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RESEARCH PAPER

Promoting black oat and ryegrass growth via *Azospirillum brasilense* inoculation after corn and soybean crop rotation

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Abstract

Inadequate management of nitrogen fertilization, due to high costs, compromises the nutrition and productivity of winter forage plants, highlighting the need to adopt sustainable alternatives, such as using nitrogen-fixing bacteria. This research evaluated the growth promotion of black oat (*Avena strigosa*) and ryegrass (*Lolium multiflorum*) plants inoculated with *Azospirillum brasilense* rhizobacteria. The experiments were carried out over two consecutive harvests in Santa Maria, Brazil. The sowing density was 300 viable seeds per square meter for black oats and 100 viable seeds per square meter for ryegrass. A dose of 5 ml per kg of seeds from the consortium was used as an inoculant. Following sowing, the seeds were incorporated into the soil through light harrowing. At the beginning of tillering, 50 kg of N ha^{-1} was applied to create treatments with and without nitrogen. Plant emergence, plant height, number of leaves and tillers were evaluated. In the first year, an inoculation response was observed for the number of black oat plants emerged m^{-2}. In the second year (corn residue), an inoculation response was observed for the number of leaves and height of black oats. Under soybean straw for oat leaf number and ryegrass tiller number. The current research highlights the effectiveness of inoculation with *Azospirillum brasilense* in promoting significant improvements in the morphological components of forage crops. Following corn, an increase in the number of leaves and tillers was observed, as well as in the height of black oats. Similarly, following soybeans, there was an increase in the number of black oat tillers and the number of ryegrass leaves. The results emphasize the importance of inoculation as an effective practice to enhance crop development and performance in agricultural systems, improving sustainability and productivity under soybean and corn residues.

Keywords

Biological nitrogen fixation, diazotrophic bacteria, sustainability, *Avena strigosa* S., *Lolium multiflorum* L.

Introduction

Sustainable food production challenges involve increasing productivity with less environmental impact (Garrett et al. 2017)and increasing anthropogenic contributions to climate change (IPCC 2014). The soybean cultivated area in Brazil is 43 million hectares. Of this total, in the last two decades, an area of approximately 15 million hectares has been used in succession (winter) with black oat (*Avena strigosa* S.), in intercropping or not, with ryegrass (*Lolium multiflorum* Lam.) (Conab 2019). Each species provides a significant amount of green biomass throughout the cultivation cycle. Black oats are chosen for their earliness. Ryegrass stands out due to its ease of natural reseeding and its production of high-quality forage in substantial volumes, even reaching peak production in advanced stages (Mittelman et al. 2010).

In the integrated crop and livestock production system (ILP), the rapid establishment of forage species plays a crucial role, enabling the anticipation of the entry of animals and minimizing the forage shortage, in addition to avoiding the lack of soil cover. Producers in this field face various challenges, including integrated pasture and pesticide residue management, pest and disease control, animal waste management, nutrient cycling and export, forage planning, land use adaptation, and high production costs associated with nitrogen fertilization. Nitrogen fertilizer is commonly used to increase forage production. However, its indiscriminate use of nitrogen decreases soil quality and productivity (Zou et al. 2018) and increases production costs and adverse environmental impacts (Espinoza et al. 2020). Nitrogen loss causes ecological and environmental problems through nutrient loss, emission of greenhouse gases, groundwater contamination by nitrates, and the eutrophication of surface waters (Huang et al. 2014; Smith and Siciliano 2015). In this sense, efficient nitrogen fertilization management will increase forage productivity and mitigate adverse economic and environmental impacts.

Furthermore, sustainability can be achieved by understanding interactions between symbiotic bacterial communities (Ali et al. 2019). Therefore, searching for sustainable and economically viable alternatives becomes essential to optimize this system's productivity and efficiency while mitigating the risks associated with conventional fertilization. A feasible alternative to optimize the cultivation of plants from the Poaceae family (wheat and corn) and reduce dependence on nitrogen fertilization is the inoculation of seeds with bacteria of the species *Azospirillum brasilense*, which are considered plant growth promoters (PGPR) (Munareto et al. 2019; Müller et al. 2021; Lima et al. 2022). Furthermore, these bacteria can increase soil fertility by fixing atmospheric nitrogen (Day and Döbereiner 1976; Ali et al. 2023; Mehmood et al. 2023). Moreover, these bacteria also produce auxins, cytokinins, ethylene and gibberellins (Cassán and Zorita 2016), which increase the number of root hairs and soil exploitation.

However, most studies conducted on *Azospirillum brasilense* inoculation focus predominantly on crops aimed at grain production. Research involving the intercropping of oats and ryegrass and alternatives to mitigate problems with nitrogen losses and using *Azospirillum brasilense* are still limited (Brum et al. 2021). However, studies that permeate crop-livestock integration from a sustainability perspective are essential for more efficient agriculture.

This research evaluated the growth promotion of black oat (*Avena strigosa*) and ryegrass (*Lolium multiflorum*) plants inoculated with *Azospirillum brasilense* rhizobacteria.

Materials and methods

Experimental area

The experiments were conducted in two agricultural seasons (2012 and 2013), in Santa Maria, Brazil, at geographic coordinates 29°41'52.53"S, 54°02'34.29"W, altitude of 195 meters. The area used was approximately 0.34 hectares, in an ILP system. The soil had the following chemical attributes: pH (CaCl2) = 4.10; M.O. (g dm^{-3}) = 21.44; P (mg dm^{-3}) = 1.79; K (cmolc dm^{-3}) = 0.80; Cu (mg dm^{-3}) = 1.19; Fe (mg dm^{-3}) = 175.78; Zn (mg dm^{-3}) = 1.36; Mn (mg dm^{-3}) = 118.23; Al^{3+} (cmolc dm^{-3}) = 0.96; H+Al (cmolc dm^{-3}) = 6.21; Ca (cmolc dm^{-3}) = 1.82; Mg (cmolc dm^{-3}) = 1.13; SMP Index = 5.70; SB (cmolc dm^{-3}) = 3.75; V (%) = 37.65; Sat. Al (%) = 20.38. According to the Köppen classification, the predominant climate is humid tropical (Cfa) (Alvares et al. 2013). Fig. 1 represents the water balance of the experiment site in the two years of conduction.

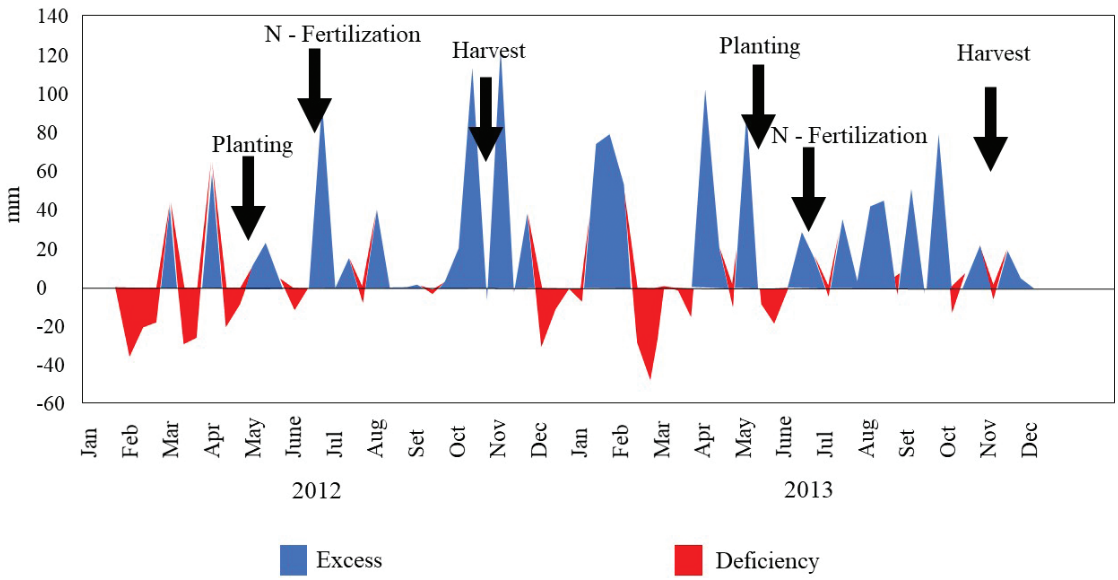

Figure 1. Water balance in 2012 and 2013. Modified from Rolim et al. (1998).

Experimental management

In both years, planting was carried out on May 17th, aiming to obtain a population composed of 300 plants m^{-2} of black oat (*Avena strigosa*) and 100 plants m^{-2} of ryegrass (*Lolium multiflorum*). Sowing was carried out by broadcast, incorporating the seeds into the soil using a harrow with discs, so the seeds were 3 centimeters deep. The black oat cultivar was "IAPAR 61" and the ryegrass cultivar was "BRS Ponteio".

Experimental design

In 2012 (first experiment), treatments were distributed in a three-factorial design (2 × 2 × 5). The first factor was inoculation (with and without), the second was nitrogen doses (0 and 50 kg ha^{-1}), and the third was pasture management. Inoculation with *Azospirilum brasilense* was carried out shortly before sowing at a dose of five ml of inoculant containing 2.0×10^{8} colony-forming units ml^{-1} for each kg of seeds in the consortium. The second factor was the management of nitrogen fertilization, which consisted of applying 0 and 50 kg ha^{-1} of nitrogen in the form of urea (45% nitrogen). Nitrogen was used at the beginning of tillering. The third factor was pasture management, where no grazing (SP); grazing with continuous stocking, without height control (PC); and intermittent grazing carried out every 21 days, leaving a residual height of 10 cm at the exit of the animals (A10); pasture height of 20 cm at the animals' exit (A20) and pasture height of 30 cm at the animals' exit from (A30). In the 2013 experiment (second experiment), two factors were added: the first was the influence of the no grazing (SP) and continuous grazing (PC) treatments carried out in 2012; the second was straw from two summer crops (soybeans and corn). The experimental units were isolated with an electric fence. The animals used for grazing were "Corriedale sheep". The experimental design was randomized blocks with sub-sub-divided plots and three replications. Each experimental unit had dimensions of 8 × 4 m, totaling an area of 32 m^2.

Evaluated variables

To evaluate the establishment of black oat and ryegrass plants in the consortium, the number of emerged plants m^{-2}, height, total number of leaves and tillers were counted. In 2012, the number of plants emerged was recorded on the dates of: May 29, 2012; June 4, 2012; June 11, 2012; June 19, 2012; June 27, 2012 and July 2, 2013. On June 26, 2012, the first nitrogen application was carried out. From this, the treatments also differed by the nitrogen application factor, going from two to four treatments: (1) without inoculation + 0 kg of N ha^{-1}, (2) without inoculation + 50 kg of N ha^{-1}, (3) with inoculation + 0 kg N ha^{-1} and (4) with inoculation + 50 kg N ha^{-1}. On July 2, 2012, five plants per plot of each species in the consortium were marked. Subsequently, the marked plants' height, number of leaves and tillers began to be measured until the animals entered for grazing, according to the methodology described by Costa et al. (2012). The number of tillers reflects the total number of culms on each marked plant. These evaluations were carried out on the following dates: July 12, 2012; July 18, 2012; July 25, 2012 and August 3, 2012.

The evaluation procedure in 2013 was the same, but there were changes regarding treatments. In the first year after the last grazing, carried out in October, the consortium was desiccated, using five L ha^{-1} of Glyphosate-based herbicide, with subsequent sowing of soybean and corn crops in all experimental units. Therefore, in addition to the inoculation and nitrogen factor, there are also factors related to the predecessor crop and continuous grazing and no grazing in the previous winter. The number of emerged plants was counted on the following dates: May 22, 2013; May 29, 2013; June 06, 2013; and June 16, 2013. On June 28, 2013, the initial measurements of plant height, leaf number, and tillers were conducted. Following this assessment, nitrogen was applied. The second and final evaluation took place on August 6, 2013. For this evaluation, the factors evaluated then included the addition of nitrogen application or not.

Statistical analysis

The analysis of variance was carried out using the Soc statistical package (Embrapa 1997). When the F value was significant, a mean comparison test and breakdown of factors and regressions were carried out. The chosen test was Duncan, with a 5% probability of error and the regressions were analyzed up to the third degree, choosing the highest significant degree (Steel et al. 1997).

Results

First experiment

After 45 days from the beginning of emergence, the number of black oat and ryegrass plants was 323 and 65 on average. The number of established plants indicates that for black oast, the use of azospirillum brasilense significantly increased the number of plants by 10%. However, for ryegrass, this relationship was not significant (Table 1). The number of emerged plants for black oat is represented by the equation NP = -82.7709+9.9372*DAP (r^2 = 0.87) and

Table 1. Number of emerged plants m^{-2} of black oat and ryegrass 45 days after planting. First experiment.

Treatments	Black oats	Ryegrass
With inoculation	335.51 a*	66.84
Without inoculation	304.99 b	63.95
0 kg of N ha^{-1}	325.37	60.44
50 kg of N ha^{-1}	322.57	67.15
Mean	323.51	64.91
CV (%)	19.92	33.89

*averages followed by the master letter do not differ from each other using the F test at 5% error probability.

for ryegrass by the equation NP = -24.5141+2.7975*DAP (r^2 = 0.93) (DAP is days after planting).

As nitrogen was applied one day before the evaluation, the effect of nitrogen was not observed due to the short time the plants had to use it (Malavolta 2006). However, this evaluation shows the probable beginning of the association between the bacteria and black oat plants. In this evaluation, the number of inoculated m^{-2} emerged plants was greater than that of non-inoculated plants. Applying 50 kg ha^{-1} of nitrogen increased the number of leaves and tillers of black oats and ryegrass and the height of black oat plants, with no effect on the height of ryegrass plants (Fig. 2). In treatments under continuous grazing in 2012, a greater number of emerged plants m^{-2} was observed compared to treatments under a non-grazing system, whether inoculated or not.

treatments, no difference was observed. Where *Azospirillum brasilense* was applied, there was a tendency for an improvement in the number of plants (2012). Unlike what occurred in 2012 and what occurred under the corn straw, in 2013, the inoculation with *Azospirillum brasilense* showed a greater number of ryegrass plants emerged m^{-2}, under the soybean canopy.

The results presented in Fig. 3b–d may be linked to the type of predecessor cultivation. When ryegrass was grown under corn redidue, there was no such difference. Observing Fig. 3c it is clear that there was an effect only from inoculation. The grazing system used in 2012 also did not influence this variable for ryegrass. Through Fig. 3d it is observed that the grazing system used in 2012 did not influence the number of emerged plants m^{-2} of black oat, under soybean stubble. At the end of the evaluations, it was observed that

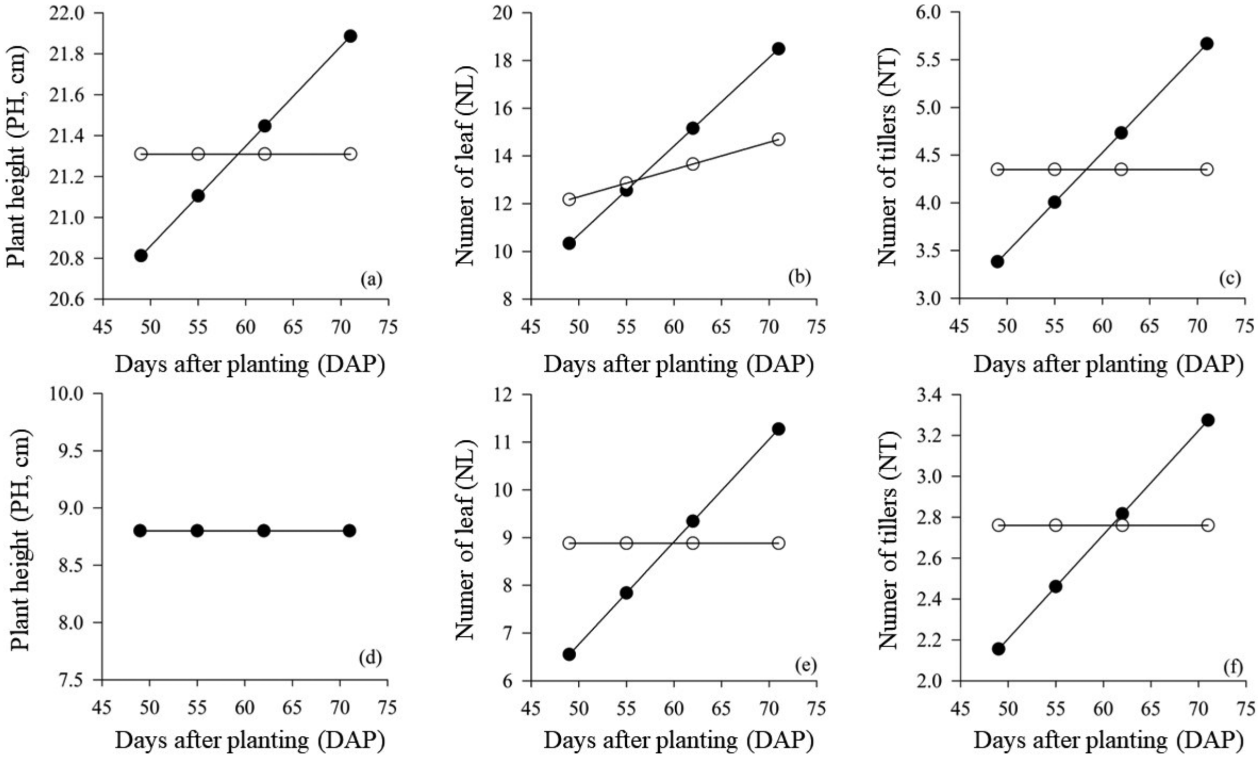

Figure 2. Nitrogen application on (**a**) plant height (PH), (**b**) total number of leaves (NL) and (c) total number of tillers (NT) of black oat and (**d**) plant height, (**e**) total number of leaves (NL) and (**f**) total number of tillers (NT) of ryegrass. Circle filled in black is 50 kg ha^{-1} of nitrogen. Unfilled circle is equivalent to 0 kg ha^{-1} of nitrogen. Regression equations for 50 kg of nitrogen ha^{-1} in black oats: PH = 18.4211+0.0488*DAP (r^2 = 0.80), NL = -7.8244+0.3706*DAP (r^2=0, 96) and NT = -1.7028+0.1037*DAP (r^2 = 0.96). Regression equations for 0 kg nitrogen ha^{-1} in black oats: PH = 21.31, NL = 6.5778+0.1142*DAP (r^2 = 0.83), NT = 4.35. Regression equations for 50 kg of nitrogen ha^{-1} in ryegrass: NF = -3.9715+0.2147*DAS (r^2 = 0.98) and NA = -0.3356+0.0508*DAS (r^2 = 0 ,94). Regression equations for 0 kg nitrogen ha^{-1} in ryegrass: NL = 8.88 and NT = 2.76. Ryegrass plant height (PH) = 8.80. First Experiment.

Second experiment

Inoculation with *Azospirillum brasilense* influenced the emergence and establishment of ryegrass plants (Fig. 3), both in grazing management and in the use of rhizobacteria. At the end of the evaluations, the number of ryegrass plants emerged was lower where there was no inoculation in a non-grazing system in 2012 (Fig. 3). For the other

the number of plants emerged, whether inoculated or not, was similar. Just as happened under corn stubble, no inoculation response was observed under soybean residue.

The use of *Azospirillum brasilense* was statistically superior for the number of black oat leaves under corn residue (Table 2). This fact indicates that the association between plant and bacteria occurred, but was influenced by the type of previous cultivation, and this fact may be

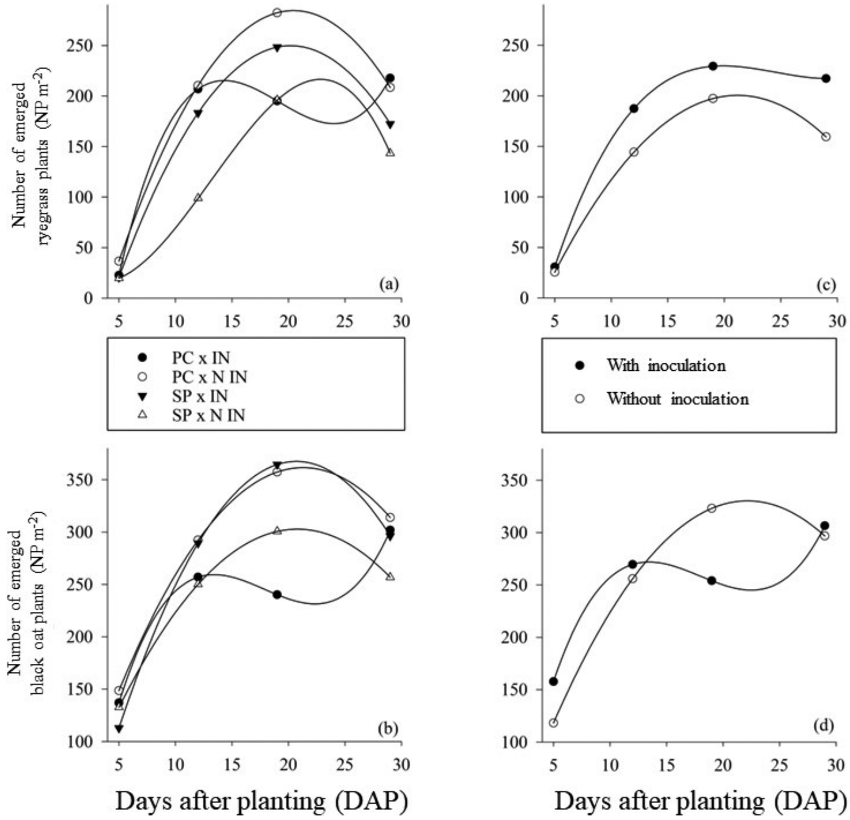

Figure 3. Interaction between inoculation and grazing system on the number of emerged plants m^{-2} of ryegrass under corn residue (**a**). PC × IN: NP = -122.6617+71.1405*DAP-4.2414*DAP^2+0.0790*DAP^3; r^2 = 0.99; PC X N IN: NP = -2.2457+34.1680*DAP-0.8024*DAP^2; r^2 = 0.96; SP × IN: NP = -75.2048+42.7721*DAP-1.0332*DAP^2; r^2 = 0.99; SP × N IN: NP = 7.7053+28.3871*DAP-0.6828*DAP^2; r^2 = 0.94. Interaction between inoculation and grazing system on the number of emerged plants m^{-2} of black oat under corn residue (**b**). PC × IN: NP = -334.7404+95.8796*DAP-5.3442*DAP^2+0.0929*DAP^3; r^2 = 0.99; PC X N IN: NP = -149.9134+42.4900*DAP-1.0390*DAP^2; r^2 = 0.98; SP × IN: NP = -154.9704+40.1075*DAP-0.9937*DAP^2; r^2 = 0.99; SP × N IN: NP = 36.9644-12.8310*DAP+2.1551*DAP^2-0.0547*DAP^3; r^2 = 0.99. Effect of inoculation on the number of emerged plants m^{-2} of ryegrass under soybean residue (**c**). Inoculated: NP = -186.8333+54.1696*DAP-2.2894*DAP^2+0.0310*DAP^3 r^2 = 0.99; Not inoculated: NP = -99.4109+28.3284*DAP-0.6691*DAP^2 r^2 = 0.99. Effect of inoculation on the number of emerged plants m^{-2} of black oat under soybean residue (d). Inoculated: NP = -83.0384+65.8779*DAP-3.9114*DAP^2+0.0725*DAP^3; r^2 = 0.99; Not inoculated: NP = -23.4922+31.9170*DAS-0.7198*DAP^2; r^2 = 0.98. Note: IN: inoculated; N IN: not inoculated; PC: continuous grazing; SP: no grazing. Second Experiment.

related to the C/N ratio of the remaining material. It was observed that under corn residue (Table 3), the height of inoculated black oat and ryegrass plants was higher than that of non-inoculated plants. Under soybean residue, there was a superior inoculation response to the number of black oat tillers and ryegrass leaves. Under corn residue, a significant effect of nitrogen was observed for plant height, number of leaves of black oats, plant height, number of leaves, and tillers of ryegrass. Under soybean stubble, a positive effect of nitrogen was observed on black oat plant height and plant height, number of leaves and tillers.

Discussions

The irregular emergence can be attributed to the way the seeds are incorporated into the soil (light harrowing). This generates different depth gradients in seed deposition and different soil-seed contact qualities. As there was no difference between treatments, it is inferred that the association between *Azospirillum brasilense* and plants was not captured by the statistical test. This non-uniformity may have interfered with the ability to express the emergence of plants regarding the use of Azospirillum brasilense.

Despite showing non-uniformity plants at the end of 45 days, the population was adequate. However, for up to 45 days, there was no difference between the application or non-application of nitrogen (Table 1). In a more uniform environment, it is possible to clearly observe the effect of the association with cereals, both on germination (Bertoncelli et al. 2023), root size and volume (Brum et al. 2021), and plant establishment (Brum et al. 2021), increased grain mass and productivity (Martin et al. 2022).

The greatest development of plants with the use of *Azospirillum brasilense* occurs due to the rapid interaction of bacteria with the plants, promoting hormone production and nitrogen fixation. This is due to the bacteria creating a gradient in the rhizosphere in which, closer to the root,

Table 2. Effects of inoculation with *Azospirillum brasilense* and grazing carried out in 2012 on plant height (PH), number of leaves (NL) and number of tillers (NT) of black oat and ryegrass grown under corn and soybean residue. Second experiment.

Treatments	Corn residue					
	Black oat			Ryegrass		
	PH	NL	NT	PH	NL	NT
With Inoculation	10.68	6.73 a*	1.77	6.02	3.83	2.66
No inoculation	10.57	5.10 b	1.41	5.44	3.06	2.17
Continuous Grazing	10.78	6.43 a	1.68 a	5.83	3.97	3.05 b
No Grazing	10.47	5.40 b	1.50 b	5.63	3.72	3.37 a
Média	10.63	5.91	1.59	5.73	3.85	3.21
CV (%)	13.23	21.22	27.69	16.3	22.46	38.74
Treatments	**Soybean residue**					
	Black oat			**Ryegrass**		
	PH	**NL**	**PH**	**NL**	**PH**	**NL**
With Inoculation	12.56	7.48	2.33	7.46	4.55	1.35
No inoculation	13.72	6.32	1.96	6.46	4.27	1.14
Continuous Grazing	13.68	7.36 a*	2.24	7.23 a	4.51	1.17
No Grazing	12.6	6.44 b	2.05	6.70 b	4.32	1.32
Mean	13.14	6.90	2.15	6.96	4.41	1.25
CV (%)	16.78	24.81	31.83	19.42	18.29	30.66

*averages followed by the master letter do not differ from each other using the F test at 5% error probability.

Table 3. Effect of inoculation with *Azospirillum brasilense*, grazing carried out in 2012 and nitrogen application on plant height (Ph), number of leaves (NL) and number of tillers of black oat and ryegrass grown under corn and soybean residue. Second experiment.

Treatments	Residue Corn					
	Black oat			Ryegrass		
	PH	NL	NT	NL	PH	NT
With inoculation	22.47 a*	10.96	3.80	13.11 a	8.86	2.46
Without inoculation	20.85 b	9.13	3.07	12.18 b	8.03	2.18
Continuous Grazing	23.15	10.88	3.63	14.26	9.52	2.69
No Grazing	20.16	9.21	3.24	11.03	7.37	1.96
50 kg ha^{-1} of nitrogen	23.22 a	10.63 a	3.66	14.26 a	9.52 a	2.64 a
0 kg ha^{-1} of nitrogen	18.10 b	8.72 b	2.92	8.99 b	6.00 b	1.61 b
Mean	21.66	10.05	3.43	12.65	8.45	2.32
CV (%)	11.82	39.61	39.95	24.8	48.2	59.64
Treatments	**Soybean residue**					
	Black oat			**Ryegrass**		
	PH	**NL**	**NT**	**NL**	**PH**	**NT**
With inoculation	34.27	10.3	3.62 a	23.41	10.31 a	2.75
Without inoculation	37.83	9.41	3.22 b	24.37	9.20 b	2.34
Continuous Grazing	37.05	10.76	3.8	23.97	11.88	2.87
No Grazing	35.06	8.94	3.04	20.82	8.42	2.22
50 kg ha^{-1} of nitrogen	38.47 a*	10.20	3.57	24.76 a	10.85 a	2.84
0 kg ha^{-1} of nitrogen	30.57 b	9.07	3.07	17.00 b	7.25 b	1.87
Mean	36.05	9.85	3.42	22.39	9.75	2.55
CV (%)	18.73	37.80	35.06	29.64	69.27	80.42

* averages followed by the master letter do not differ from each other using the F test at 5% error probability.

there is a greater concentration of inoculated bacteria. While, at greater distances there is a greater diversity of microorganisms (Wang et al. 2022). The application of nitrogen did not interfere with the number of emerged plants, as expected, since nitrogen fertilization was carried out under cover (Table 1).

The response to inoculation varies between species. This variation occurs because there is specificity in the interaction between plants and microorganisms (Morais et al. 2012). Another factor to be considered are soil and climate conditions such as pH, temperature, humidity, climatic variations, which modify the efficiency of the association (Martin et al. 2023). In this sense, it appears that for some variables the results between species did not follow the same response pattern, when for some it was significant and for others it was not (Tables 2, 3).

When one wishes to study the effects of nitrogen-fixing bacteria, it is necessary to understand the impact of mineral nitrogen on plant physiology. Ammonium (NH4+) originating from mineral fertilizer formulations is preferentially absorbed by plants in relation to nitrate (NO3-). This ammonium in high concentration reduces the activity of the bacteria's nitrogenase enzyme complex, causing a reduction in the rate of FBN (Carvalho et al. 2016). In this sense, because *Azospirillum brasilense* is a multifunctional rhizobacteria, one can consider the fact that the main function established in this research was to promote growth. The nitrogen fixation process is included in this functionality (Tables 1–3, Figs 1–3).

Generally, the maximum number of plants is reached between 15 and 25 DAP (Fig. 3). Regarding the use of inoculation with *Azospirillum brasilense*, it is generally observed that when this rhizobacteria is applied, the results are superior to not applying it. This reinforces what was mentioned by authors such as Morais et al. (2012) and Müller et al. (2021), in which the plant-bacteria association is dependent on a complex specificity between them.

Soybean plants form symbiosis with bacteria of the *Bradyrhizobium* genus. The result of symbiosis is the formation of NH3+, which is translocated by plants via the xylem, in the form of amides and ureides (Ahemad and Kibret 2014). The nitrogen present in soybean crop residues, when mineralized, did not appear in a form harmful to the nitrogenase complex. This nitrogen released by soybeans may then have been incorporated by the bacteria, providing energy for them and increasing the efficiency of the association.

The decomposition of soybean residue is more accelerated when compared to corn, therefore the rate of nitrogen release is different (Yoshiki et al. 2013). It is possible that the slower decomposition of corn residue influenced the plant to allow association with the microorganism. This result is always expected when using nitrogen fertilizer, due to the numerous functions performed by nitrogen in the plant (Yuan et al. 2014). In this case, nitrogen fertilization improved the results of components that are directly related to the production of the plants in the consortium. As for future projections of this research, a significant expansion of sustainability in forage systems is envisioned.

This perspective is grounded in the observation of a substantial increase in the number of leaves and the height of plants, especially in the case of black oats. The positive interaction between black oat plants and *Azospirillum brasilense* is notable, given the rapid development of these plants, resulting in early attainment of maximum productivity compared to ryegrass, which has slower growth. This growth promotion occurs due to *Azospirillum brasilense* establishing multiple mechanisms on oat plants. The primary mechanisms concern free nitrogen fixation, production of phytostimulators, solubilization and absorption of nutrients and phytoremediation action. As secondary mechanisms, the inhibition of pathogen growth, systemic induction of resistance, and competition for iron ions stand out (Brambilla et al. 2022). Furthermore, there arises the concrete possibility of replacing nitrogen fertilization in the evaluated amounts, representing a significant advancement in more sustainable agricultural and economic practices. PGPRs have gained attention because they can be used as biofertilizers and useful in bioremediation techniques, which in turn can reduce chemical dependency in agriculture (Dasgupta et al. 2023).

Concurrently, a substantial reduction in environmental contamination due to residue leaching and/or surface runoff is foreseeable. In the interim, the rhizosphere exhibits favorable characteristics for enhancing the biodiversity of plant growth-promoting microorganisms, suggesting an enhancement of the environmental and agricultural benefits associated with the research in question. In summary, the future prospects of this research not only corroborate the viability of more sustainable agricultural practices but also point towards a scenario of greater ecological resilience and long-term agricultural productivity.

Conclusions

The use of *Azospirillum brasilense* in the treatment of seeds from the black oat and ryegrass intercrop increases the number of emerged plants (10%), leaves (32%) and the height (7%) of black oat plants in succession to the corn crop. In succession to soybeans, inoculation also increases the number of black oat tillers (12%) and the number of ryegrass leaves (12%).

Author's contributions

All authors contributed equally to the writing of the paper and were involved in the overall planning and supervision of the work. All authors have read and agreed to the published version of the manuscript.

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