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Precrops and N-fertilizer impacts on soybean performance in tropical regions of Brazil

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ABSTRACT. Precrops have different growth patterns, nitrogen (N) requirements, and production of residues varying in amounts and quality that may affect the N-clycling and the soybean (*Glycine max L. Merrill*) cropped in succession. This study aimed to evaluate the effect of precrops and N fertilization on soybean performance. An experiment was conducted in Londrina, Paraná State, Brazil, with six precrops treatments: fallow, ruzigrass (*Urochloa ruziziensis*), showy rattlebox (*Crotalaria spectabilis*), corn (*Zea mays*) without or with 80 kg ha^{-1} of N at topdressing as urea, and wheat (*Triticum aestivum*). Subplots consisted of two levels of N fertilization at soybean sowing: 0 and 30 kg ha^{-1} of N as ammonium nitrate at sowing. *Urochloa ruziziensis* as precrops increased the soybean yield (5,171 kg ha^{-1}) when compared with corn (4,346 kg ha^{-1}) and fallow (4,467 kg ha^{-1}). In 2016/17, N fertilization of soybean with 30 kg ha^{-1} of N at sowing, although increasing the initial plant growth (745 kg ha^{-1} with vs. 662 kg ha^{-1} without), impairs nodulation (100 mg pl^{-1} with vs. 130 g pl^{-1} without) and does not increase grain yield. Oil and protein concentrations in soybean grains are not influenced by precrops and N fertilization at sowing. We found that the use *Urochloa ruziziensis* as cover crop in soybean precrops is a good recommendation option in tropical regions of Brazil, because increasing the yield of soybean grown in succession. N fertilization at in soybean sowing it should not be recommended even in the presence of a large amount of straw.

Keywords: *Glycine max*; no-tillage system; cover crops; growth; oil and protein concentrations in grains; off-season crops.

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Introduction

Grain cultivation in Brazil has predominantly been performed under no-tillage system (NT), which reduces production costs and soil erosion, and improves various soil physical, chemical, and biological attributes, increasing grain yield and reducing losses due to water stress (Franchini et al., 2012). NT is based on low soil mobilization, permanent soil cover, and crop diversification (Denardin, Kochhann, Faganello, & Cogo, 2014), where the shoot and root biomass input improves the soil quality and yield stability (Nogueira et al., 2014; Balbinot Jr., Franchini, Debiasi, & Yokoyama, 2017; Balbinot Jr. et al., 2020). The maintenance of mulching on the soil surface is critical to NT sustainability, as it reduces thermal amplitude and evaporative water losses, protects soil from erosion and reduces the incidence of weeds (Balbinot Jr., Moraes, Pelissari, Dieckow, & Veiga, 2008; Carvalho, Carvalho, Neto, & Teixeira, 2013), increasing the yield of the crop in succession (Balbinot Jr. et al., 2017; Balbinot Jr. et al., 2020).

Soybean (*Glycine max* (L.) Merrill) and corn (*Zea mays* L.) are the main crops under NT in Brazil. Soybean occupied about 36.8 million hectares in the 2019/20 season (CONAB, 2020), being the most important crop of Brazilian agribusiness. In 2018, Brazil exported approximately 89 million Mg of soybean grains, which generated incomes of US$ 35.65 billion (CONAB, 2019a).

A significant increase in the succession soybean/second crop corn has been observed in the last decade (CONAB, 2019b), with 12.3 million ha cultivated with corn in the soybean off-season (IBGE, 2019). Application of mineral N is essential for increasing the second crop corn yield (Pavinato, Ceretta, Girotto, & Moreira, 2008). Nevertheless, in the last seasons, many farmers have suppressed N fertilization in corn, aiming to reduce costs and the risk inherent to the activity facing unfavorable climate factors, especially drought. However, supressing N fertilization of the second crop corn may negatively impact the soybean cropped afterwards. Mineral N application to precrops increases nutrient cycling and reduces the straw C/N ratio, increasing the nutrient cycling in the soil to the soybean in succession (Tanaka et al., 2019).

Wheat is an winter crop widely used as precrops of soybean in Brazil, cultivated in 1.9 million ha in 2019, especially in the South region (IBGE, 2019). But, higher soybean yields in succession to wheat than after corn or fallow have usually been observed, although there are no studies proving this fact.

In Brazil, soil cover crops like *Urochloa ruziziensis* (Loss, Pereira, Giácomo, Perin, & Anjos, 2011) and *Crotalaria spectabilis* (Carvalho et al., 2013) have been used as cover crops to improve soil quality. The cultivation of such covers in the fall/winter improves several soil physical (Balbinot Jr. et al., 2011) and microbiological attributes (Nogueira et al., 2014). In several regions, fallow is common in soybean off-season, resulting in soil degradation and infestation of weeds (Balbinot Jr. et al., 2008; Carvalho et al., 2013). In this case, crops sown after fallow may have lower yield compared with sowing after cover or cash crops. Still, the impact of fallow on soybean yield compared with cash or or cover crops in the off-season has not been adequately clarified.

The use of legume cover crops such as *Crotalaria spectabilis* may increase soil organic N stocks due to biological N_2 fixation (BNF) (Smith, Gross, & Robertson, 2008). An efficient BNF, along with soil mineral N, eliminates the need for mineral N fertilizer in soybean (Mugendi, Gitonga, Cheruiyot, & Maingi, 2010). The soybean yield with efficient nodulation can be equivalent to more than 300 kg ha^{-1} of mineral N (Zilli, Campo, & Hungria, 2010; Hungria & Mendes, 2015). However, there are constant concerns on the possible positive impacts of mineral N fertilization on soybean sown on straw of grasses with high C/N ratio. Application of 30 kg ha^{-1} of N usually increases soybean growth at the early stages, but with no increases in grain yield (Franchini, Balbinot Jr., Debiasi, & Conte, 2015a; Werner, Balbinot Jr., Ferreira, Debiasi, & Franchini, 2016). These studies provide evidence that even under high amount of straw with high C/N ratio, mineral N fertilization in soybean can be dismissed.

This study aim to assess the effect of precrops and mineral N fertilization on soybean performance.

Material and methods

Experimental area

The experiment was carried out from March 2016 to March 2018, in Londrina, Paraná State, Brazil (23°11′37″ S and 51°11′03″ W, with 630 m a.s.l.). Climate is classified as rainy subtropical (Cfa) according to Köppen's classification. The rainfall and temperature data during the experimental periods are shown in Figure 1.

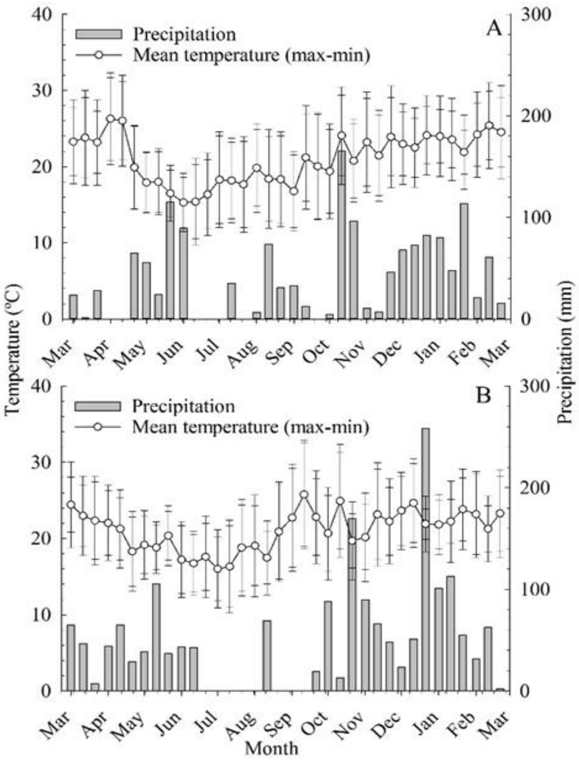

Figure 1. Rainfall and mean, maximum and minimum air temperatures, during the experimental periods: A– March 2016 to March 2017 and B – March 2017 to March 2018.

The soil is classified as Oxisol ("Latossolo Vermelho distroférrico", in the Brazilian classification; or Rhodic Eutrudox, in the USA classification) with the following chemical characteristics and granulometric fractions at the 0–20 cm layer: C (Walkley Black) 17.8 g dm^{-3}, pH $CaCl_2$ 5.1, H^+ + Al^{3+} (SMP) 5.2 $cmol_c$ dm^{-3}, K^+ (Mehlich 1) 0.85 $cmol_c$ dm^{-3}, P (Mehlich 1) 36.9 mg dm^{-3}, Ca^{2+} (KCl) 4.41 $cmol_c$ dm^{-3}, and Mg^{2+} (KCl) 1.52 $cmol_c$ dm^{-3}, 710 g kg^{-1} clay, 82 g kg^{-1} silt, and 208 g kg^{-1} sand. This soil had been under the no-tillage system for 15 years, with soybean or maize in the spring/summer season and wheat or second crop maize in the fall/winter.

Experimental design and management

The experimental design was in randomized blocks in a split-plot arrangement with five replications. Plots (5.0 × 8.0 m) consisted of six precrops treatments: 1 - fallow (no cultivation between soybean seasons), 2 - ruzigrass (*Urochloa ruziziensis*), 3 - showy rattlebox (*Crotalaria spectabilis*), 4 - second crop corn without N fertilizer as topdressing, 5 – second crop corn receiving 80 kg ha^{-1} of N fertilizer (urea) as topdressing, and 6 - wheat (*Triticum aestivum*). Subplots (2.5 × 8.0 m) consisted of soybean cropped in succession without or with (30 kg ha^{-1} of N as ammonium nitrate). The treatments were conducted on the same plots and subplots for two cropping seasons to check the cumulative effects.

Corn, *Urochloa ruziziensis*, and *Crotalaria spectabilis* were sown in mid-March and wheat at the end of April in both cropping seasons. Corn (hybrid AG 9010 YG) was sown in rows spaced 0.90 m apart, with 60,000 plants ha^{-1}. *Urochloa ruziziensis*, *Crotalaria spectabilis*, and wheat (cultivar BRS Gralha Azul) were sown with an interrow spacing of 0.17 m, with 50, 40, and 60 seeds m^{-2}, respectively. Sowing fertilization consisted of 260 and 300 kg ha^{-1} of the formulated fertilizer 08-28-16 (N-P_2O_5-K_2O) for corn and wheat, respectively. *Urochloa ruziziensis*, *Crotalaria spectabilis*, and fallow treatments did not receive fertilizer at sowing or topdressing. Corn with 80 kg ha^{-1} of N at topdressing received urea (45% N) at the V6 stage (six expanded leaves). Wheat was not fertilized with topdressing N. Corn and wheat were harvested in mid-September, and in October the area was desiccated with glyphosate (1,080 g a.i. ha^{-1}) for soybean sowing under no-till system (NT).

Soybean (cv. BRS 1010 IPRO), with indeterminate growth type and maturity group 6.1, was used in crop season 2016/17 and 2017/18. Sowing was done in the first half of October spaced was 0.45 m between rows, and density of 320,000 plants ha^{-1}. Before sowing, seeds were treated with Standak Top® (1 mL kg^{-1}) and Gelfix 5® liquid inoculant containing *Bradyrhizobium elkanii* strains SEMIA 587 and SEMIA 5019 (2 mL kg^{-1}). Fertilization consisted of 350 kg ha^{-1} of 0–20–20 (N–P_2O_5–K_2O). The subplot with mineral N received ammonium nitrate (34% N) as broadcast at the sowing time. The control of diseases, pests, and weeds followed the technical recommendations for soybean in Brazil (Embrapa, 2013).

Assessments

Remaining straw dry matter and grain yield by precrops

Twenty days before soybean sowing, straw was collected in 1 m^2 randomly set per plot. The material was oven-dried at 65°C until constant weight, then weighed and expressed in kg ha^{-1}. Corn and wheat grain yields were determined in the central area of the plots, excluding borders and expressed in kg ha^{-1} at 13% moisture.

Soil nitrate and ammonium concentrations

Soil samples were taken at V3, R2, and R5.3 of soybean developmental stages, at the 0.0–0.1 m layer with a steel auger in the useful area of the subplots. A sample formed by five subsamples was taken in the interrow and extracted for mineral N with potassium sulfate solution (0.5 mol L^{-1}). Ammonium was determined in the extract by colorimetry in a Lambda-25 UV-Vis spectrophotometer (Searle, 1984); nitrate was determined by the delta-absorbance method (APHA, AWWA, & WEF, 2006), with readings at 220 and 275 nm.

Soybean plant density and growth

Plant density was evaluated at the V3 stage in two lines of 6 m in the useful area of the plot (2.7 m^2). Shoot dry matter was assessed at V5 based on the plants collected within 2 m in two rows of the useful area in each subplot. Plants were cut close to the ground level and dried at 65°C until constant weight, weighed and expressed in kg ha^{-1} N (Kjeldahl) concentration was determined in the shoots, and N accumulation per hectare was based on the plant biomass. The leaf area index (LAI) was determined at the R5.1 stage with a LI-COR® LAI-2200 plant canopy analyzer, based on five readings in different positions in the canopy, three in the row and two in the interrow in the useful area of the plot. Plant height and first pod insertion height were evaluated at harvest maturation (R8) using ten plants per subplot.

Soybean nodulation and ureides

Attributes related to BNF were assessed only in the 2016/17 cropping season at R2 stage. The number and mass of dry nodules were determined in five plants per subplot. Nodules were counted and weighed on an analytical scale after oven drying (60ºC), and data expressed, respectively, as number of nodules per plant and milligrams per plant. Petioles of five plants were washed with deionized water and crushed with ethanol to determine the concentration of ureides (allantoin and allantoic acid). The extract was stored in microtubes at -15°C (Vogels & Drift, 1970; Hungria & Araujo, 1994) until colorimetric analysis in a UV-vis spectrophotometer (Genesys 10 UV) at a wavelength of 535 nm. The data were expressed in μmol g^{-1}.

Grain yield, yield components, and oil and protein concentrations in grains

Grain yield was estimated by harvesting three rows of 6 m (8.1 m^2) per subplot, and expressed in kg ha^{-1} with standard moisture at 13%. Ten plants were collected to evaluate the number of pods per plant; number of grains per pod, estimated by the ratio between total grains to total pods; and thousand-grain weight (g), by weighing 100 grains in four replications on an analytical scale.

Oil and protein concentrations were determined in whole and cleaned grains, according to Heil (2010). Oil and protein productivity per hectare were also calculated by multiplying grain yield by the respective oil and protein concentrations.

Statistical analysis

The dataset was subjected to tests of normality (Shapiro-Wilk) and homogeneity of variances (Hartley). Then, the analysis of variance with F-test ($p \leq 0.05$) was applied. The means were compared by the Tukey's test ($p \leq 0.05$) when a significant effect of treatments was found. Pearson linear correlation was also performed between variables ($p \leq 0.05$).

Results and discussion

Remaining straw production by precrops

In the 2016/17 cropping season, temperatures in June were lower than in the subsequent season (Figure 1), which favored wheat growth over other species since wheat has higher tolerance to cold. Thus, in the 2016/2017 cropping season, wheat produced straw as much as *Urochloa ruziziensis* (Table 1). Frost occurred in June 2016 killed *Crotalaria spectabilis* plants, so that the plant biomass was completely decomposed when soybean was sown in October. Legumes usually produce straw with a low C/N ratio, which favors biomass decomposition (De Sousa et al., 2019). The absence of remaining straw dry matter in the fallow treatment (Table 1) is attributed to the low weed seed bank in the experimental area.

Table 1. Remaining straw dry matter on the soil surface produced by precrops at 20 days before soybean sowing and grain yield of corn and wheat.

Season	*Urochloa ruziziensis*	*Crotalaria spectabilis*	Corn 0 N	Corn 80 N	Fallow	Wheat	CV (%)
Remaining straw dry matter (kg ha^{-1})							
2016/17	3,430 ab	-	2,462 b	2,764 ab	-	4,753 a	33.8
2017/18	2,902 cd	1,545 d	5,010 ab	6,382 a	-	2,673 cd	23.6
Grain yield (kg ha^{-1})							
2016/17	-	-	4,150	4,655	-	3,650	-
2017/18	-	-	5,988	6,305	-	2,764	-

Means followed by the same letter in the row do not differ one another by the Tukey's test at 5% significance. Corn 0 N = corn without topdressing N fertilization. Corn 80 N = corn with 80 kg ha^{-1} of N as urea at topdressing. ns = not significant.

In the 2017/18 cropping season, corn that received 80 kg ha^{-1} of N produced 313, 139, and 120% more straw than *Crotalaria spectabilis*, wheat, and *Urochloa ruziziensis*, respectively, not differing from corn cultivated without N at topdressing. The higher air temperature between May and July compared with the previous season favored corn growth. Therefore, a significant variation was observed between both cropping seasons regarding remaining straw production by pre crops, mainly due to different climate conditions. Brazil has a predominance of no-till system in all grain-producing regions, and the high production of straw is one of the bases for the success of this system (Franchini et al., 2012).

Nitrate and ammonium concentrations in soil

No interaction was observed between precrops and N fertilization in soybean for inorganic N concentrations in the soil (Table 2). The precrops did not affect nitrate and ammonium concentrations at 0.00–0.10 m soil layer in the three samplings during the soybean cycle. Thus, the present results did not confirm the hypothesis that immobilization by the low C/N straw of gramineous such as corn, wheat, and *Urochloa ruziziensis* reduces the inorganic N concentration in the soil compared with fallow or *Crotalaria spectabilis* cultivation. Moro, Crusciol, Nascente, and Cantarella (2013) found no effect of soil cover crops (*Crotalaria spectabilis*, *Urochloa ruziziensis*, *U. brizantha*, *U. humidicola*, and *U. decumbens*) on the nitrate and ammonium concentrations at the 0.00-0.20 m soil layer.

Table 2. Nitrate-N (NO_3^-–N) and ammonium-N (NH_4^+–N) concentrations in the soil (0.00–0.10 m layer) at three soybean growth stages as a function of precrops and N fertilization at the soybean sowing, 2016/17 cropping season.

Treatments	V3		R2		R5.3	
	NO_3^-–N (mg kg^{-1})	NH_4^+–N (mg kg^{-1})	NO_3^-–N (mg kg^{-1})	NH_4^+–N (mg kg^{-1})	NO_3^-–N (mg kg^{-1})	NH_4^+–N (mg kg^{-1})
Urochloa ruziziensis	15.4 ns	5.6 ns	19.2 ns	4.4 ns	19.9 ns	7.4 ns
Crotalaria spectabilis	16.9	5.4	18.2	2.6	18.9	7.0
Corn 0 N	17.7	2.8	17.4	4.7	19.3	7.8
Corn 80 N	14.3	6.4	17.7	4.6	19.2	6.9
Fallow	16.9	12.1	15.4	3.1	18.1	6.5
Wheat	16.9	11.1	15.3	4.4	18.9	6.5
CV (%)	17.0	54.0	17.0	62.9	22.0	19.0
Soybean 0 N	15.3b	6.6 ns	16.6 ns	4.2 ns	19.2 ns	6.9 ns
Soybean 30 N	17.3a	7.9	17.8	3.7	18.9	7.1
CV (%)	15.1	62.0	16.0	49.9	17.0	24.0
Interaction	ns	ns	ns	Ns	ns	ns

Corn 0 N = corn without topdressing N fertilization. Corn 80 N = corn with 80 kg ha^{-1} of N as urea as topdressing. ns = not significant.

N fertilization at soybean sowing increased the soil nitrate concentrations at V3 stage, but this effect disappeared in later stages. It shows that the effect of N fertilization on nitrate elevation is transient due to its fast dynamics in the soil. N fertilization in soybean did not affect the ammonium concentrations in the three samplings since the nitrification process quickly converts ammonium into nitrate in the soil. Santos et al. (2013) found fast nitrification of ammonium added as landfill leachate to a similar soil, in which more than 90% of the added ammonium was converted into nitrate within two weeks.

Soybean plant density, growth, and nodulation

No interaction was observed between precrops and soybean N fertilization for growth and nodulation (Tables 3 and 4). Soybean plant density at V3 (DEN) was not affected by treatments. In the 2016/17 cropping season, the shoot dry matter at V5 (SDM) was higher when cultivated after corn, irrespectively of the N use as topdressing, compared with the cultivation after wheat, *Crotalaria spectabilis* and fallow, but not differing from *Urochloa ruziziensis* (Table 3). In the 2017/18 cropping season, precrops did not affect soybean growth, showing that the impact of precrops on soybean growth in the vegetative period depend on the season, especially related to climate conditions. LAI at R5.1 was not influenced by the fall/winter crops in both seasons. The lower growth of soybean plants at the V5 stage after wheat, *Crotalaria spectabilis*, and fallow in the 2016/17 cropping season did not reduce LAI at R5.1, the beginning of grain filling.

The N concentrations in the shoots of soybean grown after corn that received N, and fallow, were higher than in plants grown after wheat (Table 3), as also observed for N accumulation.

The application of 30 kg ha^{-1} of N at soybean sowing increased SDM and NAC at V5 in the 2016/17 cropping season, but did not in the 2017/18 season (Table 3). The application of mineral N at soybean sowing may result in higher initial growth, when the biological N_2 fixation (BNF) process is still not fully operational (Hungria et al., 2006), but usually after this period, the amount of fixed N is enough for adequate soybean growth (Zilli et al., 2010).

Table 3. Plant density at V3 (DEN), shoot dry matter at V5 (SDM), N concentration in soybean shoots at V5 (NCS), N accumulation at V5 (NAC), and leaf area index at R5.1 (LAI) of soybean sown after precrops and N fertilization at sowing.

Treatments	2016/17					2017/18		
	DEN	SDM	NCS	NAC	LAI	DEN	SDM	LAI
	pl. m^{-2}	kg ha^{-1}	%	kg ha^{-1}	-	pl. m^{-2}	kg ha^{-1}	-
Urochloa ruziziensis	31.5 ns	712 ab	3.4 ab	24.2 bc	7.9 ns	25.9 ns	545 ns	7.0 ns
Crotalaria spectabilis	32.0	630 b	3.5 ab	22.0 c	7.7	24.4	579	6.9
Corn 0 N	32.0	810 a	3.4 ab	27.5 b	7.6	25.1	508	7.2
Corn 80 N	29.6	849 a	3.7 a	31.4 a	7.6	25.5	531	7.2
Fallow	30.6	639 b	3.6 a	23.0 bc	7.6	25.1	451	6.9
Wheat	29.4	579 b	3.2 b	18.5 d	7.8	24.7	476	7.2
CV (%)	11.0	16.4	7.4	12.2	3.5	14.3	34.1	9.0
Soybean 0 N	30.9 ns	662 b	3.5 ns	23.2 b	7.7 ns	245 ns	507 ns	7.0 ns
Soybean 30 N	30.8	745 a	3.5	26.1 a	7.7	258	523	7.1
CV (%)	7.6	19.9	7.1	13.5	3.2	12.5	27.6	8.8
Interaction	ns	ns	ns	ns	Ns	ns	ns	ns

Means followed by the same letter in the column do not differ one another by the Tukey's test at 5% significance. Corn 0 N = corn without topdressing N fertilization. Corn 80 N = corn with 80 kg ha^{-1} of N as urea as topdressing. ns = not significant.

Precrops did not affect the number of nodules per plant at R2 (NN) (Table 4). However, nodule dry matter (NDM) increased in soybean grown after wheat and *Urochloa ruziziensis* compared with *Crotalaria spectabilis*. Mineral N fertilization at soybean sowing reduced the number and mass of nodules, indicating a negative effect on BNF. Application of inorganic N in soil impairs nodulation at different phenological stages (Saturno et al., 2017) disturbing the molecular signaling between plants and rhizobia, delaying or decreasing the nodule occupation process (Nishida & Suzaki, 2018). On the other hand, the N application as topdressing (80 kg ha^{-1}) to the corn crop did not affect soybean nodulation (NN or NDM), indicating that mineral N fertilization in corn has no significant effects on nodulation of soybean grown in the sequence because the residual effect of N applied to corn is null, as indicated by the concentrations of ammonium and nitrate in the soil at different stages of soybean cycle (Table 2).

Table 4. Number of nodules (NN), nodule dry weight (NDW), and ureides concentration (URD) of soybean sown after precrops and N fertilization at sowing in the 2016/17 cropping season.

Treatments	NN	NDW	URD
	un. pl^{-1}	mg pl^{-1}	μmol g^{-1}
Urochloa ruziziensis	28.8 ns	140 a	21.4 c
Crotalaria spectabilis	26.4	90 b	29.1 ab
Corn 0 N	25.1	111 ab	26.6 ab
Corn 80 N	29.4	120 ab	30.4 a
Fallow	27.1	100 ab	30.6 a
Wheat	29.1	150 a	24.8 bc
CV (%)	22.5	28.3	13.3
Soybean 0 N	29.8 a	130 a	27.3 ns
Soybean 30 N	25.6 b	100 b	27.0
CV (%)	25.9	34.3	11.7
Interaction	ns	ns	ns

Means followed by the same letter in the column do not differ one another by the Tukey's test at 5% significance. Corn 0 N = corn without topdressing N fertilization. Corn 80 N = corn with 80 kg ha^{-1} of N as urea at topdressing. ns = not significant.

Soybean cropped after corn that received 80 kg ha^{-1} of N showed 22.6 and 42% higher ureides concentration at R2 than plants cropped after wheat and *Urochloa ruziziensis*, respectively. Ureides are the main form of fixed N transported via xylem (Hungria et al., 2006), being a good indicator of BNF efficiency, which concentration is proportional to BNF efficiency (Cerezini, Fagotti, Pipolo, Hungria, & Nogueira, 2017). However, factors like drought increase the concentration of ureides in plant, due to its non-metabolization in the shoot, and may cause a negative feedback on the BNF in nodules. There was a positive correlation between nodules per plant and nodule dry weight (0.55). In other words, treatments that promoted the number of nodules also promoted higher nodule mass. However, there was no correlation between ureides and the number or mass of nodules, highlighting the complexity of measuring BNF in soybean.

Soybean height and first pod insertion height

In the 2016/17 cropping season, soybean in succession to corn that received N showed higher height than plants grown after wheat (Table 5). In the 2017/18, soybean grown after *Crotalaria spectabilis* presented higher height than plants cropped after corn without N in topdressing, fallow, and wheat. In both seasons, wheat reduced the height of soybean cropped thereafer. This effect is probably unrelated to the temporary N immobilization for decomposition of wheat straw since the inorganic N concentration in the soil did not change among the precrops (Table 2). In addition, the soybean cultivar BRS 1010 IPRO shows high growth in height, and the reduction in growth after wheat was not limiting for grain yield since the plants still reached almost 1 m. Height reduction could reduce soybean yield in short genotypes. Soybean grown after wheat also had a lower first pod insertion height. In the 2016/17 cropping season, first pod insertion height (FPH) in soybean sown after wheat was close to 10 cm, which may cause losses during the harvesting (Kang et al., 2017).

Table 5. Plant height (PH) and first pod insertion height (FPH) of soybean plants as a function of precrops and N fertilization at sowing.

Treatments	2016/17		2017/18	
	PH cm	FPH cm	PH cm	FPH cm
Urochloa ruziziensis	106.4 ab	13.0 ab	118.9 ab	18.2 a
Crotalaria spectabilis	106.8 ab	14.0 ab	121.2 a	14.8 b
Corn 0 N	109.8 ab	17.8 a	112.1 b	16.3 ab
Corn 80 N	118.7 a	14.9 ab	116.6 ab	14.9 b
Fallow	110.2 ab	13.3 ab	112.9 b	14.8 b
Wheat	98.4 b	10.4 b	112.3 b	15.1 b
CV (%)	10.1	33.5	4.6	11.7
Soybean 0 N	107.9 ^{ns}	12.9 ^{ns}	113.2 b	15.8 ^{ns}
Soybean 30 N	108.9	14.9	119.1 a	15.6
CV (%)	7.9	46.7	4.2	13.0
Interaction	ns	ns	Ns	ns

Means followed by the same letters do not differ one another by the Tukey's test at 5% significance. Corn 0 N = corn without topdressing N fertilization. Corn 80 N = corn with 80 kg ha^{-1} of N as urea at topdressing. ns = not significant.

In the 2017/18 cropping season, N fertilization at soybean sowing increased plant height compared with no N fertilization, unlike the 2016/17 cropping season (Table 5), indicating that the effects of mineral N at crop sowing on plant height are variable among seasons. However, mineral N application did not change the first pod insertion height in both cropping seasons. In Brazil, some farmers have used 20 to 30 kg ha^{-1} of N at sowing in areas with uneven surface to increase the FPH and, consequently, reduce harvest losses. However, the results presented here demonstrated no effect of N fertilizer on FPH of soybean, as also previously verified (Silva et al., 2011; Werner et al., 2016), in addition to reduction in nodulation (Saturno et al., 2017).

Grain yield and oil and protein concentrations

In the 2016/17 cropping season, grain yield, oil and protein concentrations, and oil and protein productivities were not influenced by precrops (Table 6). On the other hand, in the 2017/18 season, grain yield, and oil and protein productivities were higher in soybean grown after *Urochloa ruziziensis* compared with sown after corn, irrespectively of N at topdressing, and fallow, not differing from sowing after *Crotalaria spectabilis* and wheat. Because the area had been managed under a well-conducted no-tillage system before the experiment was set up, the effect of precrops was observed only in the second year as an accumulated effect. These results highlight the increase of soybean yield grown after cover crops like *Urochloa ruziziensis* and *Crotalaria spectabilis*, or even wheat, in comparison to corn, which predominates in grain production systems in Brazil. The benefits of cover crops are cumulative over time. The long-term use of winter cover crops can improve soil water dynamics and physical properties (Basche et al., 2016; De Moraes et al., 2016; Blanco-Canqui & Jasa, 2019). In addition, cover crops increase nutrient cycling (Crusciol, Nascente, Borghi, Soratto, & Martins, 2015) and improve the soil microbiome (Kim, Zabaloy, Guan, & Villamil, 2020). On the other hand, continuous winter fallow reduces soil health in the NT (Balbinot Jr. et al., 2011).

In both seasons, N fertilization at soybean sowing had no effect on grain yield, oil and protein concentrations, and oil and protein productivity (Table 6). The higher initial growth of soybean due to mineral N did not increase grain yield. These results strengthen again that mineral N fertilization is unnecessary for soybean, increasing costs without benefits to the crop.

N fertilization at soybean sowing was not effective, regardless of the cover crop, as also observed Werner et al. (2016) and Franchini, Balbinot Jr., Debiasi, and Conte (2015b). In addition, N fertilizer can negatively affect nodulation, thus reducing grain yield (Kaschuk, Nogueira, Luca, & Hungria, 2016; Saturno et al., 2017). Consequently, soybean inoculation with elite *Bradyrhizobium* spp. strains, in addition to N mineralized form the soil organic matter, is enough to meet the soybean requirements for N, even for high yields (Mugendi et al., 2010; Zilli et al., 2010; Hungria & Mendes, 2015; Mourtzinis et al., 2018).

Table 6. Grain yield (YLD), oil concentration (OIL), oil productivity (OPD), protein concentration (PRT), and protein productivity (PPR) of soybean grains sown after precrops and N fertilization at sowing.

Treatments	2016/17					2017/18				
	YLD	OIL	OPD	PRT	PPR	YLD	OIL	OPD	PRT	PPR
	kg ha^{-1}	%	kg ha^{-1}	%	kg ha^{-1}	kg ha^{-1}	%	kg ha^{-1}	%	kg ha^{-1}
Urochloa ruziziensis	4,499 ^{ns}	22.0 ^{ns}	993 ^{ns}	35.7 ^{ns}	1,608 ^{ns}	5,171 a	23.6 ^{ns}	1,218 a	33.4 ^{ns}	1,726 a
Crotalaria spectabilis	4,321	21.5	930	35.7	1,545	4,639 abc	23.1	1,072 ab	34.2	1,587 ab
Corn 0 N	4,223	21.3	899	35.9	1,516	4,346 c	22.9	998 b	33.8	1,468 b
Corn 80 N	4,345	21.4	930	36.2	1,571	4,430 bc	22.9	1,014 b	34.3	1,522 b
Fallow	4,476	21.9	981	35.2	1,576	4,467 bc	23.1	1,035 b	33.7	1,503 b
Wheat	4,560	21.4	980	35.5	1,617	5,047 ab	22.9	1,159 ab	34.1	1,720 a
CV (%)	6.5	4.5	7.2	2.5	6.9	10.4	4.1	12.0	3.9	9.5
Soybean 0 N	4,379 ^{ns}	21.6 ^{ns}	947 ^{ns}	35.9 ^{ns}	1,572 ^{ns}	4,626 ^{ns}	22.9 ^{ns}	1,064 ^{ns}	33.9 ^{ns}	1,570 ^{ns}
Soybean 30 N	4,429	21.6	958	35.5	1,572	4,740	23.2	1,101	33.9	1,605
CV (%)	8.1	4.1	10.4	2.4	8.1	8.0	4.1	7.7	3.7	9.8
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Means followed by the same letters do not differ from one another by the Tukey's test at 5% significance. Corn 0 N = corn without topdressing N fertilization. Corn 80 N = corn with 80 kg ha^{-1} of N as urea at topdressing. ns = not significant.

Yield components

No interaction was observed between precrops and N fertilization in soybean for all yield components (Table 7). In 2016/17, thousand-grain weight was higher in soybean grown after corn receiving N at topdressing in relation to *Crotalaria spectabilis*, corn without N, and wheat, although this difference had no impact on grain yield (Table 6). In 2017/18, soybean grown after *Urochloa ruziziensis* presented higher number of pods per plant than plants grown after corn without N, which partially explains the higher grain yield.

Mineral N fertilization in soybean did not affect any yield component (Table 7), reinforcing the no need of N fertilization in soybean (Mourtzinis et al., 2018).

Table 7. Pod per plant (POD), number of grains per pod (GPP), and thousand-grain weight (TGW) of soybean sown after precrops and Nfertilization at sowing.

Treatments	2016/17			2017/18		
	POD	GPP	TGW	POD	GPP	TGW
	un. pl^{-1}	un. pod^{-1}	g	un. pl^{-1}	un. pod^{-1}	g
Urochloa ruziziensis	46.7 ^{ns}	2.3 ^{ns}	148.0 ab	60.8 a	2.1 ^{ns}	153.5 ^{ns}
Crotalaria spectabilis	42.3	2.2	139.0 b	59.7 ab	2.1	143.4
Corn 0 N	44.0	2.4	139.2 b	54.6 b	2.1	141.1
Corn 80 N	41.1	2.1	167.2 a	59.3 ab	2.2	151.4
Fallow	41.9	2.3	145.0 ab	62.1 a	2.1	146.7
Wheat	51.4	2.2	137.5 b	59.0 ab	2.2	155.4
CV (%)	17.6	10.8	12.6	10.9	6.9	7.9
Soybean 0 N	44.5 ^{ns}	2.3 ^{ns}	144.1 ^{ns}	57.9 ^{ns}	2.1 ^{ns}	145.7 ^{ns}
Soybean 30 N	44.6	2.1	147.8	60.6	2.2	151.5
CV (%)	19.8	13.6	9.4	17.6	5.8	7.7
Interaction	ns	ns	ns	ns	Ns	ns

Means followed by the same letters do not differ one another by the Tukey's test at 5% significance. Corn 0 N = corn without topdressing N fertilization. Corn 80 N = corn with 80 kg ha^{-1} of N as urea at topdressing. ns = not significant.

Conclusion

Ruzigrass (*Urochloa ruziziensis*) as a cover crop in soybean precrops is an option in tropical regions of Brazil, increasing the yield of soybean grown in succession compared to corn and fallow. Despite increases in the initial plant growth, N fertilization (30 kg ha^{-1} of N at sowing) impairs nodulation and does not increase grain yield and should not be used in soybean. Precrops and N fertilization at sowing do not affect oil and protein concentrations in soybean grains.

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References

APHA, AWWA, & WEF. (2006). *Standard methods for the examination of water and wastewater* (21st ed.). Washington, DC: American Public Health Association.

Balbinot Jr., A. A., Franchini, J. C., Debiasi, H., & Yokoyama, A. H. (2017). Contribution of roots and shoots of *Brachiaria* species to soybean performance in succession. *Pesquisa Agropecuária Brasileira*, *52*(8), 592-598. DOI: https://doi.org/10.1590/s0100-204x2017000800004

Balbinot Jr., A. A., Moraes, A., Pelissari, A., Dieckow, J., & Veiga, M. (2008). Formas de uso do solo no inverno e sua relação com a infestação de plantas daninhas em milho (*Zea mays*) cultivado em sucessão. *Planta Daninha*, *26*(3), 569-576. DOI: https://doi.org/10.1590/S0100-83582008000300012

Balbinot Jr., A. A., Santos, J. C. F. D., Debiasi, H., Coelho, A. E., Sapucay, M. J. L. D. C., Bratti, F., & Locatelli, J. L. (2020). Performance of soybean grown in succession to black oat and wheat. *Pesquisa Agropecuária Brasileira*, *55*, 1-9. DOI: https://doi.org/10.1590/S1678-3921.pab2020.v55.01654

Balbinot Jr., A. A., Veiga, M. D., Moraes, A. D., Pelissari, A., Mafra, A. L., & Picolla, C. D. (2011). Winter pasture and cover crops and their effects on soil and summer grain crops. *Pesquisa Agropecuária Brasileira*, *46*(10), 1357-1363. DOI: https://doi.org/10.1590/S0100-204X2011001000032

Basche, A. D., Kaspar, T. C., Archontoulis, S. V., Jaynes, D. B., Sauer, T. J., Parkin, T. B., & Miguez, F. E. (2016). Soil water improvements with the long-term use of a winter rye cover crop. *Agricultural Water Management*, *172*, 40-50. DOI: https://doi.org/10.1016/j.agwat.2016.04.006

Blanco-Canqui, H., & Jasa, P. J. (2019). Do grass and legume cover crops improve soil properties in the long term?. *Soil Science Society of America Journal*, *83*(4), 1181-1187. DOI: https://doi.org/10.2136/sssaj2019.02.0055

Carvalho, W. P., Carvalho, G. J., Neto, D. O. A., & Teixeira, L. G. V. (2013). Desempenho agronômico de plantas de cobertura usadas na proteção do solo no período de pousio. *Pesquisa Agropecuária Brasileira*, *48*(2), 157-166. DOI: https://doi.org/10.1590/S0100-204X2013000200005

Cerezini, P., Fagotti, D. S. L., Pipolo, A. E., Hungria, M., & Nogueira, M. A. (2017). Water restriction and physiological traits in soybean genotypes contrasting for nitrogen fixation drought tolerance. *Scientia Agricola*, *74*(2), 110-117. DOI: https://doi.org/10.1590/1678-992x-2016-0462

CONAB. (2019a). *Portal de informações: Importação/Exportação por País*. Brasília, DF: CONAB. Retrieved on Feb. 11, 2019 from https://portaldeinformacoes.conab.gov.br/index.php/comercio-exterior-por-pais

CONAB. (2019b). *Série Histórica da Área Plantada, produtividade e Produção: Milho 2ª Safra*. Brasília, DF: CONAB. Retrieved on Feb. 11, 2019 from https://www.conab.gov.br/info-agro/safras/serie-historica-das-safras

CONAB. (2020). *Série Histórica da Área Plantada, produtividade e Produção: Soja*. Brasília, DF: CONAB. Retrieved on Apr., 27, 2020 from https://www.conab.gov.br/info-agro/safras/serie-historica-das-safras

Crusciol, C. A., Nascente, A. S., Borghi, E., Soratto, R. P., & Martins, P. O. (2015). Improving soil fertility and crop yield in a tropical region with palisadegrass cover crops. *Agronomy Journal*, *107*(6), 2271-2280. DOI: https://doi.org/10.2134/agronj14.0603

De Moraes, M. T., Debiasi, H., Carlesso, R., Franchini, J. C., Silva, V. R., & da Luz, F. B. (2016). Soil physical quality on tillage and cropping systems after two decades in the subtropical region of Brazil. *Soil and Tillage Research*, *155*, 351-362. DOI: https://doi.org/10.1016/j.still.2015.07.015

Denardin, J. E., Kochhann, R. A., Faganello, A., & Cogo, N. P. (2014). Agricultura conservacionista no Brasil: uma análise do conceito a adoção. In L. F. C. Leite, G. A. Maciel, & A. S. F. Araújo (Eds.), *Agricultura conservacionista no Brasil* (p. 23-41). Brasília, DF: Embrapa.

De Sousa, D. C., Medeiros, J. C., Lacerda, J. J. J., Rosa, J. D., Boechat, C. L., De Sousa, M. N. G., ... Mafra, Á. L. (2019). Dry mass accumulation, nutrients and decomposition of cover plants. *Journal of Agricultural Science*, *11*(5), 152-160. DOI: https://doi.org/10.5539/jas.v11n5p152

Empresa Brasileira de Pesquisa Agropecuária [Embrapa]. (2013). *Tecnologias de produção de soja - Região Central do Brasil 2014* (Sistemas de Produção, 16). Londrina, PR: Embrapa Soja.

Franchini, J. C., Debiasi, H., Balbinot Jr., A. A., Tonon, B. C., Farias, J. R. B., Oliveira, M. C. N., & Torres, E. (2012). Evolution of crop yields in different tillage and cropping systems over two decades in southern Brazil. *Field Crops Research*, *137*, 178-185. DOI: https://doi.org/10.1016/j.fcr.2012.09.003

Franchini, J. C., Balbinot Jr., A. A., Debiasi, H., & Conte, O. (2015a). Desempenho da soja em consequência de manejo de pastagem, época de dessecação e adubação nitrogenada. *Pesquisa Agropecuária Brasileira*, *50*(12), 1131-1138. DOI: https://doi.org/10.1590/S0100-204X2015001200002

Franchini, J. C., Balbinot Jr., A. A., Debiasi, H., & Conte, O. (2015b). Crescimento da soja influenciado pela adubação nitrogenada na cultura, pressão de pastejo e épocas de dessecação de *Urochloa ruziziensis*. *Revista Agroambiente On-line*, *9*(2), 129-135. DOI: https://doi.org/10.18227/1982-8470ragro.v9i2.2611

Heil, C. (2010). *Rapid, multi-component analysis of soybeans by FT-NIR Spectroscopy*. Madison, US: Thermo Fisher Scientific.

Hungria, M., & Araujo, R. S. (1994). *Manual de métodos empregados em estudos de microbiologia agrícola*. Brasília, DF: Embrapa-Serviço de Produção e Informação.

Hungria, M., Franchini, J. C., Campo, R. J., Crispino, C. C., Moraes, J. Z., Sibaldelli, R. N. R., ... Arihara, J. (2006). Nitrogen nutrition of soybean in Brazil: contributions of biological N_2 fixation and N fertilizer to grain yield. *Canadian Journal of Plant Science*, *86*(4), 927-939. DOI: https://doi.org/10.4141/P05-098.

Hungria, M., & Mendes, I. C. (2015). Nitrogen fixation with soybean: the perfect symbiosis? In F. De Bruijn (Ed.), *Biological nitrogen fixation* (p. 1009-1024). Hoboken, NJ: Wiley-Blackwell. DOI: https://doi.org/10.1002/9781119053095.ch99

Instituto Brasileiro de Geografia e Estatística [IBGE]. (2019). *Levantamento sistemático da produção agrícola*. Brasília, DF: IBGE. Retrieved on Feb. 10, 2019 from https://www.sidra.ibge.gov.br/tabela/1618

Kaschuk, G., Nogueira, M. A., Luca, M. J., & Hungria, M. (2016). Response of determinate and indeterminate soybean cultivars to basal and topdressing N fertilization compared to sole inoculation with *Bradyrhizobium*. *Field Crops Research*, *195*, 21-27. DOI: https://doi.org/10.1016/j.fcr.2016.05.010

Kang, B. K., Kim, H. T., Choi, M. S., Koo, S. C., Seo, J. H., Kim, H. S., ... Lee, J. D. (2017). Genetic and environmental variation of first pod height in soybean [*Glycine max* (L.) Merr.]. *Plant Breeding and Biotechnology*, *5*(1), 36-44. DOI: https://doi.org/10.9787/PBB.2017.5.1.36

Kim, N., Zabaloy, M. C., Guan, K., & Villamil, M. B. (2020). Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biology and Biochemistry*, *142*, 1-14. DOI: https://doi.org/10.1016/j.soilbio.2019.107701

Loss, A., Pereira, M. G., Giácomo, S. G., Perin, A., & Anjos, L. H. C. (2011). Agregação, carbono e nitrogênio em agregados do solo sob plantio direto com integração lavoura pecuária. *Pesquisa Agropecuária Brasileira*, *46*(10), 1269-1276. DOI: https://doi.org/10.1590/S0100-204X2011001000022

Moro, E., Crusciol, C. A. C., Nascente, A. S., & Cantarella, H. (2013). Teor de nitrogênio inorgânico no solo em função de plantas de cobertura, fontes de nitrogênio e inibidor de nitrificação. *Pesquisa Agropecuária Tropical*, *43*(4), 424-435. DOI: https://doi.org/10.1590/S1983-40632013000400003

Mourtzinis, S., Kaur, G., Orlowski, J. M., Shapiro, C. A., Lee, C. D., Wortmann, C., ... Ross, W. J. (2018). Soybean response to nitrogen application across the United States: A synthesis-analysis. *Field Crops Research*, *215*, 74-82. DOI: https://doi.org/10.1016/j.fcr.2017.09.035

Mugendi, E., Gitonga, N., Cheruiyot, R., & Maingi, J. (2010). Biological nitrogen fixation by promiscuous soybean (Glycine max L. Merril) in the central highlands of Kenya: response to inorganic fertilizer soil amendments. *World Journal of Agricultural Sciences*, *6*(4), 381-387.

Nishida, H., & Suzaki, T. (2018). Nitrate-mediated control of root nodule symbiosis. *Current Opinion in Plant Biology*, *44*, 129-136. DOI: https://doi.org/10.1016/j.pbi.2018.04.006

Nogueira, M. A., Telles, T. S., Fagotti, D. S. L., Brito, O. R., Prete, C. E. C., & Guimarães, M. F. (2014). Indicators of soil quality in the implantation of no-till system with winter crops. *Revista Ciência Agronômica*, *45*(5), 990-998. DOI: https://doi.org/10.1590/S1806-66902014000500014

Pavinato, O. S., Ceretta, C. A., Girotto, E., & Moreira, I. C. L. (2008). Nitrogênio e potássio em milho irrigado: análise técnica e econômica da fertilização. *Ciência Rural*, *38*(2), 358-364. DOI: https://doi.org/10.1590/S0103-84782008000200010

Santos, C. A., Panchoni, L. C., Bini, D., Kuwano, B. H., Carmo, K. B., Silva, S. M. C. P., ... Nogueira, M. A. (2013). Land application of municipal landfill leachate: Fate of ions and ammonia volatilization. *Journal of Environmental Quality*, *42*, 523-531. DOI: https://doi.org/10.2134/jeq2012.0170

Saturno, D. F., Cerezini, P., Moreira, P. D. S., Oliveira, A. B. D., Oliveira, M. C. N. D., Hungria, M., & Nogueira, M. A. (2017). Mineral nitrogen impairs the biological nitrogen fixation in soybean of determinate and indeterminate growth types. *Journal of Plant Nutrition*, *40*(12), 1690-1701. DOI: https://doi.org/10.1080/01904167.2017.1310890

Searle, P. L. (1984). The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. A review. *Analyst*, *109*(5), 549-568. DOI: https://doi.org/10.1039/AN9840900549

Silva, A. F., Carvalho, M. A. C., Schoninger, E. L., Monteiro, S., Caione, G., & Santos, P.A. (2011). Doses de inoculante e nitrogênio na semeadura da soja em área de primeiro cultivo. *Bioscience Journal*, *27*(3), 404-412. DOI: https://doi.org/200.19.146.79/index.php/biosciencejournal/article/view/8067

Smith, R. G., Gross, K. L., & Robertson, G. P. (2008). Effects of crop diversity on agroecosystem function: crop yield response. *Ecosystems*, *11*(3), 355-366. DOI: https://doi.org/10.1007/s10021-008-9124-5

Tanaka, K. S., Crusciol, C. A. C., Soratto, R. P., Momesso, L., Costa, C. H. M., Franzluebbers, A. J., ... Calonego, J. C. (2019). Nutrients released by Urochloa cover crops prior to soybean. *Nutrient Cycling in Agroecosystems*, *113*(3), 267-281. DOI: https://doi.org/10.1007/s10705-019-09980-5

Vogels, G. D., & Drift, C. V. D. (1970). Differential analyses of glyoxylate derivatives. *Analytical Biochemistry*, *33*(1), 143-157. DOI: https://doi.org/10.1016/0003-2697(70)90448-3

Werner, F., Balbinot Jr., A. A., Ferreira, A. S., Debiasi, H., & Franchini, J. C. (2016). Soybean growth affected by seeding rate and mineral nitrogen. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *20*(8), 734-738. DOI: https://doi.org/10.1590/1807-1929/agriambi.v20n8p734-738

Zilli, J. E., Campo, R. J., & Hungria, M. (2010). Eficácia da inoculação de *Bradyrhizobium* em pré-semeadura da soja. *Pesquisa Agropecuária Brasileira*, *45*(3), 335-338. DOI: https://doi.org/10.1590/S0100-204X2010000300015