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Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review

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Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review

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Abstract

Although relationships among soybean (*Glycine max* [L.] Merr) seed yield, nitrogen (N) uptake, biological N_2 fixation (BNF), and response to N fertilization have received considerable coverage in the scientific literature, a comprehensive summary and interpretation of these interactions with specific emphasis on high yield environments is lacking. Six hundred and thirty-seven data sets (site–year–treatment combinations) were analyzed from field studies that had examined these variables and had been published in refereed journals from 1966 to 2006. A mean linear increase of 0.013 Mg soybean seed yield per kg increase in N accumulation in above-ground biomass was evident in these data. The lower (maximum N accumulation) and upper (maximum N dilution) boundaries for this relationship had slopes of 0.0064 and 0.0188 Mg grain kg^{-1} N, respectively. On an average, 50–60% of soybean N demand was met by biological N_2 fixation. In most situations the amount of N fixed was not sufficient to replace N export from the field in harvested seed. The partial N balance (fixed N in above-ground biomass − N in seeds) was negative in 80% of all data sets, with a mean net soil N mining of −40 kg N ha^{-1}. However, when an average estimated below-ground N contribution of 24% of total plant N was included, the average N balance was close to neutral (−4 kg N ha^{-1}). The gap between crop N uptake and N supplied by BNF tended to increase at higher seed yields for which the associated crop N demand is higher. Soybean yield was more likely to respond to N fertilization in high-yield (>4.5 Mg ha^{-1}) environments. A negative exponential relationship was observed between N fertilizer rate and N_2 fixation when N was applied on the surface or incorporated in the topmost soil layers. Deep placement of slow-release fertilizer below the nodulation zone, or late N applications during reproductive stages, may be promising alternatives for achieving a yield response to N fertilization in high-yielding environments. The results from many N fertilization studies are often confounded by insufficiently optimized BNF or other management factors that may have precluded achieving BNF-mediated yields near the yield potential ceiling. More studies will be needed to fully understand the extent to which the N requirements of soybean grown at potential yields levels can be met by optimizing BNF alone as opposed to supplementing BNF with applied N. Such optimization will require evaluating new inoculant technologies, greater temporal precision in crop and soil management, and most importantly, detailed measurements of the contributions of soil N, BNF, and the efficiency of fertilizer N uptake throughout the crop cycle. Such information is required to develop more reliable guidelines for managing both BNF and fertilizer N in high-yielding environments, and also to improve soybean simulation models.

Keywords: soybean, nitrogen, biological N_2 fixation, nitrogen fertilization, high yield

1. Introduction

Soybean (*Glycine max* [L.] Merr) yields have steadily increased in the past 30 years due to a combination of genetic and management improvement. The annual rate of yield increase averages 31 kg ha^{-1} in the United States (Specht et al., 1999) and 28 kg ha^{-1} globally (Wilcox, 2004). Although soybean use for biodiesel production may require expansion of land area devoted to soybean in some parts of the world, such an expansion is not likely in North America because of increased competition for land by the rapidly rising corn ethanol industry. Hence, yield increases will become the major source for sustaining further increases in soybean production, particularly in North America. The design of soil and crop management strategies that fully exploit the climatic and genetic yield potential of soybean remains a key challenge to achieve this goal.

Soybean yield potential has been defined as the maximum yield of a crop cultivar grown in an environment to which it is adapted, with nutrients and water non-limiting, and pests and diseases effectively controlled (Evans, 1993). In the U.S. Corn Belt region, soybean yield potential has been estimated to be in the range of 6–8 Mg ha^{-1} (Cooper, 2003, Specht et al., 1999). In order to achieve high yield potential, soybean must sustain high photosynthesis rates and accumulate large amounts of N in seeds. Nitrogen exists in leaves primarily as ribulose biphosphate carboxylase/oxygenase and there is generally a strong relationship between N per unit leaf area and photosynthesis (Sinclair, 2004). Therefore, the crop should have a canopy that enables full light interception and sufficient storage of N in leaves to maintain a non-N-limited photosynthetic apparatus for converting incoming radiation into new biomass and eventually grain yield.

Biological N_2 fixation (BNF) and mineral soil or fertilizer N are the main sources of meeting the N requirement of high-yielding soybeans. However, antagonism between nitrate concentration in the soil solution and the N_2 fixation process in the nodules is the main constraint the crop faces in terms of increasing N uptake (Streeter, 1988) when no other abiotic stress that reduce BNF activity occurs, e.g. soil moisture (Purcell et al., 2004), soil pH (Parker and Harris, 1977) or soil temperature (Soares Novo et al., 1999). Maximum N_2 fixation occurs between the R3 and R5 stages of soybean development (Zapata et al., 1987), and any gaps between crop N demand and N supply by N_2 fixation must be met by N uptake from other sources. If the overall N supply does not meet soybean requirements, the crop will remobilize N accumulated in leaves to the grain, which diminishes the photosynthetic capacity of the canopy and thus limits yield potential. Van Kessel and Hartley (2000) suggested that N_2 fixation will increase in high-yielding environments since the nitrogenase, located in the nodules, will adjust its activity to the demand of the legume (Mengel, 1994). However the generally observed reduction in N_2 fixation activity between the R5 and R7 stages (Zapata et al., 1987) could lead to a shortage of N during seed-filling in high-yielding environments.

Applying fertilizer-N has been proposed as an aid for increasing available N in the soil. Studies of nodulated soybeans showed significant yield response to frequent N additions when the N_2 fixation apparatus could not meet N demand (Thies et al., 1995). However, yield response of soybean to fertilizer N has been inconsistent at economically acceptable levels (Barker and Sawyer, 2005, Gan et al., 2003, Schmitt et al., 2001). An important research question is whether fertilizer N can alleviate N limitations without compromising the N_2 fixation capacity of the crop and doing so in a cost effective manner. Those studies reporting no increase in grain yield assumed that the crop simply substitutes the N it ordinarily would have derived from BNF with N from fertilizer (Deibert et al., 1979), or that more N translocation from vegetative reserves occurs when applied N lowers the rate of N_2 fixation (Herridge et al., 1984). Although it is generally believed that late N applications at reproductive stages (i.e., R3 to R5) should theoretically increase yields in high-yielding environments, the empirically measured responses in grain yield to fertilizer-N applied at late R-stage is not universal (Barker and Sawyer, 2005, Gutiérrez-Boem et al., 2004). Whereas early application of even small amounts of N often results in temporary suppression of nodule establishment and subsequent activity (Hungria et al., 2005a), an early-season N-deficiency may delay early crop growth and thus the development of an efficient nodulation system. Overall, the contradictory results obtained in N fertilization studies do not provide clear evidence as to whether N fertilization is required to complement the N supply from BNF to achieve soybean yields that approach yield potential levels.

A number of reviews have been published on N_2 fixation in legumes (Chalk, 1998, Giller and Cadisch, 1995, Hardarson and Atkins, 2003, Herridge and Danso, 1995, Peoples et al., 1995b, Unkovich and Pate, 2000; van Kessel and Hartley, 2000) and soybean in particular (Hungria et al., 2005a, Hungria et al., 2006, Keyser and Li, 1992). However, these summaries were mostly qualitative and did not emphasize the role of N_2 fixation and inherent soil fertility in high-yielding soybean systems. Likewise, many studies evaluating the response of soybean to N fertilization show conflicting results that make it difficult to draw a general conclusion about soybean response to N fertilizer. As average soybean yields continue to climb towards yield potential ceilings and potential changes in soil N supply may occur due to factors such as conservation tillage or global warming and effects on soil organic matter content, a contextual analysis of the relevant past and recent literature was deemed crucial to gain a better understanding of the foregoing issues and questions.

The objective of this review was to conduct a meta-analysis of published data to evaluate the relationships among soybean seed yield, N uptake, N_2 fixation, and the yield response to N fertilization. Particular emphasis was placed on ensuring that data from high-yielding environments, which we define as soybean seed yields greater than 4.5–5 Mg ha^{-1}, were included in this analysis.

2. Data sources and analysis

2.1. Data sources

A database derived from 108 studies that included a total of 637 data sets (site–year–treatment combinations) was compiled from field studies on N_2 fixation and N fertilization in soybean published in scientific journals from 1966 to 2006. The data comprised a wide range of soils, climatic conditions, genotypes, and crop management practices. Countries (or regions) represented included Argentina (2 data sets), Brazil (11), Australia (91), Austria (32), Canada (102), China (43), France (8), Greece (12), India (26), Indonesia (2), Japan (18), Nigeria (9), the Philippines (2), Romania (12), Thailand (99), the USA (164) and Zambia (4). Because some studies on N fertilization did not concurrently quantify N_2 fixation and other studies did not evaluate response to N fertilizer when quantifying N_2 fixation, data analyses were performed for two data sub-sets: Data set A was used to examine relationships between N uptake, N_2 fixation and grain yield; Data set B was used to evaluate yield response to fertilizer-N. Data set A comprised 61 studies in which N_2 fixation was quantified. Several different techniques were used: (1) the N difference method (Dashti et al., 1998, Israel and Burton, 1997, Israel and

Mikkelsen, 2001, Saxena and Chandel, 1997, Thies et al., 1995, Wagner and Zapata, 1982, Weber, 1966); (2) Ureides determination in the xylem sap (Gan et al., 2002, Guafa et al., 1993, Herridge, 1982, Herridge et al., 1990, Hughes and Herridge, 1989, Hungria et al., 2003, Peoples et al., 1995a, Reis et al., 2002, Takahashi et al., 1992, Yinbo et al., 1997, Ying et al., 1992, Zotarelli, 2000); (3) ^{15}N dilution and abundance techniques (Afza et al., 1987, Alvarez et al., 1995, Alves et al., 2006, Amarger et al., 1979, Bergersen et al., 1989, Bergersen et al., 1992, Boddey et al., 1990, Chapman and Myers, 1987, Coale et al., 1985, Danso et al., 1987, Eaglesham et al., 1982a, Eaglesham et al., 1982b, George and Singleton, 1992, Guffy et al., 1989, Hardarson et al., 1984, Hardarson et al., 1989, Kucey et al., 1988a, Kucey et al., 1988b, Kundu et al., 1996, Munyinda et al., 1988, Rennie et al., 1982, Rennie et al., 1988, Sisworo et al., 1990, Takahashi et al., 1991, Toomsan et al., 1995, Vasilas and Fuhrmann, 1993, Wheatley et al., 1995, Yoneyama et al., 1990, Zapata et al., 1987) and (4) acetylene reduction method (Bezdicek et al., 1978, Muldoon et al., 1980, Semu and Hume, 1979). In addition, data of N uptake and grain yields measured in a high-yield experiment conducted at the University of Nebraska–Lincoln from 1999 to 2004 were included (Setiyono et al., 2007). Data set B included 67 studies in which the effect of N fertilization, including different N amounts, timing of application, fertilizer sources and placement of fertilizer, on soybean yield was studied. Experimental details of these experiments are summarized in Appendix A.

Only field experiments with inoculated soybeans that reported good control of pests and weeds and with measured grain yield harvested from at least 5 m^2 were included. Grain yield from all studies was reported on an area basis and adjusted to a standard moisture content of 0.13 kg H_2O kg grain^{-1}. Total N uptake was typically measured in above-ground biomass near the R7 stage, when the soybean crop reaches maximum dry matter and N uptake. Therefore, N uptake and N_2 fixation values reported in this paper refer to above-ground biomass. It is important to mention that the absolute values of these variables may be underestimated since N in roots, nodules and abscised leaves are not or only partially included in the calculation. Other calculated variables included N harvest index (NHI = N amount in seed/total N uptake in above-ground biomass) and a partial N balance (N balance = N fixed − N amount in seeds). A positive partial N balance suggests a net increase in soil N after seed harvest, whereas a negative value indicates a net soil N depletion. This partial balance was calculated using above-ground N, since the contribution of below-ground N was not measured in the studies included in this review. In order to at least demonstrate the relevance of below-ground N, the partial N balance was also calculated including the contribution of below-ground biomass assuming that 24% of total N uptake is located in the roots (Rochester et al., 1998).

2.2. Data analysis

Descriptive statistics were used to summarize Data set A. The interquartile range (IQR, 25–75% percentiles), which represents 50% of all observations centered around the median, was used to describe the most frequent values for each variable. The number of cases for each variable was different, because not all variables were measured (or reported) in all the studies. Quantile regression techniques were used to model empirical relationships among variables (Cade and Noon, 2003, Koenker and Hallock, 2001). Relationships such as the one between seed yield and total N uptake tend to be scattered, including data points that represent a wide range of cultivars, environments, and management. The Blossom software (Cade and Richards, 2001) was used to model the envelopes depicting the maximum and minimum expression of a dependent variable in the range of the independent variable (the 0.99 and 0.01 quantile boundary lines). Data set B was analyzed for grain yield response to N fertilization. Data from studies that used foliar N fertilization were analyzed separately from those with only soil-applied N. Each group was further sub-divided according to N application timing, i.e., either before or after the R3 stage (Fehr and Caviness, 1977), primarily because it is after R3 when soybean N demand reaches its seasonal peak (Hanway and Weber, 1971). Studies in which a significant yield response to applied N occurred ($P < 0.05$) were designated as "N-responsive", and the response was quantified in terms of absolute (ΔY) and relative (%) yield increase over a control without N addition. The agronomic efficiency (AE, kg grain kg N applied^{-1}) was estimated as AE = ΔY/N rate, but only for N rates below 100 kg ha^{-1}, based on the assumption that this represents the maximum N rate at which N application is justified in most agronomic situations. For experiments in which different N rates were tested, the maximum AE was reported. In each N fertilization experiment, the maximum grain yield in each site–year was used as a measure of the yield potential for each particular situation. It was assumed that this value represented the maximum attainable yield, limited only by abiotic and biotic factors – other than N – in each specific study.

A simple economic analysis of N fertilization was performed by calculating the gross return above fertilizer cost (GRC) as: GRC (US$) = (Δgrain yield × price of grain) − (N rate × price of N). The 2002–2006 average prices of soybean and fertilizer-N in the USA (0.223 and 0.41 US$ kg^{-1}, respectively) were used for the baseline analysis, but we also assessed several scenarios of soybean to N price ratios. This analysis is only intended to provide a general discussion of the economic scope for N fertilization in soybean, recognizing that actual responses and profit margins may vary widely.

3. Relationships between grain yield, nitrogen uptake and nitrogen fixation

Grain yields ranged from 0.58 to 5.89 Mg ha^{-1} with an IQR of 1.98–3.34 Mg ha^{-1} (Table 1). Total N uptake averaged 219 kg ha^{-1}, with an IQR of 154–280 kg ha^{-1}and a maximum of 485 kg ha^{-1}. The linear relationship between grain yield and total N uptake with above-ground biomass had a slope of 12.7 kg grain kg^{-1} N (Figure 1). The lower and upper boundaries of this relationship had slopes of 6.4 and 18.8 kg grain kg^{-1} N, respectively. The upper boundary line (maximum N dilution) is indicative of N being the main limiting factor for yield, such that the ratio of grain yield per unit of N uptake is as large as it can be, thereby resulting in a presumably maximal internal use efficiency for the given yield level. The lower boundary line (maximum N accumulation) represents those experimental situations in which N is likely at maximum accumulation in the plant, which implies that grain yield is limited by factors other than N, resulting in the lowest internal N efficiency (Janssen et al., 1990, Witt et al., 1999). For comparison only, the range observed in this analysis showed lower internal efficiencies of N than those reported for rice, wheat and maize, which showed values ranging from 20 to 40 kg grain kg^{-1} N for the maximum accumulation boundary and 64 to 106 kg grain kg^{-1} N for maximum dilution (Janssen et al., 1990, Liu et al., 2006, Pathak et al., 2003, Witt et al., 1999). The higher concentration of N in soybean tissue and a large amount of protein and oil in its seeds, i.e., more investment of energy per unit of grain yield than cereals, account for the relatively lower internal N efficiency in soybean (Amthor et al., 1994, Sinclair and de Wit, 1975). On average, a soybean crop yielding

Table 1. Summary statistics of a meta-analysis of data published in the literature relative to N uptake and N_2 fixation in soybean and variables related to N nutrition in soybean

Variable	n	Maximum	75%	Mean[a]	25%	Minimum
Grain yield (Mg ha^{-1})	458	5.89	3.34	2.69	1.98	0.58
N content in grain (%)	289	8.08	6.75	6.34	6.00	3.84
N content in residues (%)	159	3.17	1.55	1.21	0.80	0.25
Total N uptake (kg ha^{-1})	480	485	280	219	154	44
N amount in seeds (kg ha^{-1})	323	353	184	155	122	41
N amount in residues (kg ha^{-1})	202	168	74	59	30	11
N harvest index	216	0.97	0.82	0.73	0.64	0.38
N_2 fixation (kg ha^{-1})[a]						
All N	555	337	152	111	61	0
No N	(337)	(337)	(163)	(125)	(76)	(0)
N_2 fixation (%)[a,b]						
All N	505	98	69	52	36	0
No N	(316)	(98)	(74)	(58)	(46)	(0)
Partial N balance (kg ha^{-1})[c]	321	110	−5	−40	−64	−279
	(321)	(204)	(41)	(−4)	(−38)	(−279)

The data were collected from experiments spanning a wide range of management and environmental conditions (Data set A). Note that statistics on N uptake and the N balance do not add up because the number of data sets included in this analysis varied.

[a] All N refers to summary statistics for all data sets, including treatments with or without fertilizer-N. Values in parenthesis (no N) are the summary statistics for control treatments (data sets without fertilizer N application).

[b] N_2 fixation as percentage of total N uptake.

[c] Partial N balance = N fixed in above-ground biomass − N removed with grain. Values in parenthesis were calculated including an assumed average N contribution from below-ground biomass of 24% (Rochester et al., 1998).

5 Mg ha^{-1} accumulates about 400 kg N ha^{-1} in its above-ground biomass, and that N must be provided from indigenous soil resources, the biological fixation process, and/or fertilizer.

Unkovich and Pate (2000) suggested that N uptake and N_2 fixation are often underestimated because below-ground N (BGN), including N in roots, nodules, exudates and rhizodeposition, is not taken into account. Assuming that 24% of total plant N is allocated in below-ground parts (Rochester et al., 1998), average N uptake and N_2 fixation in our dataset would increase to 288 and 164 kg ha^{-1}, respectively, which also increases the N requirement of the crop per unit yield. However, no attempts were made in our review to estimate BGN by applying a single factor to convert above-ground biomass into whole plant N because partitioning of N between both strata is likely to have a large variation in the wide range of environmental conditions evaluated in this review. Below-ground allocation of N (and biomass) can vary widely by genotype, environment, soil type and management. In well-managed irrigated soybean crops, for example, below-ground biomass is only about 15% of above-ground biomass (D. Walters, University of Nebraska, unpublished data). Applying a single partitioning factor may lead to a bias in the estimation of the real contribution of BGN in each environment. Better measurements are needed in future studies, as well as models that accurately simulate the partitioning of N to below-ground parts based in above-ground measurements.

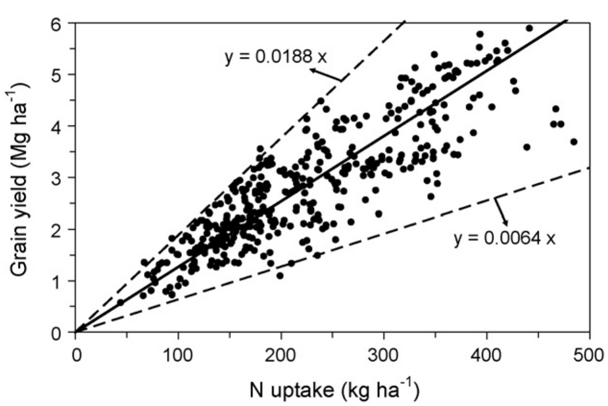

Figure 1. Relationship between grain yield (13% seed moisture content) and nitrogen uptake in above-ground biomass in soybean. The solid line is the average fit of the data, with a slope of 0.0127 Mg grain kg^{-1} N. Dashed lines show the boundaries of maximum N dilution (upper) and maximum N accumulation (lower) of N. Values shown refer to N in total above-ground biomass.

The variability in seed N concentration was much narrower than that of total seed N. For example, N concentration in soybean seeds averaged 6.34% with an IQR of 6–6.75% (37.5–42.2% protein content on a 13% moisture basis), which represents a 13% range compared to the average (Table 1). In contrast, the IQR for total seed N content was 122–184 kg ha^{-1}, which represents a 51% range. Nitrogen concentration in vegetative biomass (leaves plus stems) at R7 stage averaged 1.22%, but varied widely, with an overall range of 0.25–3.17% and an IQR of 0.8–1.55%. Hence, partitioning of N between vegetative biomass and seeds varied widely too. A larger N harvest index is expected in soybeans compared to other legumes, since a great amount of N is mobilized to the grain in relation to that remaining in the residues (Lawn, 1989). Mean nitrogen harvest index (NHI) was 0.73 with an IQR of 0.64–0.82 (Table 1). Ayaz et al. (2004) reported similar values in chickpea and lupine which had a lower N content in both in seeds and residues, but greater NHI was reported in peas, which have a lower N concentration in seeds and a greater N content in the residues. Variation in yields and NHI greatly impacted the amount of N left in crop residues and hence N availability and N management in subsequent crops (Bergersen et al., 1992, Bundy et al., 1993).

On average, N_2 fixation accounted for 52% of total N uptake (IQR between 36 and 69%; Table 1), but the proportion of fixed N decreased with increasing fertilizer-N additions (Figure 2). For those data from experiments in which soybeans received less than 10 kg N ha^{-1} as fertilizer, this relative contribution of N_2 fixation increased to 58% (IQR between 46 and 74%). In absolute

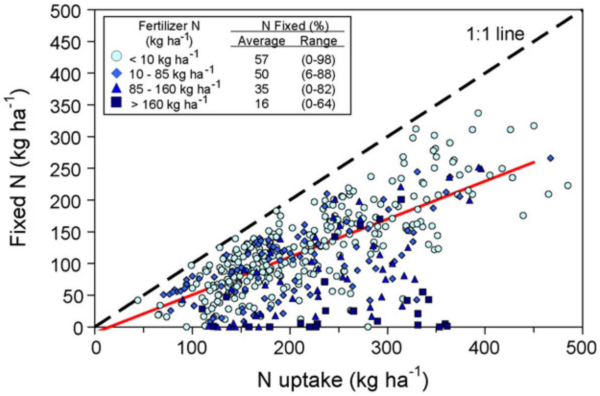

Figure 2. Relationship between N_2 fixed by soybean and nitrogen uptake in above-ground biomass. The dashed 1:1 line represents values for which all N uptake would be expected to be derived from biological N_2 fixation. Data were divided into four different categories of applied N fertilizer as denoted by the symbols. The solid line is the best linear fit for N fertilizer rates of less than 10 kg ha^{-1} ($y = 0.66x - 19$; $R^2 = 0.59$). Values shown refer to N in total above-ground biomass.

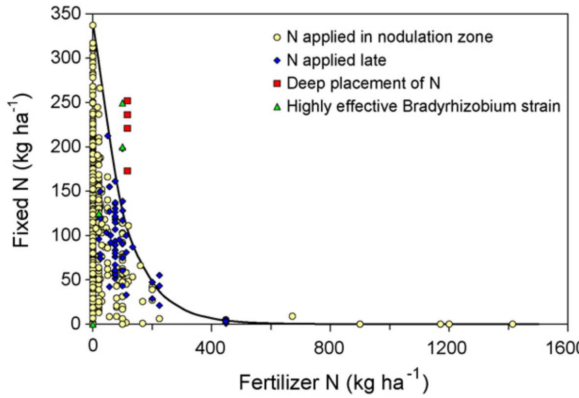

Figure 3. Relationship between nitrogen fixed by soybeans and N applied as fertilizer. The curve ($y = 337\ e^{-0.0098x}$) shows the maximum level of N_2 fixation as a function of fertilizer-N rate in those experiments in which N was applied pre-plant or during early growth in the nodulation zone (upper few cm to 20 cm of soil), or on the soil surface at later growth stages. Values shown refer to N in total above-ground biomass.

terms, N_2 fixation ranged from 0 to 337 kg N ha^{-1} with an average of 111 and 61–152 kg N ha^{-1}as the IQR (Table 1). The lowest values were associated with soils with high nitrate contents in the nodulation zone, or were observed in experiments in which the normal development of the N_2 fixation process was constrained by soil acidity (Parker and Harris, 1977), the presence of ineffective *Bradyrhizobium* strains (Israel and Burton, 1997), or water deficits (Purcell and King, 1996). Maximum N_2 fixation values of up to 360–450 kg N ha^{-1} have been suggested by several authors (Giller, 2001, Rennie et al., 1988, Unkovich and Pate, 2000). Although N_2 fixation can conceivably be managed to achieve its maximum potential by using technologies that enhance BNF (e.g. the use of a high quality inoculant, a high yielding soybean variety, excellent crop establishment, no water limitations and adequate supply of other nutrients beyond N itself), it was difficult to ascertain from the studies examined in this review the degree to which the study investigators optimized the BNF-only treatments in their experiments. A maximum N_2 fixation in above-ground biomass of 450 kg N ha^{-1} reported for soybean by Rennie et al. (1988) was not considered the highest value because the experiment average was 191 kg N ha^{-1}, and the authors did not provide any explanation for what may have caused this unusually high value. Thus, we consider 337 kg N ha^{-1} reported by Herridge (1982) as the more reliable published maximum biological N_2 fixation value for soybean.

Nitrogen fixation showed a positive relationship with N uptake across all data sets, although the data points in this relationship were widely scattered and the relationship tended to decrease with increasing fertilizer-N amounts (Figure 2). Including only data sets with fertilizer rates of less than 10 kg N ha^{-1}, a linear regression resulted in a slope of 0.66 kg N ha^{-1} from N_2 fixation per kg N uptake ha^{-1} ($r^2 = 0.64$, $P < 0.001$). The regression equation also included a negative intercept, implying some minimum level of plant growth and associated N uptake (ca. 29 kg ha^{-1}) to support any N_2 fixation. In absolute terms, the average difference between total N uptake and N_2 fixation (deviation from the 1:1 line) becomes larger as yield levels increase. When less than 85 kg N ha^{-1} was applied as fertilizer and N uptake levels were above 350 kg N ha^{-1}, the proportion of N derived from biological fixation ranged from 35 to 86%, most likely due to differences in soil N supply capacity. Highest values came from studies conducted in Brazil, where adapted *Rhizobium* strains have been selected for tropical conditions and soil mineral N content is relatively low (Alves et al., 2003, Hungria et al., 2005a, Hungria et al., 2005b).

Because N inputs may occur from atmospheric deposition or from irrigation water and because estimates of gaseous or leaching losses of N were not available in most studies, only a partial N balance could be computed. The partial N balance was negative in about 80% of all data sets, averaging—40 kg N ha^{-1}, when only above-ground N was only taken into account. However, the true net loss was less than that since N in roots and nodules was not accounted for. Assuming that 24% of total N uptake may come from below-ground (Rochester et al., 1998), the average N balance increased to −4 N ha^{-1}, with an IQR of −38 to 41 kg N ha^{-1}. In this situation, only 51% of all data sets showed negative values. These observations suggest that soybean may be considered as a neutral crop in relation to its N contribution to the soil. However, a large negative N balance would be expected in high-yielding soybeans, because the harvested grain would lead to a large amount of N removal. In the high-yield experiment at Lincoln, Nebraska (Setiyono et al., 2007), for example, soybean yield averaged 5 Mg ha^{-1} (13% basis) in a 6 year period from 1999 to 2005, with an average grain N removal of 63.4 kg N per Mg yield (i.e., 276 kg N ha^{-1}). For comparison, the average grain N removal in the data reports reviewed here was 155 kg N ha^{-1}, which was incurred at an average yield level of 2.69 Mg ha^{-1} (Table 1).

Given the fact that, in most situations, growing soybean does not result in a net N input to the system, it is likely that the soybean N credit used in many N fertilizer recommendations (e.g. for maize in the USA) does not represent a net contribution of N from the atmosphere to the system. Instead, the greater amount of available N when maize is planted after soybeans (compared to that available in maize after maize monocultures) may represent mineralization of soybean residue N and enhanced mineralization of soil organic matter (priming effect) because soybean residue has a lower C:N ratio than maize stover (Green and Blackmer, 1995). Although it is treated as a “credit” in fertilization recommendations, it is not technically a N gain for the system. However, negative to neutral N balances (Table 1) are mainly a concern in those areas where soybeans are planted as a monoculture, primarily because the progressive N loss will be detrimental for the sustainability of the system over time.

4. Nitrogen fixation and N fertilization

A negative exponential relationship was observed between N fertilizer rate and N_2 fixation when N was applied in the top 0–

Table 2. Magnitude of grain yield response to N fertilization (Data set B)

No.	Yield response to N fertilizer					No yield response to N fertilizer		
	Y_0	n	Y_N	ΔY	AE	Y_0	n	Y_N
Nitrogen applied before R3								
2	1.93–3.59	7/12	2.21–4.09	0.28–0.50	3.5–4.9	2.74–3.19	5/12	2.92–3.35
3	–	0/8	–	–	–	2.21–3.01	8/8	2.41–2.87
4	–	0/12	–	–	–	1.17–1.13	12/12	1.07–1.16
5	2.56–2.60	12/12	3.08–3.24	0.28–0.64	4.3–5	–	0/12	–
6	–	0/54	–	–	–	3.73	54/54	3.78
7	2.79–3.44	3/6	3.71–4.39	0.83–0.85	–	2.79–3.44	3/6	3.21–4.20
8	1.45	1/9	1.68	0.23	2.0	2.87–3.88	8/9	2.96–4.00
9	3.31	1/12	3.64	0.33	6	2.71–3.52	11/12	2.90–3.54
10	–	0/4	–	–	–	3.65	3/3	3.24
11	–	0/6	–	–	–	2.92	6/6	3.13
12	–	0/4	–	–	–	2.78–4.59	4/4	2.44–3.90
14	0.75–2.64	4/8	0.90–3.30	0.24–0.66	3.2–8.8	0.75–2.64	4/8	0.82–2.79
15	1.15–2.02	6/6	1.36–2.55	0.24–0.53	4.8–10.6	–	0/6	–
16	–	0/4	–	–	–	1.56–2.02	4/4	1.60–2.15
17	–	0/4	–	–	–	2.51–3.58	4/4	2.60–3.82
18	–	0/1	–	–	–	4.43	1/1	4.31
19	–	0/6	–	–	–	2.99–3.29	6/6	3.28–3.31
21	–	0/3	–	–	–	2.81	3/3	2.94
22	2.55–2.86	2/6	2.65–3.00	0.09–0.15	2.5–3.3	2.54–3.03	4/6	2.73–3.04
23	–	0/4	–	–	–	4.30–4.60	4/4	4.50–4.70
24	–	0/24	–	–	–	3.22–3.73	24/24	3.34–3.81
25	1.60–4.33	12/20	2.03–4.74	0.15–1.21	5.6–16.1	2.95–3.40	8/20	3.49–3.54
26	2.73	1/2	3.22	0.49	–	3.14	1/2	2.99
27	–	0/1	–	–	–	1.46	1/1	1.86
28	2.82–4.18	2/2	3.26–4.51	0.32–0.44	–	–	0/2	–
29	–	0/13	–	–	–	1.48–3.72	13/13	1.39–3.62
30	2.31	1/35	2.68	0.37	–	2.13–3.68	34/35	2.17–3.84
31	–	0/48	–	–	–	2.46–3.91	48/48	2.37–3.97
32	3.25	1/9	3.84	0.58	7.2	3.44–4.06	8/9	3.37–4.33
33	–	0/1	–	–	–	2.88	1/1	2.69
34	0.99	2/6	1.64	0.65	8.7	0.99	4/6	1.29
35	–	0/24	–	–	–	–	24/24	–
36	1.78–1.91	3/3	1.84–2.02	0.10–0.24	2–4.8	–	0/3	–
37	–	0/2	–	–	–	2.34–2.50	2/2	1.40–2.54
38	2.20–3.10	12/12	2.80–4.00	0.60–0.90	8–12	–	0/12	–
39	1.42–4.24	2/4	2.28–5.28	0.86–1.05	–	3.20–3.48	2/4	3.48–3.59
40	–	3/3	–	–	–	1.90–2.41	3/3	1.55–2.41
41	–	0/6	–	–	–	2.05–3.26	6/6	2.30–3.41
42	–	0/2	–	–	–	1.29–2.90	2/2	1.11–2.40
43	–	0/28	–	–	–	1.21–3.07	28/28	1.52–3.24
44	2.19–2.69	3/50	2.62–3.01	0.31–0.54	3.6	2.19–3.83	47/50	2.19–3.86
46	1.32–2.89	7/20	1.49–3.00	0.22–0.98	4.0–17.5	2.49–3.69	13/20	1.56–3.66
47	1.94	1/9	2.30	0.36	–	1.68–3.13	8/9	1.68–3.48
48	1.94	2/6	2.12	0.17	6.9	2.07–2.10	4/6	2.14–2.20
49	1.20–3.09	14/26	1.41–3.48	0.21–0.61	–	1.20–3.09	12/26	1.48–3.28
50	1.81–3.12	12/24	2.39–3.62	0.28–1.40	6.1–9.0	2.47–3.44	12/24	2.38–3.40
52	2.80–3.72	5/6	2.76–4.60	0.36–0.88	–	1.24	1/6	2.00
53	–	0/24	–	–	–	1.90–3.52	40/40	1.74–3.68
54	3.43	1/14	4.25	0.82	–	2.98–4.37	13/14	3.25–4.25
56	1.41–2.19	6/6	2.06–2.84	0.29–.66	2.9–13.1	–	0/6	–
57	–	0/2	–	–	–	2.78	2/2	2.54–2.58
Nitrogen applied after R3								
1	2.87	1/1	3.91	1.03	26	–	0/1	–
13	–	0/36	–	–	–	2.33–5.05	36/36	2.06–5.58
14	0.75–2.64	2/2	0.93–2.97	0.18–0.33	2.4–4.3	–	0/2	–
15	–	0/6	–	–	–	1.15–2.02	6/6	1.21–2.20
17	–	0/4	–	–	–	2.51–3.58	4/4	2.40–3.73
20	2.83–3.11	2/39	3.43–3.67	0.56–0.61	–	2.03–3.93	37/39	1.81–3.90
31	–	0/12	–	–	–	2.46–3.91	12/12	2.35–4.09
44	–	0/2	–	–	–	3.08	2/2	3.09–3.17
45	3.76–4.84	31/64	4.17–5.51	0.20–1.75	10–44	2.35–2.89	33/64	2.89–3.23
46	1.32–2.89	4/10	1.61–2.80	0.23–0.79	4.1–14.2	1.85–3.71	6/10	1.99–3.69
51	–	0/8	–	–	–	3.43	8/8	3.47
53	–	0/20	–	–	–	1.90–3.52	20/20	1.73–3.78
55	–	0/12	–	–	–	3.61–4.58	12/12	3.63–4.62
57	–	0/1	–	–	–	2.78	1/1	2.53

Information presented here was divided according to the application of N fertilizer before and after R3 stage. Variables: No., literature source, see Appendix A for references; Y_0, grain yield in the control (no N fertilizer added, Mg ha^{-1}); Y_N, maximum grain yield in the N-fertilized treatments (Mg ha^{-1}, or range of yields with N application if there was more than one N rate with a positive yield response); ΔY, grain yield increase to N application (Mg ha^{-1}); AE, maximum agronomic N use efficiency (kg grain kg N^{-1}) for N rates <100 kg N ha^{-1}, at N rates higher than 100 kg N ha^{-1}, AE is not presented (dash); n, number of treatments or sites in which a grain yield response (or no response) to fertilizer N was observed over total number of treatments or sites tested.

20 cm of soil or on the soil surface (Figure 3). The scatter in this relationship arises from variation in N supply derived from indigenous sources (net soil N mineralization, irrigation, atmospheric deposition), and possibly other factors affecting growth and N_2 fixation (e.g. soil pH, drought). Unfortunately, initial soil nitrate content was not measured in many of the published studies. To account for differences in the total supply of mineral N from soil and fertilizer sources, a boundary line for the 0.99 quantile was fitted to represent the maximum attainable level of N_2 fixation as a function of fertilizer-N rate. When no N fertilizer was added, the maximum amount of N_2 fixation reached 337 kg ha^{-1}, and as a consequence of the exponential decline, a maximum N_2 fixation of 129 and 17 kg N ha^{-1} would be expected if 100 and 300 kg ha^{-1} of fertilizer-N were to be applied to the upper soil layer, respectively.

Although breeding for nitrate tolerant genotypes has been suggested as a means to avoid the inhibitory effect of nitrates on N_2 fixation (Streeter, 1988), this strategy has been unsuccessful because the selected lines have low grain yields (Betts and Herridge, 1987, Herridge and Rose, 1994, Raffin et al., 1995). Figure 3 also shows several data points that do not follow the general trend, corresponding to experiments in which the fertilizer-N was placed at or below 20 cm depth, which is below the main zone of active nodules, or experiments in which a highly effective *Bradyrhizobium* strain was used. These cases suggest two possible avenues to increase N uptake at high N levels. In the former experiments large amounts of N were fixed at relatively high N fertilizer rates of 100 kg N ha^{-1} (Takahashi et al., 1991, Takahashi et al., 1992). It appears that nitrate inhibition has a local effect, and the inhibition may decrease when the nitrate concentration in the area surrounding the nodules is not increased (Arrese-Igor et al., 1997, Streeter, 1985). Since deep soil placement of N is only feasible before planting or during early development stages, utilizing a slow-release N source, such as polymer-coated urea, may increase the likelihood that N_2 fixation is not inhibited. The efficacy of such practice needs further research, particularly with regard to making supplementary N available during the seed filling period.

5. Grain yield response to N fertilization

Table 2 summarizes results from experiments in which N fertilizer was applied to soil, mostly as granular N on the surface or incorporated in the upper soil layer containing most nodules (i.e., 0–20 cm) (Data set B). Grain yield ranged from 0.7 to 5.6 Mg ha^{-1}. Positive response to fertilizer N was observed in about half of the published studies. The average yield increase from N fertilizer in these N-fertilizer responsive studies was 0.52 Mg ha^{-1} ($n = 154$), and the magnitude of the response did not significantly differ among N rate categories of 0–50, 50–100 and >100 kg N ha^{-1} (data not shown). However, the mean response at low N rates (<50 kg N ha^{-1}) was somewhat greater (0.64 Mg ha^{-1}) when considering only those treatments in which N was applied after R3 stage ($n = 40$). No attempt was made to separate the effects of fertilization timing because of the small number of field experiments with high N rates (>100 kg N ha^{-1}).

Agronomic efficiency of applied N varied widely due to large variation in indigenous soil N supply, N rates, application methods, and other factors affecting yield responses to N (Table 2). In N-responsive experiments, soil application of N fertilizer before the R3 stage mostly resulted in AE of 2–10 kg grain kg^{-1} of fertilizer N. The largest AE was observed at N rates less than 50 kg ha^{-1}. It is noteworthy that AE averaged 8.7 kg grain kg^{-1} of fertilizer N when less than 50 kg N ha^{-1} was applied before R3, but averaged a substantive three times greater (24 kg grain kg^{-1} N) when N was applied after R3 stage. The latter value is comparable to the AE of applied N in well-managed cereal crops (Ladha et al., 2005). Generally speaking, little quantitative information exists about the relative contributions of recovery efficiency (RE = increase in N uptake per kg N applied) and physiological efficiency (PE = increase in grain yield per kg increase in N uptake) to the AE of applied N in soybean. RE values were calculated in only 6 of the 20 studies in which AE was estimated (Table 2), and these had values ranging from 0.12 to 0.96 kg plant N kg^{-1} fertilizer N. There was no clear differentiation in RE between early or late applications of N, although in one study RE increased from 0.18 to 0.74 kg kg^{-1} when N was applied late as compared to early (Gan et al., 2002).

Foliar fertilization has been proposed to avoid the inhibitory effect of soil nitrate on nodule activity. A summary of experiments in which this technique was studied is shown in Table 3. Many of these studies evaluated the application of nutrient mixtures, which included nutrients other than N, making it difficult to evaluate N response *per se*. A response to N fertilization was observed in 5 out of the 11 studies. The AE was only calculated for three studies in which the net N effect could be evaluated. At comparable N rates, AE of foliar-applied N was generally lower than that achieved with soil-applied fertilizer delivered after the

Table 3. Magnitude of grain yield response to foliar fertilization (Data set B)

No.	Yield response to N fertilizer					No yield response to N fertilizer		
	Y_0	n	Y_N	ΔY	AE	Y_0	n	Y_N
Nitrogen applied before R3								
60	2.62–3.33	12/159	2.96–4.06	0.36–0.79	–	2.43–4.37	147/159	2.65–4.66
61	2.62–3.82	9/135	2.96–4.20	0.28–0.49	–	2.43–4.37	126/135	2.34–4.67
Nitrogen applied after R3								
55	–	0/12	–	–	–	3.61–4.58	12/12	3.39–4.56
58	–	–	–	–	–	3.83	2/2	3.62
59	2.23–3.85	22/57	2.42–5.34	0.13–1.04	5.3–10.6	1.68–2.98	35/57	2.12–3.25
62	–	0/16	–	–	–	2.14–3.11	16/16	1.85–2.92
63	–	0/70	–	–	–	1.71–3.33	70/70	1.56–3.29
64	–	0/21	–	–	–	2.28–4.16	21/21	2.00–3.76
65	–	0/3	–	–	–	2.52–3.94	3/3	2.79–4.08
66	2.40	2/6	2.75	0.35	2.9	2.88	6/10	3.02
67	2.60–2.63	2/8	2.96–3.22	0.33–0.62	7.8–8.2	2.62–3.59	6/8	2.59–3.37

Information was divided according to the application of N fertilizer before and after R3 stage. Variables: No., literature source, see Appendix A for references; Y_0, grain yield in the control (no N fertilizer added, Mg ha^{-1}); Y_N, maximum grain yield in the N-fertilized treatments (Mg ha^{-1}, or range of yields with N application if there was more than one N rate with a positive yield response); ΔY, grain yield increase to N application (Mg ha^{-1}); AE, maximum agronomic N use efficiency (kg grain kg N^{-1}) for N rates <100 kg N ha^{-1}, at N rates higher than 100 kg N ha^{-1}, AE is not presented (dash); n, number of treatments or sites in which a grain yield response (or no response) to fertilizer N was observed over total number of treatments or sites tested.

R3 stage (Table 2), and it ranged from 2.9 to 10.6 kg grain kg^{-1} N. A major limitation of foliar fertilization is the difficulty of applying larger quantities of N because of risk of leaf injury (Parker and Boswell, 1980, Poole et al., 1983a). Considering this risk of injury and the low AE, it is unlikely that foliar application of N can be reliably contribute to significant yield increases in soybean unless these limitations can be alleviated with improved foliar N formulations.

Yield increases due to N application were observed in a wide range of environments in studies with control yields (without N application) ranging from about 1 to 5 Mg ha^{-1}. Overall, the range in the relative response to fertilizer N was 5–40% and in absolute terms was 0.11–1.75 Mg ha^{-1} (Figure 4a). No relationship between the N-induced yield responses and the respective control yield levels was evident in the graphed data (Figure 4b).

One of the concerns about N fertilization, especially for the management of high-yielding soybean, is to know the grain yield level at which the potential contribution of the N_2 fixation system becomes insufficient (if it ever does) relative to meeting the N requirements of a crop. To address this question, data in Table 2 and Table 3 were used to relate the relative yield response to N fertilization to the maximum grain yield measured in each site–year as a measure of the attainable yield potential (Figure 5). In those published cases in which the grain yield of the unfertilized control plot was equal to the yield in plots with N application (i.e., no yield response to N fertilization), the relative yield value was 1. Figure 5 shows that responsive sites (relative yield less than 1) in which grain yield potential was below 4.5 Mg ha^{-1} were mainly experiments in which yields were low in absolute terms because of constraints other than N that affected normal crop growth, nodulation functioning or both. In these environments,

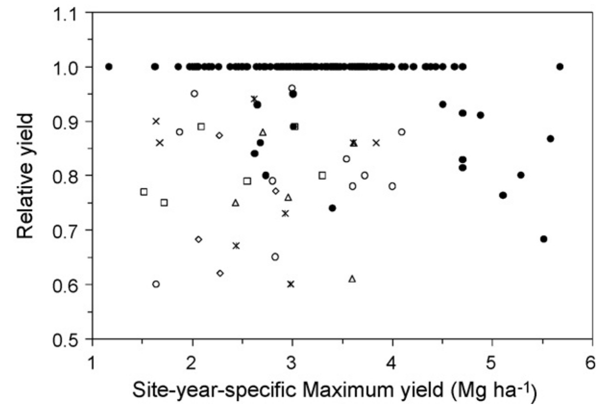

Figure 5. Relative soybean yield (yield in control plot/yield in fertilized plot) in different N treatments in relation to the maximum grain yield for each site–year. *Symbols*: Experiments in which crops had no constraints for normal growth (●). Crops with constraints for normal growth: low soil pH (*); low efficient *Rhizobium* strains (□); low temperature (◊); low fertility soils (◍); no soybean history (Δ); drought (×).

N application probably helped to overcome environmental constraints that may have limited the supply of N or its uptake by the crop. Examples of such constraints include poor establishment of the nodule system (Ham et al., 1975, Israel and Burton, 1997), extremely low soil-N at planting (Al-Ithawi et al., 1980, Starling et al., 1998, Taylor et al., 2005, Wood et al., 1993), plant water stress (Chen et al., 1992, Purcell and King, 1996, Reese and Buss, 1992), soil pH problems (Parker and Harris, 1977), low soil temperature (Soares Novo et al., 1999), or an absence of (native) *Bradyrhizobium* arising from a cropping history without legumes (Bodrero et al., 1985b).

Conversely, there were only 12 data sets in which maximum yields at the test sites were greater than 4.5 Mg ha^{-1}, which we assume to be representative of high-yielding environments. In this particular subset of data, there were only three studies in which N supply from soil fertility and biological N_2 fixation were sufficient to fully meet crop N demand at such high yield levels; in the remaining nine studies there was a significant response to fertilizer-N that tended to increase with the maximum site–yield level (Figure 5). In these high-yielding environments with no visually apparent constraints, the observed yield responses to applied N would suggest that the increased sink size and N requirement of a high-yielding soybean surpassed the N supply naturally available from soil and biological N_2 fixation.

Another issue is the timing of N application. Results from several field studies suggest that application of fertilizer during reproductive stages, and most particularly after R3, can increase seed yield. Across all site–years and yield levels in this review, the advantage of applying N late as compared to applications before R3 stage (see ΔY in Table 2 and Table 3) is not clear. Still, most of the 11 data points that defined the responsive high-yielding sites in Figure 5 came from experiments in which N was applied after R3, indicating a tendency for extra N during grain filling under high-yield conditions. This analysis included only a few studies conducted at high yield levels (Parker and Harris, 1977, Ray et al., 2005, Thies et al., 1995, Wesley et al., 1998). Published data for soybean yields >5 Mg ha^{-1} are scarce and there are no reported measurements of N uptake and N_2 fixation from field studies in which soybean yields approach the yield potential.

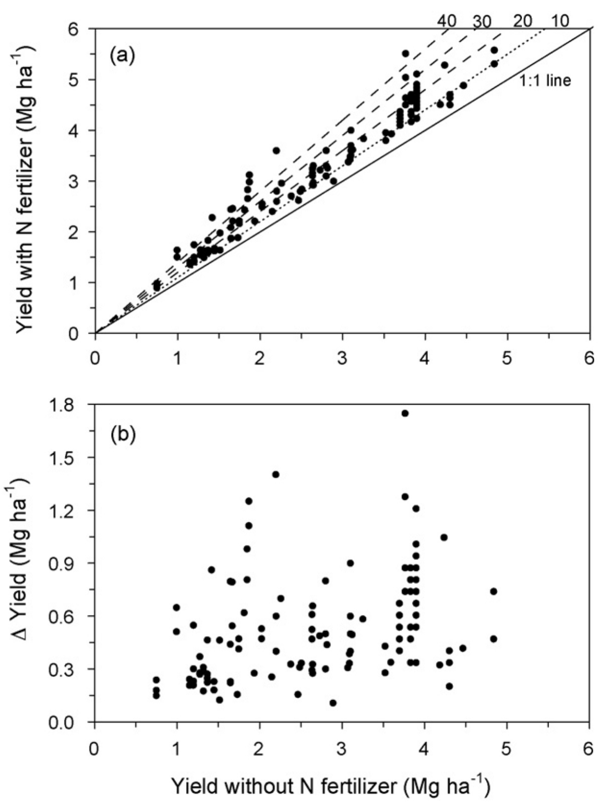

Figure 4. Relationship between grain yield in control plots (no N fertilizer added) to grain yield in N fertilized plots (a) and the yield response (ΔY) to N fertilizer in soybean (b). This figure summarizes only those experiments in which N fertilizer additions increased yields significantly (Table 2). Dashed lines in the upper panel are isolines of relative yield increase (%) over the control.

6. Inoculation and grain yield potential

Technology in inoculant production has evolved towards products that can routinely and efficiently enhance N_2 fixation in field conditions at a low cost per unit area (Hungria et al., 2005b, Lupwayi et al., 2000, Stephens and Rask, 2000). Such improvements include the proper selection of the strain, the use of a suitable carrier, and quality control during the formulation and storage of the inoculant and its application in the field. The evaluation of such technology is beyond the scope of this paper.

Sterile-peat and liquid inoculants are promising technologies that may assure a large number of viable rhizobia on a per seed basis without contamination. Hume and Blair (1992) found a higher number of *Bradyrhizobium* viable cells, when using these types of inoculants in soils with low nitrate content in sites where grain yield approached 3.2 Mg ha^{-1}. Revellin et al. (2000) subsequently demonstrated a higher number of nodules per plant and a larger response to inoculation with liquid inoculants in experiments where grain yield approached 4.7 Mg ha^{-1}, though this response was attained via comparison of inoculated versus uninoculated treatments on a field that had no previous soybean cropping history. Other sets of experiments in tropical soils without soybean history showed increases in N_2 fixation and nodule number when using sterile-peat or liquid inoculants (Singleton et al., 2002, Thao et al., 2002) but these responses were observed at sites where the grain yields were no greater than 2.5 Mg ha^{-1}. In soils with several years of soybean history, no increases in the proportion of N derived from fixation were observed when comparing the sterile-peat inoculated treatment with the non-inoculated treatment, and the authors concluded that N supply from the natural *Rhizobium* population was adequate (Hungria et al., 2006). The maximum yield in these experiments were no greater than 3.5 Mg ha^{-1}, and the response to reinoculation was observed in 4 of 12 experiments, but no response was observed when 10^{-5} cells of Rhizobia g^{-1} soil were in the soil, which is typically exceeded in fertile soils with a history of soybean production.

7. Nitrogen fertilization. Is it economical?

It is evident from the literature that BNF can provide the majority of the required N supply for soybean unless there are soil restrictions for a normal nodule activity. In some cases, however, there is a yield response to additional N. For soybean producers, however, the key issue is whether additional N fertilizer applications result in greater profit. To examine this issue, we used the average 2002–2006 prices for fertilizer N and soybean (0.223 and 0.41 US\$ kg^{-1} for N and soybean, respectively) as a baseline, in an environment that yields 5 Mg ha^{-1} without applied fertilizer N. Under an inexpensive N price scenario assuming N fertilizer cost of half the assumed baseline cost and a conservative AE of 5 kg grain per kg N applied, the GRC was U\$S 46 ha^{-1}. Alternatively, under a high soybean price scenario in which the average soybean price is 1.5 times the 2002–2006 average, the GRC increased to U\$S 63 ha^{-1}. In a less favorable scenario for N fertilization (i.e., half the average soybean price − 1.5 times the average N price), AE would have to increase by 20% for N fertilization to be profitable at all. These examples suggest that, in most cases, N fertilization would only be profitable where N_2 fixation is not able to meet the total N demand of high-yielding soybeans and when the soybean to N price ratio is large. Although soybean prices have risen dramatically in recent months, N prices have risen too, resulting in soybean to N price ratios that still would not favor N applications in many environments.

8. Conclusions and perspectives on future research needs

On average, 50–60% of soybean N demand is met by biological N_2 fixation across a wide range of yield levels and environments and the proportion of plant N derived from fixation decreases with increasing inputs of N fertilizer. In most situations the amount of N fixed by soybean is not enough to replace N export from the field with grain, or is at best close to neutral if N from below-ground parts is included. However, because of lacking data, we were unable to properly assess the real contribution of below-ground N and its variation. Generally speaking, more studies on the partitioning of N and the contribution of N fixation in above and below-ground parts need to be conducted for grain legumes such us soybean. Only then we will be able to develop functional relationships that describe above and below-ground biomass and N in response to genotype, soil and climate variations in order to quantify the total N supply by soybean to the system. Hence, the questions of whether the N demand of high yielding soybeans can be solely met by symbiotic N_2 fixation and how the BNF system can be managed to achieve full yield potential with minimal additional N input are crucial to the economic and environmental sustainability of soybean production systems. The quantitative analysis of this review suggests that the capacity of the symbiotic N supply from soybean nodules to meet crop N demand in high yielding environments (grain yields above 5 Mg ha^{-1}) remains particularly uncertain given the paucity of reliable field measurements near these yield potential levels.

It is clear from the reviewed data that high yielding soybean requires large amounts of N to support both above-ground biomass and high protein seed. The published reports do not clearly point the way to understanding whether a more efficient nodule system or supplementary N fertilization is needed to routinely achieve high yield potential in different environments. Clearly BNF is the most sustainable and lowest cost source of N, and in many cases there is no response to added N. On the other hand, there are also a number of studies that document a response to additional N, and our rudimentary economic analysis suggests that it would be profitable to apply this additional N in some cases. Hence, the issues of when, where and why soybean sometimes responds to applied N remains an important research issue.

In the published research we reviewed, the yield response of soybean to N fertilizer application depends on the yield potential of the production environment and any abiotic or biotic constraints that reduce crop growth and associated N demand. When such constraints exist, the development of rhizobia strains able to fix N_2 under stress conditions appears to be the most feasible way to secure the required N supply (Alves et al., 2003, Hungria and Vargas, 2000). On the other hand, well-nodulated soybean crops without growth constraints and managed at yields levels above 4.5 Mg ha^{-1} are more likely to respond to N fertilization. There is some evidence to suggest that techniques to provide additional N during grain filling without decreasing nodule activity are the most likely route for attaining a yield response to N fertilization in such systems. Promising options include deep placement of (slow-release) fertilizer below the nodulation zone, or applying N during reproductive stages in high-yielding environments.

Future research should be directed towards a more precise quantification of the contributions of soil N, BNF and fertilizer N at key growth stages when the crop is grown at yield potential levels. Conducting detailed measurements of the uptake efficiency of applied N at different development stages and for different N application methods will be of particular importance for understanding the reasons for lack of response, or response, to fertilizer-N. Such research would provide critical information for assessing the practicality and efficiency of different N management strategies for providing supplementary N without reducing N_2 fixation. In addition, this new knowledge would be valuable to improve models that simulate soybean growth and yield as related to the N supply from both BNF and applied fertilizer.

Appendix A. Nitrogen rates, sources and other treatment factors evaluated in different studies on nitrogen fertilization in soybeans (Data set B).

No.	Reference	N rates (kg ha⁻¹)	N source[a]	Factors involved[b]
Studies with soil application of N fertilizers				
1	Afza et al. (1987)	0, 60	U	FT
2	Al-Ithawi et al. (1980)	0, 56, 112	AN	Y, WR
3	Amarger et al. (1979)	0, 80	AN	G
4	Beard and Hoover (1971)	0–168	AS	FT
5	Bhangoo and Albritton (1976)	0–448	AN	Y
6	Bharati et al. (1986)	0, 135, 270	U	T
7	Brevedan et al. (1978)	0, 168, 336	AN	Y, FT
8	Chen et al. (1992)	0–180	AN	L
9	Cooper and Jeffers (1984)	0–220	AA	G, S
10	Dadson and Acquaah (1984)	0–160	U	P
11	Deibert et al. (1979)	0–134	SN	FM
12	Eaglesham et al. (1982b)	0, 25, 100	AS	G
13	Freeborn et al. (2001)	0–168	UAN	FT, Y
14	Gan et al. (2002)	0, 75	U	FT, G
15	Gan et al. (2003)	0, 50	U	G, FT
16	Guafa et al. (1993)	0, 50	–	WR, G
17	Gutiérrez-Boem et al. (2004)	0, 50, 100	U	L, FT
18	Ham and Caldwell (1978)	0, 30	AN	FP
19	Herridge and Brockwell (1988)	0–300	AN	I
20	Israel and Burton (1997)	0, 150	AN	G, L, Y
21	Johnson et al. (1975)	0–448	CA	-
22	Judy and Murdock (1998)	0, 37, 45	UAN	Y
23	Koutroubas et al. (1998)	0, 120, 240	AN	Y, I
24	Mendes et al. (2003)	0–40	U	Y, T
25	Parker and Harris (1977)	0–201	AN	Y, L
26	Purcell and King (1996)	0, 336	AN	WR
27	Ramesh et al. (2002)	0, 23, 30	–	–
28	Ray et al. (2005)	0, 320	AN	Y, L, G
29	Reese and Buss (1992)	0, 28	UAN	Y, FT
30	Sanders (1994)	0, 200	AN	L, I
31	Schmitt et al. (2001)	0, 84	U, US	L, FT, FP
32	Semu and Hume (1979)	0–200	AN	Y, L
33	Seneviratne et al. (2000)	0, 46	U	I
34	Sistachs (1982)	0–75	U	FT
35	Slater et al. (1991)	0, 134	UAN	WR, G, P, Y
36	Starling et al. (1998)	0, 50	AN	G, L
37	Tancogne et al. (1991)	0, 150	U	WR
38	Taylor et al. (2005)	0–100	AN	L, PD, G
39	Thies et al. (1995)	0, 1414	U	L
40	Touchton and Rickerl (1986)	0, 16	AN	PF
41	Vasilas and Ham (1984)	0, 20, 100	AS	L
42	Vasilas and Ham (1985)	0, 100	AS	T
43	Weber (1966)	0–168	AN	Y
44	Welch et al. (1973)	0–1800	AN	Y, FT, T, PD, G
45	Wesley et al. (1998)	0, 20, 40	UAN, AN, U, U+	L, FS
46	Wood et al. (1993)	0, 34, 56	AN	L, G, FT
47	Wu and Harper (1991)	0, 200	U	G, Y
48	Ying et al. (1992)	0, 25, 50	U	PF
49	Ham et al. (1975)	0, 224	U, US, AN	Y, L, G, FS
50	Bodrero et al. (1985b)	0–200	U	Y, L
51	Barker and Sawyer (2005)	0, 45, 90	U, US	Y-L-FP-FS
52	Thies et al. (1991)	0, 1800	U	L
53	Hungria et al. (2006)	0, 30, 50	U	FT, L, Y
54	Zilli et al. (2006)	0, 200	U	I
55	Bodrero et al. (2004)	0, 40, 80	U	FT, L
56	Soares Novo et al. (1999)	0, 50, 100	U	L
57	(Hungria et al., 2005a) and (Hungria et al., 2005b)	0, 30, 50	U	FT, I
Studies with foliar application of liquid fertilizers				
55	Bodrero et al. (2004)	0, 10, 20	N solution (20%)	FT, L
58	Boote et al. (1978)	0, 28	U	N rates
59	Garcia and Hanway (1976)	0–160	U	FT, rates
60	Haq and Mallarino (1998)	0, 0.8, 2.4	NPK mixtures	FT, rates
61	Haq and Mallarino (2000)	0–7.1	NPK mixtures	Y, L, FT, FS
62	Parker and Boswell (1980)	0–84	U	Rates, L
63	Poole et al. (1983a)	0–96	U	FT, rates
64	Poole et al. (1983b)	0–24	U	Biuret, FT, TD
65	Sesay and Shibles (1980)	0, 80	U	G
66	Syverud et al. (1980)	0–135	U	Rates, Y
67	Bodrero et al. (1985a)	0, 40, 80	U, NPK mixtures	G, Y, L

[a] AA, Anhydrous ammonia; AN, ammonium nitrate; AS, ammonium sulfate; C, calcium cyanamide; CA, calcium nitrate; SN, sodium nitrate; U, urea; U+, urea with urease inhibitor; US, slow release urea (polymer coated; UAN, urea ammonium nitrate solution.

[b] FP, Fertilizer placement; FS, fertilizer sources; FT, fertilizer timing; G, genotypes; I, inoculation treatments; L, locations; P, phosphorus rates; PD = planting dates; PF = Previous fertilization; T = Tillage; TD, time of day; WR, water regimes; S, spacing; Y, years.

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