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Responses of *Azospirillum brasilense* on *Brachiaria brizantha* under different soil amendments

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Abstract. The objective of this study was to evaluate the responses of *Brachiaria brizantha* to inoculation with *Azospirillum brasilense* in different soil amendments. Four soil amendment treatments were evaluated (Control, liming, combination of NPK fertilizers and liming + combination of NPK fertilizers), associated or not with the inoculant *Azospirillum*, in a completely randomized design, with five replicates per treatment. The experimental units consisted of plastic pots with a capacity of 12 liters, which were filled with 10 dm^3 of sandy clayey loam soil. For soil correction, higher productions were found for the variables SPAD indexes, chlorophyll b content, carotenoids and chlorophyll a/b ratio. The soil correction factor provided greater production of green matter, dry matter, and leaf area index when using liming with NPK when compared to the control treatment. There was an increase in root volume and weight for the type of soil correction. The use of liming and combination of NPK fertilizers promoted greater development of plant height, tiller density, stem and leaf elongation, quantity and average final leaf size. The use of *Azospirillum brasilense* inoculation associated with soil correction is recommended, as it provides positive responses on the production of the aerial part and roots of *Brachiaria brizantha*.

Key words: *Azospirillum brasilense*, acidic soil, fertilization, inoculant.

INTRODUCTION

The intensification of Brazilian livestock farming in recent decades has required sustainable pasture management strategies, especially those formed by *Brachiaria brizantha*, a species widely used due to its adaptability and high forage productivity (Santos et al., 2021). However, this deterioration, in many cases, is directly linked to low soil fertility and inadequate management practices, compromising both the productivity and sustainability of animal production systems (Mata et al., 2024).

In this scenario, the search for solutions that recover and increase soil fertility has become essential, with chemical correction and the use of bioinputs emerging as promising alternatives. This search for new approaches is intensified by the need to reduce dependence on mineral fertilizers, which generate high costs and considerable environmental impacts when used in excess (Santos et al., 2021). According to Alvarez & Ribeiro (1999), liming has as its main consequences the elevation of soil pH and the neutralization of toxic aluminum, incorporation of calcium and magnesium, the increase in cation exchange capacity, with the release of negatively charged sites of soil colloids, which allows the attraction of other nutrients, reducing loss by leaching, also favoring the increase in microbial activity and the release of nutrients from soil organic matter.

Fertilization with nitrogen, phosphate and potassium is a fundamental pillar for the support and growth of forage grasses. These macronutrients do not act in isolation; they are responsible for the constitution of vital organic compounds and the structural development of plants (Costa et al., 2025). Furthermore, the influence of NPK extends to several physiological processes: it modulates plant metabolism, optimizes water use efficiency and regulates carbohydrate translocation throughout the plant. In practical terms, this fertilization results in better growth and development of tillers, in addition to directly impacting the size and number of leaves and stems, culminating in a more robust and nutritious biomass for grazing (De Morais et al., 2016).

Among the vast spectrum of plant growth-promoting bacteria, *Azospirillum brasilense* has stood out as a protagonist in the agricultural scenario, demonstrating promising results in several crops. The ability of this bacterium to optimize the assimilation of nutrients by plants, combined with its action in the production of phytohormones, contributes to a more vigorous and efficient development (Cruz et al., 2022). The inoculation of Brachiaria brizantha seeds with *Azospirillum brasilense* strains has been the subject of several studies in recent years, demonstrating significant agronomic benefits, especially when associated with soil correction and fertilization practices (Hungria et al., 2020).

Therefore, this study aimed to evaluate the productive characteristics and quantify photosynthetic pigments, morphogenic structure of *Brachiaria brizantha* as a function of fertilization treatments and use of the bacterium *Azospirillum brasilense* during two growth cycles. We hope to identify the ideal combination of fertilizers and soil correction, associated with inoculation with *Azospirillum brasilense*, to optimize both the biochemical and productive parameters of *Brachiaria brizantha* to improve the biochemical and productive parameters of *Brachiaria brizantha*.

MATERIALS AND METHODS

Experimental details and trataments

The experiment was conducted in a greenhouse, located at the State University of Southwest Bahia, Juvino Oliveira *Campus*, located at the following coordinates: 15°38'46" south latitude, 40°15'24" west latitude, average longitude of 280 m, in the municipality of Itapetinga–BA, from September 2019 to February 2020, with a maximum temperature of 44.0 °C and a minimum temperature of 21.0 °C throughout the experimental period. The research was conducted in a completely randomized design, in a 2×4 factorial design, five fertilization treatments (Control, liming, NPK, KPK + liming), associated or not with the use of *Azospirillum brasilense*, with five replications.

The experimental units consisted of 12-liter plastic pots filled with 10 dm³ of sandy-clay loam soil. The soil was collected at a depth of 0 to 20 cm and its chemical characteristics are described in Table 1.

Table 1. Chemical analysis of experimental soil

pH	mg dm^{-3}	cmol$_c$ dm^{-3} of soil								%		mg dm^{-3}
(H_20)	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+	S.B.	t	T	V	m	OM
4.5	1	0.1	0.7	0.7	1.4	6.6	1.5	2.9	9.5	16	48	26

SB = Sum of bases; t = Effective CEC; T = Total CEC; V = Base saturation; m = Aluminum saturation; M.O = Organic matter.

The pots subjected to the soil correction factor had limestone applied 30 days before planting, with the application of 25.6 g of calcitic limestone with PRNT 82% per pot, determined by the exchangeable Al and Ca + Mg method (Alvarez Vênegas /Ribeiro, 1999).

$$NC\ (t\ ha^{-1}) = Y * \left[Al^{3+} + \left(\frac{mt * t}{100}\right)\right] + [X - (Ca^{2+} + Mg^{2+})] * 100/PRNT \quad (1)$$

where NC = limestone requirement; Y = factor that varies with the soil's acidity buffering capacity and can be defined according to the texture; mt = maximum aluminum saturation tolerated by the crop; t = effective cation exchange capacity; X = calcium and magnesium requirement of the crop; $PRNT$ = relative total neutralization power of the limestone to be applied.

The seeds used in the inoculant treatment were soaked for one hour in a solution containing *Azospirillum brasilense*, following the manufacturer's recommendation of 100 mL of the product (AzoTotal) for 5 kg of seeds, 12 seeds were used per pot, at an approximate depth of 1 cm. At the beginning of each experimental period, *Azospirillum brasilense* was re-inoculated by applying the solution directly to the soil surface via spray; the dose per experimental unit corresponded to approximately 1 mL of the product.

For the chemical fertilization factor, the P and K sources were applied at the time of standardization cutting, planting, with 110 kg ha^{-1} of P_2O_5, (3.06 g of triple superphosphate per pot) and 60 kg ha^{-1} of K_2O (0.52 g of potassium chloride per pot), respectively. The N source (urea) occurred with the application of 100 kg ha^{-1} in split form, in two applications, the first application after the first uniformization cut and the second application after the second uniformization cut (0.57 g pot^{-1}).

Seeds of *Brachiaria brizantha* cv. Marandu, with 80% cultural value, were used. Twenty days after emergence (DAE), non-vigorous or non-homogeneous plants were removed, keeping only four plants in each experimental unit (replicates). At 40 DAE, the first uniformity cut was performed at a height of 15 cm above ground level, followed by the first application of nitrogen fertilization, and at 68 DAE, the second uniformity cut was performed with the application of the second dose of nitrogen fertilization. After distributing the fertilization treatments, monitoring and evaluation of the plants began during two growth cycles of 28 days each, when the grass reached an average height of 30 cm.

To determine the water-holding capacity of the soil, three pots containing dry soil were initially weighed and then saturated with water for three days. After draining the excess water, the pots were weighed again and the difference in weight between the wet and dry soil was determined as the maximum water-holding capacity of the soil, which was 80%. This value was then used as a reference to maintain the water levels of the experimental units close to the retention capacity, with the buckets being weighed daily and the water replaced when necessary (Dutra et al., 2025).

Plant analysis

At the end of each growth cycle, a fully expanded leaf was collected from each experimental unit and placed in envelopes made of aluminum foil and immediately stored on ice, which were taken to the laboratory for determination of the chlorophyll a, chlorophyll b and carotenoid contents, where 0.03 g of the fresh mass (FM) of the collected leaf was placed in a glass vial containing 5 mL of dimethyl sulfoxide and wrapped in aluminum foil for 72 hours. Then, a reading was performed on the spectrophotometer to quantify the photosynthetic pigments according to Wellburn (1994) and values expressed in mg g^{-1} FM. In addition to reading the leaves using the SPAD method.

$$Chlorophyll\ a = (12.19 * A665) - (3.45 * A649) \quad (2)$$

$$Chlorophyll\ b = (21.99 * A649) - (5.32 * A665) \quad (3)$$

$$Carotenoids = [1{,}000 * A480 - (2.14 * Chlorophyll\ a) - (70.16 * Chlorophyll\ b)]/220 \quad (4)$$

Subsequently, the aerial part of the plants was cut at 15 cm above the ground and separated into stem/pseudo-stem and leaf blade, then weighed in natural matter. All leaf blades were scanned using an HP G2710 photo scanner at 300 dpi resolution and subsequently analyzed using the ImageJ® program, with the leaf area (LA) calculated and its value expressed in cm^2 pot^{-1}. Based on the AF data, the leaf area index (LAI), specific leaf area (SLA, cm^2 g^{-1}) and leaf area ratio (LAR, cm^2 g^{-1}) were calculated according to the equation defined by Cairo et al. (2008).

The morphological fractions were grouped and dried in a forced circulation oven at 55 °C for 72 h, and were subsequently weighed to obtain the dry mass production values of the aerial part. The residue, corresponding to the 0–15 cm soil extract, was also weighed to determine the dry mass production values of the residue. Where the production data were presented in g of DM/pot. The pots were disassembled and then the root volume (RV) of each experimental unit was determined, using a graduated cylinder with a known volume of water, where the root was introduced and through the difference, the VR was obtained, then the roots were taken to a forced circulation oven at 55 °C for 72 h to determine the production of dry root biomass.

To evaluate the bromatological composition, the levels of crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose and lignin were determined according to Detmann et al. (2012).

The estimated total digestible nutrient contents (TDN) were calculated according to Capelle et al. (2001) with the following equation:

$$NDTest = 74.49 - 0.5635 * FDA \quad (5)$$

To investigate plant growth, two tillers per pot were carefully selected and identified with colored tapes (green and orange). After a uniformity cut, the development of these tillers was monitored every three days throughout the growth period. The following characteristics were evaluated: Stem length; Number of leaves; Leaf length and width.

Using the data obtained during the evaluations, the following characteristics were calculated:

– Phyllochron (day/leaf): inverse of TapF;

– Number of live leaves: determined as the fraction of total leaves that did not show signs of senescence;

– Leaf lifespan: estimated considering the time between the appearance of the leaf apex and the first sign of senescence of the blade, therefore, the time that the leaf remained green;

– Leaf senescence rate

– Leaf appearance rate (TApF, leaves/tiller/day): obtained by dividing the number of leaves that appeared on the marked tillers of each pot by the regrowth period;

– Leaf elongation rate (LER, cm/tiller/day): calculated by the difference between the final and initial leaf lengths, divided by the measurement interval;

– Leaf/leaflet width (LF, cm): average width of the leaf blades or of the three leaflets (which make up the Java leaf) fully expanded;

– Final leaf length (CF, cm): obtained by measuring the fully expanded leaves, from their ligule to the leaf apex;

– Stalk elongation rate (TAlC, mm/tiller/day): obtained by the difference between the final and initial length of the stalk of each tiller, measured from soil level to the height of the ligule of the youngest leaf, divided by the interval of measurements;

– Final stem length (FSL, cm);

– Total plant length (TPL, cm);

– Number of tillers: The number of tillers was counted in terms of number of tillers per pot and per plant.

Statistical analysis

The data obtained were analyzed using the SAS - Free Statistical Software statistical program (SAS INSTITUTE, 2015). Analysis of variance was performed, considering types of soil corrections, use of inoculant and the interaction between types of soil corrections and use of inoculant as sources of variation. Comparisons were performed using the Tukey test. $P = 0.01$ was adopted.

RESULTS AND DISCUSSION

No significant effect ($P > 0.01$) was found for the INOCULANT × CORRECTION interaction and use of inoculant (Table 2) on the SPAD index, chlorophyll a, chlorophyll b, carotenoids, total chlorophyll and chlorophyll a/b ratio.

However, a significant value ($P < 0.01$) was observed for the type of soil correction only in the variables SPAD index, chlorophyll b content, carotenoids and chlorophyll a/b ratio. Duarte et al. (2020), when evaluating the use of *Azospirillum brasilense* inoculation in *Brachiaria ruziziensis* with different doses of N (0; 50 and 100 kg ha^{-1}), observed a reduction in chlorophyll levels in the treatment with inoculant and 100 kg ha^{-1} of N. This observation converges with studies that demonstrate that nitrogen doses greater than 50 kg ha^{-1} can inhibit the activity of bacteria in the rhizosphere, highlighting the complex interaction between nitrogen fertilization and the action of growth-promoting microorganisms.

In the present study, no difference ($P > 0.01$) was observed between treatments with and without inoculant for the variables studied in (Table 2). A possible explanation is the proximity of the experimental units, which may have facilitated the transfer of microorganisms between treatments. In fact, Oliveira et al. (2007) warn of the possibility of 'infection' of treatments when using the spraying reinoculation method, due to the proximity of the vessels. This cross-contamination may have homogenized the soil microbiota, masking the effects of inoculation and compromising the detection of significant differences between treatments.

Table 2. Effect of *Azospirillum brasilense* inoculation and different soil amendments on the SPAD index (SPAD), chlorophyll a content (CLOA), chlorophyll b content (CLOB), carotenoids (CARO), total chlorophyll (CLOT) and chlorophyll a/b ratio (RZAB) in *Brachiaria brizantha* cv. Marandu

Variable	Inoculant		Correction				*P*-value		
	Without	With	S/ CORR	CAL	NPK	CAL+ NPK	INOC	CORR	INOC × CORR
SPAD	27.0a	28.0a	26.7a	26.6a	28.0a	28.8a	0.0831	0.0238	0.3234
CLOA	1.881a	1.936a	1.976a	1.862a	1.935a	1.861a	0.4080	0.5366	0.0334
CLOB	0.041a	0.042a	0.038b	0.041ab	0.043a	0.045a	0.3862	0.0002	0.3367
CARO	0.062a	0.060a	0.069a	0.060b	0.058b	0.055b	0.2208	< 0.0001	0.1361
CLOT	1.922a	1.978a	2.013a	1.903a	1.978a	1.906a	0.4024	0.5711	0.0329
RZAB	46.36a	46.69a	53.05a	45.31b	45.71b	42.040b	0.8554	0.0012	0.2720

S/ CORR = No soil correction; CAL = Use of liming; NPK = Use of NPK; CAL+NPK = Use of liming and NPK; INOC = Inoculant; CORR = Type of soil correction; INOC×CORR = interaction between factors. Means followed by the same letter in a line do not differ from each other by Tukey's test ($P > 0.01$).

The interaction between inoculant and soil correction type was not significant ($P > 0.01$) for green matter production (PMG), dry matter production (PDM) and leaf area index (Table 3). However, the plants that received the inoculation presented higher and significant values ($P < 0.01$) than the treatments without inoculant. Corroborating the results obtained by Oliveira, Oliveira & Barioni Junior (2007), the authors worked with inoculation of *Azospirillum brasilense* in *Brachiaria brizantha* under different doses of nitrogen (0, 150, 200 and 300 kg ha^{-1}), and concluded that the treatment without nitrogen application and using inoculant produced more forage than the control treatment.

Table 3. Effect of *Azospirillum brasilense* inoculation and different soil amendments on green matter production (PMG), dry matter production (PDM) and leaf area index (LAI) in *Brachiaria brizantha* cv. Marandu

Variable	Inoculant		Correction				*P*-value		
	Without	With	S/ CORR	CAL	NPK	CAL + NPK	INOC	CORR	INOC × CORR
PMG[1]	50.5b	65.1a	14.0d	36.9c	81.2b	99.1a	0.0002	< 0.0001	0.0348
PDM[1]	13.2b	17.0a	3.5d	9.6c	20.9b	26.4a	0.0001	< 0.0001	0.0702
IAF	2.4b	3.0a	0.8d	2.0c	3.6b	4.4a	0.0008	< 0.0001	0.0518

[1]g pot^{-1}. S/ CORR = No soil correction; CAL = Use of liming; NPK = Use of NPK; CAL+NPK = Use of liming and NPK; INOC = Inoculant; CORR = Type of soil correction; INOC × CORR = interaction between factors. Means followed by the same letter in a line do not differ from each other by Tukey's test ($P > 0.01$).

The result found by the authors highlights the N fixation capacity promoted by the inoculation of *A. brasilense*. According to Taiz & Zeiger, (2009), nitrogen is classified as the nutrient that is most deficient in plants, since it is found in several components of the plant cell, including amino acids, proteins and nucleic acids. Nitrogen participates in the formation of aerial and root tissues, and acts directly in the photosynthesis process by constituting chlorophyll, promoting the dark green color of the leaf. Therefore, it is the nutrient most used, absorbed and exported by forage grasses (Primavesi et al., 2006).

The soil correction factor had a statistically significant effect ($P < 0.01$) on all three measured variables (Table 3), leading to an approximate 600% increase in green biomass, dry biomass, and leaf area index when liming was applied in combination with NPK fertilization, compared to the untreated control. This increase can be explained by the improved nutrient availability and the neutralization of excess hydrogen ions (H^+) in the soil due to liming, which in turn enhances root development. Correspondingly, root volume and weight were significantly greater in the limed treatment than in the treatment without soil correction.

The use of NPK fertilization affects the plant's development and production process, since these minerals participate in the synthesis of organic compounds, tillering, quantity of leaves, efficiency of water use, aiding their metabolism and growth (Costa, 2004). No effect was observed for the interaction between the use of inoculant and types of soil correction ($P > 0.01$) on the bromatological chemical composition (Table 4). However, an effect of the use of the inoculant ($P < 0.01$) was observed on the content of acid detergent insoluble protein (IPAD).

Table 4. Effect of *Azospirillum brasilense* inoculation and different soil amendments on the chemical composition of *Brachiaria brizantha* cv. Marandu

Variable (%)	Inoculant		Correction				*P*-value		
	Without	With	S/ CORR	CAL	NPK	CAL + NPK	INOC	CORR	INOC × CORR
DM	23.5a	24.3a	22.8a	24.0a	23.8a	24.9a	0.0934	0.0307	0.0211
MM	7.1a	7.7a	9.2a	7.2b	6.9b	6.3b	0.2191	0.0013	0.2999
CP	8.0a	7.7a	8.4a	8.2a	7.3b	7.5b	0.0140	< 0.0001	0.1659
EE	4.9a	4.6a	3.6c	4.5b	5.1b	6.0a	0.1782	< 0.0001	0.4048
NDF	63.8a	65.1a	68.7a	62.8b	64.5ab	61.7b	0.3400	0.0063	0.9974
FDA	27.7a	28.1a	30.0a	27.6b	27.3b	26.7b	0.4563	0.0030	0.2908
LIG	5.8a	6.0a	5.8a	6.0a	5.9a	5.8a	0.5308	0.8993	0.6714
IPAD	12.2a	9.9b	12.9a	12.0a	9.7b	9.6b	< 0.0001	< 0.0001	0.7826
IPND	1.7a	1.5a	2.1a	1.6b	1.4b	1.4b	0.0172	< 0.0001	0.9463
NDFP	62.0a	63.5a	66.5a	61.2a	63.1a	60.4a	0.2717	0.0151	0.9966
TDN	58.9a	58.6a	57.6b	58.9a	59.1a	59.4a	0.4563	0.0030	0.2908

DM = dry matter; MM = mineral matter; CP = crude protein; EE = ether extract; NDF = neutral detergent insoluble fiber; FDA = acid detergent insoluble fiber; LIG = lignin; IPAD = acid detergent insoluble protein; IPND = neutral detergent insoluble protein; FDNP = NDF corrected for protein; TDN = total digestible nutrients. S/ CORR = Without soil correction; CAL = Use of liming; NPK = Use of NPK; CAL + NPK = Use of liming and NPK; INOC = Inoculant; CORR = Type of soil correction; INOC × CORR = interaction between factors. Means followed by the same letter in a line do not differ from each other by Tukey's test ($P > 0.01$).

The type of soil correction using liming and fertilization promoted a slight reduction in the fibrous composition and a better value of total digestible nutrients when compared to the treatment without correction. The same did not occur with the protein fraction, which presented lower CP values. According to Cecato et al. (2004), the reduction in the protein fraction is also related to the interaction of the plant with light, photoperiod, humidity and temperature, which accelerate metabolic activity, resulting in a decrease in the amount of photoassimilates and metabolites in the cellular content, directing the energy converted in photosynthesis into structural carbohydrates, mainly lignin.

The INOCULANT × CORRECTION interaction did not show a significant effect ($P > 0.01$) for root volume, root fresh weight, root dry weight and root dry matter (Table 5).

Table 5. Effect of *Azospirillum brasilense* inoculation and different soil amendments on root production in *Brachiaria brizantha* cv. Marandu

Variable	Inoculant		Correction				*P*-value		
	Without	With	S/ CORR	CAL	NPK	CAL + NPK	INOC	CORR	INOC × CORR
RVOL	200.5a	177.5b	88.0c	134.0b	280.0a	254.0a	0.0031	< 0.0001	0.1452
RPF	180.7a	174.3a	81.7c	130.6b	254.6a	243.0a	0.4682	< 0.0001	0.9359
RPS	23.5a	21.6a	9.1b	14.8b	33.1a	33.3a	0.2786	< 0.0001	0.7056
RMS	12.5a	14.2a	15.2a	11.3a	13.3a	13.6a	0.5208	0.7867	0.3276

RVOL = Root volume, in mL; RPF = Root fresh weight, in g pot^{-1}; RPS = Root dry weight, in g pot^{-1}; RMS = Root dry matter, in (%); S/ CORR = Without soil correction; CAL = Use of liming; NPK = Use of NPK; CAL + NPK = Use of liming and NPK; INOC = Inoculant; CORR = Type of soil correction; INOC × CORR = interaction between factors. Means followed by the same letter in a line do not differ from each other by Tukey's test ($P > 0.01$).

However, a positive effect ($P < 0.01$) was observed for the type of soil correction, directly influencing root volume and weight. In general, in Brazil, soils have a high acidity level, with high levels of aluminum and manganese, associated with calcium, magnesium and phosphorus deficiency, promoting a low rate of rooting and absorption of water and nutrients, common limitations found in acidic soils.

According to Alvarez & Ribeiro (1999), liming has as its main consequences the elevation of soil pH and the neutralization of toxic aluminum, incorporation of calcium and magnesium, the increase in cation exchange capacity, with the release of negatively charged sites of soil colloids, which allows the attraction of other nutrients, reducing loss by leaching, also favoring the increase in microbial activity and the release of nutrients from soil organic matter.

The use of the inoculant showed a significant ($P < 0.01$) and lower value for root volume when compared to the treatment without inoculant. It was expected that the use of the inoculant would promote an increase in root development and volume, since these bacteria increase the synthesis of hormones that stimulate plant growth, mainly of roots, by increasing the absorption of nutrients and water (Dobbelaere et al., 2002; Bashan et al. 2004).

Barassi et al. (2008) reported that as a result of the root development promoted by Azospirillum, the plants presented, among other benefits, a greater biomass production. In the present study, significant and positive values were obtained only for the production of the aerial part, as reported in (Table 3).

Rampim et al. (2020) inoculated *Azospirillum brasilense* in corn and obtained a reduction in the aerial part and greater root production, results contrary to those of the present study. Pinc et al. (2020) working with the inoculation of Azospirillum brasilense in *Urochloa brizantha* cv. Marandu under different nitrogen doses (0, 50 and 75 kg of N ha^{-1}), did not observe a difference in root dry mass and found a significant result for aerial part dry mass with the highest production when nitrogen was used.

Regarding the effects on morphogenesis, no significant result ($P > 0.01$) was observed for the INOCULANT × CORRECTION interaction (Table 6). The use of the inoculant did not have a positive effect on any of the variables studied in (Table 6). The type of soil correction had a significant effect ($P < 0.01$) for all variables in (Table 6), except for the number of live leaves, which did not present a significant value ($P > 0.01$) for any of the factors tested. The use of liming plus NPK promoted greater plant development in height, tiller density, stem elongation, leaf elongation, quantity and average final leaf size.

Table 6. Effect of *Azospirillum brasilense* inoculation and different soil amendments on the development and growth of *Brachiaria brizantha* cv. Marandu

Variable	Inoculant		Correction				*P*-value		
	Without	With	S/ CORR	CAL	NPK	CAL + NPK	INOC	CORR	INOC × CORR
ALTPE	68.2a	72.2a	61.0b	64.2b	77.1a	78.5a	0.0263	< 0.0001	0.0548
ALTD	39.4a	41.8a	32.8c	37.4b	46.9a	45.3a	0.0179	< 0.0001	0.0205
PERF	24.6a	22.4b	10.2c	17.4b	31.8a	34.6a	0.0045	< 0.0001	0.0220
TALC	0.3a	0.3a	0.2c	0.3bc	0.4a	0.4ab	0.2157	< 0.0001	0.3172
TALF	3.0a	3.2a	1.8c	2.7b	3.9a	4.0a	0.0332	< 0.0001	0.3454
TAPF	0.11a	0.11a	0.09c	0.10b	0.13a	0.13a	0.1638	< 0.0001	0.4975
FIL	10.4a	9.8a	12.3a	11.1b	8.5c	8.5c	0.0348	< 0.0001	0.6193
NFV	4.2a	4.0a	4.2a	4.3a	4.0a	3.8a	0.4024	0.2936	0.3402
DVF	42.5a	38.3a	50.0a	46.4a	33.4b	31.8b	0.0477	< 0.0001	0.2701
TSEF	0.5a	0.6a	0.4b	0.4b	0.6ab	0.8a	0.0547	0.0002	0.9293
CFL	36.3a	35.4a	30.1b	33.3b	39.7a	40.5a	0.3338	< 0.0001	0.7124

ALTPE = Stretched plant height, in cm; ALTD = Canopy height, in cm; PERF = Tiller density, in nº/pot; TALC = Stalk elongation rate, in cm/day; TALF = Leaf elongation rate, in cm/day; TAPF = Leaf appearance rate, in nº of leaves per day; FIL = Phyllochron, in day/leaf; NFV = Number of live leaves; DVF = Leaf lifespan, (FIL*NFV); TSEF = Leaf senescence rate, in cm/day; CFL = Average final leaf length, in cm. S/ CORR = Without soil correction; CAL = Use of liming; NPK = Use of NPK; CAL + NPK = Use of liming and NPK; INOC = Inoculant; CORR = Type of soil correction; INOC × CORR = interaction between factors. Averages followed by the same letter in a line do not differ from each other according to the Tukey test ($P > 0.01$).

The interaction (INOCULANT × CORRECTION) was significant ($P < 0.01$) for the leaf:stem ratio (Table 7). This result can be explained by the delayed growth of plants in the control treatment, as a consequence of low soil fertility.

Table 7. Effect of *Azospirillum brasilense* inoculation and different soil amendments on the leaf:stem ratio in *Brachiaria brizantha* cv. Marandu

Inoculant	Correction type			
	No correction	Liming	NPK	Liming + NPK
Without	8.6Aa	5.0Abc	3.7Abc	3.0Ac
With	3.7Ba	3.9Aa	2.6Aa	2.9Aa
CV(%)	23.06			

CV(%) = Coefficient of variation in percentage. Means followed by the same capital letter in the column and lowercase letter in the row do not differ from each other according to the Tukey test ($P > 0.01$).

Results also observed by PMG, PDM (Table 3) and morphogenesis (Table 7), where in general, the control treatment presents lower values of production and plant development, mainly in plant height, stem length, leaf length, tillering and a longer leaf lifespan. The lifespan of a leaf is determined by the emergence of new leaves and the senescence of existing leaves. Thus, it is understood that *Brachiaria brizantha* in low fertility soil, Control and the use of inoculant, cannot enhance its development, especially the emergence of leaves, due to the lack and availability of minerals necessary for its metabolism.

The use of inoculant was lower for the leaf:stem ratio within the control treatment, as it promoted greater plant height, mainly due to the higher rate of stem elongation, even though this result had no statistical difference ($P > 0.01$), the amount of stem available above the cutting line influenced the calculation. Duarte et al. (2020) found an increase in stalk production with some BPCPs and also found that the leaf:stem ratio remained equal to or higher than in the treatment without inoculant. In general, BPCPs increase the production of phytohormones that are responsible for root and shoot production, thus providing greater leaf and stalk production (Dobbelaere, et al. 2002; Taiz & Zeiger 2009).

CONCLUSIONS

The combined application of *Azospirillum brasilense* and soil correction, specifically through liming combined with NPK fertilization, demonstrated a significant impact on the physiology of *Brachiaria brizantha*, grown in a controlled environment. In particular, notable stimulation in the production of the aerial part and root system was observed, indicating optimization of plant growth and development.

In contrast, the bromatological composition and photosynthetic pigments of *Brachiaria brizantha* showed less expressive changes. These results suggest that inoculation with *Azospirillum brasilense*, in synergy with soil correction, directs its effects mainly towards increasing plant biomass, with a secondary impact on the nutritional quality and photosynthetic capacity of the plant.

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