM	RM
Env [Environment]	9	2928.14***	3971.295***	772.31	2359.84**	137.19*	107.55	2.12
Rep(Env)	18	259.21**	213.707*	271.585***	283.0659*	10.77	8.04	0.28
VT [Variety]	1	486.7	164.79	917.50*	1976.59*	12.98	9.84	0.22
VT * Env	9	98.94	40.24	150.94	226.46	20.28	14.68	0.55
N [Nitrogen]	2	2606.36***	2257.87***	417	4440.86***	18.33	17.95	0.18
VT * N	2	44.02	14.35	236.88*	147.48	25.79*	17.89*	0.96*
S [Sulfur]	1	0.02	487.66	813.506*	29.69	15.23	14.1	0.02
VT * S	1	5.62	369.56*	17.84	457.53*	1.31	1.09	0.01
N * S	2	68.43	12.62	17.06	56.99	7.13	5.57	0.3
VT * N * S	2	28.38	2.44	80.61	90.78	21.21	14.52	0.66
Residual Error	180	74.48	69.69	67.52	67.23	8.03	6.01	0.2

*, **, *** = significant at ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$), respectively, tested according to Carmer et al. (1989).

†SOV = source of variation, df = degrees of freedom, AN = average nodules per plant, Sm = average small nodules per plant, M = average medium nodules per plant, L = average large nodules per plant, PM = average plant mass, SM = average shoot mass, RM = average root mass per plant.

Table A40. Df equation and error term of F test for the analysis of the factorial experiment combined over all environments.

SOV[†]	df[‡]	Equation	Error term
Env [Environment]	9	Env-1	
Env(rep)	24	Env(r-1)	
VT [Variety]	1	VT-1	VT*Env
VT * Env	9	(VT-1)(Env-1)	Residual
N [Nitrogen]	2	N-1	N*Env
N * Env	18	(N-1)(Env-1)	Residual
VT * N	2	(VT-1)(N-1)	VT*N*Env
VT * N * Env	18	(VT-1)(N-1)(Env-1)	Residual
S [Sulfur]	2	S-1	S*Env
S * Env	18	(S-1)(Env-1)	Residual
VT * S	2	(VT-1)(S-1)	VT*S*Env
VT * S * Env	18	(VT-1)(S-1)(Env-1)	Residual
N * S	4	(N-1)(S-1)	N*S*Env
N * S * Env	36	(N-1)(S-1)(Env-1)	Residual
VT * N * S	4	(VT-1)(N-1)(S-1)	VT*N*S*Env
VT * N * S * Env	36	(VT-1)(N-1)(S-1)(Env-1)	Residual
Residual Error	408	Env[(VT*N*S)-1](r-1)	
Total	611	[(VT)(N)(S)(Env)(r)]-1	

[†]SOV=Source of variation.

[‡]df = degrees of freedom. Reps varied by environment. Environments at county locations had 3 reps and Fargo environments had 4 reps.

Table A41. Df equations and error term of F test for the analysis of the first nodulation experiment combined over all environments.

SOV[†]	df[‡]	df equation	Error term
Environment (Env)	9	Env-1	Env(rep)
Env(rep)	18	Env(r-1)	
Variety (VT)	1	VT-1	VT * Env
VT * Env	9	(VT-1)(Env-1)	Error (a)
Error (a)	18	Env(VT-1)(r-1)	
Nitrogen (N)	2	N-1	N * Env
N * Env	18	(N-1)(Env-1)	
VT * N	2	(VT-1)(N-1)	VT * N * Env
VT * N * Env	18	(VT-1)(N-1)(Env-1)	
Sulfur (S)	1	S-1	S * Env
S * Env	9	(S-1)(Env-1)	Residual
VT * S	1	(VT-1)(S-1)	VT * S * Env
VT * S * Env	9	(VT-1)(S-1)(Env-1)	Residual
N * S	2	(N-1)(S-1)	N * S * Env
N * S * Env	18	(N-1)(S-1)(Env-1)	Residual
VT * N * S	2	(VT-1)(N-1)(S-1)	VT * N * S * Env
VT * N * S * Env	18	(VT-1)(N-1)(S-1)(Env-1)	Residual
Residual Error	180	[(VT)(Env)][(N)(S)-1](R-1)	
Total	335		

[†]SOV=Source of variation.

[‡]df = degrees of freedom.

Table A42. Brief summary of soybean response to sulfur fertilization.

Authors	Life stage	Parameter	Soil Type/Medium	Response
Chen et al., 2005	Maturity	Yield	Wooster silt loam, Typic Fragiudalf	11.6% yield increase
Ganeshamurthy and Reddy, 2000	Reproductive	Nodulation	Leeray fine, smectitic, Typic Haplustert	Increased nodule number and dry weight
	Maturity	Yield		40% yield increase
Hussain et al., 2011	Maturity	Dry Matter	Sandy loam	26% dry matter increase
		Yield		20% yield increase
Sharma and Sharma, 2014	Reproductive	Nodulation	Sandy loam	Increased nodule number and weight
		Root growth		Increased root length and weight
Bellaloui et al., 2011	Maturity	Protein	Silty clay loam	Increased protein concentration up to 30%

Table A43. Water table level of naturally drained (NAD) and controlled tile drained (CTD) environments at NW22 for each measurement date in 2015 from 21 May to 14 Aug.

Date	NAD		CTD
		------------------cm------------------	
21-May	-36.98		-53.88*
		LSD = -10.09	
28-May	-54.20		-76.01*
		LSD = -11.66	
09-Jun	-45.06		-72.01*
		LSD = -10.31	
23-Jun	-49.15		-78.58*
		LSD = -10.49	
06-Jul	-92.01		-99.25*
		LSD = -4.69	
27-Jul	-84.77		-90.11*
		LSD = -4.95	
14-Aug	-94.11		-95.44ns

*, ns = significant at ($p \leq 0.05$), and nonsignificant, respectively.
†Values represent cm below soil surface.