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Plant growth- promoting bacteria inoculation and co-inoculation effects on Palisade grass (*Urochloa brizantha* cv. Marandu) in ferralsol in Amazonas State, Brazil

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Abstract

Deforestation is primarily driven by three main factors the conversion of the Amazon rainforest into pastureland, agriculture, and mining, as well as land invasion. These pastures are mostly degraded, and it is necessary to restore their productive capacity. Microbial inoculants can be used to increase nutrient use efficiency. The objective of this study was to evaluate the effects of inoculation and co-inoculation of the bacteria *Rhizobium anhuiense* (RZ), *Azospirillum brasilense* (AZ) and *Pseudomonas fluorescens* (PF) on the growth of the grass *Urochloa brizantha* cv. Marandu. The field experiment with inoculants in *U. brizantha* was carried out in a randomized block design with four replications. The treatments consisted of different doses of NPK and types of inoculation. The variables used in the evaluation were plant height, number of tillers, productivity, and foliar NPK content. The results showed an interaction between fertilizer doses and inoculation treatments for variables such as number of tillers and productivity. Co-inoculation positively influenced productivity and plant nutrient content in some combinations and fertilizer doses. These findings suggest that inoculation and co-inoculation with different bacteria used different fertilizer

Abbreviations: AZ, *A. brasilense*; DSM, dry shoot mass; KDSM, potassium dry shoot mass; NDSM, nitrogen dry shoot mass; PDSM, phosphorus dry shoot mass; PF, *P. fluorescens*; RZ, *R. anhuiense*.

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doses, increasing the number of tillers, dry mass (P and K), and yield of Palisade grass as observed in co-inoculation with AZ + RZ.

Plain Language Summary

The main cause of deforestation in the Amazon rainforest is converting land into pasture, which degrades over time. In order to prevent further deforestation, it is crucial to restore and maintain the productivity of these pastures. In this study, we evaluated the use of different bacteria as inoculants to improve the nutritional value of Palisade grass. This grass is cultivated on over 100 million ha in Brazil. Our findings show that using these bacteria alone or in combination with various fertilizer doses increases plant productivity while reducing the need for fertilization. Our results demonstrate that these bacteria are an effective solution for restoring pastures and reducing fertilizer use.

1 | INTRODUCTION

The Amazon rainforest is the largest tropical forest in the world, occupying 49.5% of Brazil's territory. This area is equivalent to about 42% of the European continent, with a deforested area equivalent to the combined area of Germany and the Czech Republic. The main cause of deforestation in the Amazon is the conversion of land use to livestock production. This conversion from forest to pasture accounts for more than 83% (365,888 km^2) of the total deforested area. Deforestation occurred in low fertility soils as Ferrasols. (Sanchez & Cochrane, 1980). These Ferrasols have low natural fertility and high acidity and occupy most areas in the Amazon (Teixeira et al., 2010). As a result, the conversion of the Amazon rainforest to cattle pasture leads to ecosystem degradation. A few years after the forest is converted to pasture, the process of agricultural and biological degradation begins (Dias-Filho, 2017).

In agricultural degradation, pasture yield decreases, and the weed population increases (Buschbacher et al., 1988; Dias & Griffith, 1998; Fearnside, 1996). In the case of biological degradation, the pasture area decreases drastically along with the decrease in nutrients available in the soil, such as phosphorus (P) and nitrogen (N) (Garcia-Montiel et al., 2000). To recover degraded pastures, it is necessary to increase soil fertility with industrial fertilizers containing N and P (Noronha et al., 2010; Oliveira et al., 2005). However, industrial fertilizers cause several problems, such as environmental pollution, wherein in general only 55% applied N is used by plants and the rest is lost through leaching, erosion, and soil gas emissions (Liu et al., 2010; Smil, 1999). Tropical soils are the largest source of N_2O emissions, followed by cultivated soils (Tian et al., 2020). Phosphate fertilizers are minerals treated with sulfuric acid at high temperatures (Vassilev & Vassileva, 2003). This processing of insoluble phosphate leads to the depletion of phosphate rock reserves (Sattari et al., 2012, 2014). Another problem is that most phosphate fertilizers accumulate in the soil due to their low mobility, which is controlled by factors such as pH and aluminum (Al) and iron (Fe) oxides (Lei et al., 2024). Therefore, it is necessary to develop substitutes for these fertilizers or to increase their efficient use.

Biofertilization uses microorganisms to increase nutrient availability and assimilation in host plants (Vessey, 2003). The best example of biofertilization is biological nitrogen fixation (BNF) using diazotrophic bacteria. An example of these bacteria is rhizobia, where BNF occurs through symbiosis with legumes. For grasses, N supply during BNF occurs in association with bacteria that colonize the outer and inner parts of the roots (Ladha et al., 2022). BNF in grasses is carried out by different bacterial genera, such as *Azospirillum*, *Burkholderia*, *Gluconacetobacter*, and *Herbaspirillum* (Rosenblueth et al., 2018). Another example is the solubilization of mineral phosphates by *Bacillus* species in corn (Bini et al., 2026). In addition, plant growth-promoting bacteria (PGPB) can induce plant growth by producing regulators such as auxins, cytokinins, and gibberellins (Dobbelaere et al., 2003; Steenhoudt & Vanderleyden, 2000).

The inoculation of diazotrophic bacteria as biofertilizers has been successfully tested on various agricultural crops such as soybeans (*Glycine max*), beans (*Phaseolus vulgaris*), cowpeas (*Vigna unguiculata*), fodder peanuts (*Arachis pintoi*), and guandu beans (*Cajanus cajan*). In grasses, *Azospirillum* is used in maize and *Gluconacetobacter* in sugarcane. Another good example of biofertilization is the solubilization of mineral phosphate by PGPB, which occurs during the synthesis of low molecular weight organic acids such as citric acid, which bind to hydroxyls and carboxyls forming chelates and acidi-

fying the soil (Gamalero & Glick, 2011). Organic phosphorus is mineralized during the hydrolysis reaction of phosphate esters catalyzed by enzymes produced by microorganisms (Rodriguez & Fraga, 1999). This phosphate solubilization is carried out by microorganisms such as rhizobia, *Azospirillum*, *Bacillus*, and *Pseudomonas*, as observed in chickpea, wheat, and maize crops (Karimzadeh et al., 2020; Rudresh et al., 2005; C. A. Oliveira et al., 2009; Turan et al., 2012).

Phytostimulation occurs when PGPB produces and modulates plant growth-inducing substances such as auxins, cytokinins, and gibberellins (Dobbelaere et al., 2003; Steenhoudt & Vaderleyden, 2000; de Garcia Salamone et al., 2005). The main auxin produced by PGPBs is indoleacetic acid, which affects host plant rooting, nutrient uptake, and root exudation, as observed in rice cultivation (Steenhoudt & Vanderleyden, 2000; Mahanty et al., 2017; Yanni & Dazzo, 2010; Hahn et al., 2016; Hungria et al., 2021). PGPB can be applied by single or co-inoculation. Inoculation consists of adding the PGPB in a vehicle (liquid medium or solid substrate such as peat), straight to the seeds, or in the sowing furrow, or at post-emergence (Andrade et al., 2019; Santos et al., 2019). Co-inoculation consists of mixing PGPB bacteria (Hungria et al., 2013). For the restoration of pastures and degraded areas in the Amazon, inoculation and co-inoculation of PGPBs can be used in grasses such as *Urochloa brizantha* (brachiaria, Palisade grass), which occupies 60% of the pastures in the Amazon (Dias-Filho & Andrade, 2019). A pastureland area in the Amazon region has approximately 56 million ha, where 64% of this surface area is degraded (MAPBIOMAS, 2026). In the state of Amazonas, degraded pastures account for approximately 298,000 ha. Despite the magnitude of this problem, no studies to date have evaluated the use of microbial inoculation and co-inoculation as strategies for pasture recovery in this region. The objective of this study was to evaluate the effects of inoculation and co-inoculation of *Rhizobium anhuiense* (RZ), *Azospirillum brasilense* (AZ), and *Pseudomonas fluorescens* (PF) on the promotion of forage growth in Palisade grass (*U. brizantha* cv. Marandu).

Core Ideas

- Growth-promoting bacteria in Palisade grass were used as multifunctional inoculants.
- Bacteria inoculation increases agronomic efficiency.
- Co-inoculation improves nutritional efficiency.

TABLE 1 Soil chemical characterization at 0- to 20-cm depth in Manaus Ferrasol in 2021.

Parameters	
pH[a]	5.22
C (g kg^{-1})	21.07
Organic matter (g kg^{-1})	36.24
P (mg dm^{-3})	3.00
K ($cmol_c$ dm^{-3})	0.13
Na^+ ($cmol_c$ dm^3)	0.02
Ca^{2+} ($cmol_c$ dm^{-3})	1.62
Mg^{2+} ($cmol_c$ dm^{-3})	1.24
Al^{3+} ($cmol_c$ dm^3)	0.05
H + Al ($cmol_c$ dm^{-3})	2.01
SB ($cmol_c$ dm^{-3})	3.01
ECEC	3.06
CEC	5.03
V (%)	59.96
m (%)	1.63

Note: SB = sum of bases (Ca + Mg + K + Na); ECEC = effective cation exchange capacity (SB + Al^{3+}); CEC = cation exchange capacity in pH 7.0 [SB + (H + Al)]; *V* = bases saturation (SB/CEC × 100); *m* = aluminum saturation (100 × Al)/CEC).
[a]pH in water.

2 | MATERIALS AND METHODS

2.1 | Experimental area description

The experiment was carried out in the experimental field of Embrapa Amazônia Ocidental in Manaus, Amazonas, Brazil (02°53′36.11″ S, 59°58′18.62″ W) on soil classified as Ferrasol in an Af climate in 2021. The experimental area was conventionally prepared by plowing and harrowing, after which four composite soil samples were taken to determine the macronutrients in the experimental area (Table 1).

Subsequently, 2 metric ton of dolomitic limestone were incorporated to correct the soil pH in the area where *U. brizantha* cv. Marandu was grown. During the experiment, average air and soil temperatures were 26.43°C and 27.68°C, respectively. Average humidity was 89.50%, average rainfall was 12.61 mm, and average evaporation was 1.60 mm (see Supporting Information 1).

2.2 | Bacteria used and inoculant preparation

The strains are provided by from and are stored in the Diazotrophic and Plant Growth-Promoting Bacteria Culture Collection of Embrapa Soja (WFCC Collection #1213 and WDCM Collection #1054) in Londrina, Paraná, Brazil. The inoculant containing the bacterial strains was incubated in a specific liquid culture medium and incubated for 2 days in a bacteriological oven at 28°C, shaking at 100 rpm. Yeast-mannitol-agar YMA medium (Hungria et al., 2016) was used

TABLE 2 Palisade grass inoculation and co-inoculation treatments.

Doses NPK (%)	Treatments							
0 (0-0-0 kg ha^{-1})	SI	AZ	RZ	PF	AZ + RZ	AZ + PF	RZ + PF	AZ + RZ + PF
50 (50-40-40 kg ha $^{-1}$)	SI	AZ	RZ	PF	AZ + RZ	AZ + PF	RZ + PF	AZ + RZ + PF
100 (100-80-80 kg ha^{-1})	SI	AZ	RZ	PF	AZ + RZ	AZ + PF	RZ + PF	AZ + RZ + PF

Abbreviations: AZ, *Azospirillum brasilense* inoculant; RZ, *Rhizobium anhuiense* inoculant; PF, *Pseudomonas fluorescens* inoculant; SI, no inoculant.

for the growth of RZ (*VP 16*), DYGS (dextrose, yeast extract, glycerol, and salts) medium (Fukami et al., 2018) was used for the growth of AZ (*CNPSo 2083 and CNPSo 2084*), and Trypticase Soy Brorth medium was used for PF (*CNPSo 2719* and *CNPSo 2799*) (Döbereiner et al., 1999). The concentration of the inoculants was adjusted in a spectrophotometer at 600 nm, at an optical density of 0.6, reaching an approximate concentration of 10^8 CFU mL^{-1}.

2.3 | Experimental setup

The experiment was conducted using a randomized blocks design, with four replicates per treatment, giving a total of 96 experimental units of 9 m^2 (3 m × 3 m). The factors evaluated were NPK doses (0% = 0N-0P-0K, 50% = 50N-40P-40K, and 100% = 100N-80P-80K) and the bacterial strains (Table 2). The inoculated seeds were sown in each experimental plot in 2021. Fertilizers were applied after sowing. The N application was divided into two doses: 50% at sowing and 50% 30 days afterward (Malavolta et al., 1997). The N source used was ammonium sulfate $(NH_4)_2SO_4$), the phosphate source was single superphosphate (SPS), and the potassium (K) source was potassium chloride (KCl).

Plant height was assessed after 90 days using a millimeter ruler, where five points were measured in a sampling area of 1 m^2, obtaining the average height of the pasture as described by Costa & Queiroz (2017) and counting the number of tillers per plant, productivity, and leaf N, P, and K contents. To determine yield, a 1 m^2 sample of fresh mass was collected and dried in an oven at 65°C for 72 h. After this procedure, the water content was determined. The dry shoot mass (DSM) produced was then determined. The DSM was adjusted based on the number of plants per plot using a rule of three calculation (Schmidt et al., 2001; Silva et al., 2014). Plant biomass was harvested 90 days after sowing and establishment of the pasture. After drying, the forage was chopped to obtain foliar N, P, and K contents according to the method proposed by Malavolta et al. (1997). The N, P, K, and DSM was determined by the formula:

$$\text{DSNutrient} = \text{DSM} \times \% \text{ leave nutrient},$$

where DSNutrient is the dry shoot mass of nutrient (N, P, and K), and DSM is the dry shoot mass.

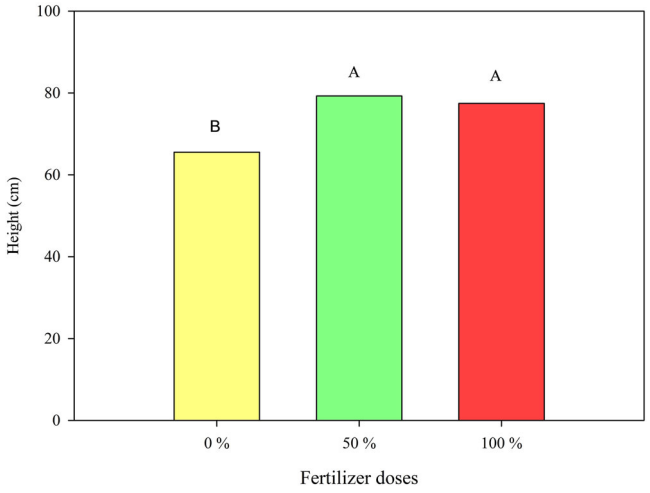

FIGURE 1 Fertilizer doses effect in plant height during the Palisade grass establishment phase after 90 days. Means followed by the same letter do not differ according to the Scott–Knott test ($p < 0.10$).

2.4 | Statistical analysis

The results obtained were subjected to an analysis of variance (Fisher, 1935) and to the Scott–Knott test for separation of means (Scott & Knott, 1974). The factors (treatment, fertilizer dose) were analyzed individually when they there was no interaction between them. The normality of the residuals was assessed using the Shapiro–Wilk (Shapiro & Wilk, 1965) and Anderson–Darling (Anderson & Darling, 1952) tests, while the homogeneity of the variance of the residuals was assessed using the Brown–Forsythe test (Brown & Forsythe, 1974). The variables were also analyzed using Pearson's correlation (Pearson, 1931). Analyses were performed using the R program (R Core Team, 2024).

3 | RESULTS

The results showed an interaction between fertilizer doses and inoculation and co-inoculation treatments for the following variables: NDSM (N in dry shoot mass), PDSM (P in dry shoot mass), KDSM (K in dry shoot mass), and yield (Table 3).

The height of *U. brizantha* cv. Marandu plants ranged from 65.53 to 79.27 cm (Figure 1). The fertilizer doses of 50% and 100% showed plants with greater height than the treatments

TABLE 3 Analysis of variance of height, number of tillers, nitrogen in dry shoot mass, phosphorus in dry shoot mass, potassium in dry shoot mass, and yield in *Urochloa brizantha* cv. Marandu was inoculated and coinoculated with growth-promoting bacteria during the pasture establishment phase.

Variation Source	Height	Tillers	NDSM	PDSM	KDSM	Yield
F-test *p*-values						
Fertilizer dose (A)	**0.02313**	**0.00001**	**0.000128**	0.00006	**0.00014**	**0.000041**
Treatment (B)	0.74887	0.32710	0.313160	0.24780	0.20726	0.263628
A × B	0.55525	**0.05882**	**0.037983**	**0.03824**	**0.08132**[a]	**0.031015**

[a]<0.1The significance level can be considered at $p < 0.1$ for field trials, according to the Brazilian Ministry of Agriculture (MAPA, 2011).
Note: The values in bold were considered significant at $p < 0.1$.

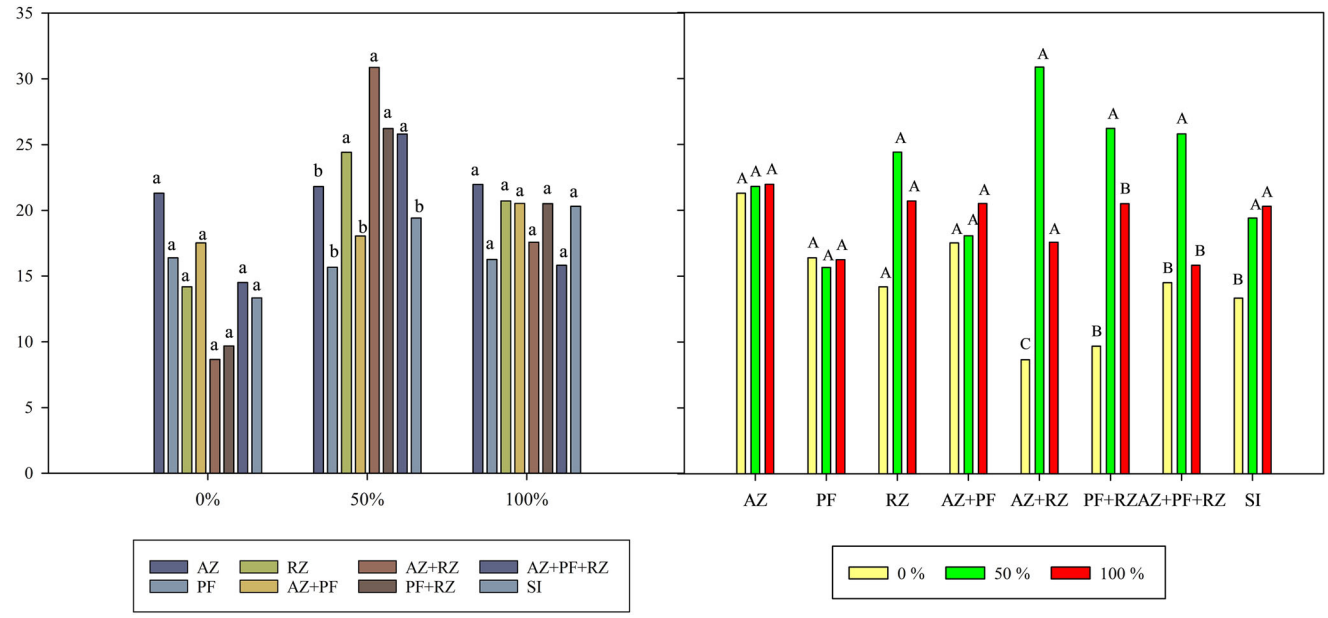

FIGURE 2 Tillers' number inoculated and coinoculated with growth-promoting bacteria during the Palisade grass establishment phase after 90 days. SI = non-inoculated, AZ = *A. brasilense* inoculant, RZ = *R. anhuiense* inoculant, and PF = *P. fluorescens* inoculant. Means followed by the same letter do not differ according to the Scott–Knott test ($p < 0.10$). Lowercase letters within the same fertilizer dose. Capital letters within the same treatment at different fertilizer doses.

without fertilizer, but they did not differ from each other. The fertilizer doses of 50% and 100% were 18% and 28% higher than the 0% dose, respectively.

The number of tillers per plant varied between 8.66 and 30.87. The number of tillers did not differ among the treatments at the 0% and 100% fertilizer doses. However, at the 50% dose, the RZ inoculation treatment and the co-inoculation treatments (AZ + RZ, RZ + PF, and AZ + RZ + PF) exhibited a higher number of tillers. At a fertilizer dose of 50%, the inoculated RZ and co-inoculated RZ, AZ + RZ, RZ + PF, and AZ + RZ + PF treatments increased the number of tillers by 25.81%, 59.04%, 35.08%, and 32.92%, respectively. This increase was greater than that observed in the non-inoculated treatments (Figure 2a). The treatments inoculated with AZ, PF, and RZ, and co-inoculated with AZ + PF and non-inoculated presented the same number of tillers regardless of the fertilizer dose. In turn, the treatments co-inoculated with AZ + RZ, PF + RZ, and AZ + PF + RZ presented different numbers of tillers at different fertilizer doses (Figure 2b).

The NDSM of *U. brizantha* cv. Marandu varied between 11.07 and 97.69 kg ha^{-1} (Figure 3a). There were no differences between treatments at 0% and 100% fertilizer doses. At the 50% fertilizer dose, the NDSM was significantly higher in the AZ, AZ + RZ, and non-inoculated (SI) treatments than in the other inoculation combinations. The AZ, PF, and RZ inoculated treatments and AZ + PF and AZ + PF + RZ co-inoculated treatments showed no differences in NDSM regardless of fertilizer dose. Meanwhile, the AZ + RZ co-inoculated treatment at a 50% fertilizer dose showed a 782% increase in NDSM compared to the 0% fertilizer dose. Meanwhile, the co-inoculated treat-

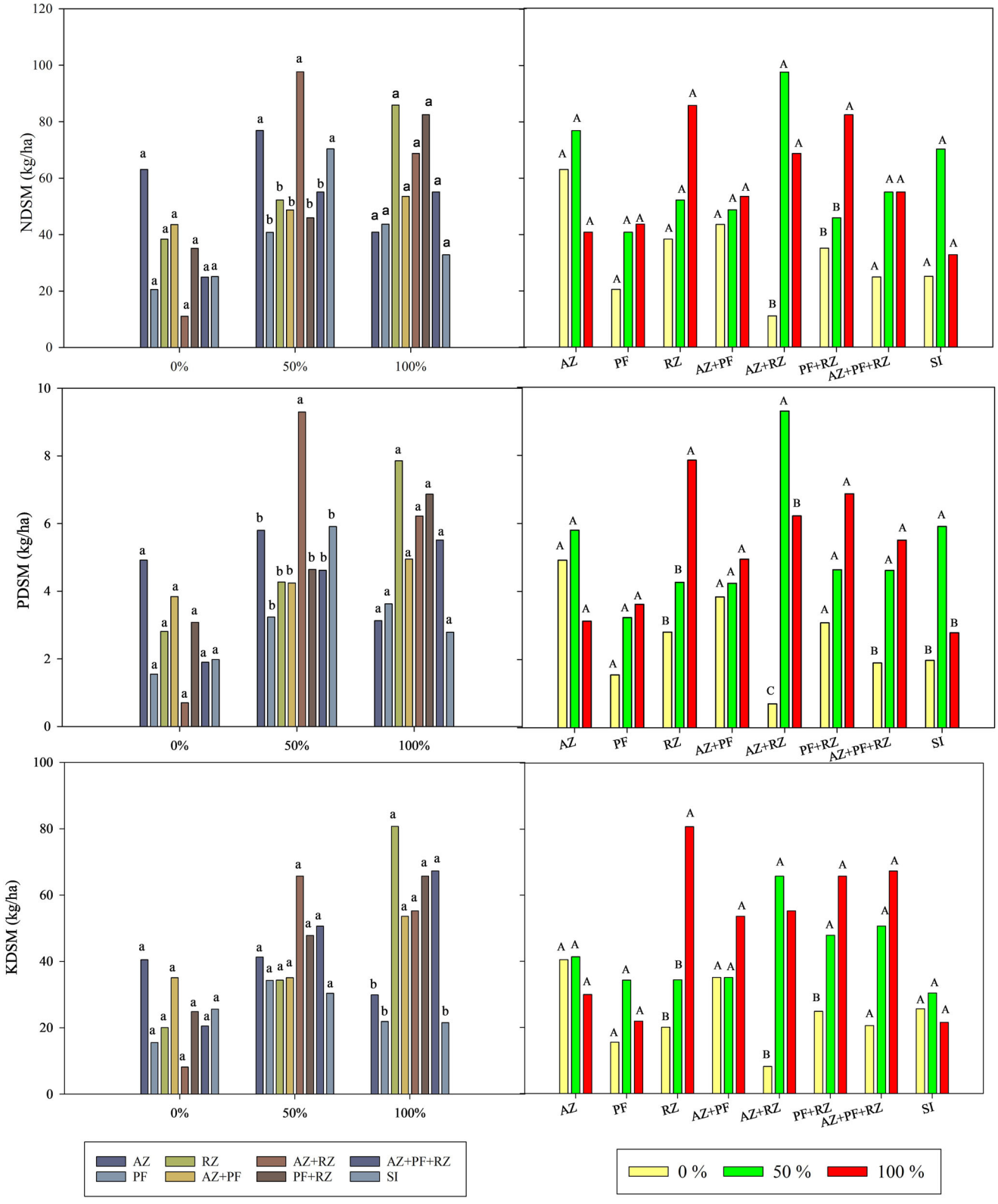

FIGURE 3 Nitrogen, phosphorus, and potassium in dry shoot mass inoculated and coinoculated with growth-promoting bacteria during the Palisade grass establishment phase after 90 days. SI = non-inoculated, AZ = *A. brasilense* inoculant, RZ = *R. anhuiense* inoculant, and PF = *P. fluorescens* inoculant. Means followed by the same letter do not differ according to the Scott–Knott test ($p < 0.10$). Lowercase letters within the same fertilizer dose. Capital letters within the same treatment at different fertilizer doses.

ment with RZ + PF at a fertilizer dose of 100% increased NDSM by 134% when compared to the fertilizer dose of 0%. (Figure 3b)

PDSM varied between 0.7 and 9.30 kg ha^{-1} (Figure 3c). The AZ and PF inoculated treatments and the AZ + PF and RZ + PF co-inoculated treatments did not show differences in PSDM at different fertilizer doses. In the different fertilizer doses, the treatment inoculated with RZ resulted in an increase of almost three times in PDSM at the 100% fertilizer dose compared to the 0% dose. Concurrently, the AZ + RZ co-inoculation resulted in an approximate 12-fold increase in PDSM at the 50% fertilizer dose compared to the 0% dose. Furthermore, the co-inoculated treatment AZ + RZ + PF increased PDSM twofold at the 50% dose and threefold at the 100% fertilizer dose. The other inoculated and co-inoculated treatments did not demonstrate significant differences in response to the various fertilizer doses (Figure 3d).

KDSM varied between 8.19 and 80.76 kg ha^{-1}. The inoculated, co-inoculated, and non-inoculated treatments demonstrated no differences at fertilizer doses of 0% and 50%. At the 100% fertilizer dose, the inoculated RZ treatment and co-inoculated AZ + PF, AZ + RZ, RZ + PF, and AZ + PF + RZ treatments exhibited higher KDSM than the other treatments but did not differ from each other (Figure 3f). These treatments at the 100% fertilizer dose showed 214%, 109%, 115%, 156%, and 162% more than the non-inoculated treatment at the 0% fertilizer dose. (Figure 3e). The AZ, PF, AZ + PF, AZ + PF + RZ, and non-inoculated treatments demonstrated no differences in the different fertilizer doses. In turn, the plants co-inoculated with AZ + RZ, PF + RZ, and AZ + PF + RZ obtained higher KDSM at 50% and 100% fertilizer doses. Meanwhile, the treatment inoculated with RZ presented higher KDSM at the 100% fertilizer dose. (Figure 3f).

Palisade grass yield varied between 743.38 and 4801.32 kg ha^{-1}. The inoculated, co-inoculated, and non-inoculated treatments did not show significant differences at fertilizer doses of 0% and 100%. At a fertilizer dose of 50%, the AZ + RZ co-inoculated treatment showed higher yields than the other treatments, which did not differ from each other. This treatment had a 44% higher yield than the non-inoculated treatment at this fertilizer dose (Figure 4a). It was observed that the inoculated AZ and PF treatments, the co-inoculated AZ + PF and AZ + PF + RZ treatments, and the non-inoculated treatment did not show differences in yield at the different fertilizer doses. In turn, the treatments inoculated with RZ and co-inoculated with RZ + PF showed higher yields only at the 100% fertilizer dose. Meanwhile, the treatment co-inoculated with AZ + RZ showed higher yields at 50% and 100% fertilizer doses (Figure 4b).

Plant height showed a positive correlation with yield, NDSM, and KDSM. The number of tillers was positively correlated with PDSM and KDSM. Yield showed a positive correlation with NDSM, PDSM, and KDSM. NDSM was positively correlated with PDSM and KDSM. PDSM was positively correlated with KDSM. (Figure 5).

4 | DISCUSSION

The present study evaluated the effects of inoculation and co-inoculation of AZ, PF, and RZ on promoting the growth of Palisade grass at different fertilizer doses. The findings indicated an interaction between inoculation and co-inoculation with varying fertilizer doses, except for the height variable. In addition, the variables exhibited a positive correlation between each other.

The observed increase in the height of Palisade grass was attributed to the utilization of fertilizers, as reported by Apollon et al. (2022). Due to their effects on the processes of elongation and photosynthesis, the macronutrients N, P, and K provided by the fertilizers affected the height of Palisade grass. Height increased due to the stimulation of elongation by N fertilizers, as observed in other grasses such as rice and wheat (Wu et al., 2023). Furthermore, both N and K directly affect leaf growth by interfering with photosynthesis. K controls stomatal function, regulating CO_2 flow, while N is present in thylakoids, which are responsible for light capture and electron transport (Pettigrew, 2008; Evans & Clarke, 2018). In turn, the P provided by fertilizers increases the efficiency of photosynthesis (Reich et al., 2009) as observed in *Randia canthioides* and *Cryptocarya concinna* in China (Zhu et al., 2014).

The number of tillers, NDSM, PDSM, and yield of Palisade grass were affected by the interaction between inoculation and co-inoculation with 50% fertilizer level. Concurrently, the interaction exerted an effect on KDSM, reaching a response twofold at a fertilizer dose of 100%. The number of tillers of Palisade grass increased with inoculation with RZ and co-inoculation with AZ + RZ and RZ + PF at the 50% fertilizer dose. The results obtained were similar to those observed on the number of tillers of Palisade grass with inoculation in the transition zone between the Cerrado and the Amazon (Leite et al., 2018) and co-inoculation with AZ and *Rhizobium tropici*. The increase in the number of tillers was probably because of the production of plant growth regulators, such as auxins and cytokinins, by the inoculated and co-inoculated microorganisms. Different studies reported the production of auxins and cytokinins by *Azospirillum* spp. (Cassán et al., 2014; Karimi et al., 2021), *Pseudomonas* spp. (Bakaeva et al., 2020; P. Singh et al., 2022), and *Rhizobium* spp. (Yang et al., 2022). This was due to the action of auxins and cytokinins, which are responsible for cell division processes that directly affect the tillering of Palisade grass (Gopalakrishnan et al., 2015; Ullah et al., 2018). Probably, protocooperation occurred between microbial species in co-inoculation (AZ + RZ and RZ + PF), with mutual benefit for microbial growth. Pro-

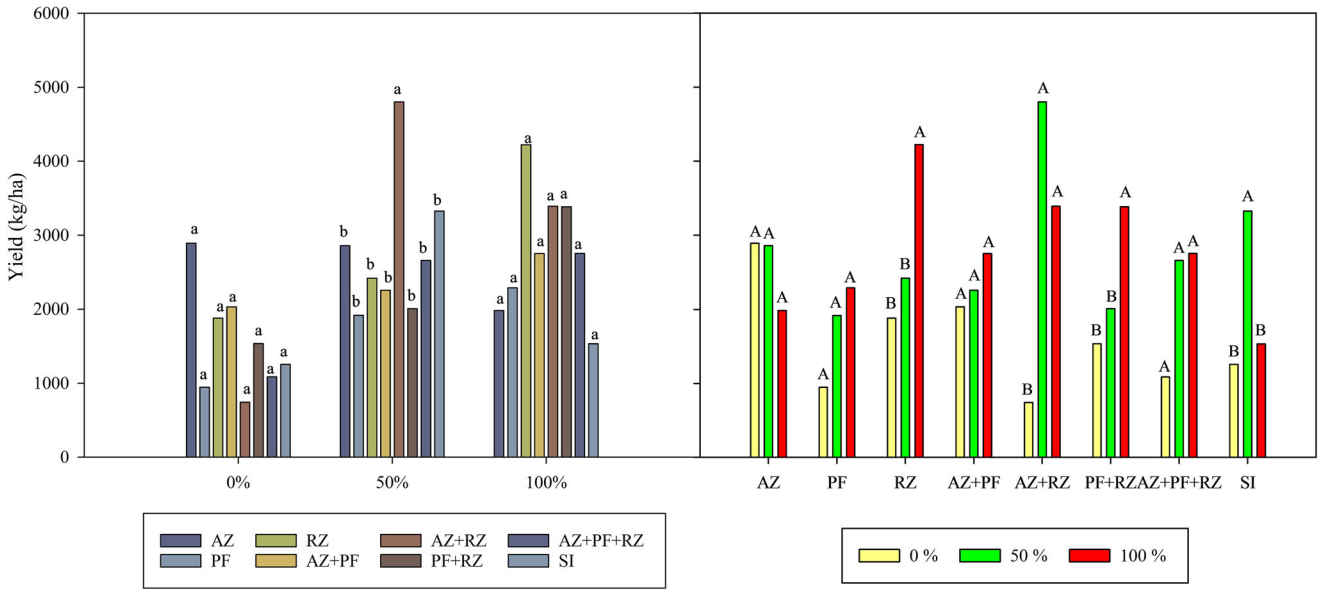

FIGURE 4 Yield inoculated and coinoculated with growth-promoting bacteria during the Palisade grass establishment phase after 90 days. SI = non-inoculated, AZ = *A. brasilense* inoculant, RZ = *R. anhuiense* inoculant, and PF = *P. fluorescens* inoculant. Means followed by the same letter do not differ according to the Scott–Knott test ($p < 0.10$). Lowercase letters within the same fertilizer dose. Capital letters within the same treatment at different fertilizer doses.

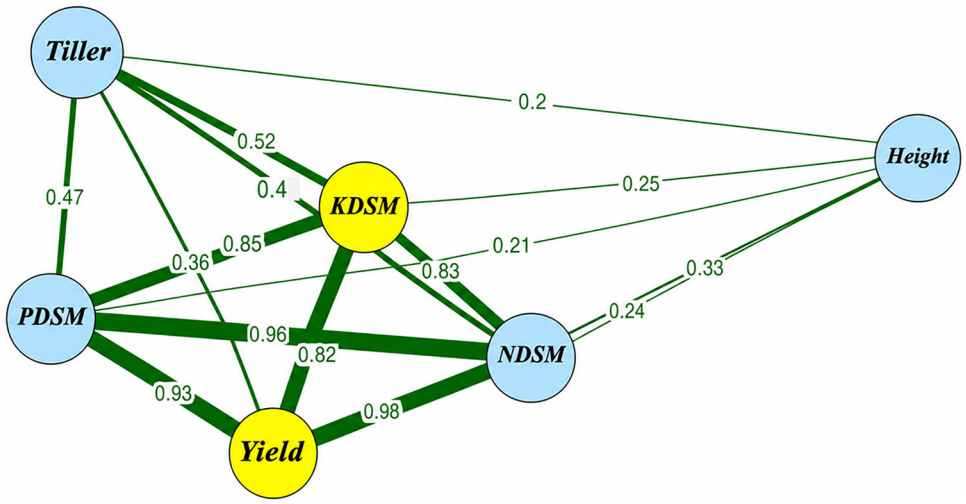

FIGURE 5 Pearson correlation of different variables in Palisade grass (*U. brizantha* cv. Marandu). KDSM, potassium dry shoot mass; NDSM, nitrogen dry shoot mass; PDSM, phosphorus dry shoot mass.

tocooperation involves the coexistence of different microbial populations and allows their growth over long periods (Singh et al., 2019). In co-inoculation with AZ + PF and AZ + PF + RZ, competition for nutrients occurred and presumably one of the species became predominant (Roell et al., 2019). In this particular instance, the co-inoculation technique did not result in a synergistic enhancement of plant growth (Trabelsi & Mhamdi, 2013). The tillers' number increased due to fertilization, where N directly influences cell multiplication, while P acts on cell division and expansion processes (Faria et al., 2018; Prystupa et al., 2003; C. C. F. Silva et al., 2009).

The NDSM of Palisade grass did not increase with AZ inoculation and AZ + RZ co-inoculation at the different fertilizer dose, and it did not differ from the SI treatment at the same dose. This outcome is incongruent with the results observed by Dos Santos et al. (2019), wherein NDSM exhibited an increase following the inoculation of AZ in Palisade grass within the Brazilian Cerrado. This outcome is also incongruent with the observations reported by other researchers, who found that the concurrent inoculation of AZ and *R. tropici* resulted in an augmentation of NDSM in Palisade grass. The outcomes of co-inoculation diverge from those obtained

through AZ in conjunction with alternative bacterial species, such as *Bacillus subtilis*, in corn cultivation (Galindo et al., 2025) and *Bradyrhizobium japonicum* in soybean cultivation, where an augmentation in N was observed in plants (Hungria et al., 2022). The results were inconsistent due to the negative effect of nitrogen fertilization on inoculation and co-inoculation, which contradicts other findings. The microbial population exhibited a negative correlation with increasing doses of nitrogen fertilizer, as evidenced by the inoculation of *Gluconacetobacter diazotrophicus* in sugarcane starting at 100 kg N ha^{-1} (Yadav et al., 2009).

The enhancement in PDSM and yield of Palisade grass co-inoculated with AZ and RZ is likely attributable to synergistic interactions among microorganisms (Wang et al., 2019). The observed effects on PDSM are likely attributable to the capacity of *Azospirillum* and *Rhizobium* species to produce phytohormones, such as auxins, and to facilitate phosphate solubilization (Giri et al., 2025; Hungria et al., 2021; Kang & Seo, 2022). The co-inoculation of AZ, RZ, and PF did not result in synergistic effects. The absence of synergy in this co-inoculation process may have been caused by antagonism between PF and the other microorganisms. The antagonism of PF is attributable to the production of substances such as the antibiotic 2,4-diacetyl-phloroglucinol, which has been shown to inhibit the in vitro growth and colonization of wheat seeds by AZ (Maroniche et al., 2018). It has been demonstrated that other antagonistic substances produced by *Pseudomonas*, such as hydrogen cyanide (Rikame & Borde, 2022; Patel et al., 2024) and phenazines (Zeng et al., 2023), have been shown to inhibit microbial growth. It is therefore possible that these substances may have affected the growth of *Azospirillum* and *Rhizobium* used in this study.

The observed increase in KDSM in Palisade grass inoculated with *Rhizobium* and in co-inoculated treatments can be attributed to different mechanisms, such as auxin production and K solubilization. Inoculation with *Rhizobium* may have increased K absorption indirectly through the production of auxins, which promote root growth, as observed in rice culture with the strain VP16 of RZ (Osório Filho et al., 2014) and increase nutrient absorption (De Souza et al., 2013). Furthermore, some bacteria belonging to the genus *Rhizobium* have been observed to possess the capacity for potassium solubilization, as evidenced by studies on *Rhizobium pusense* (Meena et al., 2015). The K solubilization occurred through the action of organic acids (tartaric, oxalic, and citric acids) produced by bacteria (Sattar et al., 2019). It has been demonstrated that *Azospirillum* and *Pseudomonas* also produce auxins (Chen et al., 2017; Sun et al., 2025). Concurrently, there is mounting evidence of K solubilization by *Pseudomonas* species (Barin et al., 2022; Sharma et al., 2024). *Azospirillum* probably indirectly affected K uptake. The indirect effects of *Azospirillum* were due to its ability to biologically fix N, as observed in Palisade grass inoculated with this microorganism (Moreira et al., 2020). Presumably, the other microorganisms (*Rhizobium* and *Pseudomonas*) influenced KDSM due to auxin production and K solubilization.

The Palisade grass yield increased by the co-inoculation of *Azospirillum* and other bacteria such as PF, *B. subtilis*, and *B. japonicum*. An increase in yield was observed in other crops such as corn, soybean and Palisade grass (Galindo et al., 2025; Hungria et al., 2021, 2022; Terra et al., 2024). In fact, yield increased by bacterial plant hormones production as auxins. The auxins change root structure and promote plant growth and nutrition (Hungria et al., 2021).

The interaction between inoculation and co-inoculation with varying fertilizer doses was observed, with the most significant effects noted at the 50% dose level in specific variables, including the number of tillers, PDSM, and yield in Palisade grass. However, we observed that this interaction was different in KDSM at the 100% dose. At a fertilizer dose of 50%, the co-inoculation of RZ, AZ + RZ, RZ + PF, and AZ + RZ + PF may have nutrient uptake through auxin production and phosphate solubilization, which increased the number of tillers, PDSM, and yield of *U. brizantha* cv. Marandu. In the context of common bean cultivation, an enhancement in grain productivity was documented with a 50% dose of NPK fertilizer and co-inoculation with *R. tropici* + *B. subtilis* and *R. tropici* + AZ + PF (Mortinho et al., 2022). Greater inoculation efficiency has also been observed in other studies at suboptimal nitrogen (N) doses in corn co-inoculated with AZ and *Bradyrhizobium japonicum* (Oliveira et al., 2022; Guimarães et al., 2026) also observed higher amounts of NPK in DSM in co-inoculation with AZ and PF in Ruzi grass (*Urochloa ruziziensis*). In the case of inoculation with *Rhizobium* and treatments co-inoculated with AZ + PF, AZ + RZ, and PF + RZ with a 100% fertilizer dose, KDSM accumulation occurred due to growth-promoting mechanisms such as auxin production by *Azospirillum*, *Rhizobium*, and *Pseudomonas*, which increase root production and the absorption capacity of nutrients such as K (Fahde et al., 2023; Vargas et al., 2017; Y. Wu et al., 2022). In addition, the solubilization of potassium (K) by various species of *Pseudomonas*, which facilitate the supply of K to plants, also contributed to this accumulation. Furthermore, we observed that NDSM was not affected by inoculation and co-inoculation at any of the doses tested. The differential outcomes of inoculation and co-inoculation at varying fertilizer doses can be attributed to variations in growth rate, influenced by nutrient availability, as per the principle of microbial growth restriction (Yamagishi & Hatakeyama, 2025). This principle posits that the availability of a specific nutrient can impair the proliferation of other growth factors. In the absence of fertilizer, the macronutrients nitrogen (N), phosphorus (P), and potassium (K) proved ineffective in stimulating microbial and palisade grass growth, as evi-

denced by the lack of efficacy observed among the inoculated and co-inoculated treatments across the variables evaluated as NDSM, PDSM, and KDSM. At a fertilizer dosage of 50%, no N deficiencies were observed. Presumably, AZ + RZ co-inoculation prevented P from being the limiting nutrient in Palisade grass yield, where the most scarce nutrient limits productivity by Liebig's law of the minimum (Echavarría-Heras et al., 2023).

The response to inoculation varied with fertilizer level and microbial combination. At *50% NPK*, increases were observed in tillering, phosphorus accumulation, and yield, particularly with AZ + RZ. At *100% NPK*, some effects were observed for potassium accumulation, while under *0% fertilization*, no clear benefits were detected. These results indicate that the effects of PGPB depend on fertilization level and do not support broad recommendations for large-scale use, reinforcing the need to combine inoculation with appropriate nutrient management.

5 | CONCLUSIONS

The results of inoculation and co-inoculation at different fertilizer doses allow us to understand their effects on Palisade grass yield. These findings of this study showed that height was influenced by fertilizer doses. In turn, the interaction between inoculation, co-inoculation, and fertilizer doses affected the number of tillers, NDSM, PDSM, KDSM, and yield. Besides, the number of tillers increased with the inoculation of RZ and with co-inoculation at different fertilizer doses. NDSM was not affected by inoculation and co-inoculation treatments, while KDSM responded to inoculation with RZ and co-inoculation at a fertilizer dose of 100%. In addition, co-inoculation of AZ and RZ allowed a significant increase in PKDSM and Palisade grass yield. These findings suggest that co-inoculation with AZ and RZ has potential to be used, particularly under reduced fertilization conditions as part of strategies for the recovery of degraded pastures in the Amazon. However, further research is needed on different soils and evaluations of Palisade grass yield using inoculation and co-inoculation of plant growth-promoting microorganisms.

AUTHOR CONTRIBUTIONS

Sara Rodrigues Batista: Formal analysis; investigation; writing—original draft. **Cláudia Majolo**: Formal analysis; methodology; supervision; writing—original draft. **Rogério Perin**: Formal analysis; methodology; validation. **Marco Antonio Nogueira**: Formal analysis; funding acquisition; writing—review and editing. **Mariangela Hungria**: Resources; visualization; writing—review and editing. **Christiane Abreu Oliveira-Paiva**: Formal analysis; writing—review and editing. **Enílson Luíz Saccol de Sá**: Formal analysis; writing—review and editing. **Christoph Müller**: Writing—review and editing. **Maria Teresa Gomes Lopes**: Writing—review and editing. **Aleksander Westphal Muniz**: Conceptualization; methodology; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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