



## Role of N<sub>2</sub> fixation in the soybean N credit in maize production

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### Abstract

Many studies have shown that maize (*Zea mays* L.) requires less fertilizer N for optimum yield when grown in rotation with soybean [*Glycine max* (L.) Merr] than when grown in monoculture, which is referred to as the 'soybean N credit' in the maize growing areas of the United States. Because the specific source of this soybean N credit is unclear, our objective was to determine the role of nodules and N<sub>2</sub> fixation as a contributing source of the soybean N credit. Our research approach was designed to separate the effect of symbiotic N<sub>2</sub> fixation from other rotational effects, as the treatments included: maize grown after nodulated (N<sub>2</sub> fixing) soybean and maize grown after non-nodulated (non N<sub>2</sub> fixing) soybean. A separate experiment examined maize grown after maize. For each previous crop, maize was grown the following year with varying rates of fertilizer applied N. In both years, the yield differences between nodulated and non-nodulated soybean as the previous crop were much smaller than the apparent yield decrease associated with continuous maize. Although small in magnitude, maize following non-nodulated soybean accumulated less total N, was paler in leaf color, and yielded less than maize following nodulated soybean in the more favorable year of 1999, while most of these differences were not observed in 2000. These findings indicate that soybean nodules and N<sub>2</sub> fixation, while having a certain role, are not the major determinants of the soybean N credit.

### Introduction

Over-fertilization\* of maize fields in the upper Midwest could lead to excess dissolved nutrients (in particular N) in the Mississippi River, thereby contributing to a hypoxic zone along the Louisiana-Texas shelf of the Gulf of Mexico, which kills or drives away aquatic bottom dwellers (Rowe, 2001). In addition, excessive applications of N fertilizer decrease farm earnings, as fertilizer is one of the largest operating costs for maize production. These environmental and economic concerns emphasize the need to accurately predict the fertilizer N requirements of a maize crop under various production conditions.

The type of cropping system is notably an important variable to take into account, as maize grown in rotation with soybean usually requires less fertilizer N to attain an economic optimum yield than does maize

following maize. Therefore, a so-called soybean N credit is commonly used in the Midwest in order to adjust fertilizer N recommendations downward when maize is grown following soybean (Kurtz et al., 1984). Estimates of soybean N credits vary widely according to years and locations, and values ranging from 22 to 210 kg N ha<sup>-1</sup> have been reported (Bundy et al., 1993). Others have even reported instances of negative (i.e., requiring more N) soybean credits (Meese, 1993). This variability casts doubt as to the accuracy of estimates of the soybean N credit, and subsequently as to the reliability of N recommendations for maize grown in rotation with soybean (Klocke et al., 1999).

Uncertainty also exists regarding the source or reason for the soybean N credit, and while many people ascribe to the notion that the soybean N credit in some way involves N, others have suggested non-N related factors as possible explanations for the beneficial effects of including soybean in the rotation.

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Some non-N related possibilities include interrupting the release of maize autotoxic compounds (Anderson and Cruse, 1995), an improved control over various weeds, insects, and pathogens (Lipps, 1988; Varvel and Peterson, 1990; Ratcliffe et al., 2000), or to a better seedbed (Raimbault and Vyn, 1991). Differences in C/N ratios and total biomass between maize and soybean residues could also effect N availability to the subsequent maize crop by altering the rates of soil mineralization or immobilization (Aulakh et al., 1991; Green and Blackmer, 1995; Kaboneka et al., 1997). Our earlier work supports this view as we found that net mineralization of soil N was influenced by both the quality (C:N ratio) and quantity of residues from the previous crop (Gentry et al., 2001). The improved growth of maize following soybean, i.e. the soybean N credit, was largely the result of a decrease in net soil mineralization in continuous maize production. However, by comparing N<sub>2</sub>-fixing (nodulated) and non N<sub>2</sub>-fixing (non-nodulated) soybean as the previous crop we also concluded that N<sub>2</sub>-fixation plays at least some role in the soybean N credit, presumably from the decomposition of N-rich nodules and/or soybean residues (Gentry et al., 2001).

The N<sub>2</sub>-fixing ability of soybean has long been hypothesized as an explanation for the soybean N credit, in part because of reports that a growing soybean crop can release some of its symbiotically-fixed N during the same season (Brophy and Heichel, 1989). Martin et al. (1990) have shown that this leakage is responsible for transfer of N from soybean to intercropped maize plants, and we showed a higher level of inorganic N in the soil immediately adjacent to a previously nodulated soybean row (Gentry et al., 2001). We extend our earlier work on the importance of previous crop residues and soybean nodules as sources of the soybean N credit (Gentry et al., 2001) with additional field studies assessing the impact of nodules on the magnitude of the soybean N credit and on the N requirement of the subsequent maize crop. Our objective was to determine the role of the nodules and N<sub>2</sub> fixation as a contributing source of the soybean N credit. Our approach was to compare the effect of a nodulated (N fixing) and a non-nodulated (non-N fixing) soybean isolate as the previous crop, on the growth and N requirement of the following maize crop.

## Materials and methods

### *Field site, cultural practices and treatment arrangement*

The experiments were conducted at the Department of Crop Sciences Research and Education Center in Champaign, IL during the 1999 and 2000 growing seasons on plots that had the appropriate previous crop treatments established during the prior seasons (i.e. 1998 and 1999). The slope was less than 1% with a downward gradient of the field from south to north. Analysis of soil samples prior to the start of the experiments indicated that all plots within the experimental area were composed of a single soil type; a Drummer silty clay loam (fine-silty, mixed mesic Typic Haplaquolls) (Gentry et al., 2001). The range in percent organic matter for the 0–10 cm depth was 4.5–5.0% (mean of 4.7%), whereas the average percent organic matter for the 10–30 and 30–50 cm depths was 4.5 and 3.2%, respectively. The total N in the top 50 cm of soil was 8,915 kg N ha<sup>-1</sup>. Soil tests indicated that sufficient levels of P and K were present for optimal yields. Daily precipitation was recorded within 1 km of the site at the Illinois Climate Network, and the departure from the 30 year average in 1999 and 2000 is presented in Figure 1.

In years when soybean was the crop, a nodulated cultivar (Williams 82, maturity group III), and a non-nodulated isolate of this cultivar that is unable to perform symbiotic N<sub>2</sub> fixation, were planted at a density of 350,000 plants ha<sup>-1</sup>. In all cases where maize was grown, Pioneer hybrid 33A14 was planted at a density of 92,200 plants ha<sup>-1</sup>. Each experimental unit (of maize or nodulated or non-nodulated soybean) consisted of a six-row plot, with rows 6.1 m long and spaced 0.76 m apart. Primary tillage was done in the fall with a field cultivator, followed by a secondary tillage with a finishing tool in the spring. All maize plots received an in-furrow application of Aztec (cyflurhin/tebupirimphos) at a rate of 2.4 kg a i ha<sup>-1</sup> to control Western corn rootworm larvae (*Diabrotica sp.*), and an application of Dual II Magnum (S-metolachlor/benoxacor) at a rate of 2.3 kg a i ha<sup>-1</sup> and Aatrex (atrazine) at a rate of 1.7 kg a i ha<sup>-1</sup> for weed control. The maize cultivar used (Pioneer 33A14) was a bt variety, so European corn borer (*Ostrinia nubilalis*) control was not necessary.

In order to achieve the previous crop and N fertilizer treatments, the field was divided into four sections (A, B, C and D) as shown in Figure 2. For the first re-

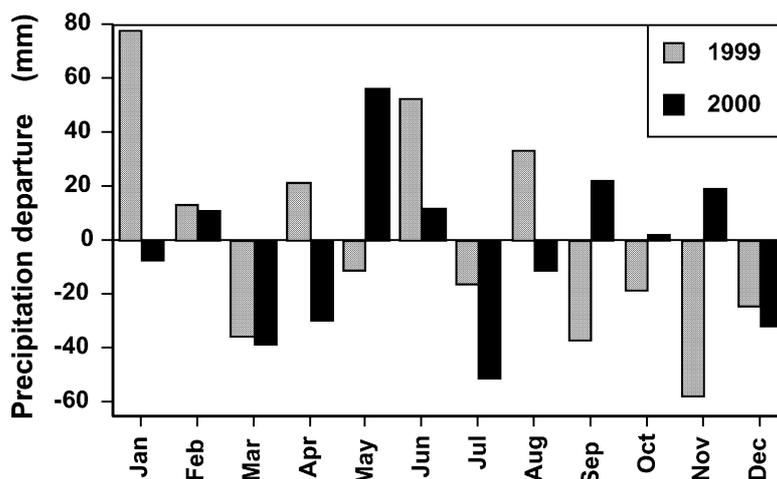


Figure 1. Precipitation at Champaign, IL in 1999 and 2000, expressed as the departure from the 30 year (1961–1990) average.

petition, the experiments were conducted on sections A and B established during the 1998 growing season, and used for treatments during 1999, whereas the second repetition was conducted on sections C and D that were established in 1999 and used for treatments in 2000. Within each section, experimental units were arranged in a randomized complete block design with either three or four replications.

Maize was planted in section A on 20 May 1998 (Figure 2), and fertilizer N was uniformly applied on 2 June as ammonium sulfate at a rate considered optimal maximum yield at this site ( $168 \text{ kg N ha}^{-1}$ ). This section was combine-harvested on 12 October 1998. In section B, nodulated and non-nodulated soybean plots were planted 25 May 1998. They did not receive any fertilizer N application, and were harvested on 24 September for the nodulated soybean plots, and 21 September for the non-nodulated soybean plots. For the following growing season (1999), maize was planted on 3 May in both sections A and B to give three previous crop scenarios: (1) maize following maize, (2) maize following nodulated soybean, and (3) maize following non-nodulating soybean. Eight equally distributed rates of fertilizer N (0, 34, 67, 101, 135, 168, 202 and  $235 \text{ kg N ha}^{-1}$ ) were applied to the maize following maize plots, while because of the limited number of soybean plots installed in 1998, only the first six rates (0, 34, 67, 101, 135 and  $168 \text{ kg N ha}^{-1}$ ) were applied to the maize following either type of soybean. All fertilizer rates were hand applied on 16 May as granular ammonium sulfate, with a fertilizer rate randomly assigned to a plot within

each block. Immediately following application the fertilizer was incorporated with a field cultivator.

The two experiments (maize following maize, and maize following either type of soybean) were repeated a second time during the 1999 and 2000 growing seasons, over four blocks established in sections C and D (Figure 2). In 1999, maize was planted 3 May in section C, received a uniform N fertilizer rate of  $168 \text{ kg N ha}^{-1}$  as granular ammonium sulfate on 16 May, and was harvested 22 September. Over the same year in section D, both types of soybean plots were planted 12 May, did not receive any fertilizer application, and were combine-harvested 17 and 10 September for nodulated soybean, and non-nodulated soybean plots, respectively. Maize was planted the following growing season (2000), on 28 April in both sections C and D. Eight equally distributed N rates (0, 34, 67, 101, 135, 168, 202 and  $235 \text{ kg N ha}^{-1}$ ) were applied on the maize following maize treatment, while due to the smaller number of plots with soybean as the previous crop, only seven N rates (0, 34, 67, 101, 135, 168 and  $202 \text{ kg N ha}^{-1}$ ) were applied on maize following either type of soybean. The different fertilizer rates were randomly assigned to a plot within each block, and were hand-applied as granular ammonium sulfate on 16 May and followed by immediate incorporation with a field cultivator.

#### Plant measurements

Several growth and N status measurements were made during vegetative and reproductive development of the maize plants that had either maize, nodulated soybean,

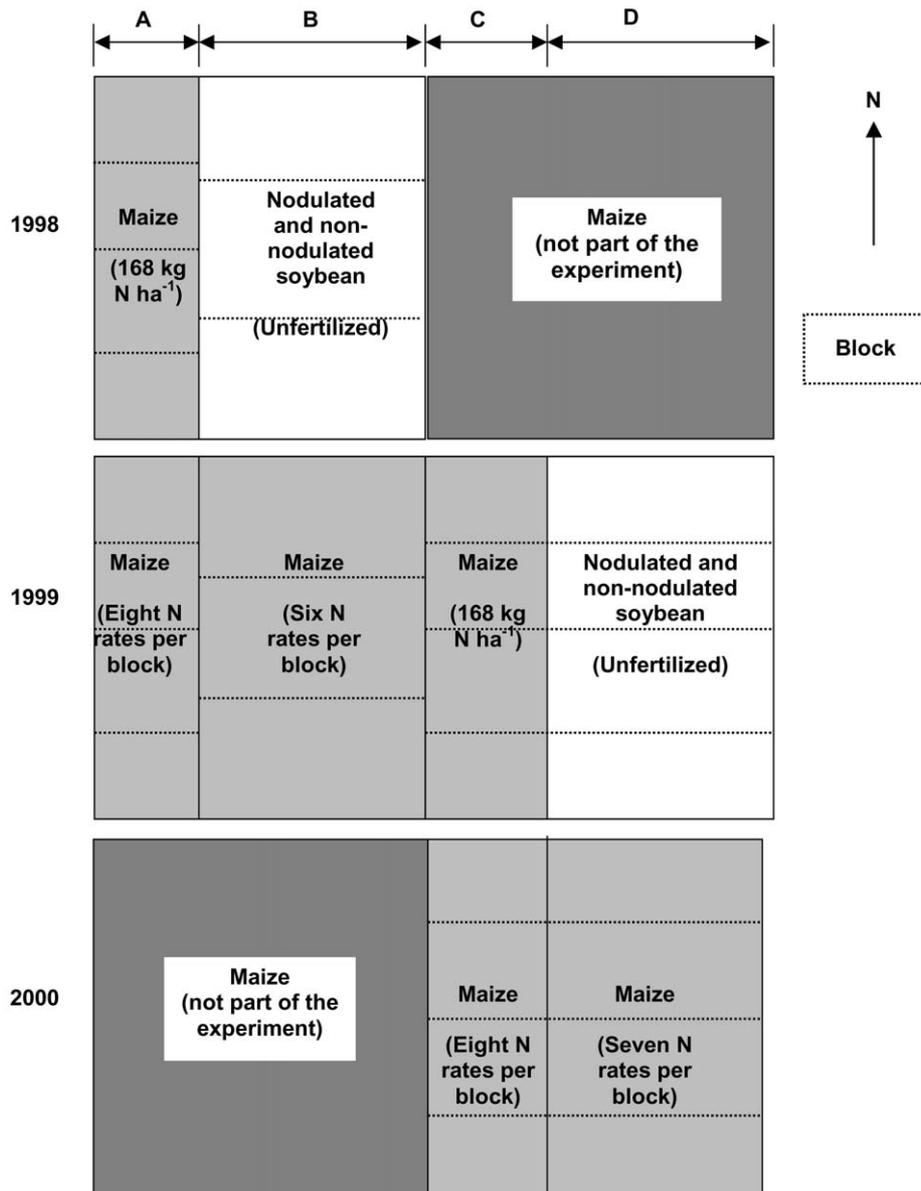


Figure 2. The field arrangement and yearly cropping sequence used to obtain the previous crop and N rate treatments.

or non-nodulated soybean as the previous crop (sections A and B for 1999, and C and D for 2000). In both experimental years, the number of plants in the center two rows of each plot was counted at the V10 growth stage (Ritchie et al., 1997) to obtain the average plant population (stand) per plot. Leaf greenness was measured at the V15 growth stage with a handheld chlorophyll meter (SPAD-502) on the last fully developed leaf from thirty randomly selected plants. Leaf area index (LAI) was measured at the V8 growth

stage in 1999 and the V10 growth stage in 2000 using a LiCor 2000 LAI-meter. In order to obtain the most accurate LAI estimates, two measurements were made at three different distances from the stalk base, with one taken 0.05 m away from the stalk, another in the middle of the row (0.4 m away from the stalk), and a third one between those two points (0.2 m from the stalk). This procedure gave six different LAI measurements that were averaged to obtain the overall LAI for each plot.

To account for non-N effects related to maize insect damage, plots were monitored throughout the study for European corn borer, and no damage was visually observed in either of the years. The level of corn rootworm larval injury was also assessed in 1999 and 2000 by digging three plants (from rows 2 and 5) from the 168 kg N ha<sup>-1</sup> treatment at the R3 growth stage and rating their roots based on the Iowa State system (Hills and Peters, 1971).

At the R6 growth stage (which occurred on 27 August 1999 in sections A and B, and 7 September 2000 in sections C and D), four representative plants per plot were selected from rows 2 and 5. These plants were divided into stover (leaves and stalks), reproductive support fractions (consisting of husk, shank, tassel, and cob), and grain. The stover fraction was weighed fresh, shredded and a sub-sample was dried in a forced-draft oven at 80 °C for 72 h, after which the dry weight of the stover was determined. The entire reproductive fraction was dried in a forced draft oven at 80 °C for 72 hours before weighing, while the dry weight of shelled grain was determined by subtracting the moisture as measured with a grain moisture meter. After drying in a forced draft oven at 80 °C for one week, a sub-sample of 300 kernels was weighed to determine the individual kernel weight, and the kernel number per plant calculated by dividing total grain dry weight by the individual kernel dry weight. All dried plant fractions were then ground to pass through a 2 mm mesh screen and analyzed for total N using a combustion technique (Fissons NA 2000 N Analyzer). Based on these measurements, we calculated the dry weight, N concentration and N content of each fraction (stover, reproductive-support, and grain). The total accumulation of N by whole plants (above-ground portions) was determined by summing values for the three fractions. The final grain yield was determined by hand harvesting all plants in the two center rows (i.e., rows 3 and 4) when the grain had dried to an acceptable moisture (on 18 September 1999 for sections A and B, and on 22 September 2000 for sections C and D). Hand-harvesting was necessary in order to avoid harvesting rows 2 and 5 on which destructive measurements had been conducted during the season.

Plots containing soybean as the previous crops (section B in 1998 and section D in 1999) were also sampled to determine whole plant N accumulation (above-ground portions) by harvesting all plants in a 50 cm section of row at the late R6 growth stage (6 September 1998 and 17 September 1999 for nodulated soybean, and 3 September and 10 Septem-

ber for non-nodulated soybean), dividing into stover, pods, and seeds, and analyzing each fraction for N as described above. Grain yield of the previous crop was determined by combine-harvesting all rows in plots from sections A and B in 1998, and from sections C and D in 1999, and adding back the seed weight of plant samples used to determine whole plant N accumulation. Based on these measurements, the previous-crop grain yields in 1998 and 1999 were 2.3 and 2.9 Mg ha<sup>-1</sup> for nodulated soybean, 1.1 and 0.8 Mg ha<sup>-1</sup> for non-nodulated soybean, and 8.5 and 8.3 Mg ha<sup>-1</sup> for maize.

### *Statistical analysis*

Because the previous crop treatments were confounded with field locations (see Figure 2), measurements made on maize following maize, and on maize following either type of soybean were statistically analyzed as separate experiments. Also due to differences in the number of N levels evaluated, and in some cases the number of replications, years were also analyzed separately. For each experiment and year, plant parameters were analyzed by analysis of variance (with nodulation type and N rate as treatments for maize following soybean, and N rate only as the treatment for maize following maize), and by regression to determine the N response functions. Summary tables of these analyses are presented in Tables 1 and 2. Statistical differences at the 5% level were considered significant, and in cases where a presented trend or tendency is not within this probability level the actual *P* value is given.

Despite the field confounding, we believe the impact of field location on the measured variables was minor, as all experimental plots were located within 50 meters of each other in a field with little reported variability from east/west (Gentry et al., 2001). All cultural practices were also conducted at the same time on both sides of the field. Thus, for comparative purposes the data for all treatments (maize after maize, maize after nodulated soybean, and maize after non-nodulated soybean) are presented on the same figures. Any apparent differences between maize after maize and maize after soybean discussed in the text are based on clearly obvious differences as indicated on the Figures, and/or as differences in magnitude of the respective values of at least 20%

Table 1. Analysis of variance summary for the two experiments (maize after nodulated or non-nodulated soybean, and maize after maize) in 1999 and 2000

		Leaf Chlorophyll (SPAD units)	LAI (ratio)	Grain yield (Mg/ha)	Plant density (no./ha)	Kernel number (no./plant)	Kernel weight (mg/kernel)	Plant N Accumulation (kg/ha)	
Source of variation		1999							
	df								
Soy/maize	Block	2	*	NS	NS	NS	*	*	
	Soybean type (T)	1	**	**	NS	NS	**	**	
	N rate (N)	5	**	**	**	NS	**	**	
	T × N	5	NS	**	NS	NS	NS	NS	
	C.V., %		4.1	5.8	10.3	11	7.7	4.8	15.3
Maize/maize	Block	3	NS	NS	**	NS	*	NS	
	N rate	7	**	**	**	NS	**	**	
	C.V., %		3.7	9.1	12.8	15.7	8.6	4.3	20.1
		2000							
Soy/maize	Block	3	**	**	NS	NS	NS	*	
	Soybean type (T)	1	**	NS	NS	NS	NS	NS	
	N rate (N)	6	**	**	**	NS	**	NS	**
	T × N	6	NS	NS	NS	NS	NS	NS	
	C.V., %		3.2	9.3	9.4	5.9	11.8	5.9	14.7
Maize/maize	Block	3	NS	NS	**	NS	NS	NS	
	N rate	7	**	**	**	NS	**	NS	**
	C.V., %		4.5	13.6	9.6	9.3	13.4	9.1	20.9

\*, \*\* Significant at the 0.05 and 0.01 probability levels, respectively.

## Results

The degree of root damage was assessed in order to account for non-N related rotational effects caused by corn rootworm larval feeding. Using the Iowa State 1–6 rating scale (Hills and Peters, 1971), these values for maize after maize, maize after nodulated soybean, and maize after non-nodulated soybean plots were 2.4, 2.0 and 2.0 in 1999, and 1.9, 2.1 and 2.2 in 2000. These values are all within 0.5 units of each other, indicating there was negligible difference between the previous crops on the degree of corn rootworm larval damage to the following maize crop. In addition, there were no other obvious visual differences in the root systems that could be attributed to the previous crop.

The amount of N fertilizer applied affected maize leaf greenness and LAI during the vegetative growth stages in both years (Figure 3, Table 1). Although increasing the N rate clearly increased LAI in the maize following maize experiment, the effect of N rate on LAI was much more tempered for maize following soybean. Only a moderate effect of N supply on LAI was observed in 1999, and then only for the maize following non-nodulated soybean (Figure 3, Table 1);

while in 2000, there was a fairly large and similar impact of N supply on LAI of maize following either the nodulated or non-nodulated soybean. In contrast to LAI, the relative leaf chlorophyll was highly influenced by fertilizer N addition in both years regardless of the previous crop (Figure 3). Compared to nodulated soybean as the previous crop, non-nodulated soybean resulted in paler leaves on the following maize crop in both years of the study, although the differences among these treatments were larger in 1999 than in 2000 (Figure 3, Table 1). While the SPAD chlorophyll values were especially low for N deficient plants in the maize following maize experiment, leaf chlorophyll of these plants rapidly increased with incremental increases in the N supply, and ultimately achieved values as high as plants in the maize following soybean experiments (Figure 3).

While weather conditions were generally conducive to optimal maize growth throughout the 1999 growing season, below-average precipitation occurred in July of 2000 (Figure 2), when the crop was flowering. This difference may help to explain why the grain yields were generally higher (maximum of

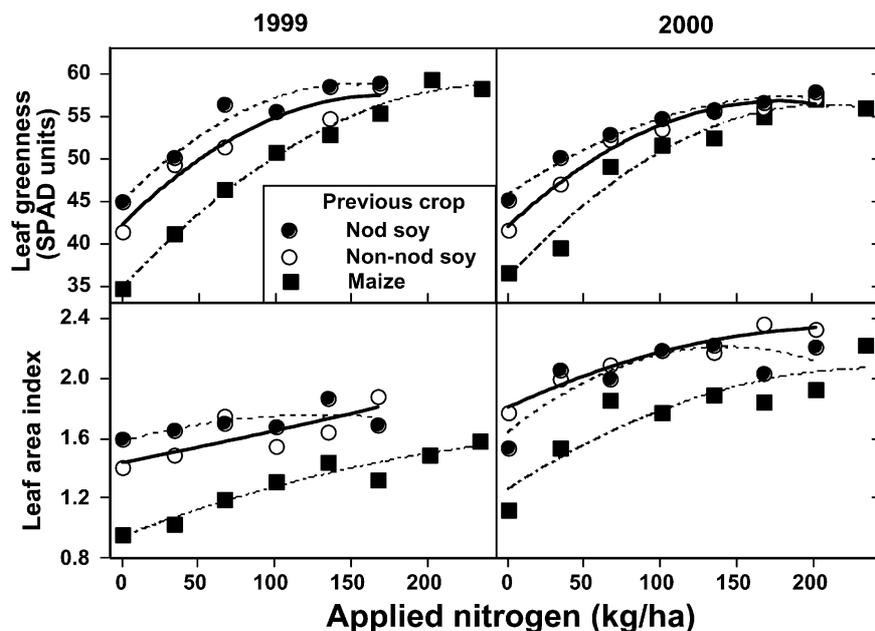


Figure 3. Effect of previous crop and rate of applied N on the level of leaf chlorophyll and leaf area index of corn. Leaf chlorophyll was measured at the V15 growth stage for both years, and leaf area index was measured at the V8 and V10 growth stages in 1999 and 2000, respectively. Maize was grown in 1999 and 2000 on plots that had either nodulated soybean (nod soy), non-nodulated soybean (non-nod soy), or maize in 1998 and 1999. Regression equations and R-square values are given in Table 2.

10.9 Mg ha<sup>-1</sup> vs 9.0 Mg ha<sup>-1</sup>) in 1999 than in 2000 (Figure 4). Interestingly, however, yield of the unfertilized maize crop was relatively similar between the two years and did appear to be influenced by the previous crop. Averaged over the two years, the zero N plots of maize after nodulated soybean yielded 5.9 Mg ha<sup>-1</sup> compared to 4.9 Mg ha<sup>-1</sup> for maize after non-nodulated soybean and 3.0 Mg ha<sup>-1</sup> for maize after maize. Yields in the maize following maize experiment were lower than maize following either type of soybean, and this difference could not be overcome with additional increments of N supply (Figure 4). The presence of a non-nodulated rather than a nodulated soybean as a previous crop impaired the yield performance of the subsequent maize crop in the more favorable environment of 1999, while a similar tendency ( $P = 0.12$ ) was also observed in 2000 (Figure 4, Table 1). In 2000, the yield response to N was quadratic (Table 2) with 159 kg N ha<sup>-1</sup> maximizing yield of maize after nodulated soybean, 233 kg N ha<sup>-1</sup> for maize after non-nodulated soybean, and 295 kg N ha<sup>-1</sup> for maize after maize. Conversely, in 1999, the yield responses to N were linear and the highest N rate evaluated always resulted in the highest yields.

Analysis of the components responsible for grain yield showed important variations in their response to addition of fertilizer N, previous crop, and year (Figure 5, Table 1). As expected, plant density was not influenced by the amount of N fertilizer applied in either year; however, it was influenced by the year. In contrast, for both years of the study, additional fertilizer N applications led to an increase in the number of kernels per plant for all previous crop treatments. While N fertilization resulted in heavier individual kernels in 1999, especially for the maize following maize experiment, the rate of fertilizer applied N did not influence individual kernel weight in 2000 for any of the previous crop treatments (Figure 5, Table 1). Similarly, the N<sub>2</sub> fixation capability of the previous soybean crop only influenced individual kernel weight of the following maize crop in 1999, where maize following non-nodulated soybean had lighter individual kernels than maize following nodulated soybean.

The total accumulation of N in above-ground plant parts was positively influenced by the addition of fertilizer N in both years, with the magnitude of response to N being greater in 1999 than in 2000 (Figure 6). Having a nodulated rather than non-nodulated soybean as the previous crop also resulted in the accumulation of more N by maize plants in 1999, but not in 2000.

Table 2. Regression equations and  $R^2$  values for the effect of N rate on different plant parameters for three previous crop treatments in 1999 and 2000

Plant parameter	Previous crop	1999		2000	
		Regression equation <sup>†</sup>	$R^2$	Regression equation <sup>†</sup>	$R^2$
Leaf chlorophyll (SPAD units)	Nod soy	$45 + 0.18x - 5.8 \times 10^{-4}x^2$	0.80	$46 + 0.12x - 3.1 \times 10^{-4}x^2$	0.83
	Non-nod soy	$42 + 0.18x - 5.0 \times 10^{-4}x^2$	0.85	$42 + 0.17x - 4.6 \times 10^{-4}x^2$	0.91
	Maize	$35 + 0.19x - 3.8 \times 10^{-4}x^2$	0.95	$36 + 0.19x - 4.6 \times 10^{-4}x^2$	0.88
LAI	Nod soy	$1.62 + 9.5 \times 10^{-4}x$	0.27	$1.64 + 7.9 \times 10^{-3}x - 2.7 \times 10^{-5}x^2$	0.39
	Non-nod soy	$1.62^{\ddagger}$		$1.86 + 2.6 \times 10^{-3}x$	0.37
	Maize	$0.99 + 2.6 \times 10^{-3}x$	0.74	$1.36 + 3.4 \times 10^{-3}x$	0.52
Grain yield (Mg/ha)	Nod soy	$6.50 + 2.9 \times 10^{-2}x$	0.73	$5.63 + 3.5 \times 10^{-2}x - 1.1 \times 10^{-4}x^2$	0.65
	Non-nod soy	$5.25 + 3.4 \times 10^{-2}x$	0.95	$4.75 + 3.5 \times 10^{-2}x - 7.6 \times 10^{-5}x^2$	0.85
	Maize	$3.18 + 2.7 \times 10^{-2}x$	0.81	$3.01 + 3.3 \times 10^{-2}x - 5.6 \times 10^{-5}x^2$	0.85
Plant density (no./ha)	Nod soy	$70629^{\ddagger}$		$83967^{\ddagger}$	
	Non-nod soy	$71299 - 199x + 1.46x^2$	0.49	$84453^{\ddagger}$	
	Maize	$65672^{\ddagger}$		$73626^{\ddagger}$	
Kernel number (plant)	Nod soy	$331 + 1.75x - 4.5 \times 10^{-3}x^2$	0.81	$287 + 1.49x - 4.3 \times 10^{-3}x^2$	0.50
	Non-nod soy	$274 + 3.59x - 1.5 \times 10^{-2}x^2$	0.87	$218 + 2.69x - 8.7 \times 10^{-3}x^2$	0.76
	Maize	$186 + 2.46x - 5.7 \times 10^{-3}x^2$	0.87	$184 + 2.69x - 6.9 \times 10^{-3}x^2$	0.77
Kernel weight (mg/kernel)	Nod soy	$271 + 0.23x$	0.50	$267 - 9.1 \times 10^{-2}x$	0.15
	Non-nod soy	$255 + 0.25x$	0.51	$264 - 1.9 \times 10^{-2}x$	0.29
	Maize	$223 + 0.53x - 1.1 \times 10^{-3}x^2$	0.74	$264 - 8.9 \times 10^{-2}x$	0.36
N accumulation (kg/ha)	Nod soy	$102 + 0.83x$	0.68	$103 + 0.35x$	0.53
	Non-nod soy	$87 + 0.70x$	0.80	$87 + 0.43x$	0.65
	Maize	$65 + 0.82x$	0.78	$68 + 0.41x$	0.59

<sup>†</sup>equation = plant parameter in the given units; x = fertilizer N rate, kg ha<sup>-1</sup>.

<sup>‡</sup>Indicates the plant parameter averaged across N rates since linear and quadratic regression models were not significant at the  $P = 0.05$  level.

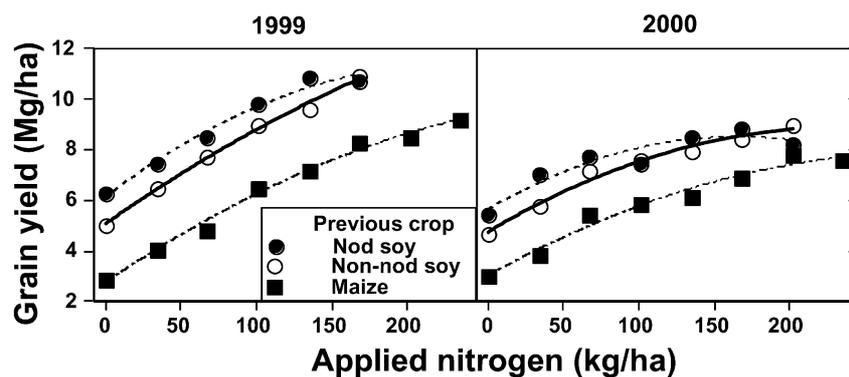


Figure 4. Effect of previous crop and rate of applied N on grain yield of maize. Regression equations and R-square values are given in Table 2. Cropping details as given in Figure 3.

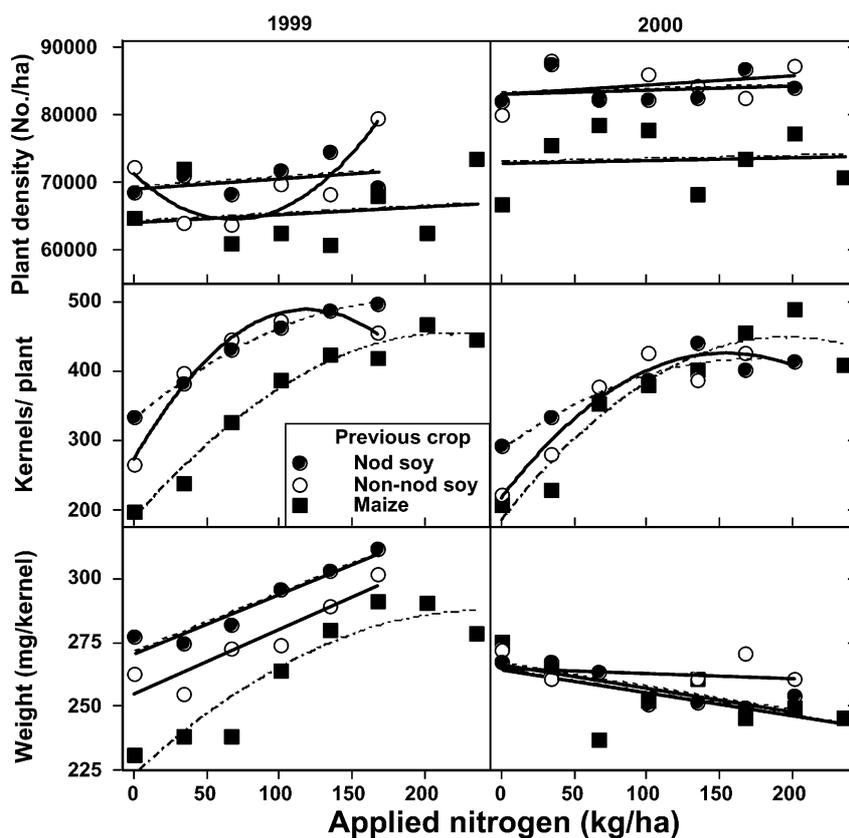


Figure 5. Effect of previous crop and rate of applied N on plant density, kernel number per plant, and individual kernel weight of maize. For both years, stand was measured at the V10 growth stage, kernel number at the R6 growth stage, and kernel weight at the R6 growth stage. Regression equations and R-square values are given in Table 2. Cropping details as given in Figure 3.

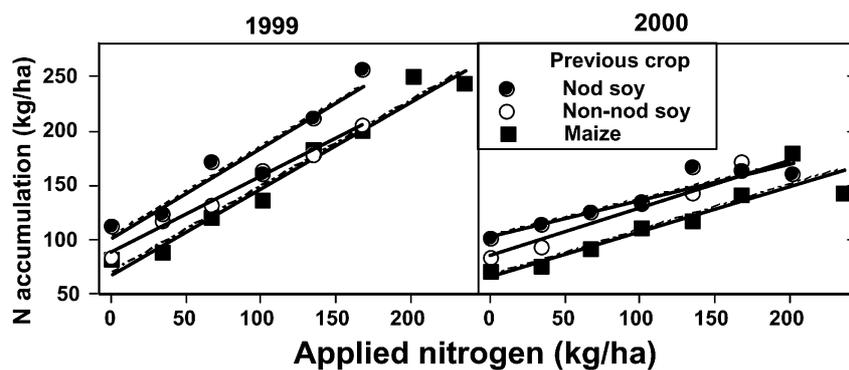


Figure 6. Effect of previous crop and rate of applied N on total N accumulation by above-ground plant parts at the R6 growth stage. Regression equations and R-square values are given in Table 2. Cropping details as given in Figure 3.

## Discussion

The impact of previous crop and the rate of N fertilization on grain yield was consistent with the growth observations made during the plant's vegetative development. Increasing the rate of fertilizer N generally increased LAI, and clearly increased leaf chlorophyll, and the overall patterns of response to N supply were relatively similar to the response of grain yield to N (Figures 3 and 4). Vegetative growth was also influenced by the previous crop, as maize following nodulated soybean had greener leaves than maize following non-nodulated soybean; which were both greener than the values obtained in the maize following maize experiment. Previous work has shown that SPAD chlorophyll readings are a good indication of leaf N status (Schepers et al., 1992), and the close association between the treatment-induced differences in leaf chlorophyll (both as a result of N rate and the previous crop) and final grain yield observed in this study (Figures 3 and 4) suggests that N and/or N availability plays an important role in the soybean N credit. Similarly, maize grown after non-nodulated soybean yielded less than maize after nodulated soybean (significant in 1999 with a similar trend in 2000) (Figure 4), which was associated with a lower leaf chlorophyll and with a lower accumulation of N by above-ground plant parts (Figures 3 and 6). Ennin and Clegg (2001) also showed a higher concentration of leaf chlorophyll and a greater accumulation of plant N when maize followed soybean than when it followed maize, which they attributed to increased N availability. Thus, we believe that N<sub>2</sub> fixation by nodules is at least partially responsible for the soybean N credit. A higher level of inorganic N in the soil immediately adjacent to where nodulated, but not non-nodulated soybean had grown the previous season (Gentry et al., 2001) also supports this view.

The yield differences between maize growing after either nodulated or non-nodulated soybean appeared to be much smaller than the yield decrease associated with maize growing after maize (Figure 4). The relatively small impact of nodulation condition of the previous soybean crop on the growth and yield of the subsequent maize crop was in spite of the fact that there was a considerable difference in grain yield and plant N accumulation between nodulated and non-nodulated soybean. Nodulated soybean yielded 1.2 Mg ha<sup>-1</sup> more than non-nodulated soybean in 1998 and 2.1 Mg ha<sup>-1</sup> more in 1999. Nodulated soybean also accumulated 73 kg ha<sup>-1</sup> more plant N than

non-nodulated soybean in both years (117 kg N ha<sup>-1</sup> for nodulated compared to 44 kg N ha<sup>-1</sup> for non-nodulated soybean in 1998 and 145 kg N ha<sup>-1</sup> compared to 72 kg N ha<sup>-1</sup> in 1999), which represents the seasonal N input from N<sub>2</sub> fixation. These notable differences, and the minor impact of nodulated or non-nodulated soybean as the previous crop shows that nodules, or N derived from N<sub>2</sub> fixation, are not essential components in the beneficial aspects associated with the soybean N credit. This finding is in agreement with previous suggestions made by Maloney et al. (1999), and implies a larger role for soil processes that influence N availability (i.e., mineralization/immobilization), and/or other non-N related factors in the soybean N credit. Our previous work on the soil at this experimental site also suggested that differences in soil mineralization played a large role in the soybean N credit (Gentry et al., 2001).

The large apparent difference in yields between the maize following maize experiment and maize following either type of soybean can also be viewed as both N and non-N related factors. In 1999, the lower grain yields in the maize following maize experiment compared to maize following nodulated soybean was due to a combination of both smaller and less numerous kernels (Figures 4 and 5). Because kernel production and size is influenced by the availability of plant N (Pearson and Jacobs, 1987), we hypothesize that part of the soybean N credit observed in 1999 was attributable to N-related factors. Conversely, the apparent improvement in yield performance for maize following either type of soybean, compared to the maize after maize experiment in 2000 was mainly attributable to a difference in seedling emergence as reflected in the greater number of plants per hectare (Figure 5). Based on visual observations of the soil at planting, a greater proportion of small soil aggregates was present when soybean rather than maize was the previous crop (data not shown). Others have also reported improved soil granulation for soybean as opposed to maize as the previous crop, possibly because soybean roots deplete soil water in the surface layer more extensively than maize (McCracken et al., 1985; Copeland et al., 1993). We hypothesize that the resultant fluctuations in soil moisture could aid in loosening the soil, leading to better seed-soil contact and improved plant emergence. Why this response only occurred in 2000 is not clear but may be related to the abnormally low precipitation that occurred from Sept. 1999 to April 2000 (Figure 1). Regardless of the cause, this data suggests

that factors in addition to N availability, may also play a role in the soybean N credit.

We also used the apparent difference in yields, and yield response to fertilizer N, between the maize following maize experiment and the maize following nodulated soybean to estimate the soybean N credit using the fertilizer replacement value method (Blevins et al., 1990; Smyth et al., 1991). These calculations gave estimates of the soybean N credit of 96 kg N ha<sup>-1</sup> in 1999 and 70 kg N ha<sup>-1</sup> in 2000, which were both considerably larger than the 45 kg N ha<sup>-1</sup> that is commonly used in the Midwest to make fertilizer N recommendations (Kurtz et al., 1984). This reduction in fertilizer N application when maize follows soybean is largely responsible for the term 'soybean N credit'. While N inputs from N<sub>2</sub> fixation are clearly important in the soybean N credit, our data shows that N<sub>2</sub> fixation plays a relatively minor role. Rather, the soybean N credit seems largely the result of other factors, suggesting that a term like 'soybean rotation effect' might more accurately describe this phenomenon. These rotation effects could still be due to N availability as a result of differences in soil mineralization/immobilization, or to other factors which are not directly related to N (i.e., allelopathy, soil tilth, etc.) Regardless of the terminology used, distinguishing the components of the soybean N credit could be important in research studies investigating this credit, and in designing crop management strategies to capitalize on the benefits of a maize/soybean rotation.

## Conclusions

The increase in leaf chlorophyll, plant N accumulation, and yield of maize when nodulated rather than non-nodulated soybean was the previous crop supports the view that nodules and N<sub>2</sub> fixation are important factors in the soybean N credit. Conversely, the modest effect of the previous soybean crop's nodulation condition compared to the yield decrease associated with continuous maize shows that nodules, or N derived from N<sub>2</sub> fixation are not the major determinants of the soybean N credit.

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