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Foliar Spray Inoculation with Plant Growth Promoting Bacteria Associated with Nitrogen Doses in *Megathyrsus maximus* cv. BRS Zuri

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Abstract: This study evaluated the combined effect of foliar spray inoculation with plant growth-promoting bacteria (PGPB) and nitrogen doses on the yield, development, and nutritive value of *Megathyrsus maximus* cv. BRS Zuri. The experimental design was randomized blocks with four replications, with repeated measures in time. Foliar inoculation of two bacteria (*Azospirillum brasilense* Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084) and *Pseudomonas fluorescens* (CNPSo 2799)) and 40 and 80 kg ha^{-1} N (urea) rates, in addition to the control (without inoculation and N fertilization), were applied. In the rainy season, at the level of 40 kg ha^{-1} of N, inoculation of both *A. brasilense* and *P. fluorescens* increased, respectively, the tiller number by 33% and 25% (22 February), and the N accumulated in tissues by 42% and 25% (22 January), while in the previous year (21 February) the beneficial effects of both bacteria were observed in the percentage of leaf blade and in the true digestibility *in vitro*. When the foliar spray was inoculated with *A. brasilense* Ab-V5 and Ab-V6 and *P. fluorescens* CNPSo 2799, with 80 kg N ha^{-1}, the root system of Zuri grass increased by 61% and 30%, respectively.

Keywords: biological nitrogen fixation; *Pseudomonas fluorescens*; *Azospirillum brasilense*; root system

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1. Introduction

Forage plants, mainly represented by the genus *Urochloa* spp. (syn. *Brachiaria*) and *Megathyrsus* spp. (syn. *Panicum*), constitute the main source of food for cattle [1]. Within the genus *Megathyrsus*, *M. maximus* has been widely cultivated in tropical and subtropical regions [2], and considerable advances have been made in improving existing pastures. Zuri grass (*M. maximus* cv. BRS Zuri) is an important cultivar because of its agronomic and nutritional qualities. Besides fast growth and high biomass yield, Zuri grass regenerates well over successive cycles [3].

Fertilizers represent an alternative to potentially reduce seasonal variations in tropical forages and can increase their quality. However, commercial fertilizers are the most expensive inputs, although repeated fertilization or high amounts of N alone can cause nutrient imbalances in the soil and ultimately negatively affect forage yield and nutritional value [4]. Researchers have reported that use of growth promoters, while reducing their intake and increasing efficiency of chemical fertilizers, increases plant growth by increasing N and P absorption [5]. In sustainable agricultural systems, the use of biological fertilizers is important in increasing product production and maintaining sustainable soil fertility. Today, bio-fertilizers are considered as an alternative to chemical fertilizers to increase soil fertility and production of products in sustainable agriculture [6,7]. In addition, with the growing concern for the development of sustainable and less polluting agriculture, searching for alternatives to reduce the environmental impact of mineral fertilizers is necessary [8] without causing losses in productivity and nutritional quality [9,10].

Nitrogen fertilizers are expensive, and their industrial yield, through the traditional Haber–Bosch process, is currently responsible for 1.2% of anthropogenic CO_2 emissions [11], as it requires high amounts of fossil fuels. Therefore, the partial replacement of nitrogen fertilizers by microorganisms that perform biological nitrogen fixation (BNF) and promote plant growth through alternative approaches can reduce CO_2 emissions [12].

Rhizobacteria of the genus *Azospirillum* can associate with grasses and enhance crop growth and productivity through several mechanisms, including the yield of phytohormones, BNF, phosphorus solubilization, and increased root development. In addition, they can increase plant resistance and attenuate stress caused by biotic and abiotic factors, such as attack by phytopathogens, radiation and excessive temperature, salinity, and drought [12–14].

In *Urochloa brizantha* and *U. ruziziensis*, the inoculation of Ab-V5 and Ab-V6 strains of *A. brasilense* associated with doses of N-fertilizer, promoted significant increases in forage mass yield, with the highest increases observed for the treatments with bacteria associated with a dose of 40 kg N ha^{-1} [15]. The research on sunflower plant showed that simultaneous use of Azotobacter, Azospirillum, Pseudomonas, and U.S. cadmium increased the grain yield [16]. Furthermore, a study with the inoculation of *Pseudomonas fluorescens* in *U. decumbens* showed an increase in the elongation rate of stems and the number of leaves per tiller [17]. This factor may increase the leaf:stem ratio, a desirable characteristic in animal feed because it may provide a material of greater nutritional value. Plant height, dry weight, and dry leaves of corn plants increased by inoculation with Azospirillum bacteria [18], while fresh weight of the aerial part of the plant, leaf number, and corn plant height increased by the inoculation of its seeds with the bacteria of the genus Pseudomonas [19].

This study hypothesized that foliar spray inoculation of *M. maximus* with plant growth-promoting bacteria (PGPB) associated with N doses could reduce the amount of nitrogen fertilizer and obtain high forage dry mass yield with high-value nutrition more sustainably. Therefore, the objective of this study was to evaluate the nutrition, development, nutritive value, and dry mass yield of shoots and roots of *Megathyrsus maximus* cv. BRS Zuri after foliar spray inoculation with PGPB associated with doses of N.

2. Materials and Methods

2.1. Location and Climatic Conditions

The experiment was conducted in a forage *M. maximus* cv. BRS Zuri field, which was established two years ago (2018) in Araçatuba County, located in the northwest region of the São Paulo State, Brazil, altitude of 390 m, latitude 21°10′53″ S, and longitude 50°26′07″ W. The climate is defined as Aw, according to the Köppen classification [20].

The local soil is classified as Red Yellow Acrisol according to the International System [21]. Soil collections were conducted at a depth of 0–0.2 m, resulting in 15 samples for chemical analysis. The results were as follows: M.O. = 24 g dm^{-3}; pH = 4.9; 9, 1, 0.19, 1, 80, 9.8, and 1.2 mg dm^{-3} for P (phosphorus resin), S, B, Cu, Fe, Mn, and Zn, respectively; 1.9, 13, 11, 30, 1, 25.9, and 55.9 mmol$_c$ dm^{-3} for K, Ca, Mg, H + Al, Al, SB, and CEC, respectively, and base saturation V = 46.3%. The liming requirement was determined using the base saturation method to reach 70% of the cation exchange capacity [22].

Dolomitic limestone with 90% neutralizing power was used for soil correction at the end of August 2020, at the time of grass implantation in the area. For surface fertilization with phosphorus, 100 kg of P_2O_5 per hectare in the form of simple superphosphate and 60 kg ha^{-1} of K_2O in the form of potassium chloride were applied by hand broadcast one week after the grass standardization cut, and 40 and 80 kg N ha^{-1} in the form of urea in all inoculated treatments and the non-inoculated control. Notably, the fertilizations were conducted considering the weather forecast. After fertilization, it rained in the area, and irrigation was not necessary.

2.2. Experimental Design and Treatments

The experiment was a randomized block design, with repeated measures in time, replicated four times, with seven treatments. Treatment 1 (control—no N and no inoculation), treatment 2 (40 kg ha^{-1} of urea and no inoculation), treatment 3 (80 kg ha^{-1} of urea and no inoculation), treatment 4 [*Azospirillum brasilense* Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084) plus 40 kg ha^{-1} of urea], treatment 5 [*Azospirillum brasilense* Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084) plus 80 kg ha^{-1} of urea], treatment 6 [*Pseudomonas fluorescens* (CNPSo 2799) plus 40 kg ha^{-1} of urea] and treatment 7 [*Pseudomonas fluorescens* (CNPSo 2799) plus 80 kg ha^{-1} of urea].

The strains were from a selection program carried out in Brazil and are used commercially. *A. brasilense* Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084) are used as inoculants for maize (*Zea mays* L.) [23], wheat (*Triticum aestivum* L.) [23], *Urochloa* spp. (*Brachiaria* spp.) [15] and coinoculation of soybean [*Glycine max* (L.) Merrill] [24] and bean (*Phaseolus vulgaris* L.) [24], and *P. fluorescens* is used in maize [25].

The inoculants were produced at Embrapa Soja Soil Biotechnology Laboratory (Londrina, Paraná State, Brazil), *A. brasilense* Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084) were prepared in DYGS medium [26], and *P. fluorescens* (CNPSo 2799) in TSB medium [27].

The treatments that received foliar inoculation were sprayed and fertilized with N in the form of urea every two cuts, with an interval between each cut of approximately 45 days, except in the dry season when there was a rest for the forage plants. Following the laboratory recommendation, the inoculant rates for foliar application were 300 mL ha^{-1}. Therefore, the volume of spray solution used was 200 L ha^{-1} (200 mL of distilled water per 9 m^2 plot, which contained 270 μL of the respective inoculant). The bacterial concentration of the inoculants was adjusted to 2 × 10^8 cells mL^{-1} for *A. brasilense* and 1 × 10^8 cells mL^{-1} for *P. fluorescens*. The applications were carried out under ideal temperature and humidity conditions, usually in the late afternoon, at mild temperatures.

2.3. Collections and Chemical-Bromatological Composition

During the months of the experiment, the cuts were made in February 2021, March 2021, May 2021, November 2021, January 2022, February 2022, and March 2022. February 2021, March 2021, January 2022, February 2022, and March 2022 were considered the rainy season (summer), whereas the dry season (winter) was May 2021 and November 2021. Seven cuts were conducted during the dry and rainy seasons to determine the plant's dry mass when the plots partially reached the height of 0.7 m [28] and when the shoots were harvested at 0.15 m above the soil surface. The samples to estimate the dry weight of the aerial part were collected with a sampler square with an area of 1.0 m^2, and a sickle (cleaver) was used to cut the interior plants to cut rice.

Before each cut, plant height readings were measured with a millimeter ruler, and the relative chlorophyll index (SPAD) was obtained using a SPAD-502 Plus digital chlorophyll meter (SPAD—Development of soil and plant analysis), and in both cases, readings were performed on newly expanded leaf slides. The tiller number was counted using a 0.25 m^2 circle inside each plot. Afterward, it was multiplied by four to express the number of tillers per m^2.

After harvesting, the plant material was weighed, a sample of approximately 200 g of the leaf blade fractions was separated, and the stem + sheath was identified, weighed, and dried in an oven with forced air circulation at a temperature of 55 °C for 72 h, as described by [29]. The dry material was weighed on a precision scale to quantify the yield of forage dry mass, and the samples were ground in a Wiley-type micro-mill R-TE-648 through a 1 mm sieve. The nutritive value (crude protein, neutral detergent fiber, and acid detergent fiber) was determined according to [30], whereas true in vitro dry matter digestibility (IVDMD) was determined according to [31].

The total N content was determined by sulfuric digestion followed by the semi-micro distillation Kjeldahl method [32]. Nutrient accumulation was calculated by multiplying the concentration by the amount of dry weight produced from each cut.

The root system was evaluated at the end of the experiment. The root biomass of the stratified pasture was determined at depths of 0–0.20 m through a sample per plot, using a cylindrical steel tube 0.50 m long and 0.10 m in diameter, with an opening to facilitate the stratification of the samples. The roots were separated from the soil by successive washing in running water in sieves with a 1 mm mesh until it was no longer possible to identify any soil contamination. Afterward, the root samples were dried in an oven with forced air circulation at 65 °C until constant weight.

2.4. Statistical Analysis

The data were tested for normality of errors and the variables dry mass yield, botanical composition, chlorophyll content, and chemical-bromatological composition in the model of repeated measurements over time in split plots.

For cumulative yield, data were grouped according to summer (February 2021, March 2021, January 2022, February 2022, and March 2022) and winter (May 2021 and November 2021) seasons. Base 10 logarithm transformation on root yield, the number of tillers, dry weight yield, N uptake, and N concentration was applied. The results were submitted to ANOVA, followed by the Scott–Knott multiple comparison test ($p \leq 0.10$), which was performed using the SISVAR program version 5.6 [33].

According to Normative Instruction No. 13, when the "F" test is not significant at 5% but significant at 10%, the means of the treatments must be compared using the mean test, also at the 10% level of significance [34].

3. Results

3.1. Plant Height and Tiller Number

For average plant height, the cuts for February 2021, March 2021, November 2021, January 2022, February 2022, and March 2022 were statistically significantly different (Table 1). For the February 2021 cut, the treatments fertilized with 80 kg ha^{-1} N showed higher means than those of the other treatments. In contrast, in the cuts for March and November 2021, only the control was lower than the other treatments. For the January 2022 harvest, 80 kg ha^{-1} mineral N and *P. fluorescens* CNPSo 2799 plus 80 kg ha^{-1} N had higher means. However, inoculation of *A. brasilense* Ab-V5 + Ab-V6 associated with a dose of 40 kg ha^{-1} mineral N was 5.4% higher compared to the same rate of mineral N in the absence of inoculation for the January 2022 evaluation.

February 2021 showed better results for 80 kg ha^{-1} mineral N, *A. brasilense* Ab-V5 + Ab-V6 associated with a rate of 40 kg ha^{-1} mineral N, *A. brasilense* Ab-V5 + Ab-V6 associated with 80 kg ha^{-1} mineral N and *P. fluorescens* CNPSo 2799 plus 80 kg ha^{-1} N. A positive effect of 7.8% of the treatment inoculated with *A. brasilense* Ab-V5 + Ab-V6 associated with 40 kg ha^{-1} mineral N was observed compared to the same dose of mineral N without inoculation. Regarding the evaluation of March 2022, the treatment inoculated with *P. fluorescens* CNPSo 2799 plus 80 kg ha^{-1} N was higher than that for the other treatments and was 9.3% higher than that for the treatment fertilized with the same N rate.

A significant effect for the cuts of February 2021, March 2021, November 2021, January 2022, February 2022, and March 2022 was observed for the number of tillers per m^2 (Table 1). In February 2021, only the control was lower than that for the other treatments. The treatment inoculated via the foliar spray with *A. brasilense* Ab-V5 + Ab-V6 fertilized with 40 kg ha^{-1} N produced a 45% increase in the number of tillers compared to the treatment with 40 kg ha^{-1} N and was statistically higher compared to the other treatments for the evaluation of March 2021. A similar effect was found for the evaluation of January 2022, which produced a 30% increase in the number of tillers compared to the treatment with 40 kg ha^{-1} N. However, for the cut of November 2021, the treatments of the foliar spray inoculated with *A. brasilense* Ab-V5 + Ab-V6 fertilized with 80 kg ha^{-1} N and *P. fluorescens* CNPSo 2799 fertilized with 40 kg ha^{-1} N were higher compared with other treatments and produced 21% and 13% increases in the number of tillers compared to the treatment with the dose of mineral fertilizer, respectively.

Table 1. Mean plant height and number of tillers per square meter of *Megathyrsus maximus* cv. BRS Zuri inoculated with PGPB.

	Height of the Plants (cm)						
Treatments	**Feb-2021**	**Mar-2021**	**May-2021**	**Nov-2021**	**Jan-2022**	**Feb-2022**	**Mar-2022**
Control	65 e	62 b	38	46 b	88 d	79 b	80 c
40 kg ha^{-1} of N	88 b	62 a	40	52 a	92 c	76 b	95 b
80 kg ha^{-1} of N	98 a	71 a	40	52 a	105 a	85 a	96 b
A. brasilense + 40 kg ha^{-1} of N	81 c	69 a	41	53 a	97 b	82 a	94 b
A. brasilense + 80 kg ha^{-1} of N	93 b	69 a	42	55 a	98 b	82 a	98 b
P. fluorescens + 40 kg ha^{-1} of N	74 d	68 a	40	51 a	94 c	77 b	94 b
P. fluorescens + 80 kg ha^{-1} of N	92 b	66 a	37	52 a	101 a	87 a	105 a
CV (%)	8.74	8.74	8.74	8.74	8.74	8.74	8.74
p-value	0.0001 **	0.0022 **	0.6012 ^{ns}	0.0460 *	0.0001 **	0.0003 **	0.0001 **
	Tillers Number per m^2 ***						
Control	127 b	136 b	95	104 b	130 b	96 b	123 b
40 kg ha^{-1} of N	197 a	132 b	97	105 b	119 b	114 b	166 a
80 kg ha^{-1} of N	171 a	158 b	96	105 b	122 b	122 b	171 a
A. brasilense + 40 kg ha^{-1} of N	192 a	192 a	90	99 b	155 a	152 a	131 b
A. brasilense + 80 kg ha^{-1} of N	213 a	153 b	104	127 a	111 b	102 b	150 a
P. fluorescens + 40 kg ha^{-1} of N	168 a	152 b	99	119 a	134 b	143 a	154 a
P. fluorescens + 80 kg ha^{-1} of N	167 a	163 b	92	95 b	112 b	118 b	180 a
CV (%)	6.70	6.70	6.70	6.70	6.70	6.70	6.70
p-value	0.0012 **	0.0255 *	0.5188 ^{ns}	0.0842 *	0.0373 *	0.0009 **	0.0063 **

Means with the same lowercase letters in the column are not different using the Scott–Knott test ($p \leq 0.10$). ns = not significant. * significant at 5% probability. ** significant at 1% probability. *** Tiller number data were transformed to the base 10 logarithm.

In contrast, for the February 2022 evaluation, there was a significant effect for the foliar spray treatments inoculated with *A. brasilense* Ab-V5 + Ab-V6 and *P. fluorescens* CNPSo 2799 fertilized with 40 kg ha^{-1} N compared to the other treatments, producing 33% and 25% increases in the number of tillers compared to the treatment with the same dose of mineral fertilizer, respectively. For the evaluation of March 2022, only the control treatments and *A. brasilense* Ab-V5 + Ab-V6 associated with 40 kg ha^{-1} N were inferior to the other treatments.

3.2. Forage Accumulation by Seasons

Treatments significantly affected accumulated yield of the dry mass of the shoots (DMS) for summer, winter, and total seasons (Table 2). For example, in the summer season, the treatment plants were fertilized with 80 kg ha^{-1} mineral N and the foliar spray inoculated with *A. brasilense* Ab-V5 (CNPSo 2083) + Ab-V6 (CNPSo 2084) and *P. fluorescens* CNPSo 2799 associated with fertilization with 80 kg ha^{-1} N obtained higher means of the DMS compared to the other treatments. However, as in the winter period, which corresponded to collections from May 2021 to November 2021, the averages of the accumulated DMS differed only from the negative control, with no significant differences between the other treatments.

Although there was no statistical difference, the foliar spray treatments inoculated with *P. fluorescens* CNPSo 2799 and *A. brasilense* Ab-V5 + Ab-V6 plus 40 kg ha^{-1} mineral N showed 8.5% and 4.3%, respectively, with increased DMS compared to the 40 kg ha^{-1} mineral N in the winter period.

The accumulated DMS in seven evaluations during the 14 months of the evaluation showed a significant effect for treatments fertilized with 80 kg ha^{-1} mineral N and foliar spray inoculated with *A. brasilense* Ab-V5 (CNPSo 2083) + Ab-V6 (CNPSo 2084) and *P. fluorescens* CNPSo 2799 associated with 80 kg N ha^{-1} fertilization. Although not significantly different, the treatment plants inoculated with *A. brasilense* Ab-V5 + Ab-V6 fertilized with 40 kg ha^{-1} of N produced a 4% increase than that for the fertilized treatment with 40 kg ha^{-1} mineral N in the total accumulated DMS (Table 2).

Table 2. Means of accumulated yield of dry mass of the shoots in the summer, winter, and total seasons, and root dry weight yield of *Megathyrsus maximus* cv. BRS Zuri inoculated with PGPB.

	Accumulated Yield of Dry Mass			
Treatments	**Summer ($kg\ ha^{-1}$)**	**Winter ($kg\ ha^{-1}$)**	**Total ($kg\ ha^{-1}$)**	**Roots *** ($kg\ m^{-3}$)**
Control	7861 c	2269 b	10130 c	5.3 c
40 kg ha^{-1} of N	12169 b	3675 a	15844 b	7.7 a
80 kg ha^{-1} of N	14695 a	4304 a	18996 a	5.9 b
A. brasilense + 40 kg ha^{-1} of N	12653 b	3835 a	16488 b	6.9 a
A. brasilense + 80 kg ha^{-1} of N	14502 a	3562 a	18064 a	9.5 a
P. fluorescens + 40 kg ha^{-1} of N	11701 b	3991 a	15691 b	6.3 a
P. fluorescens + 80 kg ha^{-1} of N	14130 a	3632 a	17763 a	7.7 a
CV (%)	12.43	16.25	10.95	16.94
p-value	0.0001 **	0.0043 **	0.0001 **	0.0063 **

Means with the same lowercase letters in the column are not different using the Scott–Knott test ($p \leq 0.10$). ns = not significant. ** significant at 1% probability. *** Root dry weight data were log 10 transformed.

In evaluating the root system in the 0–0.20 m layer at the end of the experiment, significant differences were observed between treatments (Table 2). The fertilized treatments with 40 kg ha^{-1} N, the foliar spray inoculated with *A. brasilense* Ab-V5 + Ab-V6, and *P. fluorescens* CNPSo 2799, both associated with 40 and 80 kg ha^{-1} mineral N, were higher than that for other treatments. Treatments inoculated with *A. brasilense* and *P. fluorescens* associated with 80 kg ha^{-1} mineral N produced 61% and 31%, respectively, increased root dry weight than that for the treatment with the highest mineral N rate with no inoculation.

3.3. Relative Chlorophyll Index and Yield of Dry Mass of the Shoots

For the relative chlorophyll index (RCI) of Zuri grass, the cuts from February 2021, May 2021, November 2021, January 2022, February 2022, and March 2022 showed significant differences between treatments (Table 3). The February 2021 cut had a higher RCI for the treatment fertilized with a dose of 80 kg ha^{-1} N and the foliar spray treatment inoculated with *A. brasilense* Ab-V5 + Ab-V6 fertilized with a dose of 80 kg ha^{-1} No mineral. For the May 2021 cut, despite the higher RCI for the fertilized treatment with 80 kg ha^{-1} mineral N, the treatments inoculated with *A. brasilense* Ab-V5 + Ab-V6 and *P. fluorescens* CNPSo 2799 fertilized with 40 kg ha^{-1} mineral N were higher compared to the treatment fertilized with the same rate in the absence of inoculation, confirming the positive effect of the inoculation.

For the November 2021 cut, only the treatment fertilized with 80 kg ha^{-1} N was higher than that for the other treatments. In contrast, for January 2022, in addition to the fertilized treatment with 80 kg ha^{-1} N, the treatments inoculated with *A. brasilense* Ab-V5 + Ab-V6 and *P. fluorescens* CNPSo 2799 fertilized with 80 kg ha^{-1} mineral N were higher. For the February 2022 cutting, only the treatments fertilized with 40 kg ha^{-1} N and those inoculated with *A. brasilense* Ab-V5 + Ab-V6 fertilized with 40 kg ha^{-1} N were lower than that for the other treatments. For the February 2022 cut, the treatment inoculated with *P. fluorescens* CNPSo 2799 fertilized with 40 kg ha^{-1} mineral N was 12.5% higher than that for the fertilized treatment with the same rate in the absence of inoculation.

For the evaluation of March 2022, only the fertilized treatment with 80 kg ha^{-1} N and the treatment inoculated with *P. fluorescens* CNPSo 2799 fertilized with 80 kg ha^{-1} mineral N were higher than that for the other treatments. Generally, mean RCI values ranged from 24 to 36 SPAD units.

The average DMS showed significant results for the cuts of February 2021, March 2021, November 2021, January 2022, February 2022, and March 2022 (Table 3). For the evaluation of February 2021, the plants of the fertilized treatments with 40 kg ha^{-1} mineral N, 80 kg ha^{-1} N, and those inoculated with *A. brasilense* Ab-V5 + Ab-V6 plus 80 kg ha^{-1} N were statistically higher than that for the others. For the March 2021 cut, the fertilized treatment with 80 kg ha^{-1} N and the inoculated treatment via the foliar spray with *A. brasilense* Ab-V5 + Ab-V6 plus 80 kg ha^{-1} N were statistically higher compared with the others.

Table 3. Averages of relative chlorophyll indices and shot dry mass yield of *Megathyrsus maximus* cv. BRS Zuri inoculated with PGPB.

	Relative Chlorophyll Indices (SPAD Units)						
Treatments	**Feb-2021**	**Mar-2021**	**May-2021**	**Nov-2021**	**Jan-2022**	**Feb-2022**	**Mar-2022**
Control	32 c	31	25 c	31 c	28 c	27 a	28 e
40 kg ha^{-1} of N	32 c	30	26 c	34 b	30 b	24 b	32 c
80 kg ha^{-1} of N	36 a	29	30 a	36 a	34 a	27 a	36 a
A. brasilense + 40 kg ha^{-1} of N	32 c	29	28 b	33 c	30 b	24 b	33 c
A. brasilense + 80 kg ha^{-1} of N	36 a	30	29 b	34 b	33 a	26 a	34 b
P. fluorescens + 40 kg ha^{-1} of N	31 c	30	28 b	32 c	31 b	27 a	30 d
P. fluorescens + 80 kg ha^{-1} of N	34 b	29	28 b	33 b	33 a	28 a	36 a
CV (%)	8.24	8.24	8.24	8.24	8.24	8.24	8.24
p-value	0.0001 **	0.4590 ^{ns}	0.0001 **	0.0001 **	0.0001 **	0.0005 **	0.0001 **
	Yield of Dry Mass of the Shoots (kg ha^{-1}) ***						
Control	1398 c	1571 b	1203	1066 b	1816 c	1812 b	1263 c
40 kg ha^{-1} of N	3313 a	1817 b	1269	2406 a	2788 b	1919 b	2333 b
80 kg ha^{-1} of N	3941 a	2060 a	1460	2844 a	3474 a	2503 a	2715 a
A. brasilense + 40 kg ha^{-1} of N	2765 b	1898 b	1263	2572 a	3381 a	2026 a	2583 a
A. brasilense + 80 kg ha^{-1} of N	3494 a	2350 a	1098	2464 a	3336 a	2231 a	3090 a
P. fluorescens + 40 kg ha^{-1} of N	2458 b	1862 b	1172	2820 a	3072 b	2176 a	2133 b
P. fluorescens + 80 kg ha^{-1} of N	2944 b	1810 b	1257	2376 a	3911 a	2442 a	3024 a
CV (%)	3.77	3.77	3.77	3.77	3.77	3.77	3.77
p-value	0.0001 **	0.0186 **	0.2991 ^{ns}	0.0001 **	0.0001 **	0.0132 **	0.0001 **

Means with the same lowercase letters in the column are different using the Scott–Knott test ($p \leq 0.10$). ns = not significant. ** significant at 1% probability. *** Dry weight yield data were transformed to the base 10 logarithm.

Although the evaluation did not show a statistical difference in November 2021, which precedes the entire dry season period (Figure 1), the plants in the treatments inoculated with *P. fluorescens* and *A. brasilense* with 40 kg ha^{-1} mineral N produced 17% and 7%, respectively, compared to the treatment with only 40 kg ha^{-1} mineral N. In the second year of evaluation, foliar spraying in January 2022 on plants inoculated with *A. brasilense* Ab-V5 + Ab-V6 was statistically higher and promoted an additional 21% compared with the treatment fertilized with 40 kg ha^{-1} mineral N without inoculation. In the evaluation of February 2022, the treatments inoculated with *A. brasilense* and *P. fluorescens* associated with 40 kg ha^{-1} N were higher compared with the same N dose without inoculation and produced a 6.0% and 13.0% increase, respectively. For March 2022, the re-inoculation with PGPB by spraying the aerial part increased *A. brasilense* and *P. fluorescens* plus 80 kg ha^{-1} N by 14% and 11%, respectively, compared with the 80 kg ha^{-1} mineral N and without inoculation. However, the treatment inoculated with *A. brasilense* fertilized and with 40 kg ha^{-1} N was significantly higher than that for the treatment with the same dose and without inoculation. It produced an 11.0% increase in the yield of the dry mass of the shoots (DMS), demonstrating the positive effect of foliar application every two cuts. For the average of the cuts, the treatments inoculated with PGPB produced 13% increased DMS compared with the fertilized treatments and without inoculation.

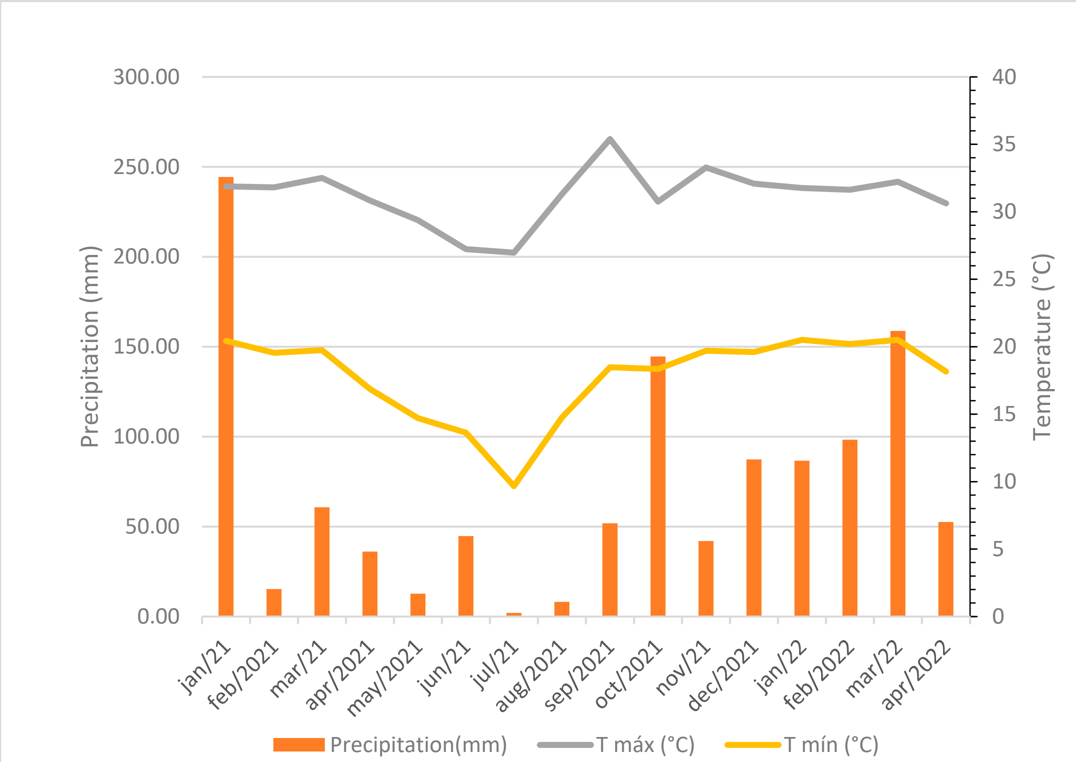

Figure 1. Maximum and minimum air temperature (°C) and monthly accumulated rainfall (mm) during the experiment from January 2021 to April 2022.

3.4. Leaf Blade and Daily Accumulation of Dry Mass of the Shoots

There was a significant effect on the leaf blade percentage for all evaluations in the experimental period (Table 4). For the February 2021 evaluation, the treatments inoculated with *A. brasilense* and *P. fluorescens* fertilized with 40 kg ha^{-1} N were higher compared with the treatment fertilized with the same dose without inoculation. In March 2021, the percentage of leaf blade was lower only for 40 kg ha^{-1} N and for *A. brasilense* Ab-V5 + Ab-V6 plus 40 kg ha^{-1} mineral N, and a positive effect of 6.8% was observed of the inoculation with *P. fluorescens* plus 40 kg ha^{-1} N compared with the treatment with the same N rate fertilization without inoculation.

In the May 2021 evaluation, the control and the treatment inoculated with *P. fluorescens* plus 80 kg ha^{-1} N were higher compared with the other treatments. In contrast, for November 2021, the control and the treatment inoculated with *A. brasilense* plus 80 kg ha^{-1} N were higher compared with the other treatments. In January 2022, the treatments inoculated with *A. brasilense* and *P. fluorescens* associated with 40 kg ha^{-1} N were higher compared with the other treatments. For the February 2022 cut, only the treatment inoculated with *A. brasilense* fertilized with 40 kg ha^{-1} N was higher compared with the other treatments.

For March 2022, the leaf blade percentage was higher in the treatment plants at 40 kg ha^{-1} N and inoculation with *P. fluorescens* with 40 kg ha^{-1} mineral N. A significant effect was observed for the evaluations of February 2021, March 2021, November 2021, January 2022, February 2022, and March 2022 for the DMS accumulation per hectare per day (Table 4). In February 2021, there was a higher accumulation of DMS for 40 kg ha^{-1} N, 80 kg ha^{-1} N, and inoculation of *A. brasilense* Ab-V5 + Ab-V6 at 80 kg ha^{-1} mineral N. In March 2021, treatments that were fertilized with 80 kg ha^{-1} of N and inoculated with *A. brasilense* Ab-V5 + Ab-V6 at 80 kg ha^{-1} mineral N were higher than that for the other treatments. For the November 2021 evaluation, only the control was low compared with the other treatments.

Table 4. Mean leaf blade percentage and average daily accumulation of dry mass of the shoots of *Megathyrsus maximus* cv. BRS Zuri inoculated with PGPB.

	Leaf Blade Percentage (%)						
Treatments	**Feb-2021**	**Mar-2021**	**May-2021**	**Nov-2021**	**Jan-2022**	**Feb-2022**	**Mar-2022**
Control	96 a	95 a	97 a	94 a	75 b	90 b	92 b
40 kg ha^{-1} of N	91 b	88 b	94 b	91 b	73 b	89 b	95 a
80 kg ha^{-1} of N	91 b	94 a	92 b	92 b	74 b	90 b	89 c
A. brasilense + 40 kg ha^{-1} of N	94 a	86 c	94 b	89 c	78 a	96 a	89 c
A. brasilense + 80 kg ha^{-1} of N	90 b	93 a	92 b	95 a	76 b	91 b	88 c
P. fluorescens + 40 kg ha^{-1} of N	94 a	94 a	93 b	92 b	79 a	91 b	95 a
P. fluorescens + 80 kg ha^{-1} of N	91 b	93 a	97 a	92 b	75 b	92 b	87 c
CV (%)	3.81	3.81	3.81	3.81	3.81	3.81	3.81
p-value	0.0002 **	0.0001 **	0.0005 **	0.0030 **	0.0001 **	0.0001 **	0.0001 **
	Daily Accumulation of Dry Mass of the Shoots (kg ha^{-1} day^{-1}) ***						
Control	40 c	37 b	21	6 b	37 c	52 b	32 c
40 kg ha^{-1} of N	95 a	43 b	23	13 a	57 b	55 b	58 b
80 kg ha^{-1} of N	113 a	49 a	26	15 a	71 a	72 a	68 a
A. brasilense + 40 kg ha^{-1} of N	79 b	45 b	23	14 a	69 a	58 b	65 a
A. brasilense + 80 kg ha^{-1} of N	100 a	56 a	20	13 a	68 a	64 a	77 a
P. fluorescens + 40 kg ha^{-1} of N	70 b	44 b	21	15 a	63 b	62 a	53 b
P. fluorescens + 80 kg ha^{-1} of N	84 b	43 b	22	13 a	80 a	70 a	76 a
CV (%)	7.82	7.82	7.82	7.82	7.82	7.82	7.82
p-value	0.0001 **	0.0186 **	0.3067 ns	0.0001 **	0.0001 **	0.0136 **	0.0001 **

Means with the same lowercase letters in the column are different using the Scott–Knott test ($p \leq 0.10$). ns = not significant. ** significant at 1% probability. *** Dry mass accumulation data were transformed to the base 10 logarithm.

In January 2022, plants from the foliar spray treatments inoculated with *A. brasilense* Ab-V5 + Ab-V6 and *P. fluorescens* plus 40 kg ha^{-1} N increased by 21% and 11%, respectively, in the DMS accumulation per hectare per day when compared to the without inoculated control and 40 kg ha^{-1} mineral N. In February 2022, the inoculated treatment with *P. fluorescens* plus 40 kg ha^{-1} N was statistically higher compared with the one fertilized with 40 kg ha^{-1} N without inoculation and produced a 13% increased DMS accumulation per hectare per day. However, in the March 2022 evaluation, a greater accumulation was obtained in the plants that received 80 kg ha^{-1} N and the treatments inoculated with *A. brasilense* and *P. fluorescens* with 80 kg ha^{-1} No mineral fertilization. The PGPB were responsible for promoting an additional 13.0% and 12.0%, respectively, for *A. brasilense* and *P. fluorescens* fertilized with 80 kg ha^{-1} mineral N when compared with the treatment only with 80 kg ha^{-1} N. Meanwhile, the treatment inoculated with *A. brasilense* associated with 40 kg ha^{-1} N was higher compared with the treatment fertilized with the same N rate and accumulated an increase of 12.0% DMS.

3.5. N Uptake and N Concentration

There was a significant effect on N uptake for February 2021, November 2021, January 2022, February 2022, and March 2022 (Table 5). In February 2021, the highest N uptake was in plants that were applied 80 kg ha^{-1} N and those inoculated with *A. brasilense* Ab-V5 + Ab-V6 plus 80 kg ha^{-1} mineral N. For November 2021, the highest uptake of N occurred in plants with 80 kg ha^{-1} of N compared to the others. However, for January 2022, after the foliar spray application, there was a positive increase of 43.0% for the treatment plants inoculated with *A. brasilense* and 40 kg ha^{-1} mineral N compared with those of the treatment with the lowest mineral N rate.

For February 2022, the fertilized treatments with 80 kg ha^{-1} mineral N and inoculated with *P. fluorescens* plus 80 kg ha^{-1} N showed greater uptake of N and were higher compared with the others. In March 2022, the treatment with *A. brasilense* and *P. fluorescens* plus 80 kg ha^{-1} N and the plants fertilized with 80 kg ha^{-1} N showed significantly higher uptake compared with the others.

Table 5. Means of nitrogen uptake and total nitrogen concentration in the shoots of the grass *Megathyrsus maximus* cv. BRS Zuri inoculated with PGPB.

	N Uptake (kg ha^{-1})						
Treatments	**Feb-2021**	**Mar-2021**	**May-2021**	**Nov-2021**	**Jan-2022**	**Feb-2022**	**Mar-2022**
Control	39 d	50	34	12 e	23 d	36 b	25 d
40 kg ha^{-1} of N	88 b	59	40	88 d	40 c	41 b	61 b
80 kg ha^{-1} of N	121 a	65	40	130 a	75 a	51 a	68 a
A. brasilense + 40 kg ha^{-1} of N	80 c	63	38	78 d	57 b	41 b	53 b
A. brasilense + 80 kg ha^{-1} of N	116 a	71	38	99 b	67 b	44 b	78 a
P. fluorescens + 40 kg ha^{-1} of N	71 c	60	39	96 c	50 b	43 b	43 c
P. fluorescens + 80 kg ha^{-1} of N	95 b	63	39	99 c	83 a	49 a	67 a
CV (%)	8.37	8.37	8.37	8.37	8.37	8.37	8.37
p-value	0.0001 **	0.1689 ns	0.1284 ns	0.0001 **	0.0001 **	0.0626 *	0.0001 **
	Total N Concentration (g kg^{-1})						
Control	28 b	32	28 b	12 e	13 d	20	20 b
40 kg ha^{-1} of N	27 b	32	32 a	39 c	14 d	22	27 a
80 kg ha^{-1} of N	31 a	32	35 a	53 a	21 a	20	25 a
A. brasilense + 40 kg ha^{-1} of N	30 b	34	31 a	31 d	17 c	20	20 b
A. brasilense + 80 kg ha^{-1} of N	33 a	30	34 a	50 a	20 b	20	25 a
P. fluorescens + 40 kg ha^{-1} of N	29 b	33	33 a	36 c	16 c	20	20 b
P. fluorescens + 80 kg ha^{-1} of N	33 a	35	32 a	44 b	21 a	20	22 b
CV (%)	4.59	4.59	4.59	4.59	4.59	4.59	4.59
p-value	0.0005 **	0.3400 ns	0.0128 *	0.0001 **	0.0001 **	0.7665 ns	0.0001 **

Means with the same lowercase letters in the column are different using the Scott–Knott test ($p \leq 0.10$). ns = not significant. * significant at 5% probability. ** significant at 1% probability.

There was a significant effect on total N concentration for February 2021, May 2021, November 2021, January 2022, and March 2022. In February 2021, treatments with 80 kg ha^{-1} N and *A. brasilense* and *P. fluorescens* plus 80 kg ha^{-1} N were higher. In contrast, in May 2021, only the control was lower than that for the other treatments. For November 2021, treatments with 80 kg ha^{-1} N and *A. brasilense* plus 80 kg ha^{-1} N were high. In January 2022, treatments with 80 kg ha^{-1} N and *P. fluorescens* plus 80 kg ha^{-1} N were high.

Although higher averages were not obtained, in January 2022, the treatments inoculated with *A. brasilense* and *P. fluorescens* plus 40 kg ha^{-1} N were significantly higher than those of the same rate without inoculation. In March 2022, treatments at 40 and 80 kg ha^{-1} N and inoculation of *A. brasilense* plus 80 kg ha^{-1} N were higher compared with the other treatments.

3.6. Crude Protein and True In Vitro Digestibility of Dry Matter

There was a significant effect on the crude protein (CP) content in forage in February 2021, May 2021, November 2021, January 2022, and March 2022 (Table 6). The evaluation in February 2021 showed higher results for the treatments of 80 kg ha^{-1} mineral N, and *A. brasilense* and *P. fluorescens* plus 80 kg ha^{-1} mineral N. In February 2021, the plants in the treatment inoculated with *A. brasilense* Ab-V5 + Ab-V6 and with 80 kg ha^{-1} mineral N showed a 10% increase compared to those that received only the same rate of N without inoculation. For May 2021, only the control and the treatment inoculated with *A. brasilense* plus 40 kg ha^{-1} mineral N were lower than that for the other treatments. In November 2021, 80 kg ha^{-1} mineral N treatments had a higher CP content. However, in January 2022, the treatments with 80 kg ha^{-1} mineral N and inoculated with *A. brasilense* and *P. fluorescens* fertilized at the highest mineral N dose were higher than that for the other treatments. In March 2022, treatments with 40 and 80 kg ha^{-1} mineral N and inoculation of *A. brasilense* plus 80 kg ha^{-1} N were higher compared with the other treatments.

For true in vitro dry matter digestibility (IVDMD), the evaluation in February 2021 for treatments inoculated with *A. brasilense* and *P. fluorescens* at 40 kg ha^{-1} was higher compared with the without inoculated control at the same N rate. In the March 2021 evaluation, the IVDMD percentages were higher in the plants that received 40 kg ha^{-1} N than that in the others. In November 2021, plants inoculated with *A. brasilense* fertilized with 40 kg ha^{-1} N and *P. fluorescens* fertilized with 80 kg ha^{-1} N had a lower IVDMD percentage. In January 2022, only the control was higher compared with the other treatments. While in February 2022, the plants fertilized with 80 kg ha^{-1} N and inoculated with A. *brasilense* and with *P. fluorescens* fertilized with 80 kg ha^{-1} N showed a lower level of IVDMD.

Table 6. Means of crude protein content of true in vitro dry matter digestibility (IVDMD) of *Megathyrsus maximus* cv. BRS Zuri inoculated with PGPB.

	Crude Protein (%)						
Treatments	**Feb-2021**	**Mar-2021**	**May-2021**	**Nov-2021**	**Jan-2022**	**Feb-2022**	**Mar-2022**
Control	17 b	20	17 b	7 f	8 b	12	13 b
40 kg ha^{-1} of N	17 b	20	20 a	22 d	9 b	13	17 a
80 kg ha^{-1} of N	19 a	20	22 a	29 a	13 a	13	16 a
A. brasilense + 40 kg ha^{-1} of N	18 b	21	19 b	19 e	10 b	13	13 b
A. brasilense + 80 kg ha^{-1} of N	21 a	19	21 a	25 b	12 a	12	16 a
P. fluorescens + 40 kg ha^{-1} of N	18 b	20	21 a	21 d	10 b	12	12 b
P. fluorescens + 80 kg ha^{-1} of N	21 a	22	20 a	26 c	13 a	13	14 b
CV (%)	18.07	18.07	18.07	18.07	18.07	18.07	18.07
p-value	0.0024 **	0.2776 ^{ns}	0.0178 *	0.0001 **	0.0001 *	0.9714 ^{ns}	0.0001 **
	IVDMD (%)						
Control	68 b	72 b	73	77 a	74 a	76 a	75 a
40 kg ha^{-1} of N	68 b	75 a	72	78 a	71 b	77 a	73 a
80 kg ha^{-1} of N	66 c	70 c	73	77 a	70 b	74 b	73 a
A. brasilense + 40 kg ha^{-1} of N	70 a	68 d	71	75 b	70 b	77 a	74 a
A. brasilense + 80 kg ha^{-1} of N	67 c	68 d	74	78 a	71 b	74 b	71 b
P. fluorescens + 40 kg ha^{-1} of N	71 a	70 c	72	77 a	70 b	76 a	71 b
P. fluorescens + 80 kg ha^{-1} of N	68 b	69 d	71	76 b	70 b	74 b	70 b
CV (%)	3.59	3.59	3.59	3.59	3.59	3.59	3.59
p-value	0.0001 **	0.0001 **	0.1617 ^{ns}	0.0172 *	0.0014 **	0.0009 **	0.0001 **

Means with the same lowercase letters in the column are different using the Scott–Knott test ($p \leq 0.10$). ns = not significant. * significant at 5% probability. ** significant at 1% probability.

3.7. Neutral Detergent Insoluble Fiber and Acid Detergent Insoluble Fiber

For the neutral detergent insoluble fiber (NDF) percentage, there was a statistical difference in the fourth and seventh evaluations. The levels varied from 61% to 74% of the NDF throughout the experiment (Table 7). For November 2021, inoculation of *A. brasilense* and *P. fluorescens* fertilized with 80 kg ha^{-1} mineral N positively affected the NDF and acid detergent insoluble fiber (ADF), as these were higher compared to the results obtained with the same mineral N rate without inoculation.

Table 7. Mean neutral detergent insoluble fiber (NDF, %) and acid detergent insoluble fiber content in (ADF, %) of the shoot of the grass *Megathyrsus maximus* cv. BRS Zuri inoculated with PGPB.

	NDF (%)						
Treatments	**Feb-2021**	**Mar-2021**	**May-2021**	**Nov-2021**	**Jan-2022**	**Feb-2022**	**Mar-2022**
Control	72	73	69	65 a	71	70	70 a
40 kg ha^{-1} of N	74	72	68	61 a	72	70	72 a
80 kg ha^{-1} of N	75	73	68	60 c	72	74	71 a
A. brasilense + 40 kg ha^{-1} of N	74	77	67	64 a	73	71	64 b
A. brasilense + 80 kg ha^{-1} of N	72	74	69	61 a	72	72	72 a
P. fluorescens + 40 kg ha^{-1} of N	73	71	68	63 a	72	72	72 a
P. fluorescens + 80 kg ha^{-1} of N	74	73	70	62 b	73	71	72 a
CV (%)	9.80	9.80	9.80	9.80	9.80	9.80	9.80
p-value	0.9582 ^{ns}	0.3748 ^{ns}	0.9481 ^{ns}	0.0001 **	0.9952 ^{ns}	0.8322 ^{ns}	0.0520 *

Table 7. *Cont.*

Treatments	Feb-2021	Mar-2021	May-2021	Nov-2021	Jan-2022	Feb-2022	Mar-2022
				ADF (%)			
Control	43	39	36	33 a	38	37	34 b
40 kg ha^{-1} of N	41	38	35	30 a	39	36	36 a
80 kg ha^{-1} of N	43	40	36	29 c	39	40	37 a
A. brasilense + 40 kg ha^{-1} of N	40	41	35	32 a	39	37	32 b
A. brasilense + 80 kg ha^{-1} of N	40	40	36	30 a	39	39	36 a
P. fluorescens + 40 kg ha^{-1} of N	40	38	35	31 a	38	38	36 a
P. fluorescens + 80 kg ha^{-1} of N	41	39	37	30 b	39	38	37 a
CV (%)	9.82	9.82	9.82	9.82	9.82	9.82	9.82
p-value	0.2907 ^{ns}	0.3134 ^{ns}	0.8487 ^{ns}	0.0001 **	0.9830 ^{ns}	0.3031 ^{ns}	0.0116 *

Means with the same lowercase letters are different using the Scott–Knott test ($p \leq 0.10$). ns = not significant. * significant at 5% probability. ** significant at 1% probability.

4. Discussion

Plant growth promotion by bacteria has been associated with various microbial processes that may vary with bacterial species (e.g., Rosier et al., and Guimarães et al. [35,36]). For the two strains of *A. brasilense* used in our study, the growth promotion has been mainly attributed to the high levels of phytohormones synthesized, especially indole-3-acetic acid (IAA) [37], greatly improving root growth and the uptake of water and nutrients [37–39]. For *P. fluorescens* strain CNPSo 2799, similar to another strain from our group—CNPSo 2719—it was successfully used for seed and the foliar spray inoculation of *Urocholoa* sp. [39].

The benefits of seed inoculation with *A. brasilense* strains Ab-V5 and Ab-V6 have been broadly reported for cereal and legume grain crops [40] and grass pastures of *Urochloa* spp. [12,39,41] and *Megathyrsus* sp. [42,43]. Plant growth promotion by leaf-spraying of *A. brasilense* and/or *P. fluorescens* and/or Bacillus sp. has also been reported for grasses, including grain crops [37,44] and pastures [39], and also legume grain crops [39,45–47]. However, Fukami et al. [37] visualized and recovered a few *A. brasilense* cells in corn leaves after leaf spray inoculation, a first indication that the effects might be attributed to bacterial metabolites, and not to living cells. This was confirmed in experiments with leaf spray of cell-free metabolites [48,49]. Therefore, the effects of leaf spray of both *A. brasilense* and *P. fluorescens*, including living cells or their metabolites, could be majorly attributed to systemic signaling from shoots to roots, which would contribute to root growth and also by inducing mechanisms of resistance to abiotic and biotic stresses [48–50].

For the pasture evaluations of the seven average evaluations, the average height of the Zuri grass ranged from 0.65 to 0.77 m with a cutting interval of six weeks [28]. The average height of plants in this study was similar to the experiment with Zuri grass inoculated with PGPB, where the average height of plants ranged from 0.63 to 0.80 m [51]. Different from the results shown in this experiment, in a study with the grass *Megathyrsus maximus* cv BRS Zuri inoculated with *A. brasilense* Ab-V5 + Ab-V6 without N fertilization had a higher average plant height, showing the potential of PGPB in benefiting the development of tropical forage plants [52].

Zahir and colleagues observed an increase in the height of the 704 maize plant corn that was inoculated with Azospirillum bacteria [53]. In addition, an increase of 8.5% was reported in corn plant height, whose seeds were inoculated with Azospirillum and Pseudomonas [54].

The number of tillers had a significant effect on most evaluations. However, a positive effect was observed on the number of tillers of Marandu grass under inoculation with *A. brasilense* Ab-V5 + Ab-V6, separately, from N fertilization, at 25 and 50 kg ha^{-1} N only in the first evaluation, both in the first and second year of the crop [55].

However, some studies found different results, where they observed a positive effect on increasing the number of tillers per m^2 in treatments inoculated with PGPB. In addition, they reported higher tiller density in Zuri grass with coinoculation with *Rhizobium tropici*

CIAT 899 and *A. brasilense* Ab-V5 + Ab-V6, in most evaluations, associated with fertilization with 100 kg ha^{-1} mineral N [51].

Inoculation with *P. fluorescens* strain CNPSo 2719 increased stem elongation and leaf expansion and the number of basal tillers in *U. brizantha* [55]. In M. maximus, inoculation of *P. fluorescens* with the same strain resulted in increased shoot and root dry weight, number of tillers, and N and magnesium (Mg) uptake [43]. Furthermore, the same strain was inoculated into hybrids of Urochloa spp. showing an increase in the dry weight of shoots and roots, as well as an increase in the number of tillers [12].

Although the DMS in the winter season did not show a statistical difference, an increase in the DMS was possibly observed in the winter period for the foliar spray treatments inoculated with *P. fluorescens* and *A. brasilense*. This possibly occurs owing to the increase in the root system of the inoculated plants and the yield of phytohormones, allowing a greater development in the winter period than that in the plants without inoculation. A similar effect was reported in Mavuno grass under PGPB inoculation, where the results of the DMS in the dry season were 14.6% higher in the inoculated treatment with *A. brasilense* Ab-V5 + Ab-V6 associated with 50 kg ha^{-1} mineral N compared with the same fertilizer dose and without inoculation [51].

In a study evaluating the effect of inoculation by *P. fluorescens* in *Pennisetum clandestinum* during the winter, a considerable DMS was verified compared to those that received only N fertilization and inferred that such results were caused by the release of phytohormones [56]. A similar effect was reported in sorghum (*Sorghum bicolor*) inoculated with PGPB and subjected to water stress conditions. In addition, they observed that *A. brasilense* Ab-V5 reduced the effects of drought, showing less dead tissue [57].

The inoculation of PGPB allows positive responses to the interaction between tropical grasses, proving the potential of these microorganisms to change the physiology of plants and cause them to be more resistant to abiotic stressors [58]. Furthermore, the use of endophytic diazotrophic bacteria makes it possible for the forage to increase nutrient uptake with the yield of auxins and siderophores that promote increased root growth, being a sustainable way to higher DMS [59].

In a pot experiment, with the inoculation of *P. fluorescens* with strains CCTB 03 and ET76 fertilized with 100 kg ha^{-1} mineral N, there was an increase in root mass yield of *Urochloa ruzizienses* of 66.0% and 29.0%, respectively, compared to the without inoculated treatment. The experiment also verified the positive effect of using PGPB to increase the root system of plants, promoting a larger area of uptake of nutrients for the plants [60].

Greenhouse and field experiments were performed with brachiarias inoculated with strains Ab-V5 and Ab-V6 via seeds or leaf spray; all treatments received 40 kg ha^{-1} of N at sowing and half received a second application with 40 kg ha^{-1} of N 30 days after emergence [39]. Under greenhouse conditions, inoculation with *A. brasilense* impressively increased root traits, including biomass, tissue volume and density, total and specific length, and the incidence of root hairs in *U. brizantha* and/or *U. decumbens* (syn. Urochloa eminii) [39]. Following, field trials were performed with *U. ruziziensis* (syn. Urochloa eminii), and the benefits of seed inoculation at the pasture establishment, or leaf spray in established pastures, were confirmed, either when they received a basal level of 40 kg ha^{-1} of N, or when receiving another application of 40 kg ha^{-1} of N 30 days after seedling emergence. On average, shoot biomass increased by 22%, in addition to 13% of N and 10.4% of K concentrations in leaves [39].

RCI was close to the values shown in an experiment using Zuri grass inoculated with PGPB, which found a significant effect on RCI for the first evaluation of Zuri grass. Treatments that were reinoculated with *A. brasilense* and *P. fluorescens* were higher than those for the other reinoculated treatments, with SPAD values of 31 and 26, respectively [51].

In a study with *M. maximus* cv. Tanzania, critical levels of RCI readings of 30 to 45 were more suitable for monitoring the N nutritional status than the shoot N content in different seasons [60]. The DMS in this study is consistent with the results observed in an experiment with Zuri grass with the treatments inoculated with *A. brasilense* Ab-V5 + Ab-V6. They reported a DMS in the sixth evaluation of an increase of 42% compared to the control, and the treatment inoculated with *P. fluorescens* had a DMS in the second evaluation, an increase of 7.0% compared to the control [51].

The mean percentage of leaf blades varied from 73% to 97% throughout the experimental period. In a study with Zuri grass, the leaf blade percentages throughout the experiment were similar to the results observed in this study [51].

In an experiment with "Marandu" grass and *U. ruziziensis*, N accumulation increased by 4% to 15% under inoculation with *A. brasilense* Ab-V5 + Ab-V6 and fertilization with 40 kg ha^{-1} of N, compared with treatment with N and without inoculation [12]. The N concentration in the forage ranged from 12 to 50 g kg^{-1} throughout the experimental period. Therefore, the N content observed in this study is within the expected range for the crop, which considers adequate values in the range of 15 to 25 g kg^{-1} for forages of the *Megathyrsus* group [22]. Identifying the positive effect of PGPB associated with mineral fertilization in increasing the N content in the plant tissues of the grass is possible.

A study evaluating the effect of inoculation by *A. brasilense* in seeds of Marandu grass associated with the N use observed mean values of 15% for CP, indicating adequate pasture management [61]. The increase in the CP levels observed in studies with grasses applying N fertilization occurs because N actively participates in the synthesis of organic compounds that form the structure of the plant [62]. N availability considerably influences plant nutrition, which is reflected in forage yield and quality [63].

The increase in IVDMD occurs by increasing the nitrogenous fraction, with a consequent proportional reduction in the cell wall [64]. This is a desirable characteristic in forage plants destined to feed ruminants for better nutrition. The experimental results are different from those shown in a study with Zuri grass, where a 10% increment was observed for IVDMD for treatments reinoculated with *A. brasilense* Ab-V5 + Ab-V6 and *P. fluorescens* CCTB 03 fertilized with 100 kg ha^{-1} N compared with the treatment at the same dose of N [51].

The NDF and ADF results presented in this experiment corroborate the study by Soares Filho et al. [51] with Zuri grass, who found that *A. brasilense* Ab-V5 + Ab-V6 fertilized with 100 kg ha^{-1} N promotes a positive effect on the NDF and ADF levels compared to the fertilized treatment only at the same N rate without inoculation. The percentages of the NDF between 55% and 60% of the dry weight of the food are negatively associated with consumption [65]. Forages with a low NDF content have a higher consumption rate; therefore, NDF levels greater than 60% in the dry weight of the food have an adverse effect on feed intake, and lower values are desirable [65]. The ADF was from adequate to high depending on the cut of the grass [65]. Conversely, low ADF values mean higher energy and high digestibility [65].

5. Conclusions

In the rainy season, at the level of 40 kg ha^{-1} of N, inoculation of both *A. brasilense* and *P. fluorescens* increased, respectively, the tiller number by 33% and 25% (22 February), and the N accumulated in tissues by 42% and 25% (22 January), while in the previous year (21 February) the beneficial effects of both bacteria were observed in the percentage of leaf blade and in the true digestibility in vitro.

The root system of Zuri grass had an increase of 61% and 30%, respectively, when the foliar spray was inoculated with *A. brasilense* Ab-V5 and Ab-V6 and *P. fluorescens* CNPSo 2799, associated with 80 kg ha^{-1} mineral N.

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