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Assessing Nitrogen Limitation in Inoculated Soybean in Southern Brazil

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Core Ideas

- Soybean yield did not respond to low starter fertilizer N rates in soils with high organic matter content.
- Nitrogen limitation tended to be greater in low compared with medium-high yield levels.
- Nitrogen limitation is potentially related to lower contribution of N coming from biological nitrogen fixation (BNF) and mineralization.

ABSTRACT

Overcoming potential N limitation in soybean [*Glycine max* (L.) Merr.] is a critical factor for sustaining plant nutrient demand and improving productivity. Following this rationale, a set of studies were executed in southern Brazil with the goals of quantifying yield response to early season fertilizer N rates (up to 40 kg ha^{-1}), "starter N fertilization," and to understand if soybean seed yields are limited by N (testing a non-limiting N scenario) when grown in soils with medium to high organic matter content. The main key outcomes of this research were: (i) starter N fertilization did not increase yields compared with non-fertilized soybean, potentially highlighting the absence of an early season N limitation; and (ii) N limitation was observed when soybean yields were compared with non-limiting N scenario and it tended to be greater in low compared with medium-high yield levels, potentially connected with co-limitations on both N sources (N_2 fixation and mineralization) to satisfy soybean N demand. Producing soybean in a sustainable manner implies focus on production practices to conserve and, potentially, to increase soil organic matter on a long-term basis. Furthermore, it requires enhancing the biological N_2 fixation process for satisfying the large plant N demand for achieving high soybean yields. Future research should be focused on understanding factors governing biological N_2 fixation and N mineralization processes in soybean grown in soils with medium-high organic matter content.

Abbreviations: BNF, biological nitrogen fixation; DW, dry weight; Ndfa, nitrogen derived from the air; SOM, soil organic matter.

Soybean [*Glycine max* (L.) Merr.] is one of the most globally relevant field crop legume with a production of 340 million Mg in 2017–2018 (USDA, 2019). Brazil accounts for 35% (120 million Mg in 2017–2018) of the global soybean production, being the largest producer alongside the United States (USDA, 2019). As a source of protein and oil for humans and animals, soybean is a critical element for food security challenges. Increasing soybean seed yield instead of expanding hectarage is a key to attend global food demands (Fischer and Connor, 2018). Therefore, strategies to improve crop productivity at the farmer level should be further explored. Soybean yield potential is attained when a well-adapted variety is grown under ideal conditions, without water and nutrient limitation, and in absence of abiotic (light, salinity, heat, drought) or biotic (diseases, insects, weeds) stresses (Evans, 1993).

Nitrogen is one of the most important nutrients for soybean, primarily acquired via two sources: biological nitrogen fixation (BNF) and mineral N derived from soil organic matter (SOM) mineralization. As the C requirements for mineral N assimilation (4 kg C kg^{-1} N) is lower than BNF (6–7 kg C kg^{-1} N) (Kaschuk et al., 2009), higher amounts of mineral N provided to soybean decrease the BNF contribution (Dadson and Acquaah, 1984). Then, reducing a plausible yield-limitation caused by N in high-yield environments (>6–7 Mg ha^{-1}) (Salvagiotti et al., 2009) is a challenge, because increasing BNF might rise the energetic cost and could potentially penalize seed yield (Tamagno et al., 2018). On the other hand, applying N via fertilization might reduce soybean root nodulation and BNF process (Streeter and Wong, 1988; Kanayama et al., 1990).

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Table 1. Geographical coordinates and characterization of 0- to 20-cm soil layer from field trials conducted in 2015–2016 and 2016–2017 growing seasons in southern Brazil.†

Sites	Lat.	Long.	Mn	S	P	K	Ca	Mg	Al	H + Al	CECpH 7.0	Clay	SOM	V	pH H_2O
			mg dm^{-3}				$cmol_c$ dm^{-3}					g kg^{-1}		%	
					2015–2016 Growing season										
C. Simão 1	25°03′57.83″ S	51°50′01.98″ W	32	85	1.5	47	4.5	3.8	0.0	4.4	12.8	470	41	66	5.6
Taguá 1	25°34′26.97″ S	51°37′00.40″ W	6	21	5.3	66	5.1	2.8	0.1	3.9	11.9	400	51	68	5.4
Pinhão 1	25°43′10.94″ S	51°39′30.23″ W	3	10	25.0	60	7.1	4.4	0.0	2.8	14.4	340	47	81	6.2
Candói 1	25°36′22.39″ S	51°59′12.58″ W	4	16	8.5	97	6.3	2.6	0.0	4.9	14.0	280	60	65	5.5
Guarapuava 1	25°32′51.07″ S	51°29′50.83″ W	4	12	6.5	194	6.6	2.8	0.0	3.1	13.0	340	46	76	5.8
					2016–2017 Growing season										
C. Simão 2	25°03′47.64″ S	51°50′07.55″ W	20	31	2.6	114	6.1	4.2	0.0	5.5	16.1	470	47	66	5.6
Taguá 2	25°34′17.16″ S	51°37′05.82″ W	7	13	2.1	98	7.1	4.1	0.0	5.5	16.9	400	53	68	5.7
Pinhão 2	25°39′59.69″ S	51°42′51.09″ W	4	16	4.0	288	8.3	5.3	0.0	4.9	19.2	340	52	75	5.8
Candói 2	25°26′38.70″ S	51°54′32.81″ W	3	13	8.4	147	8.1	3.6	0.0	5.5	17.5	280	57	69	5.7
Guarapuava 2	25°32′43.68″ S	51°30′01.18″ W	9	15	7.3	231	6.5	2.8	0.1	8.7	18.6	340	50	53	5.4

† CEC, cation exchange capacity; SOM, soil organic matter; V, base saturation.

There is no consensus about N limitation in soybean and its relationship under different yield levels. For instance, Ray et al. (2006) found lack of yield response to the addition of external N at varying yield levels, whereas Cafaro La Menza et al. (2017) and Ortez et al. (2018) observed a response (but not consistent for the latter authors) to N fertilization as soybean yield improved. Lack of consistency on soybean yield response to the addition of external N is clear from the recent investigations mainly linked to the absence of characterization of soil N supply and N_2 fixation pools. Then, these N pools are critical not only to complement future research on this topic, but to better understand the factors affecting a potential yield response and exposing soybean to N limitations.

Connecting to N limitations, many studies investigated adding lower amounts of N fertilizer to soybean early in the season commonly known as starter N fertilization (usually up to 40 kg N ha^{-1}). The rationale behind this practice is to supply low amounts of N early in the season when nodules are not completely formed and N_2 fixation is not active (Abendroth et al., 2006) as well as when N derived from mineralization can be scarce under low temperatures and/or low levels of SOM (Dadson and Acquaah, 1984). Results of those studies are contradictory, showing either yield increases (e.g., Osborne and Riedell, 2006; Boroomandan et al., 2009; Gai et al., 2017) or lack of yield response to N (e.g., Hungria et al., 2006; Mrkovački et al., 2008; Josipović et al., 2011; Kamara et al., 2012; Balbinot Junior et al., 2016).

This study aimed to quantify soybean yield response to external N addition. The first objective was to evaluate the impact of low fertilizer N rates (up to 40 kg N ha^{-1}) as starter N fertilization on soybean seed yield. The second goal of this study was to understand, by providing full-N (non-limiting N scenario) to the crop, if seed yields are limited by N even when soybean plants are grown in soils with medium to high (from 41 to 60 g kg^{-1}) organic matter content.

MATERIALS AND METHODS

Field Trials

Ten field trials were performed at five locations (Campina do Simão, Taguá, Pinhão, Candói, and Guarapuava) in the Center-South region of Paraná State, Brazil, during the 2015–2016 and 2016–2017 cropping seasons (Table 1). All trials were conducted under the no-till system, varying in the time of no-till adoption ranging from short-term (2 yr) at Campina do Simão site to long-term sites at Taguá and Pinhão (>10 yr) and Candói and Guarapuava (30 yr). All sites, with the exception of Campina do Simão, have a long history of soybean production (>15 yr). The regional climate is Cfb (humid temperate climate with moderately hot summer), according to the Köppen classification, without dry season (Aparecido et al., 2016). Based on 25 yr of weather data (from 1989 to 2014), annual precipitation ranges from 1550 to 1800 mm, with the occurrence of weekly rainfall during spring–summer, and annual mean temperature ranges from 16.5 to 18.5°C (Aparecido et al., 2016). Precipitation and temperature data for each site-year is shown in Fig. 1. Soils of the trials were classified as Rhodic Hapludox (Soil Survey Staff, 2014). Across the years, the trials were conducted on the same farm at each site, but different locations within the farm; thus, each site-year was considered as independent sites.

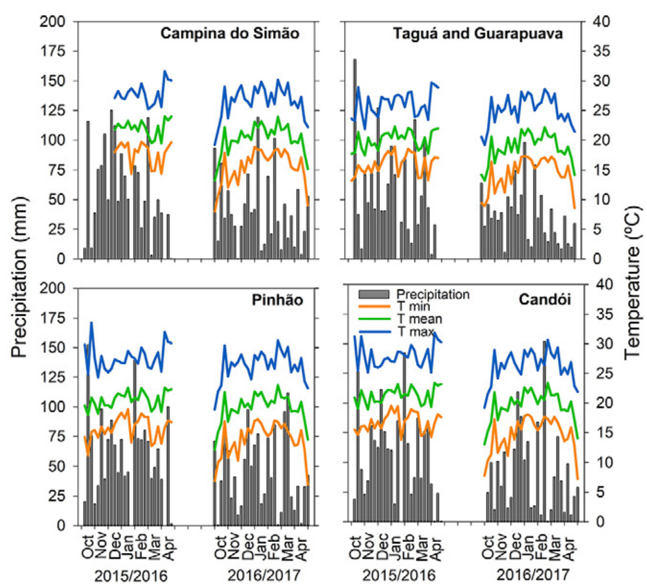

Fig. 1. Precipitation and temperature (minimum, mean, and maximum) of all the sites in 2015–2016 and 2016–2017 growing seasons.

Six treatments were evaluated: a control (without N fertilization), four starter N rates (10, 20, 30, and 40 kg N ha^{-1}) applied as urea (46% N) at sowing, and full-N fertilization (300 kg N ha^{-1} applied as urea in split application: 50% at sowing and 50% at R1 growth stage). The band fertilization method was used to apply urea at sowing, whereas the broadcast fertilization method was adopted at the R1 growth stage (Fehr and Caviness, 1977). Experiments were performed in a completely randomized block design with three or four repetitions. Plots consisted of eight planting lines spaced 40 cm apart and 5 m long.

For all trials, soybean 'BMX Apolo RR' (Don Mario 5.8i), indeterminant growth habit, was sown at 30 plants m^{-2}. Liquid inoculant containing *Bradyrhizobium elkanii* (SEMIA 5019) + *B. japonicum* (SEMIA 5079) strains was applied at a rate of 100 mL per 50 kg seeds, except for the full-N treatment. Seeds received fungicide and insecticide treatment before the sowing, which occurred between the end of October and mid-November. Soil samples were collected at sowing and their characterization (0- to 20-cm layer) is in Table 1. For all treatments, fertilization was managed as 250 kg ha^{-1} of 0–25–25 (N–P_2O_5–K_2O). Phytosanitary treatments were applied according to regional recommendations.

In the zero-N and starter N treatments, five plants per plot were collected at flowering (R1, Fehr and Caviness, 1977) growth stage and separated into the root, shoot, and nodules. Samples were dried at 65°C until constant weight had been reached. In the first cropping season (2015–2016), nodule number and dry weight (DW) were analyzed for the entire root, whereas in the second cropping season (2016–2017) nodule number and DW were obtained only from the crown root to facilitate the measurements. Nodule number and weight from the whole root in the second cropping season were estimated based on the data collected from crown root according to equations fitted by Cardoso et al. (2009). Nitrogen concentration in the shoot was determined by the Thermo Fisher Scientific CN Analyzer (Flash 200 model), and N content in the shoot was calculated by considering the shoot DW. At harvesting (from end of March to mid-April), seed yield was determined and expressed as 130 g kg^{-1} moisture content.

Statistical Analysis

Based on the objectives, data of each trial was divided into two data sets. For starter N evaluation, the control treatment (zero-N) and the starter N rates treatments were analyzed. For N limitation study, zero-N and full-N were used. Data for both tests (starter N and N limitation) were submitted to analysis of variance. For starter N, blocks within site and the interaction between site and treatment were considered as random factors. Means were compared with Tukey HSD using the lsmeans function (lsmeans R package; Lenth, 2016) at the 0.05 confidence level. For the N limitation test, a linear regression model was fitted between full-N and zero-N. In addition, the dataset was divided into terciles categorizing the sites in three yield levels according to the mean yield per site. Low (<5000 kg ha^{-1}), medium (5000–6000 kg ha^{-1}), and high (>6000 kg ha^{-1}) yield levels included three (Campina do Simão 1 and 2, and Pinhão 1), four (Taguá 1 and 2, Candói 1, and Guarapuava 1), and three (Pinhão 2, Candói 2, and Guarapuava 2) sites, respectively. Complementing the linear regression, the proportion of yield difference for full-N relative to zero-N was calculated for each yield level.

Table 2. Seed yield and its components, root nodulation, N content, shoot and root growth, and shoot/root ratio at R1 stage of soybean fertilized with starter N in 10 field trials conducted in 2015–2016 and 2016–2017 growing seasons, in southern Brazil.

Treatment	Seed			Nodule		Shoot		Root	Shoot/root
	Yield	No.	Dry wt.	No.	Dry wt.	N content	Dry wt.	Dry wt.	ratio
	kg ha^{-1}	seeds m^{-2}	mg seed^{-1}	nodules plant^{-1}	mg plant^{-1}	kg ha^{-1}			
C. Simão 1	3421e†	1842h	186b	55b	151de	53d	1200d	270d	4.8fg
C. Simão 2	4004d	2320g	171d	23d	120e	70cd	1800c	360cd	5.3efg
Taguá 1	5454b	2939d	186b	81a	206bc	127a	2520ab	420bc	6.2cde
Taguá 2	5482b	3106b	176cd	39c	233b	109ab	3030a	540a	5.8def
Pinhão 1	4853c	2659f	183b	90a	280a	99abc	2220bc	330cd	6.8bc
Pinhão 2	5921a	3201a	185b	44bc	179cd	76bcd	2070bc	450ab	4.7g
Candói 1	5491b	3038c	181bc	79a	227b	101abc	2280bc	300d	8.0a
Candói 2	6044a	3295a	184b	31cd	115e	109ab	2610ab	360cd	7.5ab
Guarapuava 1	5308b	2876e	185b	88a	239ab	117a	2520ab	390bc	6.4cd
Guarapuava 2	6137a	3182a	193a	30cd	140de	102abc	3090a	450ab	6.8bc
0 kg N ha^{-1}	5206	2846	181	57	202	93	2250	360	6.3
10 kg N ha^{-1}	5197	2822	184	53	188	86	2100	360	6.2
20 kg N ha^{-1}	5195	2821	184	56	191	98	2370	390	6.2
30 kg N ha^{-1}	5269	2876	183	58	191	104	2550	420	6.2
40 kg N ha^{-1}	5192	2863	182	56	173	101	2400	390	6.2
Site (S)	***	***	***	***	***	***	***	***	***
N rate (N)	ns‡	ns	ns	ns	ns	ns	ns	ns	ns
S × N	ns	**	ns	*	ns	ns	ns	ns	*

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

† Means with different letters within columns differ by the Tukey's test at $p \leq 0.05$.

‡ ns, not significant.

Regression models were fitted between average yield (zero-N treatment) in each site and N derived from the air (Ndfa) and SOM aiming to understand if yield variations were related to the BNF and/or mineral N derived from SOM mineralization. The Ndfa measurements were obtained at soybean R5 growth stage through by the ^{15}N natural abundance method (Shearer and Kohl, 1986) from trials conducted in the same sites during the 2017–2018 season (Ambrosini, 2019). As soil type and weather characteristics were similar between years, and minor variation in Ndfa within a site across years is reported in the literature (Alves et al., 2006), we consider that the Ndfa values can provide useful information on this analysis.

RESULTS

Starter Nitrogen Fertilization

Regardless of potential trends in several factors, fertilizer N rates (10–40 kg N ha^{-1}) applied at sowing did not influence any of the variables analyzed ($p > 0.05$; Table 2) relative to the control (no N added). On average, soybean seed yield ranged from 3421 to 6137 kg ha^{-1} among all 10 sites. Guarapuava 2, Candói 2, and Pinhão 2 sites had significantly higher seed yield and seed number than other sites (Table 2). Overall, Guarapuava 2 also had greater seed DW and shoot DW compared with the other sites (Table 2).

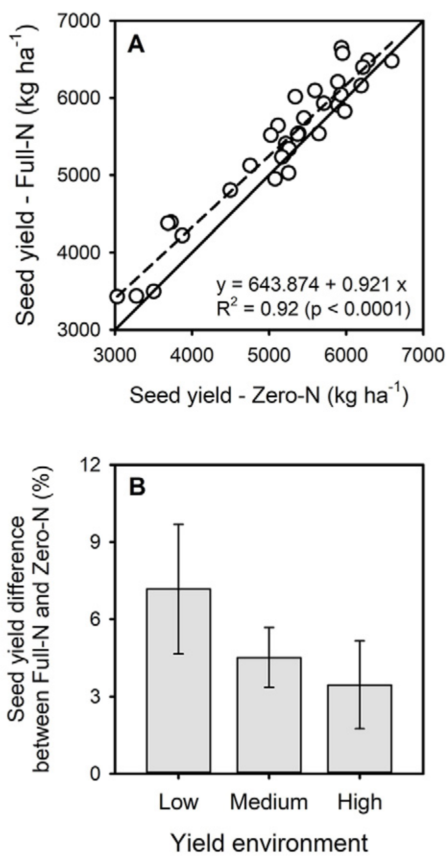

Fig. 2. Seed yield in full-N vs. zero-N (A), and percentage of seed yield difference between both treatments for low (<5000 kg ha^{-1}), medium (5000–6000 kg ha^{-1}), and high (>6000 kg ha^{-1}) yield environments (B). In (A) each data point represent a repetition of both treatments in all sites, and the diagonal solid line is a 1:1 line. In (B) each bar represents the average of all sites for each yield environment. The absence of letter means that there was no significant difference by ANOVA ($p < 0.05$) and vertical lines are the standard error.

All the plant variables were generally lower in Campina do Simão 1 and 2 (Table 2).

Interaction effects (site × N rate) were observed for seed number, nodule number, and shoot/root ratio (Table 2). As the interaction effects did not present a pattern and were agronomically irrelevant, they are shown only in the text, but not discussed. For instance, the application of 40 kg N ha^{-1} provided the lowest seed number in Campina do Simão 2 (2193 seeds m^{-2}) and the highest in Candói 2 (3644 seeds m^{-2}), whereas no N effect was observed on the other sites. For nodule number, the interaction effects were observed only in Candói 1 and Guarapuava 1. In Candói 1, the highest (95 nodules plant^{-1}) and the lowest (63 nodules plant^{-1}) number of nodules were obtained by applying 20 and 0 kg N ha^{-1}, respectively. In Guarapuava 1, the highest (97 nodules plant^{-1}) and the lowest (71 nodules plant^{-1}) values were observed by applying 30 and 10 kg N ha^{-1}, respectively. For shoot/root ratio, effects of interaction were observed only in Candói 2, where the highest ratio (9.2) was observed without N application.

Nitrogen Limitation

Full-N treatment increased seed yield by 236 kg ha^{-1} (from 5183 to 5419 kg ha^{-1}) related to zero-N ($p < 0.0001$), which represents a yield increase by 4.6% across sites (Fig. 2). Soybean yield for full-N vs. zero-N relationship presented similar slopes ($p = 0.12$) at varying yield levels (Fig. 2A), but differing only on the intercepts ($p = 0.012$) of the adjusted model. When yields were evaluated in levels (low, medium, and high), a trend was observed for greater yield under full-N relative to the zero-N (Fig. 2B), with a larger separation on yield under low yield levels (7.2% yield difference for full-N vs. zero-N). Yield components were not affected by full-N fertilization in anyone of the yield environments ($p > 0.05$; Table 3).

Soybean seed yield in zero-N treatment averaged 4068, 5384, and 6110 kg ha^{-1} in low, medium, and high yield levels, respectively. High yield levels were achieved by a combination of both high N_2 fixation and a greater N contribution derived from N mineralization (Fig. 3). For N_2 fixation, after 65% of N contribution relative to the total plant N demand, yields tend to plateau, potentially emphasizing that N demand is not limited by this factor beyond that point (Fig. 3A). Above 5000 kg ha^{-1} for soybean seed yield, the contribution of N derived from the mineralization process seems to be a larger component of increasing yields and sustaining plant N demand (Fig. 3B).

In summary, starter N fertilization with small fertilizer N rates was not an useful practice aiming to increase soybean yields, potentially highlighting the absence of N limitation early in the crop growing season. For the full-N study, N limitation tended

Table 3. Seed dry weight and number in full-N vs. zero-N for low, medium, and high yield environment.

Yield environment	N rate	Seed no.	Seed dry wt.
		seeds m^{-2}	mg seed^{-1}
Low	zero-N	2288 ns†	178 ns
(<5000 kg ha^{-1})	full-N	2394	182
Medium	zero-N	2973 ns	181 ns
(5000–6000 kg ha^{-1})	full-N	3097	182
High	zero-N	3275 ns	187 ns
(>6000 kg ha^{-1})	full-N	3370	187

† Not significant by ANOVA ($p < 0.05$).

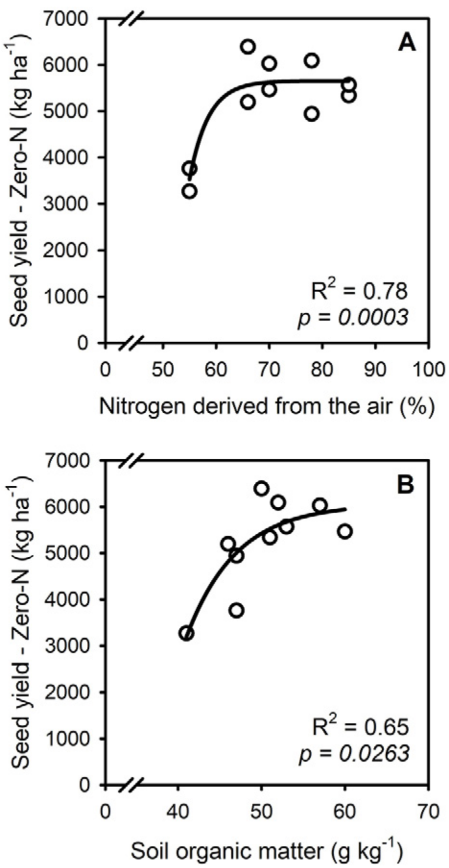

Fig. 3. Relationship between seed yield in zero-N and N derived from the air (A) and soil organic matter (B). In both figures, each data point represents the average of every single site.

to be greater in low compared with medium-high yield levels, potentially connected with co-limitations on N demand coming from both N_2 fixation and N mineralization processes.

DISCUSSION

Starter Nitrogen Fertilization

Soybean yield response to small N amounts should be expected only in N-deficient soils (Dadson and Acquaah, 1984), which is not the case of soils from the Center-South region of Paraná, southern Brazil with high SOM (SOM > 41 g kg^{-1}; Table 1) (Fontoura and Bayer, 2009). Lack of yield response to starter N is consistent with previous studies in Brazil (Jendiroba and Câmara, 1994; Mendes et al., 2003; Hungria et al., 2006; Aratani et al., 2008; Balbinot Junior et al., 2016) and around the world (Herridge and Brockwell, 1988; Mrkovački et al., 2008; Josipović et al., 2011; Kamara et al., 2012; Janagard and Ebadi-Segherloo, 2016) with diverse soil and weather conditions.

Nitrogen Limitation

Interestingly, soybean yield response to full-N fertilization tended to be greater in low than medium to high yield environments (Fig. 2A, 2B), whereas previous studies reported greater differences in yield environments above 4500 kg ha^{-1} (Salvagiotti et al., 2008, 2009). However, great N demand to sustain high seed yield is not the only issue driving N limitations in soybean. For instance, our results showed that potential problems related to N supply via BNF (Ray et al., 2006) and/or soil mineral N availability (Dadson and Acquaah, 1984; Schipanski et al., 2010) are even more relevant and were not taking into consideration in previous investigations (Cafaro La Menza et al., 2017; Ortez et al., 2018).

As for the soybean yield limitations, the Center-South region of Paraná, southern Brazil, usually does not have problems with water deficit and/or heat stresses (Fig. 1). Therefore, the main challenge for low-yielding soybean producers is adopting conservation management practices to increase SOM, such as no-till and crop rotation (Diekow et al., 2005; Bayer et al., 2009), providing adequate conditions for the BNF process (Divito and Sadras, 2014; Ferguson and Gresshoff, 2016). For high-yielding soybean producers, future improvements on BNF needs further consideration on seed yield formation and seed composition (Tamagno et al., 2018), but the alternative of supplementing the crop with N fertilization will produce an impairment on the N_2 fixation process (Streeter and Wong, 1988; Kanayama et al., 1990).

Feeding the growing world population is one of the greatest challenges for the next decades. Then, increasing crop productivity per unit area instead opening new arable lands is one of the agricultural main challenges for the near future (Fischer and Connor, 2018). However, applying high N amounts to attain the maximum yield potential is not environmentally profitable, hence, finding ways to increase BNF should be the main thought of soybean researchers in the future. Then, it is not our intention recommending N fertilization to farmers.

CONCLUSION

The main key outcomes of this research were: (i) starter N fertilization did not increase yields, potentially highlighting the absence of an early season N limitation; and (ii) N limitation tended to be greater in low compared with medium-high yield levels, potentially connected with co-limitations on both N sources (N_2 fixation and mineralization) to maintain soybean N demand. Producing soybean in a sustainable manner will imply focusing on production practices to conserve and, potentially, to increase in a long-term basis SOM and promote enhancing the BNF process for maintaining the large N demand required to achieve superior soybean yields. Future investigations should be focusing on obtaining a more complete characterization of soil, weather, and plant-related traits critical to improve the understanding of both N mineralization and N_2 fixation processes.

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