Plant Soil
DOI 10.1007/s11104-017-3279-9

REGULAR ARTICLE

Relative importance of biological nitrogen fixation and mineral uptake in high yielding soybean cultivars

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Received: 4 January 2017 / Accepted: 4 May 2017

Abstract
Backgrounds and aims Soybean yield depends on total N uptake, N use efficiency, and harvest index. Nitrogen uptake relays on biological fixation (BNF) and soil absorption. Usually, BNF is considered a yield-related process. However, there is limited information on whether maximizing percent BNF (%BNF) is actually required to maximize N uptake and yield.
Methods Seventy cultivars were evaluated for total N uptake, N use efficiency, and harvest index. Biological N fixation was determined in a subset of cultivars. The harvest index of N derived from atmosphere and from soil was also assessed.
Results Yield was positively associated with total N uptake. Highest N uptake was not linked to increased %BNF. An inverse relationship between the amount of BNF (kgBNF) and soil N absorption was observed. Harvest index of N derived from BNF was 85%, while it was 77% for N derived from soil.
Conclusions Highest total N uptake was attained by different combinations of kgBNF and mineral soil N absorption. This showed that maximizing %BNF is not required to maximize yield. High %BNF played a pivotal role in determining neutral soil N balance. This is so even though N derived from BNF was more partitioned to seeds than N derived from soil.

Keywords *Glycine max* (L) Merr. · Nitrogen · Biological fixation · Maturity group · Biomass partitioning

Introduction

Nitrogen is commonly the most limiting factor for crop growth and seed yield in the absence of water availability constraints (Gifford and Evans 1981; Hirel et al. 2007). Nitrogen availability is critical for leaf area generation and for photosynthetic carbon fixation (Sinclair and Horie 1989). At the crop level, these two processes will impact on crop growth and therefore crop productivity. In this way, seed yield can be described using the following framework that considers N uptake and use (Garnier et al. 1995; Rotundo et al. 2014):

$$\text{Seed yield}\left(\text{kg ha}^{-1}\right) = \text{N uptake} \times \text{N use efficiency} \times \text{Harvest index} \qquad (1)$$

where N uptake (kg ha^{-1}) is total N captured from emergence to physiological maturity, N use efficiency

Responsible Editor: Katharina Pawlowski.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-017-3279-9) contains supplementary material, which is available to authorized users.

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(NUE) is aboveground biomass production per unit N uptake (kg kg N^{-1}; Novoa and Loomis 1981), and harvest index (HI) is the proportion of total aerial biomass that is partitioned to seed biomass at maturity (kg seed kg biomass^{-1}; Donald and Hamblin 1976). Among these attributes, N uptake is usually the main driver of soybean yield (Sinclair and Jamieson 2006; Rotundo et al. 2014). Rotundo et al. (2014) showed that highest-yielding soybean cultivars may differ in NUE and HI but they all had the largest N uptake when compared with low yielding cultivars. A better understanding of the processes associated with increased N uptake is critical for securing future gains in seed yield.

Soybean N uptake depends on two alternative N sources, biological N fixation (BNF) and mineral soil N absorption. The relative contribution of each N source is the result of environmental conditions, agronomic management, and genetic factors. On average, the percentage of BNF (%BNF) ranges between 40 to 80% of total soybean N uptake (Salvagiotti et al. 2008). This percentage is usually reduced under water stress (Purcell et al. 2003; Sinclair et al. 2010), elevated temperatures (George et al. 1988), and high nitrate soil concentration (Salvagiotti et al. 2008, 2009). Agronomic management aimed to increase crop productivity (e.g., planting date, plant population, pest and disease control, and cultivar adaptation) is positively associated with biomass production and the amount of N derived from BNF (kgBNF) (Herridge et al. 2001; Yanyan et al. 2011; Herridge et al. 2008). Genetic factors associated with BNF depend on characteristics of the host, the strain, and their interaction. Several studies advanced in understanding host genetic mechanisms controlling BNF (Santos et al. 2013; Hwang et al. 2014; Muñoz et al. 2016). These showed that there is substantial variation in %BNF related to bacteria strain (Israel 1981) and host-strain specificity (Senaratne et al. 1987). However, for a given environment, there is limited information on whether maximizing %BNF is actually required to attain maximum N uptake in highest-yielding cultivars.

Reported maximum soybean yield ranges from 6500 to 9200 kg ha^{-1} under potential conditions (Van Roekel et al. 2015). The information regarding the relative contribution of BNF and mineral soil N absorption is scarce for environments yielding higher than 5000 kg ha^{-1} (Salvagiotti et al. 2008). Approximately, 390 kg N ha^{-1} are required to produce 5000 kg of soybean seed ha^{-1}, assuming 12.7 kg seed produced per kg of N uptake. Whether meeting this N demand relies more on BNF or on mineral soil N absorption remains to be determined.

Apparent soil N balance depends on %BNF and N harvest index (NHI) (Johnson et al. 1975; Austin et al. 2006). The last parameter is usually calculated for the whole plant N pool (i.e., Tamagno et al. 2017). However, NHI can be estimated separately for atmospheric and soil N. The idea that NHI could be different depending on the N source has not been explicitly tested.

Total N uptake may also differ among soybean maturity groups (MG) (Mastrodomenico and Purcell 2012). Late MGs reach flowering having higher accumulated N when compared to earlier MGs due to a longer vegetative period (Zeiher et al. 1982). Nitrogen capture during soybean vegetative period depends more on mineral soil N absorption than on BNF (Zapata et al. 1987). This is because nodules are completely developed only after the beginning of flowering. As such, we hypothesize that N uptake of late MG cultivars rely more on mineral soil N absorption than on BNF when compared to early ones. However, direct comparisons of contrasting MGs in terms of %BNF are scarce.

Our study had four specific objectives: (i) to evaluate the role of total N uptake, N use efficiency, and harvest index for explaining seed yield variation across cultivars, (ii) to evaluate the relative importance of BNF versus mineral soil N absorption for explaining total N uptake, (iii) to estimate HI of N derived from atmosphere and from soil, and (iv) to evaluate the relationship between %BNF and apparent soil N balance. These objectives were tested in three different maturity groups.

Materials and methods

Growing conditions and experimental design

Two field experiments were carried out during the 2012/13 growing season at Campo Experimental Villarino, located in Zavalla, Santa Fe, Argentina (33°1′ S, 60°53′ W). One experiment was conducted under rainfed conditions in a field having corn as previous year crop (Exp. 1). The other experiment was irrigated and soybean was the previous year crop (Exp. 2). Soil available N (quantified as N-NO_3^- in the upper 60 cm depth) in mid-September was 29 and 41 kg ha^{-1} for Exp. 1 and 2, respectively. Extractable P (P-Bray) was 27 and 12 mg

kg^{-1} and soil organic matter was 26.9 and 30.2 g kg^{-1}, for Exp. 1 and 2, respectively. Planting date was November 13th for both experiments. Precipitation during the growing season (October to March) was 637 mm, and irrigation was 222 mm in Experiment 2. Both fields had a long history of soybean cultivation and soil type in both experiments was a silty clay loam, Vertic Argiudoll, Roldan serie.

Treatments were arranged in a completely randomized block design with four replications in each experiment. Plots were four rows, 0.52 m apart, and 4 m long. Final plant density was adjusted to 30 plants m^{-2} after manual thinning at V1 (Fehr and Caviness 1977). Weeds were chemically controlled and hand removed whenever necessary. Pests and diseases were controlled by spraying commercially recommended soybean products.

Seventy commercial cultivars belonging to MGs III, IV and V were evaluated (Electronic Supplementary Material 1). These cultivars are fully adapted to the latitude of the experimental site (Baigorri et al. 2002). Seeds were inoculated at recommended rates with RizoLiq LLI® (Rizobacter Company, Argentina) containing *Bradyrhizobium japonicum* (strain E109) and an osmo-protector to sustain the viability of the bacteria after seed pesticide application. Compatible seed insecticide and fungicide Cruiser Advanced® (Syngenta Company, Argentina) was applied at a rate of 1 cm^3 seed kg^{-1}.

Field measurements

At physiological maturity (R7; Fehr and Caviness 1977) a 1.04 m^2 aboveground biomass sample was taken from the two central rows, bagged, and dried at 65 °C to constant weight. Total above ground biomass (kg ha^{-1}) was determined and each sample was then threshed with a stationary thresher. Seeds were weighed for seed yield determination (kg ha^{-1}). Yield was reported on a dry basis. Harvest index was calculated as the ratio between seed yield and total aboveground biomass (Donald and Hamblin 1976). Non-seed vegetative biomass was recovered after threshing from each individual sample. Seed and non-seed vegetative biomass were grounded separately and passed through a 1 mm mesh. Tissue N concentration was determined by Kjeldahl analysis (Mckenzie and Wallace 1953). Aboveground N uptake (kg ha^{-1}) was calculated by adding seed N content (kg ha^{-1}) to the non-seed vegetative biomass N content (kg ha^{-1}). Nitrogen use efficiency for biomass production (kg kg N^{-1}) was calculated as the ratio between total above ground biomass and N uptake both at physiological maturity (Rotundo et al. 2014).

Determination of biological nitrogen fixation

A subset of cultivars were selected for separately quantifying %BNF in seed and non-seed vegetative biomass (%BNF_{seed} and %$BNF_{non\text{-}seed}$, respectively) using the natural ^{15}N abundance method (Peoples et al. 1988; Peoples et al. 1989). See next section for cultivar selection criteria. Five glyphosate-resistant maize plots were planted in each block as a non-fixing reference plant. ^{15}N was determined in a continuous flow isotope ratio mass spectrometer (CF-IRMS) with an Europa 20–20 system. The %BNF was calculated using the following equation (Peoples et al. 1989):

$$\%BNF = \left(100\,\frac{\delta^{15}\text{N maize-}\delta^{15}\text{N soybean}}{\delta^{15}\text{maize-B}}\right) \tag{2}$$

where δ ^{15}N maize and δ ^{15}N soybean are the natural ^{15}N abundance of the reference and soybean plants, respectively. Parameter B was considered −1.032 corresponding to the ^{15}N natural abundance of N in soybean that relies only on BNF (Collino et al. 2015).

Calculations of N related traits

Seed N derived from biological fixation ($kgBNF_{seed}$, kg ha^{-1}) was calculated as:

$$kgBNF_{seed} = N_{seed} \times \left(\%BNF_{seed} \times 100^{-1}\right) \tag{3}$$

where N_{seed} is total seed N (kg ha^{-1}) and %BNF_{seed} is the percentage of N derived from biological fixation in seeds.

Non-seed vegetative N derived from biological fixation ($kgBNF_{non\text{-}seed}$, kg ha^{-1}) was calculated as:

$$kgBNF_{non\text{-}seed} = N_{non\text{-}seed} \times \left(\%BNF_{non\text{-}seed} \times 100^{-1}\right) \tag{4}$$

where $N_{non\text{-}seed}$ is total non-seed vegetative N (kg ha^{-1}) and %$BNF_{non\text{-}seed}$ is the percentage of N derived from biological fixation in non-seeds tissues.

Total amount of aboveground N derived from biological fixation (kgBNF, kg ha^{-1}) was calculated as:

$$\mathrm{kgBNF} = \mathrm{kgBNF}_{\mathrm{seed}} + \mathrm{kgBNF}_{\mathrm{non\text{-}seed}} \tag{5}$$

where $\mathrm{kgBNF}_{\mathrm{seed}}$ is total seed N (kg ha^{-1}) and $\mathrm{kgBNF}_{\mathrm{non\text{-}seed}}$ is total non-seed vegetative N (kg ha^{-1}), both derived from biological fixation.

Percentage of N derived from BNF (%BNF) in aboveground biomass was calculated as:

$$\%\mathrm{BNF} = \left(\mathrm{kgBNF} \times N_{\mathrm{uptake}}^{-1}\right) \times 100 \tag{6}$$

where kgBNF is the total amount of aboveground N derived from biological fixation (kg ha^{-1}) and $\mathrm{N}_{\mathrm{uptake}}$ is total N uptake in aboveground biomass.

Total mineral soil N absorption in aboveground biomass ($\mathrm{N}_{\mathrm{soil}}$, kg ha^{-1}) was calculated as:

$$N_{\mathrm{soil}} = N_{\mathrm{uptake}} - \mathrm{kgBNF} \tag{7}$$

where $\mathrm{N}_{\mathrm{uptake}}$ is total N uptake in aboveground biomass (kg ha^{-1}) and kgBNF is the total amount of aboveground N derived from biological fixation (kg ha^{-1}).

The amount of seed N derived from mineral soil absorption ($\mathrm{SeedN}_{\mathrm{soil}}$, kg ha^{-1}) was calculated as:

$$\mathrm{SeedN}_{\mathrm{soil}} = N_{\mathrm{seed}} - \mathrm{kgBNF}_{\mathrm{seed}} \tag{8}$$

where $\mathrm{N}_{\mathrm{seed}}$ is total seed N (kg ha^{-1}) and $\mathrm{kgBNF}_{\mathrm{seed}}$ is total seed N (kg ha^{-1}) derived from biological fixation.

It is important to underline that the absolute values of soil mineral N absorption and BNF were underestimated since N in roots, nodules, and rhizodeposition were not included in the estimations.

The NHI of the atmospheric source ($\mathrm{NHI}_{\mathrm{BNF}}$) was calculated as:

$$\mathrm{NHI}_{\mathrm{BNF}} = \left(\mathrm{kgBNF}_{\mathrm{seed}} \times \mathrm{kgBNF}^{-1}\right) \times 100 \tag{9}$$

where $\mathrm{kgBNF}_{\mathrm{seed}}$ is total seed N (kg ha^{-1}) and kgBNF is the total amount of aboveground N, both derived from biological fixation (kg ha^{-1}).

The NHI of the mineral soil source ($\mathrm{NHI}_{\mathrm{soil}}$) was calculated as:

$$\mathrm{NHI}_{\mathrm{soil}} = \left(\mathrm{SeedN}_{\mathrm{soil}} \times N_{\mathrm{soil}}^{-1}\right) \times 100 \tag{10}$$

where $\mathrm{SeedN}_{\mathrm{soil}}$ is seed N derived from mineral soil absorption (kg ha^{-1}) and $\mathrm{N}_{\mathrm{soil}}$ is total mineral soil N absorption in aboveground biomass (kg ha^{-1}).

The apparent N balance ($\mathrm{N}_{\mathrm{balance}}$, kg ha^{-1}) was calculated as:

$$\mathrm{N}_{\mathrm{balance}} = \mathrm{kgBNF} - \mathrm{N}_{\mathrm{seed}} \tag{11}$$

where kgBNF is the total amount of aboveground N derived from biological fixation (kg ha^{-1}) and $\mathrm{N}_{\mathrm{seed}}$ is total seed N (kg ha^{-1}; Collino et al. 2015). After that, the %BNF was correlated with the apparent N balance expressed in kg N ha^{-1} to estimate the contribution of BNF to apparent soil N balance.

Statistical analysis

Seed yield, N uptake, NUE, and HI were analyzed with mixed models using the MIXED procedure from SAS software (SAS Institute 2003, Cary, NC). Maturity group and cultivars nested within MGs were considered fixed factors. Experiments and blocks nested within experiments were considered random factors.

A cluster analysis using the package *pvclust* in the R software (R Core Team 2013) was carried out in order to identify cultivars with contrasting seed yield and N uptake. The variable N uptake level (High and Low) was incorporated into the mixed model as derived from the cluster analysis. Therefore, the analysis included the factors N uptake level, MG, N uptake level x MG interaction, and cultivars nested within N uptake level x MG interaction as fixed. Experiments and blocks nested within experiments were considered random factors.

Nitrogen source (atmospheric N or mineral soil N) was included as a new factor when analyzing NHI. Since NHI of atmospheric and mineral soil N was measured on the same experimental unit, they were not independent. To account for this lack of independence we followed the approach described by Holland (2006) and Poeta et al. (2014), and considered N source as a repeated measured factor. The least significant difference was reported at $P \leq 0.05$ for post hoc comparison.

Results

Seed yield, N uptake, NUE, and HI

Days from emergence to physiological maturity were 120, 128, and 137 for MGs III, IV, and V, respectively

($P < 0.05$). Seed yield differences among MGs were detected. However, most of the variation was explained by individual cultivar effects (Table 1). Seed yield across cultivars ranged from 3600 to 6200 kg ha^{-1}. Cultivars accounted for 78% of explained variation. Maximum yields were observed for MG IV.

Nitrogen uptake at physiological maturity across cultivars ranged from 315 to 430 kg N ha^{-1}. Cultivar effects accounted for 90% of the explained variation in total N uptake. Late maturity cultivars (MGs IV and V) had higher N uptake as compared to the early ones (MG III; Table 1). Variation in NUE and HI was explained in similar proportions by both MG and cultivar effects (Table 1). Maturity group V had 10% more NUE and 20% less HI than MGs III and IV. Across cultivars NUE values ranged from 28 to 40 kg kg N^{-1} and HI ranged from 29 to 54%.

There was no relationship between seed yield and days to physiological maturity (Fig. 1a). On the contrary, seed yield was positively associated with total N uptake (Fig. 1b) and HI (Fig. 1d). A negative correlation between NUE and yield was observed (Fig. 1c), but it explained a very low proportion of seed yield variation ($R^2 = 0.16$; Fig. 1c). Nitrogen uptake and HI explained approximately the same proportion of seed yield variation (R^2 ~0.40; Fig. 1b and d). Scattering observed in MG V is related with specific cultivars having very low HI values.

Cultivars were clustered for similar seed yield, N uptake, and NUE resulting in three groups (Clusters A, B, and C; Electronic Supplementary Material 2). Cultivars in cluster "A" had lower seed yield, N uptake and NUE than cultivars in cluster "C", while cluster "B" had intermediate values. Seed yield, N uptake, NUE, HI and the number of cultivars within each MG for these clusters are detailed in Electronic Supplementary Material 3. Fifteen cultivars were selected from clusters "A" and "C" for further determinations and analysis. These cultivars were classified in either high or low N uptake level (Table 2).

Relative importance of atmospheric vs mineral soil N for maximum yields

Seed yield and N uptake were associated with N uptake level, as expected from the cluster analysis ($P < 0.05$)

Table 1 Minimum, maximum, and average seed yield, total N uptake, N use efficiency (NUE), and biomass harvest index (HI) across 70 soybean cultivars evaluated in Experiments 1 and 2 ($n = 8$). Sources of variation are maturity groups (MG) and cultivars nested within MG. Level of significance (*P* value), least significant difference (LSD), and percent sum of squares (% SS) are reported

Maturity Group	Seed yield (kg ha^{-1})		Total N uptake (kg ha^{-1})		NUE (kg kg N^{-1})		HI (%)	
III								
Minimum	4495		315		28		44	
Maximum	5776		403		32		53	
Average	5175		358		30		49	
IV								
Minimum	4517		319		28		38	
Maximum	6197		430		34		54	
Average	5557		378		31		48	
V								
Minimum	3598		345		31		29	
Maximum	6127		430		40		46	
Average	4974		383		35		38	
Source of variation	*P* value (LSD)	%SS	*P* value (LSD)	%SS	*P* value (LSD)	%SS	*P* value (LSD)	%SS
MG	***(157)[†]	22	**(10)	10	***(0.5)	52	***(1)	59
Cultivar(MG)	***(753)	78	***(48)	90	***(2.4)	48	***(4)	41

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

[†] Least significant difference (LSD) for $P < 0.05$

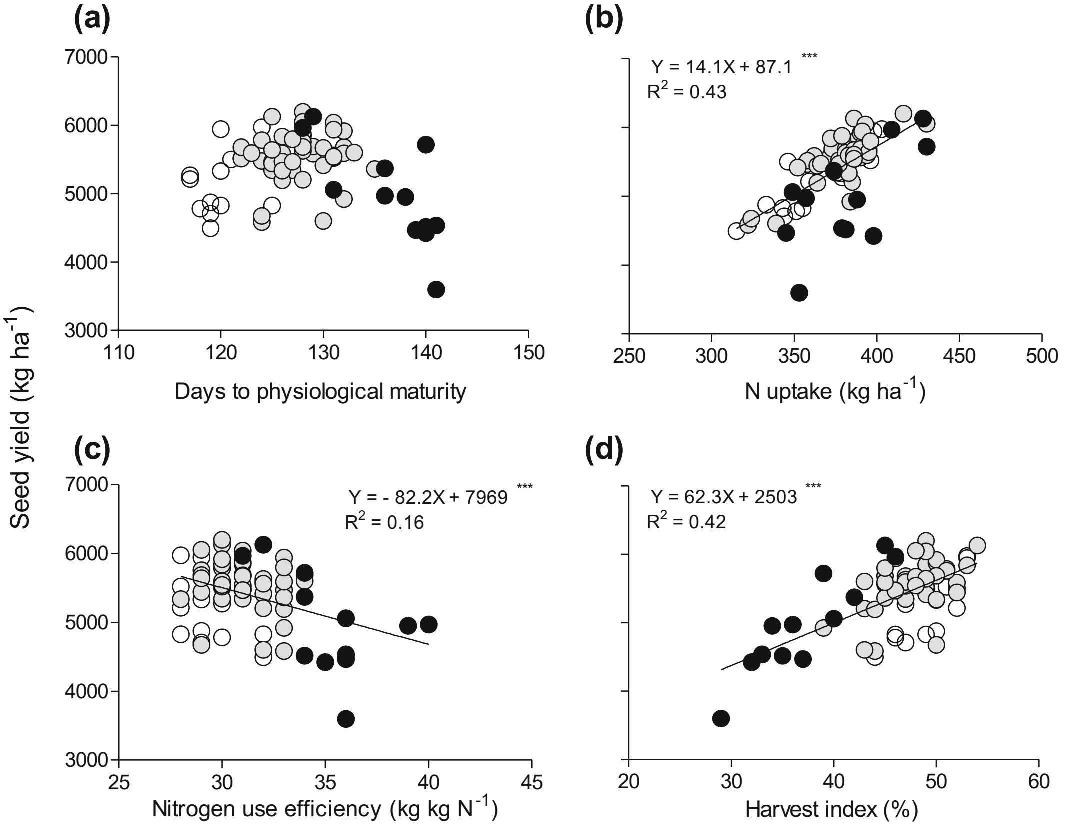

Fig. 1 Relationship between seed yield and (**a**) days to physiological maturity, (**b**) total N uptake, (**c**) N use efficiency, and (**d**) harvest index. White, grey and black circles correspond to MGs III, IV, and V, respectively. The linear model is presented whenever significant ($P < 0.05$). Each point is an average of four replicates from two experiments ($n = 8$). *** means significance at $P < 0.001$

(Table 2). No differences among cultivars within N uptake levels were observed for yield and N uptake. Maturity group was not significantly associated with any of the evaluated variables. Therefore, it was disregarded as a source of variation and removed from the statistical model. High N uptake cultivars yielded ~5800 kg ha^{-1} and low N uptake ~4600 kg ha^{-1}; this represents a 24% relative yield difference. High N uptake cultivars had ~75 kg ha^{-1} more total N uptake than low N uptake cultivars, resulting in a significant ~22% relative difference ($P < 0.05$) (Table 2).

Nitrogen uptake level explained 64% of the variation in kgBNF. Biological N fixation for high N uptake cultivars was 290 kg N ha^{-1}, while it was ~220 kg N ha^{-1} for the low N uptake ones ($P < 0.05$) (Table 2). The effect of cultivars within N uptake levels explained the remaining variation in kgBNF. The relative variation in kgBNF across cultivars was similar within each N uptake level. The difference in kgBNF within high N uptake cultivars SRM4602 and SY3.9 was 30%; within the low N uptake cultivars the difference between SPS3900 and A4423 was 28%.

Mineral soil N absorption (kg N ha^{-1}) was not different between cultivars belonging to the high or low N uptake level (Table 2). The variation in mineral soil N absorption was mostly associated with differences among cultivars within each N uptake level ($P < 0.05$). The relative variation in soil N absorption was ~50 to 40% for the high and low N uptake levels, respectively. The maximum difference was observed between cultivars SY3.9 and LDC3.8 (142 vs. 93 kg N ha^{-1}) for the high N uptake level, and between A4423 and SPS3900 (124 vs. 89 kg N ha^{-1}) for the low N uptake level ($P < 0.05$) (Table 2).

The percent of total N uptake derived from the atmosphere (%BNF) was significantly higher for cultivars belonging to the high N uptake level (71%) compared to cultivars from the low N uptake level (67%) ($P < 0.05$) (Table 2). However, most of the variation in %BNF was

Table 2 Seed yield, total N uptake, biological nitrogen fixation (kgBNF expressed as kg ha^{-1} and %BNF expressed as percentage), and soil N absorption at physiological maturity for selected cultivars classified as having high or low N uptake level. Values are average of four replicates from Experiments 1 and 2 (n = 8). Sources of variation are N uptake level and cultivar nested within N uptake level. Level of significance (*P* value), least significant difference (LSD), and percent sum of squares (% SS) are reported

N uptake level	Cultivar	Seed yield (kg ha^{-1})	Total N uptake (kg ha^{-1})	kgBNF (kg ha^{-1})	%BNF (%)	Soil N absorption (kg ha^{-1})
High	LDC3.8	5946	397	304	75	93
	SY3.9	5522	396	254	64	142
	T2137	5976	403	260	65	143
	FN4.35	5680	395	271	68	125
	NS4009	5596	394	271	70	123
	SRM4370	5642	395	295	75	100
	SRM4602	6052	430	333	77	98
	DM5.1i	5964	428	324	75	104
	SPS4x99	6127	409	297	72	112
	Average	5834	407	290	71	116
Low	ACA3939	4674	324	212	66	112
	SPS3900	4829	343	254	74	89
	A4423	4517	319	197	61	124
	ACA420	4495	315	203	64	112
	SRM4839	4468	345	239	69	105
	RA532	5059	349	236	68	112
	Average	4674	332	224	67	109

Source of variation	*P* value (LSD)	%SS	*P* value (LSD)	%SS	*P* value (LSD)	%SS	*P* value (LSD)	%SS	*P* value (LSD)	%SS
N Uptake Level	***(89)[†]	99	***(19)	88	***(19)	64	**(3)	21	n.s.	3
Cultivar (N Uptake level)	n.s.	1	n.s.	12	*(37)	36	**(6)	79	*(23)	97

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. is not significant

[†] Least significant difference (LSD) for $P < 0.05$

related to cultivar effects within each N uptake level. For example, cultivars within the high N uptake level varied from 77 to 64% (SRM4602 and SY3.9, respectively). Cultivars from the low N uptake level varied from 74 to 61% (SPS3900 and A4423, respectively).

A negative correlation between kgBNF and mineral soil N absorption was observed across cultivars within each N uptake level ($P < 0.05$) (Fig. 2). The slope of the regression was not different when comparing cultivars belonging to the high and low N uptake levels ($P > 0.05$). The slope represented a reduction of 1.4 kg of fixed N per each kg of increasing mineral soil N absorption. The y-intercept was significantly different for the high and low N uptake cultivars, associated with average differences in kgBNF between those groups.

Nitrogen harvest index of atmospheric vs soil N

The partitioning of total N to the seed (NHI) was different depending on whether N was derived from BNF or absorbed from the soil solution (N source effect, $P < 0.05$) (Table 3). Approximately 64% of the total variation in NHI was related to the N source effect. Nitrogen HI was 85 and 77% for N derived from BNF and absorbed from soil solution, respectively. This difference was observed across maturity groups, and cultivars. The effect of N source on NHI was not modified by the N uptake level ($P > 0.05$) (Table 3). However, the effect of N source was strongly dependent on each particular cultivar, as denote by a 20% of variation explained by the N source by cultivar interaction ($P < 0.05$) (Table 3). For two thirds of the cultivars, NHI was higher for N derived from BNF than for N

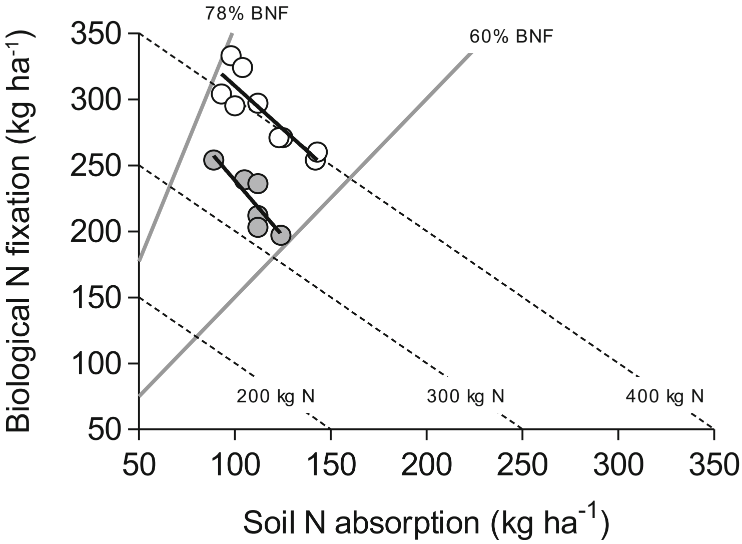

Fig. 2 Relationship between amounts of total aboveground N derived from biological fixation (kg ha^{-1}) and from soil N absorption (kg ha^{-1}). *White* and *grey symbols* represent cultivars belonging to high and low N uptake levels, respectively. Each point is an average of four replicates from two experiments (n = 8). Regression R^2 is 0.77 and 0.73 ($P < 0.05$) for the high and low N uptake cultivars, respectively. The *dotted lines* represent different isolines of total aboveground N uptake (kg ha^{-1}). The *solid lines* represent the maximum and minimum percentage of biological N fixation (%BNF)

derived from soil. For the remaining cultivars the effect of N source on NHI was not significant. Maturity group had a significant interaction with N source ($P < 0.05$) (Table 3). For MGs IV and V, NHI was higher for N derived from atmosphere compared to N derived from the mineral soil solution. The effect of N source on NHI was not observed for cultivars belonging to MG III.

Contribution of BNF to apparent soil N balance

There was a positive correlation between %BNF and the apparent soil N balance ($R^2 = 0.66$, $P < 0.05$) (Fig. 3). The parameters of the linear model were not different for cultivars in the high or low N uptake levels. The common slope for both N uptake levels was ~3 kg N ha^{-1}. This indicates that soil N balance is 3 kg N ha^{-1} less negative for each individual increase in %BNF. Approximately 80% BNF was required to attain a neutral soil N balance.

Discussion

Nitrogen uptake is a major driver of soybean seed yield. In general, N uptake has been a better seed yield predictor than biomass accumulation (Pazdernik et al. 1997; Rotundo et al. 2014). This is related with several physiological processes associated with N at the plant and canopy levels, like leaf area formation, light interception, carbon assimilation, and reserves accumulation (Sinclair and Jamieson 2008). Our results also showed that N uptake was positively associated with soybean seed yield. Ultimately, total N uptake depends on the amount of N that is biologically fixed and/or absorbed from the soil solution.

At a global scale BNF accounts for, on average, 60% of total N uptake (Salvagiotti et al. 2008). In general, most studies evaluated %BNF in situations where total N uptake was below 400 kg N ha^{-1}. For example, Salvagiotti et al. (2008) compiled published data on %BNF and had only 2% of data points above 400 kg N ha^{-1}. Collino et al. (2015) surveyed 80 production fields in Argentina and had 8% of fields above 400 kg ha^{-1} of N uptake only. Our report provides data on %BNF from nine high-yielding cultivars averaging 407 kg ha^{-1} of N uptake. Even though these high-yielding cultivars had a slightly higher %BNF compared to the low yielding ones (71 vs. 67%, respectively), there was substantial overlapping across them. This indicates that a high %BNF is not an absolute requirement for maximizing N uptake and seed yield. We observed an important intraspecific variation in high-yielding soybeans that allowed 400 kg ha^{-1} of N uptake were attained by %BNF ranging from 60 to 78%.

Table 3 Nitrogen harvest index of N derived from atmosphere or from soil solution for selected cultivars classified as having high or low N uptake level. Values are average of four replicates from Experiments 1 and 2 (n = 8). Sources of variation are nitrogen source (NS), N uptake level, maturity group (MG), and cultivar nested with N uptake level and MG interaction. Different letters in the same row indicate significant differences between N sources at $P < 0.05$. Level of significance (*P* value), least significant difference (LSD), and percent sum of squares (% SS) are reported

N uptake level	Maturity Group	Cultivar	N harvest index (%)	
			Atmospheric N	Soil N
High	III	LDC3.8	89.1a	81.1b
		SY3.9	84.1a	86.6a
		T2137	86.3a	88.3a
	IV	FN4.35	86.1a	72.3b
		NS4009	89.2a	72.2b
		SRM4370	87.6a	79.4b
		SRM4602	86.2a	73.5b
	V	DM5.1i	83.5a	72.7b
		SPS4x99	83.8a	76.3b
Low	III	ACA3939	86.4a	82.9a
		SPS3900	86.3a	76.9b
	IV	A4423	84.6a	74.9b
		ACA420	84.9a	78.9a
	V	SRM4839	81.0a	64.5b
		RA532	81.1a	80.6a
Maturity group	III		86.5a	82.6a
	IV		86.0a	75.3b
	V		82.4a	73.6b
	Average		85.4a	77.4b
Source of variation			*P* value (LSD)	% SS
Nitrogen source			***(1.7) †	63.7
NS*N uptake level			n.s.	0.1
NS*MG			**(4.1)	9.4
NS*MG*N uptake level			*(5.7)	6.1
NS*Cultivar (N uptake level * MG)			**(6.4)	20.8

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. is not significant

† Least significant difference (LSD) for $P < 0.05$

Combining our data with Salvagiotti et al. (2008) shows that this range in %BNF for attaining ~400 kg ha^{-1} of N uptake can be even wider, it ranged from 40 to 80% in non-fertilized trials (Fig. 4). Our results indicate that there is no intrinsic benefit in promoting %BNF for maximizing yield. The interchangeability between N derived from BNF or from the soil solution is evidenced as a negative correlation between these processes.

There are different mechanisms that may explain the observed negative correlation between kgBNF and mineral soil N absorption across cultivars exploring similar soil N availability and environment. Negative correlations between physiological processes can arise from genetic effects and/or from physiological constraints (Stearns 1989; Weih 2003). The genetic basis of BNF has been recently evaluated using recombinant populations (Santos et al. 2013; Hwang et al. 2014; Muñoz et al. 2016). However, no attempt has been made to explore the co-dependence of BNF and mineral soil N absorption. On the other hand, physiological constraints

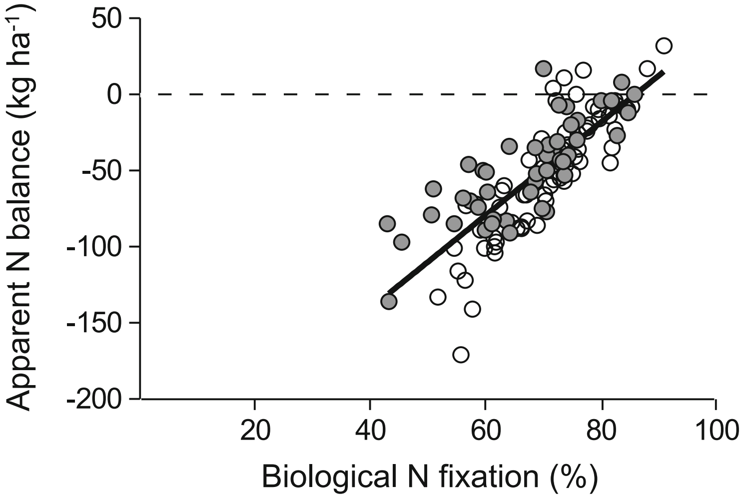

Fig. 3 Relationship between apparent soil N balance (kg N ha^{-1}) and percentage of biological N fixation (%BNF). *White* and *grey symbols* represent cultivars belonging to high and low N uptake levels, respectively

between these two processes may occur at different levels. Even though these cultivars were tested in the same environment with the same initial soil NO_3^- concentration, cultivars may differ in potential soil depth exploration (Voisin et al. 2007). This may determine differences in the actual soil nitrate (NO_3^-) concentration that is available for the cultivars. Cultivars exploring more soil volume may have less local depletion in soil NO_3^- and therefore could have reduced BNF.

The slope of the negative correlation between kgBNF and mineral soil N absorption for both the high and low N uptake cultivars was −1.4 kg N ha^{-1} (Fig. 2). This implies that for each increasing unit of N absorbed from the soil, there is a reduction in 1.4 units in kgBNF. This observation, together with the lack of genotypic variation in mineral soil N absorption, suggests that there are more chances of increasing total N uptake via increasing kgBNF than through increasing the absorption from the soil solution. Maximizing kgBNF requires adjusting management practices aimed at increasing soybean yield potential. Soybean crops having optimum planting dates, adapted maturity group, optimum sowing density,

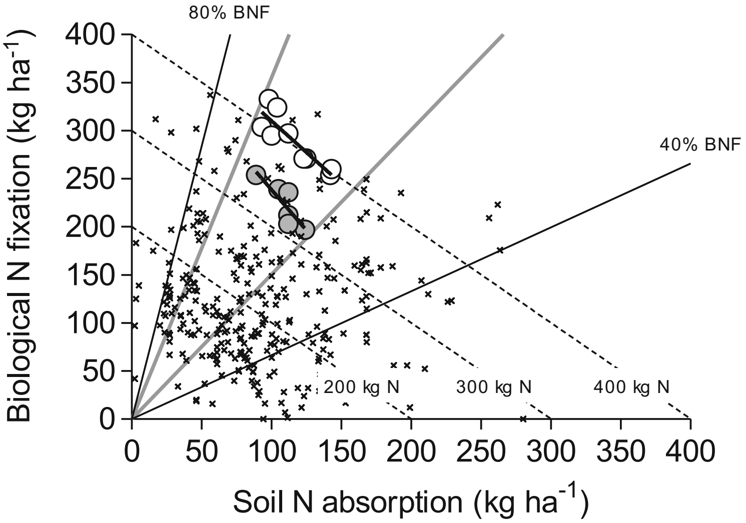

Fig. 4 Relationship between amounts of total aboveground N derived from biological fixation (kg ha^{-1}) and from soil N absorption (kg ha^{-1}). *White* and *grey symbols* represent cultivars belonging to high and low N uptake levels, respectively. Each point is an average of four replicates from two experiments (*n* = 8). Data extracted from Salvagiotti et al. (2008) are depicted with *x* symbol. The *dotted lines* represent different isolines of total aboveground N uptake (kg ha^{-1}). The *solid lines* represent the different percentages of biological N fixation (%BNF)

pest protection, and high-yielding cultivars secure a rise in the total amount of kgBNF (Voisin et al. 2007; Yanyan et al. 2011). Also, at the plant scale, kgBNF may be optimized by modifying the inducible daily rhythm in nodule activity (Cabeza et al. 2015). Daily cycles of downregulation have been observed in situations of no N-limitation (Cabeza et al. 2015). Therefore, uncoupling this downregulation phenomenon could result in a reduced trade-off between biological N fixation and mineral N soil absorption.

There was no association between MG and seed yield within the high N uptake level subset of cultivars. This is in concordance with Santachiara et al. (2017), showing that contrasting MGs can attain similar seed yields despite differences in total resource capture and use. We expected longer MGs having a reduced %BNF compared to shorter ones. However, no significant differences between MGs were observed for %BNF. Most of the variation in %BNF was accounted for cultivar effects regardless the specific MG.

Our results showed a positive correlation between %BNF and apparent soil N balance. For the total N uptake and NHI explored in our experiments, 80% BNF was required to attain an apparent soil N balance close to neutral. This is so even though we demonstrated that the NHI of the atmospheric source is higher than the mineral source (i.e., it is more likely exported to the seed). This last result is consistent with findings that showed symbiotically fixed N was more mobile during seed filling than N coming from the soil (Warembourg and Fernandez 1985). These authors suggested that a large fraction of contemporary BNF during the seed-filling period is integrated into a special pool of single storage molecules in petioles and stems which may preferentially supplies developing seeds. In general, even when remobilization from vegetative tissues (originated from both, BNF or mineral N, mainly before the seed filling period) is the most predominant source of N supply to the seed, higher seed yield has been observed in cultivars sustaining BNF until maturity (Abu-shakra et al. 1978).

Conclusions

Total canopy N uptake was positively correlated with seed yield, explaining yield differences across cultivars. Highest seed yield and N uptake were not necessarily associated with maximizing %BNF. High N uptake is a requisite for high yielding cultivar, but this N can be reached through different combinations of BNF and soil mineral uptake. The explored range of %BNF varied from 60 to 78%. The amount of N fixed (kgBNF) was negatively correlated with mineral soil N absorption. Any extra kg ha^{-1} of absorbed N from the soil solution implied a reduction in 1.4 kg ha^{-1} of N derived from BNF.

No significant differences between MGs within N uptake levels were detected in terms of %BNF. Biologically fixed N was allocated more preferentially to seeds in MGs IV and V. Even though N from BNF was more partitioned to the exported N pool, an increase in %BNF was positively associated with a less negative apparent N balance of the soil agroecosystem. Soil N neutral balance was attained at 80% biological N fixation.

Acknowledgements This research was funded by Agencia Nacional de Promoción Científica y Tecnológica, Argentina (PICT2011-1292). Authors want to thank Florencia Buldain for laboratory support.

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