

## Original Article

## Soybean crops with short duration are prone to nitrogen limitation in high-yielding subtropical environments



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## ABSTRACT

The asynchrony between soil nitrogen (N) and biological N<sub>2</sub> fixation results in N limitation in soybean crops. The crop duration can potentially alter the asynchrony and N limitation. The objective of this study was to determine the effect of soybean crop duration on N limitation. Seventeen field experiments were conducted in subtropical environments in Brazil with different crop durations: short (102–114 d), medium (115–126 d), and long (>126 d). A full-N fertilizer treatment that synchronized crop N demand and supply throughout crop development was compared with zero-N treatment. Seed yield, protein and oil concentrations, and seed weight and number were determined. The short duration crop was the only one with seed yield response to N supply (0.74 Mg ha<sup>-1</sup>; 15.5 %). When the long duration crop was fertilized with N, seed protein concentration increased without a trade-off in oil concentration. The N response on seed yield increased by ca. 48 kg ha<sup>-1</sup> per day, when crop duration was shorter than 123 d. While the protein yield response increased linearly from long to short crop duration, the oil yield response followed a bi-linear trend. We conclude that N limitation on seed yield in subtropical environments increased as the soybean crop duration decreased below 123 d, while the N limitation on seed protein concentration occurred across all crop durations. The intensification of the subtropical soybean-based cropping systems will require increasing soil N to avoid seed protein and yield reductions.

## 1. Introduction

Soybean [*Glycine max* (L.) Merr.] is the most cultivated legume crop in the world due to its capacity to provide large quantities of protein and oil for food and feed (Grassini et al., 2021). Brazil is the world's largest soybean producer (156 million Mg) and exporter (93 million Mg), generating approximately one-third of its production in subtropical climates. Brazil has the highest soybean yield potential among major soybean producing countries but also has the largest yield gap (Marin et al., 2022; USDA, 2023). Soybean in subtropical climates plays an important role in accelerating the rate of soybean yield gain. Projections indicate an increase in the world population

and income in developing countries, which will require the demand for food and energy to grow by up to 50% by 2050 (Cassman and Grassini, 2020). One of the major soybean yield gaps is related to water supply (Marin et al., 2022; Tagliapietra et al., 2021). Still in Brazil, 83% of total soybean area has a water-limited yield potential of more than 4.5 Mg ha<sup>-1</sup>. While crop management practices, such as planting date, proper selection of crop duration, and fungicide application, are already being tuned up to close soybean yield gaps, meeting the high demand for nitrogen (N) is a major challenge for increasing soybean productivity further (Giller and Cadisch, 1995; Salvagioti et al., 2008; Sinclair and Horie, 1989). In addition, the genetic improvements for high-yielding and short-duration soybean

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cultivars add complexity for meeting soybean N demand, as a larger amount of N is needed to be accumulated in a short period of time.

The soybean N requirement per unit of photosynthate produced is the highest among food crops (Sinclair and De Wit, 1976). The soybean N uptake is indeed 3.3 times larger than that of maize and 4.3 times larger than that of wheat and rice (Barracough et al., 2010; Bender et al., 2013; Salvagiotti et al., 2008; Yin et al., 2019). To meet this N demand, soybean crops rely on biological N fixation (BNF) and soil mineral N (Ns). The BNF accounts for, on average, 60% of total N uptake by the crop with the peak of supply between beginning of pod setting and seed filling, while the Ns contributes mainly during the vegetative phase of the crop when the BNF is still developing nodules and capacity to fix N (Ciampitti and Salvagiotti, 2018; Salvagiotti et al., 2008; Santachiara et al., 2017a). The amount of BNF depends on Ns contributions, as soybean crops preferentially acquire N from the soil rather than from BNF owing to the associated energetic cost of BNF, which generates a negative association (trade-off) between BNF and Ns (Connor et al., 2011; Streeter and Wong, 1998). Likewise, stresses such as water limitation and low soil temperatures can negatively affect BNF (Purcell et al., 2004; Zhang et al., 1995). Even in the absence of stresses, an asynchrony between BNF and Ns coinciding with the beginning of the maximum N demand period for soybean showed that daily crop growth and N uptake rates could be limited by N supply in high-yielding soybean crops (Cafaro La Menza et al., 2020). These N limitations can be expressed in terms of differences in seed yield, N uptake, and seed protein and oil concentrations between a crop grown with ample N supply vs. a crop relying on BNF and Ns (Cafaro La Menza et al., 2019).

Soybean N limitation has already been reported in Argentina, the United States, and Brazil, with the yield level of the environments as the main factor influencing the N limitation, which is likely to appear in environments with the yield level of more than 4.5 Mg ha<sup>-1</sup> (Ambrosini et al., 2019; Cafaro La Menza et al., 2017, 2019, 2020; Cordeiro and Echer, 2019; Orteza et al., 2019). Within high-yielding environments, soils with low Ns have the largest N limitation (Cafaro La Menza et al., 2019). While N limitation in high-yielding environments is a relatively new research topic, no other factors have been identified to influence or reduce N limitation in high-yielding soybean crops. For example, the selection of crop maturity group (MG) and planting date are crop management practices that have been used to set crop duration in order to match the critical soybean period for seed yield determination with the best possible solar radiation and temperature conditions (Grassini et al., 2021). The choice of sowing date and cultivar are factors contributing to the yield gap observed in subtropical environments (Winck et al., 2023), along with other previously identified management practices, such as fertilization, fungicide application, and plant density (Tagliapietra et al., 2021). However, N availability is a key factor contributing to the yield gap under high-yield conditions. Short crop duration will advance development stages faster than longer crop duration, setting large daily crop N demand in a short period of time. This rush of crop N demand for growth and development in short duration crops would potentially deplete the Ns before the BNF is ready to fully provide the N needed. A possible carbon shortage to sustain the BNF process may occur between R1 and R5, when vegetative and reproductive growth overlaps (Walsh et al., 1987). According to Patterson and La Rue (1983), the contribution of BNF is higher in longer duration genotypes due to the time available to fix N. This may also be related to the longer vegetative phase of long duration cultivars than short ones (Egli, 1993; Jiang and Egli, 1995; Zanon et al., 2015). Given the current scenario of (1) producers increasing the area cultivated with soybean as a second harvested crop in the subtropical environments (Follmann et al., 2019), (2) increasing adoption of high-yielding and short-MG cultivars with high daily crop growth rate that can lead to high daily N uptake demand (Heatherly, 2005; Santachiara et al., 2017b; Specht et al., 2014; Tagliapietra et al., 2021; Zanon et al., 2016), and (3) the exacerbated asynchrony between BNF and N uptake demand in high-yielding and short-duration soybean crops (Cafaro La Menza et al., 2020), we hypothesize that a short-duration

soybean crop in high-yielding subtropical environments is more limited by the N supply than a long-duration soybean crop.

Understanding the soybean N limitation in subtropical environments is necessary to ensure soybean seed yield, protein, and oil gain rates needed to meet the world's food security. Currently, there are at least 38 M ha in Brazil that can consistently achieve more than 4.5 Mg ha<sup>-1</sup> every year, and their cropping systems are being intensified by increasing the number of crops per year and decreasing the individual crop duration. The objective of this study was to determine the effects of N supply and crop duration on soybean seed yield, seed protein and oil concentration, and protein and oil yield. To address this objective, a full-N treatment that mimicked no N limitation was tested against a zero-N treatment in which the soybean crop relied on Ns and BNF across 17 high-yielding subtropical irrigated environments in Brazil with a wide range of crop duration (102–141 d).

## 2. Materials and methods

### 2.1. Field experiments

Field experiments were conducted on farmers' fields under no-tillage irrigated conditions in the State of Rio Grande do Sul, Brazil, which has the largest cultivated area of soybean in the world and has a humid subtropical climate (USDA, 2023). The experiments were conducted during three growing seasons (2018, 2019, and 2020) on high-yielding soybean (>4.5 Mg ha<sup>-1</sup>) fields strategically located to represent climate and soil variation. A randomized complete block design (RCBD) was used, with four replicates per treatment in each environment. In the present study, the 'environment' is defined as the combination of sowing date, MG, and location (Table 1). In addition, the experimental location within each field was based on yield maps from the previous year so that the experiments were placed on the highest yield spot in each environment. The management of weeds, pests, and diseases was preventive to eliminate any limiting and reducing yield factors. The seeds were treated with insecticide and fungicide, and inoculated with four doses of *Bradyrhizobium elkanii* (strains SEMIA 587 and SEMIA 5019 at a concentration of  $5 \times 10^9$  CFU mL<sup>-1</sup>).

The experiments were planted from mid-August to the end of January with a range of more than five months of sowing window, exposing the crop to different yield potential and duration (Table 1) (Tagliapietra et al., 2021; Zanon et al., 2016). Furthermore, we used an MG ranging from 5.0 to 6.8 to represent the duration length in the main sowing window from October to December used by farmers. Baseline fertilization with N, P, K, S, Mg, and Ca was calculated for each environment to achieve yield potential based on the CQFS-RS/SC 2016 guidelines (Table S1). In each environment, we had a total of eight plots (four replicates per N treatment) consisting of 6 rows and 10 m long with a 0.45 m row spacing.

### 2.2. N fertilization protocol and data collection

Two N supply treatments, full-N and zero-N, were compared across environments with different crop duration. The full-N treatment aimed to ensure non-limiting N conditions for soybean development and growth by adding N fertilizers according to the expected crop's N demand. The zero-N treatment aimed to mimic real N supply under the common crop management practices of high-yielding producer fields, depending on the BNF, Ns, and a small amount of mineral N applied as a starter at sowing (Table S1). The total N amount applied in the full-N treatment was calculated based on the N-fertilization protocol proposed by Cafaro La Menza et al. (2017). Therefore, the yield potential of each environment was simulated using the CROPGRO-Soybean model, with measured daily meteorological data over the last 15 years, which is a sufficiently long period to obtain accurate and reliable yield estimates (Boote et al., 2002; Grassini et al., 2015). This model was previously used, calibrated, and evaluated for soybean in a subtropical environment (Aramburu Merlos

**Table 1**

Description of soybean experiments and their soil characteristics (0–30 cm of soil depth) across the 17 high-yielding environments conducted in Rio Grande do Sul, Brazil during three growing seasons (2018, 2019, and 2020).

Site	Sowing date	MG	Plant population (plants m <sup>-2</sup> )	Yield potential (Mg ha <sup>-1</sup> )	Duration (d)	Soil type <sup>a</sup>	Soil texture <sup>b</sup>	Soil pH	SOC (g kg <sup>-1</sup> )	V (%)
Cruz Alta (28° 51'S, 53° 36'W and 420 m altitude)	Nov 3, 2017	5.8	27	6.7	130	Oxisols	Clay loam	5.5	30	60
	Nov 20, 2018	6.5	22	6.0	127	Oxisols	Clay loam	5.5	30	60
	Nov 17, 2019	5.5	31	6.2	116	Oxisols	Clay loam	5.0	27	58
	Nov 21, 2019	5.8	26	6.3	111	Oxisols	Clay loam	5.5	16	53
	Dec 3, 2019	6.5	22	5.5	120	Oxisols	Clay loam	5.8	22	65
Júlio de Castilhos (29° 11'S, 53° 36'W and 434 m altitude)	Oct 30, 2017	5.0	28	6.9	114	Oxisols	Clay	5.5	24	53
	Oct 30, 2017	5.1	33	7.0	114	Oxisols	Clay	5.5	24	53
	Oct 20, 2018	5.6	16	6.5	131	Oxisols	Clay	5.5	25	52
	Jan 26, 2020	5.8	42	3.6	119	Oxisols	Clay	5.1	14	51
	Jan 26, 2020	6.2	18	2.7	118	Oxisols	Clay	5.3	12	50
São F. de Assis (29° 24'S, 54° 54'W and 398 m altitude)	Nov 9, 2019	5.2	25	5.4	108	Ultisols	Silt loam	5.5	26	55
Santa Maria (29° 43'S, 53° 43'W and 95 m altitude)	Aug 17, 2018	5.0	44	5.8	102	Ultisols	Loam	5.5	22	55
	Aug 17, 2018	5.9	31	4.7	128	Ultisols	Loam	5.5	22	55
	Aug 17, 2018	6.8	44	4.6	141	Ultisols	Loam	5.5	22	55
	Nov 17, 2019	5.8	25	6.5	115	Ultisols	Loam	5.1	12	50
	Dec 17, 2019	5.0	32	4.9	106	Ultisols	Loam	5.5	13	56
Uruguaiana (29° 45'S, 56° 49'W and 90 m altitude)	Nov 20, 2018	5.8	21	6.5	123	Alfisols	Clay loam	5.0	15	49

<sup>a</sup> Brazilian Soil Classification System (Embrapa, 2013).

<sup>b</sup> Classification of the textural triangle (USDA).

Abbreviations: MG, maturity group; SOC, soil organic matter; and V, base saturation based on the Committee on Soil Chemistry and Fertility (CQFS-RS/SC, 2016).

et al., 2015; Ribas et al., 2021; Tagliapietra et al., 2021). The yield potential simulations ranged from 7.0 Mg ha<sup>-1</sup> (sowed at the end of October) to 2.7 Mg ha<sup>-1</sup> (sowed late January). Only two environments (No. 9 and 10) had lower yield potential than the 4.5 Mg ha<sup>-1</sup> and were included because of being an emerging management practice in soybean-based cropping systems in Brazil (Follmann et al., 2019). Therefore, the total amount of N applied in the full-N treatment was based on (1) yield potential of the specific environment, (2) 79 kg N required per each additional 1,000 kg of seed yield (Bender et al., 2015; Ortel et al., 2020; Ortez et al., 2019; Tamagno et al., 2017), and (3) the expected fertilizer-N use efficiency of 70%. The total amount of N was divided into five applications at V2, V4, R1, R3, and R5 (development stages based on Fehr and Caviness (1977)), with a proportion of the total N of 10%, 10%, 20%, 30%, and 30%, respectively. This split of N fertilizer was based on typical N uptake dynamics (Bender et al., 2015; Cafaro La Menza et al., 2020; Gaspar et al., 2017; Thies et al., 1995). Hence, the total amount of fertilizer broadcast between rows ranged from 305 to 790 kg N ha<sup>-1</sup>. Fertilizer applications preceded irrigation events within 24 h, with an irrigation depth of 20 mm.

Plant phenology was monitored every three days, following the scale of Fehr and Caviness (1977). At the R8 stage, an area of 2 m<sup>2</sup> in the center of each replicate was used to measure the seed yield and seed weight, which were then adjusted to 0.130 kg H<sub>2</sub>O kg<sup>-1</sup> of seed. The seeds were oven-dried until they reached a constant mass. To estimate the individual seed mass, 200 seeds were weighed in each replicate and treatment (Table S2). Then, each seed sample was ground to determine protein by the Kjeldahl method (Mckenzie and Wallace, 1953), and the oil was determined by the method of Bligh and Dyer (1959). Protein and oil concentrations were expressed based on dry weights. The protein and oil yields (Mg ha<sup>-1</sup>) were derived from the multiplication between seed protein and oil concentrations (in units of kg constituent kg<sup>-1</sup> seed) and seed yield on a dry basis (Table S2).

### 2.3. Data analysis

A combined analysis of variance (ANOVA) across environments was conducted to determine the effect of N supply and its interaction with soybean crop duration (SAS ® PROC MIXED v.9.3) (Moore and Dixon, 2015). In this analysis, the 17 environments had crop durations

ranging from 102 d to 141 d, so we divided them into three crop duration groups spaced equally by 12 d: short (102–114 d), medium (115–126 d), and long (127–141 d). Therefore, seven, seven, and three environments comprised the short, medium, and long crop duration groups, respectively. The type III test of fixed effects was used to determine whether the fixed effects (crop duration and N treatments) of the proposed model were significant. Linear regressions were used to investigate the relationship between the difference in seed, protein, and oil yields (full-N minus zero-N) and the crop duration in days using GraphPad®. Slopes, intercepts, and coefficients of determination (R<sup>2</sup>) were evaluated by the F test, and estimated values were reported accordingly.

### 3. Results

The mean seed yield in the full-N treatment was 0.29 Mg ha<sup>-1</sup> (6%) higher than that in the zero-N treatment. Notably, the N treatment significantly interacted with crop duration (Table 2, Fig. 1), suggesting that the response of seed yield to N supply was variable across crop durations. Indeed, the seed yield in the short duration crop was significantly higher in the full-N than zero-N treatment (0.74 Mg ha<sup>-1</sup>; 15.5%), while no significant yield difference between the treatments was found in the middle and long duration crops. The seed yield across the 17 environments ranged from 3.7 to 6.6 Mg ha<sup>-1</sup> for the full-N treatment and from 3.7 to 6.3 Mg ha<sup>-1</sup> for the zero-N treatment (Table S2). Moreover, the yield response of short duration crop to N supply increased at a rate of 48 kg ha<sup>-1</sup> per day when crop duration was shorter than 123 d (Fig. 1). The N fertilizer in the full-N treatment increased the individual seed weight across environments by 7.6% (+13.8 mg seed<sup>-1</sup>) in comparison to the zero-N treatment, and the seed weight had the larger response to the N supply than other yield components. When looking at seed weight responses in relation to weather variables (i.e. temperature and solar radiation), no clear relationship was found, but short duration crop tended to be at the higher end of mean solar radiation for the seed-filling period, where the largest responses were observed (Table S1). While the crop duration factor alone did not influence the yield, crop duration changed the magnitude of the seed number and weight in a nearly complementary fashion, interacting with the N treatments (Table 2).

In terms of seed quality, there was no significant interaction between the crop duration and N supply treatments on protein and oil concentrations (Table 2). The full-N treatment significantly increased the seed protein concentration and decreased the seed oil concentration by an average of 4.2 and 3.5%, respectively. Surprisingly, the crop duration had no effect on the seed protein concentration, but it did affect the seed oil concentration. The average trend shows that the long duration crop had the greatest seed oil concentrations (18.6%), followed by the shorter (16.5%) and middle (15.3%) duration crops. Although there was no significant crop duration × N interaction, there was a trend that the shorter the crop duration was, the more positive the effect of N on the seed protein concentration and the more negative the effect of N on the seed oil concentration. Overall, no significant trade-off was found between seed protein and oil concentrations across N treatments, crop duration, or environments (Fig. S2). When seed yield and quality were integrated into protein and oil yields, both variables showed significant crop duration × N interaction.

The relationship between protein yield response and crop duration clearly revealed the existence of N limitation in high-yielding sub-tropical environments (Fig. 2A). The linear trend indicated an increased N limitation toward short crop duration at a rate of 9 kg ha<sup>-1</sup> of protein per day of shortening in crop duration. This N limitation was first observed as seed protein concentration increased by 1.3, 1.5, and 1.7 percentage points in the short, medium, and long duration crops, respectively, and then the yield increased only in short duration crops (Table 2). While the protein yield showed the composite N limitation in terms of both seed yield and protein concentration, the oil yield followed a similar bi-linear trend as the seed yield in response to crop duration. Indeed, oil yields were not affected by N supply in long and medium duration crops but increased at a rate of 15 kg ha<sup>-1</sup> of oil per day of crop duration, when it was shorter than 114 d. When looking at protein yield responses in relation to weather variables (i. e. solar radiation), no clear relationship was found, but short duration crop tended to be at the higher end of the mean solar radiation for the seed-filling period, and the largest responses to N supply in protein yield were observed for short duration crops (Fig. S1). Overall, short duration soybean crops were prone to N limitation in high-yielding subtropical environments, in which N response was first denoted as increases in seed protein concentration across all crop durations and then as increases in seed yield only in crops with duration shorter than 123 d.

4. Discussion

In this study, the combined N supply from BNF and Ns did not meet the N demand required by soybean, generating an average N limitation of 6.1% in seed yield and 4.2% in protein concentration across

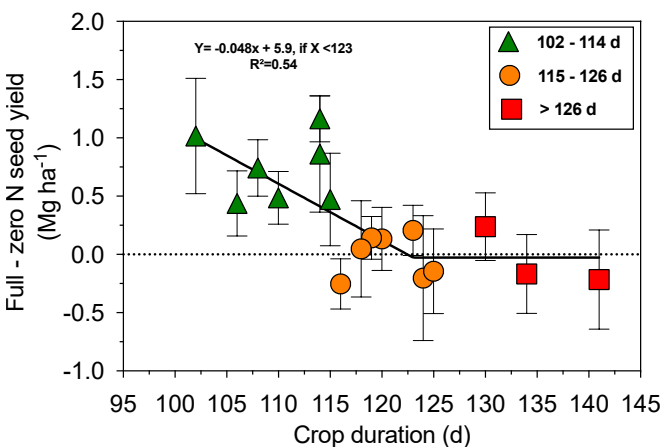


Fig. 1. Differences in seed yield between full-N and zero-N treatments in relation to the crop duration across 17 environments. Each data point represents the average seed yield for sowing date × maturity group × location × year. The fitted linear regression parameters (solid line) and coefficient of determination (R<sup>2</sup>) are shown. The dataset was separated into three groups based on crop duration: 102–114 d, 115–126 d, and >126 d.

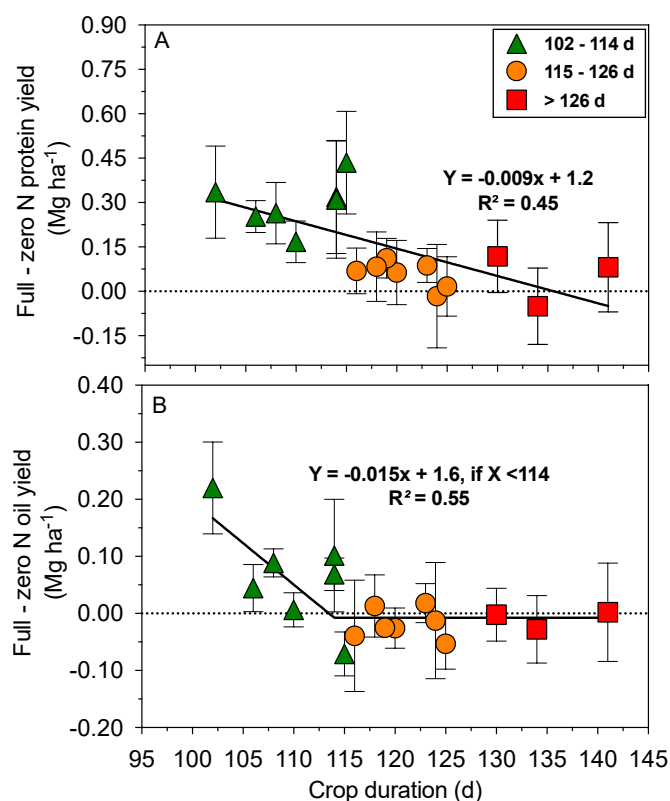
subtropical environments (Table 2). This N limitation in seed yield remains within the range previously reported by Ambrosini et al. (2019) in the State of Paraná, Brazil (4.6%), and by Cafaro La Menza et al. (2019) in Nebraska, USA (12%). Likewise, soybean N limitation found in this study in terms of protein concentration is within the range found across tropical and subtropical environments (Figueiredo Moura da Silva et al., 2023). Several studies, mostly on lower yield levels than this study, have shown inconsistency in the response to N fertilization in soybean (Mourtzinis et al., 2017). Unlike most studies arguing for positive vs. no response to N fertilization in soybean, our study explained part of the inconsistencies in N fertilization responses by grouping environments with different crop durations. This grouping and corresponding analysis were possible due to the intrinsic characteristics of subtropical soybean production in Brazil, which has not been evaluated previously. Indeed, previous research in temperate environments found that N was becoming a yield-limiting factor in high-yielding environments and that Ns played a key role in explaining the magnitude of responses across environments (Cafaro La Menza et al., 2017, 2019). Here, we found that an additional factor explaining the magnitude of N responses across environments was crop duration, in which short duration crops were prone to N limitation.

Table 2  
Soybean seed yield, seed number and weight, seed protein and oil concentrations, and protein and oil yields of different maturity groups (102–114, 115–126, and 127–141 d) under full-N and zero-N treatments.

Maturity group	N treatment	Seed yield	Seed number	Seed weight	Protein concentration	Oil concentration	Protein yield	Oil yield
(MG)		(Mg ha <sup>-1</sup> )	(m <sup>2</sup> )	(mg)	(%)	(%)	(Mg ha <sup>-1</sup> )	(Mg ha <sup>-1</sup> )
102-104	Full-N	5.52	2848	196.2	36.3	16.0	1.73	0.77
	Zero-N	4.78	2658	182.4	35.0	17.0	1.45	0.70
	Difference	0.74***	189*	13.8***	1.3*	-1.0*	0.28***	0.07**
115-126	Full-N	4.76	2729	174.6	36.5	15.5	1.49	0.63
	Zero-N	4.77	2837	168.0	35.0	15.1	1.46	0.64
	Difference	-0.01	-108	6.6*	1.5**	0.4*	0.04	-0.01
127-141	Full-N	4.71	2200	221.0	36.6	18.5	1.50	0.75
	Zero-N	4.78	2524	192.5	34.9	18.6	1.44	0.76
	Difference	-0.06	-323	28.5**	1.7	-0.1	0.06	-0.01
Overall	Difference	6.10%	4.5%	7.3%	4.2%	-3.4%	10.8%	2.6%
ANOVA								
MG		1.79	4.74*	8.47***	0.01	4.68*	1.73	3.02
N		6.36*	1.71	55.25***	14.36***	5.03*	15.93***	1.12
MG × N		11.21***	5.82**	7.10*	0.07	1.55	8.01***	3.68*

Statistical significance is indicated by \*P < 0.05, \*\*P < 0.01, and \*\*\*P < 0.001.





**Fig. 2.** Differences in protein yield (A) and oil yield (B) between full-N and zero-N treatments in relation to the crop duration across 17 environments. Each data point represents the average protein and oil yields for sowing date  $\times$  maturity group  $\times$  location  $\times$  year. The fitted regression parameters (solid line) and coefficient of determination ( $R^2$ ) are shown. The dataset was separated into three groups based on crop duration: 102–114 d, 115–126 d, and  $>126$  d.

Several studies have shown that short duration crops have high yield potential and adaptability to subtropical environments (Tagliapietra et al., 2021; Zanon et al., 2016; Zdziarski et al., 2018). In contrast, some studies in temperate environments have shown that crop duration does not alter N dynamics with respect to seed N needs from BNF and vegetative N mobilization to the seeds (Mastrodomenico and Purcell, 2012; Zeiher et al., 1982). However, these studies included soybean cultivars that mostly yielded less than  $4.5 \text{ Mg ha}^{-1}$  with limited crop N demand and did not assess the possibility of N supply shortage by including an N fertilization treatment that ensures no N limitation as a control. Based on our findings, the observed yields as a proxy of the yield potential of short duration crops in subtropical environments might be underestimated by the N limitation. This could lead to the selection of longer MG that will lower the potential of food production in the subtropical cropping systems by limiting yield, seed protein, and the possibility of having an extra crop in the rotation. The explanation behind the larger N limitation in short duration soybean crops may be related to the asynchrony between BNF and soybean N demand in high-yield environments (Cafaro La Menza et al., 2020). Short duration crops advance development stages faster than longer duration crops, imposing a large daily N demand before BNF is fully developed. These conditions may exacerbate the asynchrony between BNF and crop N demand and cause short duration crops to incur larger N limitations than long duration crops. Although BNF was not directly measured, the use of a full-N treatment in comparison with a zero-N treatment allowed us to estimate the shortage of BNF contribution under field conditions indirectly. Studies in Brazil have shown that BNF efficiency varied according to soil type, soybean cultivar, and rhizobium strains, highlighting the importance of direct BNF assessments in future research to understand the underlying mechanisms of BNF failure in fulfilling soybean N demand (Hungria

et al., 2006). While subtropical soybean producers are moving to intensify their cropping systems, integrated crop management that enhances Ns, such as cover crop or N fertilization strategies, will need to be adopted to maximize the yield potential and quality of short duration soybean crops (Tagliapietra et al., 2022). In the United States, Kendall et al. (2025) reported that applying a low dose of N in no-till systems could help increase soybean yields. Andrade et al. (2013) reported similar results in Argentina, where growers adopting crop rotation and applying higher amounts of N to the system achieved higher yields and grain protein concentrations.

We recognize that the total N-fertilizer applied in this study was not sustainable for N recommendations (both environmentally and economically). However, the fertilization protocol allowed us to supply N above the capacity of BNF and Ns together, supplying N in non-limiting conditions for achieving yield potential and enabling us to test the hypothesis of N limitation across different crop durations. Previous studies have shown that moderate N applications, such as  $135 \text{ kg N ha}^{-1}$  and  $98.4 \text{ kg N ha}^{-1}$  in Northeast China, and even lower rates around  $34 \text{ kg N ha}^{-1}$ , can lead to economically viable yield increases in soybean (Brooks et al., 2022; Córdova et al., 2020; Hao et al., 2023). Our results indicate that a short soybean duration ( $<123$  d) was prone to N limitations in high-yielding subtropical environments. This limitation seems to be directly related to rapid plant growth in a short period of time, which could have been induced by a possible carbon shortage to sustain the BNF due to the overlap of vegetative and reproductive growth (Walsh et al., 1987). Indeed, the type of N limitation shown in our study was not related to environmental constraints that could affect BNF, such as water deficit and soil temperature, which have already been studied elsewhere (Purcell et al., 2004; Ray et al., 2006; Zhang et al., 1995). Our results can be extended to most of the areas cultivated with soybean in South Brazil, North Central Argentina, and Paraguay, which use the same range of crop durations as this study. Also, these areas are currently moving toward intensification of cropping systems by increasing the number of crops per year while shortening the crop duration (Cassman and Grassini, 2020; Tagliapietra et al., 2021; van Ittersum et al., 2016). Other areas of expansion of soybean cultivation include degraded sandy soils with pastures and low indigenous N supply, which can also affect the performance of short duration soybean crops (Cafaro La Menza et al., 2017; Cordeiro and Echer, 2019). Therefore, future research aimed at increasing N availability throughout the production system is essential. This includes identifying effective management practices such as crop rotation, the use of cover crops, the optimal timing for N application, the use of slow-release fertilizers, and others to reduce the N limitation.

Two other key findings related to the N response identified in this research are noteworthy: a consistent increase in seed weight and seed protein with N fertilizer across the crop durations. While the seed number is the most important yield component across field crops, the N limitation in soybean affects, more in magnitude and significance, the seed weight across all nodes of the canopy than the seed number (Bonfanti et al., 2025). These increases in seed weight seem to be related to a larger amount of vegetative N when N fertilizer is applied, which maintains higher photosynthesis at canopy levels during the early seed filling (Cafaro La Menza et al., 2020, 2023). Moreover, this extra mobilized N helps sustain higher levels of seed protein concentration. Figueiredo Moura da Silva et al. (2023) reported that seed protein concentration was limited by N supply in tropical and subtropical environments in Brazil, resulting in a 4% relative reduction under irrigated conditions, and that this N-related limitation increased with water deficit, reaching up to 12%. Here, we stress the role and potential of tropical and subtropical environments in providing the appropriate seed protein levels to meet food security requirements and that these levels will be met by providing crops with the right and synchronized amount of N supply. A large part of the variability in seed protein and oil concentrations is usually attributed to genotype, the environment, and agronomic practices, with N fertilization being the only practice that can break the trade-off between seed protein and oil concentrations (Grassini et al., 2021). Here, we found a consistent effect of N fertilizer on seed protein and N concentrations across crop durations and an expected effect

of the crop duration on the oil concentration. However, the trade-off between seed protein and oil concentrations was inconsistent and not significant across crop durations. In addition to Arce et al. (2025), most studies addressing seed protein and oil variability and their trade-off have been conducted in temperate environments (Assefa et al., 2018, 2019; Bosaz et al., 2019; Rotundo et al., 2016). However, this research contributes to the limited body of research on seed protein and oil concentrations and yields in subtropical environments as affected by N supply and crop duration. Finally, the concomitant increase in seed weight and protein concentration without reducing the oil concentration denotes the research needs to assess seed size (i.e. sieve diameter or seed volume), as proteins, lipids, carbohydrates, and minerals have different densities, and trade-offs in seed components might be explained by changes in seed size.

## 5. Conclusions

In this study, we investigated the effect of soybean duration and N supply on soybean seed yield, protein, and oil. This work revealed that high-yielding subtropical environments were limited by N supply (i.e. seed yield and protein concentration) regardless of crop duration. In terms of the response of seed yield to N supply, short duration soybean crops were prone to N limitation, whereas long crops did not show significant yield responses to extra N. Indeed, the response of seed yield to N supply increased by ca. 48 kg ha<sup>-1</sup> per day when crop duration was shorter than 123 d, while no N limitation, in terms of seed yield, was found in soybean crop duration of more than 126 d. The N fertilization increased the seed protein concentration across crop durations, indicating that N limitation in seed protein concentration occurred across all crop durations. The individual seed weight consistently increased due to the N supply across all crop durations and environments. The intensification of the subtropical soybean-based cropping systems will require the supply of relatively large amounts of daily available N from the soil to avoid reductions in soybean seed yield and protein concentration.

## Abbreviations

BNF	biological N fixation
MG	maturity group
N	nitrogen
Ns	soil mineral N

## CRedit authorship contribution statement

**Guilherme Guerin Munareto:** writing, supervision, resources, methodology, investigation, formal analysis, data curation, and conceptualization; **Nicolas Cafaro La Menza:** writing, visualization, validation, supervision, methodology, and investigation; **Eduardo Lago Tagliapietra:** writing, methodology, investigation, funding acquisition, formal analysis, data curation, and conceptualization; **Lucia Bonfanti:** methodology, investigation, and data curation; **Cesar Eugenio Quintero:** writing, supervision, resources, and formal analysis; **Alexandre Ferigolo Alves:** resources, methodology, investigation, data curation, and conceptualization; **Nereu Augusto Streck:** writing, supervision, resources, methodology, and conceptualization; **Evandro Henrique Figueiredo Moura da Silva:** resources, methodology, and investigation; **Fabio Ricardo Marin:** writing, visualization, resources, methodology, and formal analysis; and **Alencar Junior Zanon:** writing, visualization, validation, supervision, resources, methodology, investigation, formal analysis, data curation, and conceptualization.

## Declaration of competing interest

The authors declare that they have no any competing interest. Author Alencar Junior Zanon (Editorial Board member) was not involved in the journal's review or decisions related to this manuscript.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crope.2025.06.002>.

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