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Azospirillum brasilense associated with nitrogen fertilization in *Urochloa humidicola* pastures cultivated in the Amazonian savanna

Azospirillum brasilense associado a adubação nitrogenada em pastos de *Urochloa humidicola* cultivada na savana amazônica

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Abstract: The objective of this study was to assess the impact of *Azospirillum brasilense* in conjunction with nitrogen fertilization on structural, morphogenetic, yield-related, and chemical characteristics of *Urochloa humidicola* cultivated in the Amazon savannah. Treatments consisted of five nitrogen application rates (0, 50, 100, 150, and 200 kg ha^{-1}), both with and without *A. brasilense* inoculation. The experiment was laid out in a completely randomized design with a 5×2 factorial arrangement, including four replications. The morphological composition of the pastures, morphogenetic and structural characteristics and chemical composition of the forage were evaluated. The utilization of *A. brasilense*, either alone or combined with nitrogen fertilization, did not influence the enhancement of forage yield. However, nitrogen fertilisation led to consistent increases in production, primarily through an increase in leaf and culm components and a reduction in the proportion of dead material in pastures. It also led to a linear increase in the proportions of NDF, ADF and CP in the forage. The use of *A. brasilense* does not alter the production and chemical composition of the forage, while nitrogen fertilization favors the management of pastures with *U. humidicola* grown in Amazonian savanna soil.

Key-words: Amazonian quicuio grass; Brachiaria; diazotrophic bacteria; morphogenesis; tropical pastures.

Resumo: O objetivo com este estudo foi avaliar os efeitos do *Azospirillum brasilense* associado ao uso da adubação nitrogenada sobre as variáveis estruturais, morfogênicas, produtivas e químicas de *Urochloa humidicola* cultivada na savana Amazônica. Os tratamentos foram constituídos de cinco doses de nitrogênio (0, 50, 100, 150 e 200 kg ha^{-1}) associadas a inoculação ou não de *Azospirillum brasilense*, em delineamento inteiramente casualizado, em esquema fatorial 5x2, com quatro repetições. Foram avaliadas as composições morfológicas dos pastos, as características morfogenéticas e estruturais, e a composição química da forragem. O uso de *Azospirillum brasilense*, isolado ou em associação com a adubação nitrogenada, não foi determinante para o aumento de rendimento de produção da pastagem. No entanto, a adubação nitrogenada proporcionou ganhos lineares em produção, principalmente no aumento dos componentes folha e colmo, e redução na proporção de material morto nos pastos, bem como aumentou linearmente as proporções de FDN, FDA e PB da forragem. O uso de *A. brasilense* não altera a produção e a composição química da forragem, enquanto a adubação nitrogenada favorece o manejo de pastos com *U. humidicola* cultivados em solo da savana Amazônica.

Palavras-chave: Quicuio-da-Amazônia; braquiária; bactéria diazotrófica; morfogênese; pastagens tropicais.

1. Introduction

Livestock expansion in the Amazon savannah is a growing trend, nonetheless, the prevailing approach continues to be extensive grazing on native pastures, leading to suboptimal animal growth, reduced production indices, and soil degradation [1]. Among the various forage species thriving in the Amazon, *Urochloa humidicola* stands out for its remarkable adaptability to temporary flooding, prolonged drought periods, and acidic soils with inherently low fertility [2]. These soil-climatic traits align with the characteristics of the extensive savannah areas prevalent in the Amazon. Recent years have witnessed studies showcasing encouraging outcomes from introducing agricultural crops in these environments [3, 4, 5]. However, research focusing on cultivated pasture management in these regions remains limited.

In the context of pasture management, increasing soil fertility is the most important aspect, as the productivity and structural performance of plants in the field, as well as the potential soil degradation resulting from grazing activities, significantly depend upon this aspect [6]. In this scenario, nitrogen fertilization is the practice exerting the most pronounced impact on herbage yield, enhances the structural, morphogenetic, productive, and chemical attributes of plants [7, 8], which are variables influenced by the physiological multifunctionality of this nutrient [9].

The utilization of nitrogen-fixing bacteria (NFB) has gained attention due to their effectiveness across diverse pasture types. Given the cost-intensive nature of pasture production in the Amazon savannah region, there is a great search for alternatives that minimize reliance on specific fertilizers [10, 11]. However, factors such as high temperatures-above 35°C, for instance, in studies involving legumes [12], low soil moisture during certain times of the year, and acidic soil pH values-below 5.0 as observed in legume trials [13], as well as the availability of other nutrients, can negatively affect the activity of nitrogen-fixing bacteria (NFB) and the formation of root nodules. Therefore, considering the edaphoclimatic conditions of the Amazonian savanna, it becomes essential to analyze how these factors influence the performance of associative NFB in grasses, since most existing studies focus primarily on legume associations.

Among the recognized NFB, those of the genus Azospirillum stand out because of their contributions to phytohormone production and phosphate-solubilizing enzymes. Furthermore, they reduce the required nitrogen quantities by amplifying its effects and enhancing plant resilience against biotic and abiotic stressors [10, 14]. It is important to highlight, however, that negative interactions may occur between nitrogen fertilization and NFBs, particularly when high concentrations of nitrogen are applied to the soil. This can suppress the activity of the nitrogenase enzyme, which is essential for biological nitrogen fixation, thereby reducing the formation of root nodules. This phenomenon occurs because plants, when supplied with nitrogen from fertilizers, reduce their need to establish symbiotic relationships with nitrogen-fixing bacteria, leading to a decreased production of chemical signals that attract rhizobia [15].

Against this backdrop, this research aims to evaluate the impact of nitrogen fertilization and *Azospirillum brasilense* inoculation in *U. humidicola* pastures cultivated within the Amazon savannah.

2. Material and methods

2.1 Experimental site and climatological data

The research was conducted in the central portion of the Amazon savannah, in Boa Vista, Roraima, Brazil (2º52'15.49 N and 60º42'39.89 W, 90 m a.s.l.), from August 2020 to February 2021 (Figure 1).

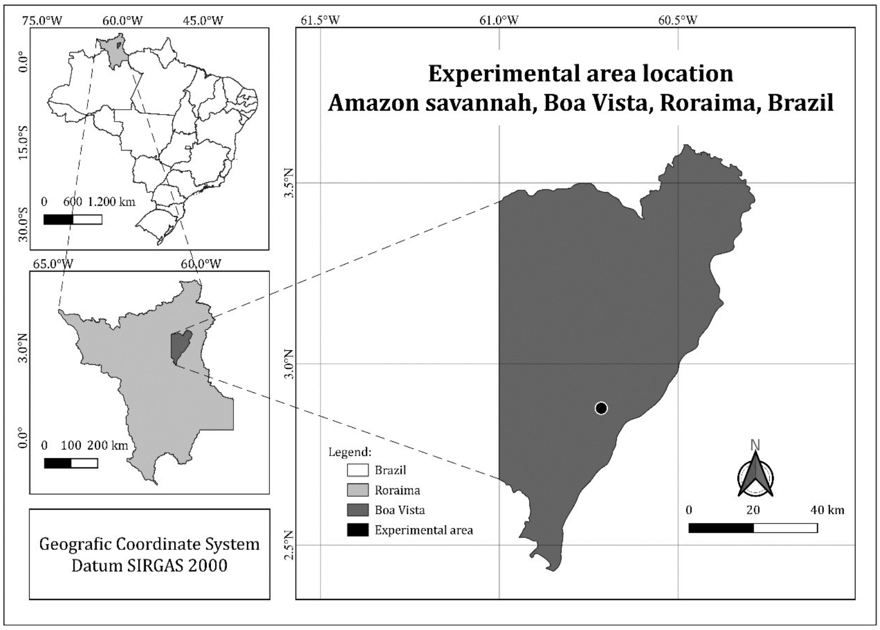

Figure 1. Study area in the State of Roraima, Northern Brazilian Amazon.

The Amazon savannah is characterized by a predominantly flat to gently undulating landscape featuring woody grassy savannah vegetation known as the savannah park type [5]. The predominant tree species in the area are caimbé (*Curatella americana* L.), murici (*Byrsonima crassifolia* (L.) H.B.K.), paricarana (*Bowdichia virgilioides* Kunth), sucuba (*Himatanthus articulathus* (Vahl) Wood), and buriti (*Mauritia flexuosa* L.) [16].

Climatically, the region falls under the Köppen classification Aw, with a rainy season from April to September and a dry season from October to March. The average annual precipitation is 1,657 mm [17]. Information on rainfall, maximum and minimum temperatures are shown in Figure 2. The data were obtained manually from an on-site weather station.

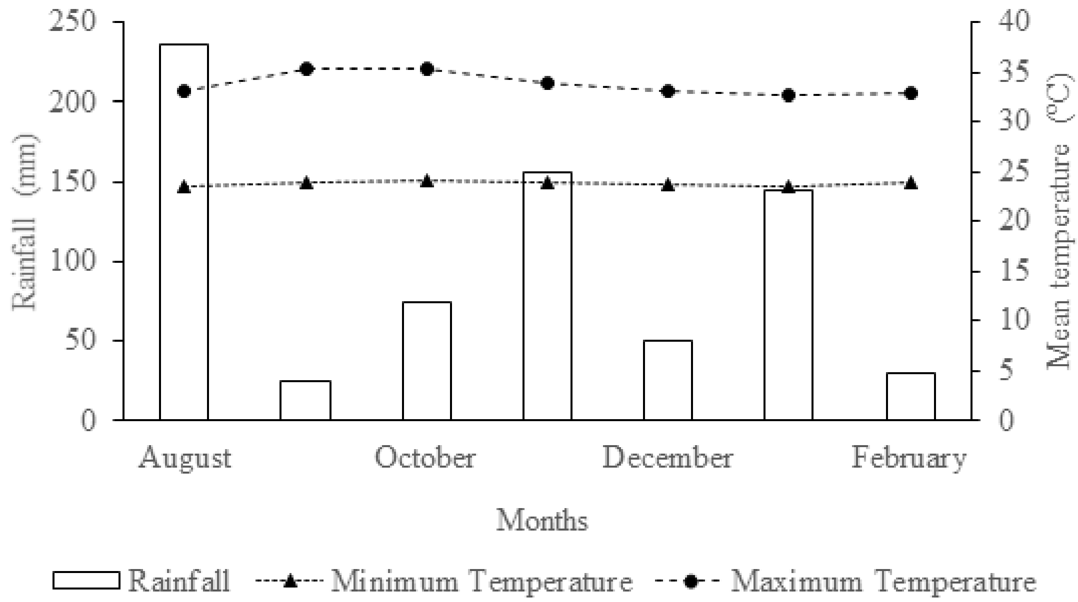

Figure 2. Total rainfall (mm) and mean daily maximum and minimum temperature (ºC) in each month of the study period (August to February 2020) in Amazon savannah, Boa Vista, Roraima, Brazil.

The soil is classified as Latossolo Amarelo distrófico according to the Brazilian Soil Classification System [18] or Xanthic Haplustox according to Soil Taxonomy [19]. The physicochemical characteristics in the 0-20 cm layer are shown in Table1.

Table 1. Soil chemical and physical properties at the experimental site.

pH	Ca	Mg	Al	H+Al	CEC	K	P	BS	Clay	Silt	Sand
	cmolc dm^{-3}						mg dm^{-3}	%			
4.40	0.48	0.12	0.56	4.00	4.66	0.06	2.4	14	21	5	74

Hydrogen potential (pH: CaCl2); calcium (Ca); magnesium (Mg); aluminum (Al); hydrogen plus aluminum (H+Al); cation exchange capacity (CEC); potassium (K); phosphorus (P: extracted by Mehlich-1); base saturation (BS).

2.2 Treatments and experimental design

The study area consisted of a *U. humidicola* cv. Comum pasture that had been established five years prior. The pasture was divided into 40 experimental units of 12 m^2 each. Management involved cutting the pasture when the plots reached a height of 20 cm, with the entire plot being cut and leaving a stubble height of 10 cm. Following a seven-month period of data collection, the regrowth period (RP) and the total number of cuts (NC) in each plot were calculated.

A completely randomized design was employed, organized as a 2×5 factorial arrangement with four replications. The first factor encompassed two levels of inoculation (with and without inoculant), while the second factor comprised five nitrogen rates (0, 50, 100, 150, and 200 kg ha^{-1}). These treatments were applied to the *U. humidicola* cv. Comum pasture. The liquid inoculant (Azototal®) utilized contained a pure culture of *A. brasilense* (strains AbV5 and AbV6), included water (solvent/support), yeast extract, ammonium chloride (source of nitrogen and vitamins), glycerol and malic acid (carbon sources), potassium phosphate, magnesium sulfate, sodium chloride, calcium chloride, ferric EDTA, copper sulfate, zinc sulfate, boric acid, sodium molybdate, and manganese sulfate as mineral sources.

The inoculant was administered as recommended by the manufacturer (Biotrop) for application on already established pastures. The dosage of Azototal used was equivalent to 300 mL/ha, applied using a handheld sprayer, with the product being sprayed directly onto the leaf blades. The application was carried out after the initial cut to standardize plant conditions at the beginning of the experiment.

Nitrogen rates were applied using urea as the source, distributed in split applications to minimize nutrient losses. Specifically, 50 kg ha^{-1} of N were applied every 15 days, starting in July. Thus, the rates of 50, 100, 150, and 200 kg ha^{-1} were divided into one, two, three, and four applications, respectively. As a result, all treatments received pre-established doses and evaluations began only after the final application in August.

To enhance base saturation to 50%, liming was conducted by applying 2.01 t ha^{-1} of limestone to the surface without overturning, as determined by the base saturation method. Phosphorus and potassium fertilization was carried out due to low availability in the soil. Potassium fertilization involved 50 kg ha^{-1} of K_2O (using potassium chloride), while phosphate fertilization utilized 30 kg ha^{-1} of P_2O_5 (triple superphosphate source) following the recommendations to the Werner a *et al.* (20) for very low levels of phosphorus and potassium in the soil and considering *U. humidicola* as having low nutrient requirements.

2.3 Forage measurements

The variables under analysis were categorized into four groups: structural variables (NT - number of tillers, PH – Pre-harvest plant height, LAI - leaf area index, and NLL - number of live leaves); morphogenetic variables (LAR - leaf appearance rate, LER - leaf elongation rate, PHY - phyllochron, LSR - leaf senescence rate, LLS - leaf lifespan, and SER - stem elongation rate); yield variables based on dry matter (L - leaf percentage, S - stem percentage, DDM - dead material percentage, and DHA - daily herbage accumulation); and chemical variables based on dry matter (NDF - neutral detergent insoluble fiber, ADF - acid detergent insoluble fiber, and CP - crude protein).

The determination of NT involved counting the total number of tillers in two randomly placed 0.25 m^2 squares within each experimental unit. This count was performed during each plot's harvesting phase. PH was measured using centimeter-graded rulers at 12 points per plot. LAI was assessed using a canopy analyzer (LAI 2000, LI-COR, Lincoln, Nebraska, USA) before harvesting. In each plot, one reading was taken above and five readings were performed at the base of the canopy, positioning the bar with the reading sensor just above the ground, always between 09h00 and 11h00.

Morphogenetic and structural variables were evaluated by selecting three tillers per plot and assessing them twice weekly during the post-harvest regrowth period. This involved measuring various aspects of leaf growth and pseudo stem length. During these assessments, measurements were taken for various parameters, including the length of expanding, fully expanded, and senescent leaf blades, as well as the combined length of the sheath and stem, referred to as the pseudo stem. With each instance of plot cutting, three fresh tillers were selected from each experimental unit for ongoing evaluations. Subsequently, utilizing these initial variables, the following variables were calculated:

LAR (leaves tiller^{-1} day^{-1}): the ratio of total leaves on the tiller to the regrowth period;

LER (cm tiller^{-1} day^{-1}): the difference between final and initial leaf lengths divided by regrowth days;

PHY (days leaf^{-1}): regrowth period divided by total leaves on the tiller;

LSR (cm tiller^{-1} day^{-1}): the ratio of average length of senescent leaves per tiller to regrowth days;

LLS (days): the time from complete leaf expansion to leaf death;

SER (cm tiller^{-1} day^{-1}): relationship between pseudostem length and regrowth period;

NLL (leaves tiller^{-1}): the average number of non-senescent expanded leaves per tiller.

Yield variables (L, S, DDM, and DHA) were determined by harvesting herbage above the 10 cm stubble. From the total herbage, subsamples were taken from each plot, weighed and separated into the morphological components of leaf blade, stem + sheaths, and dead material. Subsequently, the samples were partially oven-dried at 55ºC for 72 h to determine their respective air-dried sample content and calculations.

For the analysis of the plant's chemical composition, 500 g of samples of herbage were collected from above the 10 cm harvest stubble. These samples were then placed in an oven at 55ºC for 72 h and then ground using a Wiley mill equipped with a 1-mm sieve. The chemical components of forage were analyzed according to the Silva and Queiroz [(21)] methodologies, as follows: Dry matter (DM), crude protein (CP, DM%), neutral detergent fiber (NDF, DM%), and acid detergent fiber (ADF, DM%).

2.4 Statistical analyses

All variables were submitted to analysis of variance by F-test (P<0.05), considering the completely randomized design, organized as a 2 (with and without inoculant - I) x 5 (0, 50, 100, 150, and 200 kg ha^{-1} of nitrogen rates – N) factorial arrangement with four replications. Harvest cycles were treated as repeated measures over time and nitrogen rates, inoculant, and interactions were considered as fixed effects. When only a significant main effect was revealed for the inoculation factor, means were compared by the F test (P<0.05), and when a significant main effect was observed for N (P<0.05), polynomial orthogonal contrasts were used to test the linear, quadratic, cubic, and quartic effects. The data were analyzed using PROC MIXED procedure of SAS software (Statistical Analysis System, version 9.2). The proposed mathematical model was as follows:

Yij=μ+(inoculant-I)i+(nitrogen rates-N)j+(I x N)ij+ εij (1)

in which Yij = is the dependent variable, μ is the general mean value, and εij is the error term associate to experimental unit.

3. Results

There was no interaction between I and N (P>0.05) for the variables regrowth period (RP) and total number of cuts (NC) in the plots. Only fertilization altered these two variables, where the plots obtained the following NC and RP (days) in seven months, respectively: 0 kg N ha^{-1} = 3.71 and 57.00; 50 kg N ha^{-1} = 4.00 and 51.90; 100 kg N ha^{-1} = 4.95 and 42.50; 150 kg N ha^{-1} = 5.70 and 36.70; and 200 kg N ha^{-1} = 6.27 and 33.50.

An interaction effect (I×N) was observed (P≤0.05) only for LAR and SER (Table 2), indicating that the impact of N depended on *A. brasilense* inoculation levels. The NLL was unaffected (P>0.05) by the use of inoculant or N rates in the soil-climatic conditions of the Amazon savannah. Other variables showed significant responses (P≤0.05) only to N rates, suggesting that *A. brasilense* inoculation without N fertilization doesn't significantly alter morphological or productive aspects in *U. humidicola* cv. Comum cultivated in savannah soil.

Table 2. Summary of analysis of variance applied to structural, morphogenetic, yield-related, and chemical composition variables of *Urochloa humidicola* cv. Common subjected to *Azospirillum brasilense* (strains AbV5 and AbV6) under inoculation and nitrogen rates in Amazonian savannah soil.

Variable		P-value		
		Inoculant (I)	N rate (N)	I×N interaction
Structural	NT	0.7968	<0.0001	0.1138
	PH	0.4172	0.4351	0.4139
	LAI	0.1171	0.0039	0.7846
	NLL	0.7537	0.3348	0.1711
Morphogenetic	LAR	0.8402	<0.0001	0.0297
	LER	0.9759	<0.0001	0.1768
	PHY	0.2999	<0.0001	0.0656
	LSR	0.0705	0.0250	0.4195
	LLS	0.3361	<0.0001	0.3228
	SER	0.3130	0.0007	0.0041
Yield	L	0.4674	0.0426	0.3277
	S	0.3412	0.0005	0.3435
	DDM	0.0764	<0.0001	0.7365
	DHA	0.2185	<0.0001	0.1932
Chemical	NDF	0.4986	0.0037	0.1820
	ADF	0.5689	0.0171	0.1128
	CP	0.3851	<0.0001	0.6221

NT - number of tillers; PH - plant height; LAI - leaf area index; NLL - number of live leaves; LAR - leaf appearance rate; LER - leaf elongation rate; PHY - phyllochron; LSR - leaf senescence rate; LLS - leaf lifespan; SER - stem elongation rate; L - leaf percentage; S - stem percentage; DDM - dead material percentage; DHA - daily herbage accumulation; NDF - neutral detergent insoluble fiber; ADF - acid detergent insoluble fiber; and CP - crude protein. P≤0.05 - significant effect.

3.1 Structural variables

The increase in N doses had a significant effect (P≤0.05) on the structural variables NT and LAI and did not alter (P>0.05) the variables PH and NLL (Table 3). The non-significant effect on plant height (PH) confirms that the cuts were made at the recommended average height (20 cm).

Table 3. Effect of nitrogen rates on number of tillers (NT), plant height (PH), leaf area index (LAI), and number of live leaves (NLL) of *Urochloa humidicola* cv. Common in Amazonian savannah soil.

Variable	Nitrogen rate (kg ha^{-1})					SEM	Contrast
	0	50	100	150	200		
NT (tillers m^{-2})	589.66	689.09	774.51	885.17	919.74	21.77	L
PH (cm)	21.21	21.02	21.03	19.36	20.20	0.46	ns
LAI (m^2 m^{-2})	3.00	3.10	3.29	3.38	3.83	0.16	L
NLL	6.57	7.05	6.95	6.71	7.21	0.24	ns

SEM - standard error of the mean; L - linear effect; ns - not significant.

3.2 Morphogenetic variables

In the I×N interaction, LAR exhibited a quadratic response with increasing N rates in the presence of inoculant (Table 4). Without inoculant, LAR showed positive linear behavior. At 200 kg N ha^{-1}, pastures with inoculant had higher LAR (0.272 leaves day^{-1}) compared to those that did not receive it (0.257 leaves day^{-1}).

Table 4. Interaction effect between *Azospirillum brasilense* (strains AbV5 and AbV6) inoculation and nitrogen rates on leaf appearance rate (LAR) and stem elongation rate (SER) and the isolated effect of nitrogen rates on leaf elongation rate (LER), phyllochron (PHY), leaf senescence rate (LSR), and leaf lifespan (LLS) of *Urochloa humidicola* cv. Common in Amazonian savannah soil.

LAR	Nitrogen rate (kg ha^{-1})					SEM	Contrast
(leaves day^{-1})	0	50	100	150	200		
W inoculant	0.158	0.153	0.156	0.189	0.272 a	-	Q
W/o inoculant	0.147	0.156	0.158	0.196	0.257 b	0.0045	L
SER	Nitrogen rate (kg ha^{-1})					SEM	Contrast
(cm day^{-1})	0	50	100	150	200		
W inoculant	0.139 a	0.241 a	0.351 a	0.318 a	0.315 a	-	Q
W/o inoculant	0.175 a	0.264 a	0.294 b	0.242 b	0.171 b	0.017	Q
	Nitrogen rate (kg ha^{-1})					SEM	Contrast
	0	50	100	150	200		
LER (cm day^{-1})	0.735	0.796	0.780	0.929	1.117	0.04	L
PHY (days leaf^{-1})	7.41	7.35	7.15	5.00	4.31	0.24	L
LSR (cm day^{-1})	0.118	0.130	0.100	0.100	0.086	0.011	L
LLS (days)	45.04	48.18	49.54	32.39	28.49	1.01	Q

SEM - standard error of the mean; linear (L) and quadratic (Q) effects. Means followed by different letters in the columns differ from each other by the F test (P≤0.05).

Stem elongation rate (SER) showed responded quadratically to the N rates regardless of an inoculant presence. This variable increased up to 100 kg ha^{-1} and decreased beyond 150 kg ha^{-1} with inoculant. From 100 kg ha^{-1} onwards, the inoculated pastures exhibited higher SER. As for the other morphogenetic variables, N rates had a linear increasing effect on LER, a linear decreasing effect on PHY and LSR, and quadratic effect on LLS. The rate of 200 kg ha^{-1} provided a LLS of 28.49 days, while without any fertilization the leaves remained alive for 45 days.

3.3 Yield variables

The yield variables exhibited varying responses to the different N rates employed (Table 5), displaying a quadratic impact (P≤0.05) on L and S, along with a linear decrease (P≤0.05) in DDM, and a linear increase in DHA.

Table 5. Effect of nitrogen rates on leaf percentage (L), stem percentage (S), dead material percentages (DDM) and daily herbage accumulation (DHA) of *Urochloa humidicola* cv. Common in Amazonian savannah soil.

Variable	Nitrogen rate (kg ha^{-1})					SEM	Contrast
	0	50	100	150	200		
L (%)	47.35	41.72	39.17	43.55	42.84	1.86	Q
S (%)	37.54	43.09	47.84	48.66	49.43	1.21	Q
DDM (%)	15.56	16.74	14.46	7.63	7.18	0.88	L
DHA (kg ha^{-1} day^{-1})	299.21	403.34	416.30	545.98	593.52	48.88	L

SEM - standard error of the mean; linear (L) and quadratic (Q) effects.

Leaf percentage (L) underwent a decline with the rise in N fertilization up to the N rate of 100 kg ha^{-1}, beyond which it escalated, peaking at the maximum N rate of 200 kg ha^{-1}. Steam showcased a quadratic growth pattern, with the highest value recorded at the N rate of 200 kg ha^{-1}. Dead material exhibited a linear descent with escalating N rates, with the lowest value (7.18 %) occurring at the application of 200 kg ha^{-1} of N (Table 5). In the case of DHA, the application of N resulted in a linear rise, with increases of 45.2% and 49.6% witnessed at the application rates of 150 and 200 kg ha^{-1} of N, respectively, in comparison to pastures without fertilization.

3.4 Variables of forage chemical composition

All chemical variables displayed a positive linear trend (P≤0.05) in response to increasing N rates (Table 6). NDF for *U. humidicola* cv. Comum consistently exceeded 70% across all evaluation conditions, while the average ADF surpassed 27%. The CP content ranged from 5.25% for unfertilized pastures to 7.68% for those treated with 200 kg ha^{-1} of N, signifying a 31.6% increase.

Table 6. Effect of nitrogen rates on neutral detergent insoluble fiber (NDF), acid detergent insoluble fiber (ADF), and crude protein (CP) contents of *Urochloa humidicola* cv. Common in Amazonian savannah soil.

Variable	Nitrogen rate (kg ha^{-1})					SEM	Contrast
	0	50	100	150	200		
NDF (% DM)	71.46	71.94	72.80	72.23	74.76	0.52	L
ADF (% DM)	27.90	27.07	27.58	28.24	29.03	0.63	L
CP (% DM)	5.25	6.00	6.02	6.83	7.68	0.12	L

SEM - standard error of the mean; L - linear effect.

4. Discussion

When analyzing the variables on which the inoculant had no significant effect, either alone or in combination with nitrogen fertilization, it is possible to infer the influence of nitrogen on bacterial populations in the soil. Studies have shown that high doses of nitrogen fertilizer can negatively affect microbial populations and mobility, as well as impairing their symbiotic relationship with plants [(22)], which may result in impaired soil health and reduced plant productivity.

Excess nitrogen can alter soil pH, thereby inhibiting microbial growth [(23, 24)]. Additionally, high nitrate levels can serve as an alternative nitrogen source for plants, reducing their dependence on symbiosis and further impacting Azospirillum populations [(25, 26)]. Furthermore, nitrogen itself can reduce microbial motility, hindering their ability to colonize plant roots effectively [(27)].

Another factor that may interfere with the effectiveness of *A. brasilense*, particularly when applied via foliar spraying in forage crops, is ambient temperature. The optimal temperature range for the development of *A. brasilense* in culture medium lies between 28°C and 34°C [(28)]. However, under field conditions, temperature fluctuations may affect both the survival of the bacterium in the soil and its colonization of the rhizosphere or internal plant tissues. Therefore, a possible explanation for the limited effectiveness of *A. brasilense* in enhancing forage yield and chemical composition may be directly associated with the high maximum temperatures observed at the experimental site, which exceeded 33°C.

High temperatures can induce thermal stress in bacterial cells, compromising their viability and functionality. Some studies indicate that prolonged exposure to temperatures above 40°C may result in the loss of biological nitrogen fixation capacity and a decrease in the production of auxins such as indole-3-acetic acid (IAA) [(29)]. Moreover, excessive heat can affect the stability of commercial inoculants both during storage and after field application [(30)].

Temperature can also influence the interaction between *A. brasilense* and the host plant. Under favorable conditions, such as mild temperatures and adequate moisture, root colonization tends to be more intense, enhancing the expression of the microorganism's beneficial effects [(31)]. Conversely, under extreme temperatures, the plant may prioritize stress tolerance mechanisms, thereby reducing its receptivity to microbial colonization [(32)].

4.1 Structural variables

Urochloa humidicola cv. Comum showcased remarkable sensitivity to N fertilization, especially evident in leaf production and tillering. This echoes findings by Silva *et al.* [6] and Santos *et al.* [33] in Marandu grass (U. brizantha cv. Marandu) and Silva *et al.* [34] in Massai grass (Panicum maximum) across diverse Brazilian regions. The reported linear increases in tillering ($\hat{Y} = 363.45 + 1.102N$) by Silva *et al.* [6] and ($\hat{Y} = 614.7 + 1.201N$) by Santos *et al.* [33] underscored the N-dependent rise in these studies. A notable distinction is the heightened response magnitude in *U. humidicola* cv. Comum, especially in the context of the NT (Table 3), suggesting that the interaction between the soil and climatic conditions in the Amazon savannah and N fertilization maximizes pasture structure enhancement, particularly in tiller count.

4.2 Morphogenetic variables

Results regarding LAR under the influence of N fertilization, in conjunction with *A. brasilense* inoculation, resembled findings by Silva *et al.* [35], who reported elevated LAR values of 0.15 and 0.12 leaf day^{-1} for U. decumbens and U. brizantha respectively at an N rate of 338 kg ha^{-1}. Leaf appearance rate, a product of several factors such as sheath length, leaf elongation, and temperature, predominantly responds to N supply [36, 37]. For SER, heightened influence from N was observable at higher rates when paired with *A. brasilense*, possibly due to accelerated plant growth resulting in shading of lower leaves and basal buds. This scenario can stimulate stem elongation in search of more light. Sizeable N rates can similarly bolster LAR and SER, a phenomenon attributed to heightened tissue turnover triggered by enhanced cell production [37, 38].

As emphasized by Cruz *et al.* [39], the evaluation of morphogenetic variables provides insights into plant behavior in response to applied influences. These variables delineate the temporal-spatial expansion of plant organs, revealing their ecological adaptation and the accumulation of productive components of forage species within cultivated areas. According to Souza *et al.* [40], morphogenesis sheds light on species behavior and physiological limits, paving the way for management recommendations in alignment with pasture needs and agronomic challenges. In this context, the increase in N rates up to 200 kg ha^{-1} positively impacted *U. humidicola* morphogenesis in Amazon savannah soil, resulting in daily increments of leaf and stem development while curtailing leaf senescence and PHY. Notably, the decline in LLS with increasing N rates implies that for optimal pasture utilization, an interval of 28 to 32 days offers a green leaf-rich canopy.

The findings also establish a contrasting relationship between leaf appearance and elongation, compared with phyllochron and leaf lifespan, a trend supported by Cruz *et al.* [39]. According to Abreu *et al.* [41], the reduction in leaf lifespan and phyllochron due to N fertilization arises from the synergy between leaf emergence and elongation. This interaction leads to a shorter leaf lifespan as N levels increase, thereby accelerating the turnover rate of plant tissues through the acceleration of morphogenetic processes during vegetative growth.

The interaction between nitrogen fertilization and the presence of inoculant showed a significant effect on LAR. The quadratic response observed with increasing nitrogen rates in the presence of the inoculant suggests a potential optimization of leaf development at intermediate fertilization levels. In the absence of the inoculant, the positive linear trend in LAR indicates that nitrogen directly promotes leaf formation, although without the additional benefits of microbial stimulation.

Stem Elongation Rate (SER) exhibited a quadratic response regardless of the presence of the inoculant. However, inoculated pastures showed higher SER from 100 kg ha^{-1} onwards, highlighting the role of *A. brasilense* in enhancing vegetative growth under moderate fertilization conditions. This interaction can be strategically exploited to maximize biomass production, particularly in systems where fertilizer use is limited due to cost constraints or environmental concerns.

4.3 Yield variables

The stem (S) percentage exhibited a quadratic pattern in contrast to the trend observed in leaf (L). The augmentation in fertilization led to an exponential increase in S, while L experienced a decline until the application of 100 kg ha^{-1} of N, followed by subsequent growth. These findings suggest that beyond an N rate of 100 kg ha^{-1}, *U. humidicola* pastures tend to augment leaf production while stabilizing stem production (Table 5).

The reduction in DDM within pastures with rising N rates can be elucidated by the decrease in LSR observed in the tillers. Comparable outcomes were also noted by Silva *et al.* (34) following elevated N rates in Massai grass (P. maximum) pastures during periods of heightened luminosity and temperature. This highlights that these conditions, characteristic of the Amazon savannah, enhance the favorable impact of N rates on pastures.

Regarding DHA, Delevatti *et al.* (42) also observed a linear increase in tropical pastures due to N application. This phenomenon is mainly attributed to the rise in tillering as a response to N fertilization in tropical grass a trend also seen in our study (Table 5). Nonetheless, the magnitude of this effect is dependent on local soil-climatic conditions and the cultivated forage species (43).

4.4 Variables of forage chemical composition

Increasing N rates provided an increase in plant fiber content. Alves *et al.* (44) explained that NDF represents the cell wall nutritional fraction, comprising cellulose, hemicellulose, and lignin, while ADF encompasses the fraction of cellulose bound to lignin, a phenolic compound indigestible to animals. According to those authors, carbohydrates rich in NDF and ADF have slow degradation and passage rates through the reticulum and rumen, reducing total dry matter intake due to reticulum and rumen filling constraints, which might hinder the expression of animals' genetic potential for production. Though fiber production in our study surpassed ideal levels for animal consumption, akin to results from different studies involving Urochloa grasses (2, 42), these fiber values seem characteristic of certain species within the Urochloa genus, such as *U. humidicola*.

Despite the heightened NDF and ADF content, the increase in N doses increased the forage CP. These outcomes hold significance, as enhanced productive rates correlated with higher CP values, a primary limiting factor for dry matter intake and weight gain in animals grazing *U. humidicola* pastures (2). Nitrogen has a pivotal role in plant constituents, including chlorophylls, proteins, amino acids, enzymes, and several other essential compounds for plant maintenance and development, as emphasized by Taiz *et al.* (9). Insufficient nitrogen curbs plant growth swiftly. Responses to N rates in Urochloa pastures are widely debated in the literature, mirrored by the findings of Oliveira *et al.* (45), which indicates increased productivity and improved chemical quality, especially in protein content, due to heightened N fertilization. This aligns with the observations of Fonseca *et al.* (46) regarding the positive influence of various N sources applied to the soil on pasture protein content.

In tropical regions, like the Amazon savannah, Pereira *et al.* [(43)] underline that N fertilization for perennial grass often disregards climatic variations throughout the vegetative cycle, plant demand, as well as N availability and losses in the soil. Hence, precise fertilization management based on specific and seasonal recommendations in the region could greatly contribute to pasture establishment and management guidelines.

Overall, the application of *A. brasilense* did not notably enhance the examined variables, even when combined with N fertilization. These outcomes might be linked to the applied inoculation method and local climatic conditions. The manufacturer suggests that the *A. brasilense* strains AbV5 and AbV6 can be applied through spraying on established plants, as done in our study, or through seed inoculation, as seen in studies with positive responses. Thus, the impacts of high local temperatures, characteristic of the region, could have hindered the symbiotic process between plants and bacteria when applied via spraying. This emphasizes the need for a better grasp of how to handle this inoculant within Amazon savannah conditions.

5. Conclusion

The utilization of *Azospirillum brasilense*, applied on leaves and in established pasture independently or in conjunction with N fertilization, does not confer advantages regarding the forage accumulation and chemical composition of *Urochloa humidicola* grown in Amazon savannah soil. On the other hand, nitrogen fertilization in the range of 100 to 200 kg ha^{-1} exhibits positive effects on the structural and morphogenetic aspects of *U. humidicola* pastures cultivated in Amazon savannah soil. Additionally, this range of nitrogen applications fosters enhanced herbage accumulation. The chemical composition of *U. humidicola* cultivated in the Amazon savannah is influenced by nitrogen fertilization, leading to larger protein and fibrous content in response to the heightened N availability. For future studies, we recommend evaluating *A. brasilense* strains that are tolerant of higher temperatures, such as those found in Roraima. These strains should be combined with alternative application methods in established pastures. In the case of establishing a pasture, the strains should be applied via seed inoculation. Regarding soil analysis, we suggest performing chemical analyses at the end of the experimental period to assess the long-term effects of the treatments. Future research should explore the influence of interactions between *A. brasilense*, nitrogen application rates and other environmental factors on the ecological dynamics and economic viability in tropical pastures.

Conflict of interest statement

The authors have no conflict of interest in the manuscript.

Data availability statement

The data will be provided upon request.

Author contributions

Conceptualization: J.W. L.Castro, J. Lopes and W.L. da Silva. Análise formal: W.L. da Silva. Investigation: J.W. L.Castro, E.M. Severo, V. B. de Souza Júnior and J. L. L. Monteiro Neto. Methodology: J. Lopes and W.L. da Silva. Visualization: V. B. de Souza Júnior and N. C. da Silva. Writing (original draft): J.W. L.Castro. Writing (review and editing): W.L. da Silva.

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