SCAP
SOCIEDADE DE CIÊNCIAS AGRÁRIAS DE PORTUGAL

Residual phosphate fertilization and inoculation of *Bacillus* sp. and *Azospirillum* sp. in early development and phosphorus use efficiency of sugarcane

Fertilização residual com fosfato e inoculação com *Bacillus* sp. e *Azospirillum* sp. no desenvolvimento inicial e na eficiência do uso de fósforo na cana-de-açúcar

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ABSTRACT

Phosphorus (P) is a limiting nutrient for sugarcane development in Brazilian tropical soils. However, there is evidence that phosphate-solubilizing bacteria (PSB) can improve plant development and P acquisition. A pot experiment was conducted to investigate the interaction between residual P fertilization and inoculation of *Bacillus* sp. and *Azospirillum* sp. on early development and P use efficiency traits of sugarcane plants (*Saccharum* sp., var. RB867515) grown in a sandy clay loam Oxisol from Brazilian Cerrado. Sugarcane seedlings were transplanted into soil containing three levels of residual P availability (low, medium and high) and subjected to three inoculation treatments with PSB [control (uninoculated plants); plants inoculated with *B. megaterium* (BRM119) and *B. subtilis* (BMF2484); and plants inoculated with *A. brasilense* (Ab-V5 and Ab-V6) and *A. amazonense* (BR11145)], arranged in a 3×3 factorial with four replicates. Results: Under low and medium residual P availability, plants inoculated with *Bacillus* sp. and *Azospirillum* sp. strains have greater plant height, number of tillers, leaf area, shoot dry matter, root dry matter, P uptake efficiency and physiological P use efficiency when compared to uninoculated plants, respectively. These results show that the inoculation of *Bacillus* sp. and *Azospirillum* sp. can be a sustainable agricultural practice to be recommended for sugarcane cropping. However, *Bacillus* sp. and *Azospirillum* sp. strains have reduced phosphate solubilization and mineralization potential when applied to tropical soil with high available P concentration. Nevertheless, the physiological basis of how high soil P availability affects the phosphate solubilization capacity of these bacteria remains unknown.

Keywords: P uptake; phosphate-solubilizing bacteria; residual P; *Saccharum* sp.

RESUMO

O fósforo (P) é um nutriente limitante para o desenvolvimento da cana-de-açúcar em solos tropicais brasileiros. No entanto, existem evidências de que bactérias solubilizadoras de fosfato (BSF) podem melhorar o desenvolvimento da planta e a aquisição de P. Um experimento em vasos foi conduzido para investigar a interação entre a fertilização com P residual e a inoculação de *Bacillus* sp. e *Azospirillum* sp. sobre o desenvolvimento inicial e características de eficiência de uso de P em plantas de cana-de-açúcar (*Saccharum* sp., var. RB867515) cultivadas em um Latossolo Argiloso-Arenoso do Cerrado brasileiro. Mudas de cana-de-açúcar foram transplantadas para solo contendo três níveis de disponibilidade de P residual (baixo, médio e alto) e submetidas a três tratamentos de inoculação com BSF [controle (plantas não inoculadas); Plantas inoculadas com *B. megaterium* (BRM119) e *B. subtilis* (BMF2484); e plantas inoculadas com *A. brasilense* (Ab-V5 e Ab-V6) e *A. amazonense* (BR11145)], dispostas em um esquema fatorial 3×3 com quatro repetições. Resultados: Sob baixa e média disponibilidade residual de P, as plantas inoculadas com cepas de Bacillus sp. e *Azospirillum* sp. apresentaram maior altura, número de perfilhos, área foliar, matéria seca da parte aérea, matéria seca da raiz, eficiência de absorção de P e eficiência fisiológica de uso de P, respectivamente, quando comparadas às plantas não inoculadas. Esses resultados demonstram que a inoculação com *Bacillus* sp. e *Azospirillum* sp. pode ser uma prática agrícola sustentável recomendada para o cultivo da cana-de-açúcar. Entretanto, as cepas de *Bacillus* sp. e *Azospirillum* sp. apresentaram potencial reduzido de solubilização e mineralização de fosfato quando aplicadas em solos tropicais com alta concentração de P disponível. No entanto, a base fisiológica de como a alta disponibilidade de P no solo afeta a capacidade de solubilização de fosfato dessas bactérias permanece desconhecida.

Palavras-chave: absorção de P; bactérias solubilizadoras de fosfato; P residual; *Saccharum* sp.

INTRODUCTION

Sugarcane (*Saccharum* sp.) is one of the most important bioenergy crops grown in tropical regions of Brazil and the world, mainly due to its use in the production of biofuels and their derivatives, as well as the production of bioenergy through the combustion of its biomass (Huang *et al*., 2020). Brazil is currently the world's largest producer of sugarcane, with production of 663 million tons of stalks in the 2025/26 harvest (CONAB, 2026). However, the average Brazilian productivity in the 2025/26 harvest was only 75.4 Mg ha^{-1}, considered low when compared to the crop's yield potential, which is greater than 200 Mg ha^{-1} (Santos and Borém, 2013).

The low availability of phosphorus (P) in Brazilian tropical soils has been identified as one of the main factors limiting the development of sugarcane crops and contributing to the low stalk productivity in Brazil (Schiavo *et al*., 2018; Withers *et al*., 2018). While these soils contain substantial total P reserves (50 to 1800 mg P kg^{-1}), only a minimal portion, around 1%, is available to plants (Pavinato *et al*., 2021). This small fraction of available P (P-labile) in tropical soils is caused by the high reactivity and high adsorption capacity of P to soil constituents, especially to iron and aluminum (oxy)hydroxides (Lizcano-Toledo *et al*., 2021; Soares *et al*., 2021).

In this agricultural scenario, high rates of phosphate fertilizers have been applied to the soil to provide optimal plant development and obtain high stalk productivity, which has increased sugarcane production costs (Soltangheisi *et al*., 2019; Rein *et al*., 2021; Soares *et al*., 2021). However, the largest proportion of P applied with phosphate fertilizers is retained by soil constituents, forming the unavailable P fraction in the soil (Nunes *et al*., 2020; Johan *et al*., 2021). Therefore, the reuse of the non-labile P fraction in the soil is under discussion at a global level, with an emphasis on Brazilian agricultural production (Withers *et al*., 2018; Soares *et al*., 2025).

Among the sustainable strategies or practices that can be used to improve the absorption of the non-labile P fraction from the soil or the absorption of residual P from phosphate fertilizers, the use of phosphate-solubilizing microorganisms has stood out. These microorganisms can promote the growth of sugarcane plants through their ability to solubilize insoluble phosphates from the soil (Santos *et al*., 2018; Gonçalves *et al*., 2020; Reis *et al*., 2020; Rosa *et al*., 2020; Silva *et al*., 2023), and its use in agriculture represents a sustainable biotechnological option to reduce the application of high rates of phosphate fertilizers in sugarcane crops, which reduces production costs and minimizes environmental impact (Rosa *et al*., 2020; Silva *et al*., 2021, 2023). Therefore, the use of phosphate-solubilizing microorganisms can improve soil P use efficiency

(PUE) and is a promising strategy to improve P nutrition in sugarcane fields and optimize the agricultural exploitation of tropical soils.

Among the main phosphate-solubilizing microorganisms used in the tropical regions of Brazil, bacteria from the genera *Azospirillum*, *Bacillus*, *Burkholderia* and *Pseudomonas* have stood out (Gírio *et al*., 2015; Gonçalves *et al*., 2020; Rosa *et al*., 2020; Reis *et al*., 2020; Silva *et al*., 2023; Ibanhes-Neto *et al*., 2023). P solubilization by these bacteria is a complex process, which depends on many factors related to the microorganism-soil-plant interaction. However, among the main mechanisms associated with the phosphate solubilization capacity of these bacteria are the excretion of organic acids and production of siderophores (Sharma *et al*., 2013; Kumar *et al*., 2018; Kalayu, 2019). The excretion of organic acids into the soil, such as malic, oxalic and glutamic acid, can complex the aluminum (Al^{3+}) (accompanying ion) of aluminum phosphate ($AlPO_4$), releasing the phosphate ion (PO_4^{-3}) into the soil solution, while siderophores are chelating agents with high affinity for metals such as Al^{3+} and iron (Fe^{2+}) present in insoluble Al and Fe oxides in the soil (Sharma *et al*., 2013).

The genera *Azospirillum* sp. and *Bacillus* sp. are the main groups of bacteria used for the inoculation of a wide variety of grass species, including sugarcane. *Azospirillum brasilense* and *A. amazonense* (Syn. *Nitrospirillum amazonense*) are species of diazotrophic bacteria that promote plant growth through the fixation of atmospheric nitrogen, solubilization of inorganic phosphates and the ability to synthesize plant hormones, such as auxins, gibberellins and cytokinins (Fukami *et al*., 2018). *Bacillus megaterium* and *B. subtilis* are species of plant growth-promoting bacteria capable of solubilizing phosphate through the production of organic acids, transforming non-labile soil P into plant-assimilable fractions (Hashem *et al*., 2019; Huang *et al*., 2019; Rodrigues *et al*., 2023). However, the effect of inoculating pre-sprouted seedlings with *Azospirillum* sp. and *Bacillus* sp. in tropical soils with different levels of residual phosphate fertilization remains unknown. In this research, we tested the hypothesis that inoculation pre-sprouted seedlings with phosphate-solubilizing bacteria (*Bacillus* sp. or *Azospirillum* sp.) under conditions of lower residual phosphate fertilization could have a synergistic effect, resulting in increased P use efficiency and greater initial development of sugarcane.

This study was conducted to investigate the effect of the interaction between residual phosphate fertilization and inoculation of pre-sprouted seedlings with phosphate-solubilizing bacteria (*Bacillus* sp. or *Azospirillum* sp.) on the initial development and P-use efficiency traits of sugarcane plants (*Saccharum* sp.) grown in a sandy clay loam Oxisol from the Brazilian Cerrado region.

MATERIAL AND METHODS

Plant Growth Conditions

The experiment was conducted in a temperature-controlled agricultural greenhouse in Cassilândia, Mato Grosso do Sul, Brazil (19°05′29″S, 51°48′50″W, and 540 m altitude), in 15-L plastic pots, where the mean air temperature was 28.2 °C during the day and 25.4 °C during the night, mean relative air humidity was 72% (±6%), and midday photosynthetic photon flux density was 1,178 µmol m^{-2} s^{-1} (±217 µmol m^{-2} s^{-1}).

The soil used in the experiment was a sandy clay loam Oxisol (or Latossolo Vermelho distrófico in the Brazilian classification) with 240 g kg^{-1} of clay, collected from the plow layer in a field experiment located in the municipality of Jataí, Goiás, Brazil, where the soybean/maize succession system has been fertilized annually with 30, 60 and 120 kg ha^{-1} of P_2O_5 using simple superphosphate, for 8 years. For this bioassay under greenhouse conditions, representative soil samples were collected from each of the four replications of the field experiment. Soil chemical properties were determined by adopting standard procedures recommended by Teixeira *et al*. (2017), and the results are shown in Table 1.

The soil was fertilized with 50 mg kg^{-1} N (urea), 100 mg kg^{-1} K (potassium chloride), 15 mg kg^{-1} S (calcium sulfate), 2 mg kg^{-1} Cu (copper sulfate), 2 mg kg^{-1} Zn (zinc sulfate), and then transferred to 15-L pots and subjected to sugarcane cropping. Fertilizers were incorporated into the entire soil volume of the pots two days before transplanting the sugarcane pre-sprouted

Table 1 - Chemical properties of soil collected in a field experiment with residual phosphate fertilization

Residual phosphate fertilization	pH	OM	$P_{Mehlich-1}$	H+Al	Ca	Mg	K	CEC	V	Soil P level†
kg ha^{-1} year^{-1} P_2O_5		g dm^{-3}	mg dm^{-3}	cmol$_c$ dm^{-3}					%	
30	5.7	21	6.2	3.42	3.84	1.12	0.25	8.63	60	Low
60	5.6	23	13.8	3.18	3.68	1.10	0.26	8.22	60	Medium
120	5.6	22	21.3	3.24	3.75	1.12	0.24	8.35	61	High

pH in 0.01 mol L^{-1} $CaCl_2$ solution. OM: organic matter. CEC: cation exchange capacity. V: soil base saturation. †Class of interpretation of soil P availability extracted by Mehlich-1 for sandy clay loam soils in the Cerrado region (Sousa and Lobato, 2004).

seedlings. Each plastic pot was filled with 18 kg (±14 dm^3) of air-dried soil sieved in a 5.0-mm mesh. At 30 and 60 days after transplanting the sugarcane seedlings, 50 mg dm^{-3} of N was also applied using urea (45% N) previously diluted in pure water.

Experimental Design and Treatments

The experiment was arranged in a completely randomized block design in a 3 × 3 factorial arrangement with four replicates. Treatments consisted of three levels of residual soil P availability (low, medium and high) and three inoculation treatments with phosphate-solubilizing bacteria [control (non-inoculated plants); and plants inoculated with *Bacillus* sp. or *Azospirillum* sp.]. Each experimental unit consisted of a 15-L plastic pot containing a sugarcane seedling, with 36 pots in the experiment.

Plant material, inoculation and irrigation

Individual buds (mini-setts) 3.0 cm long, as recommended by Franco *et al*. (2020), were extracted from the middle third of stalks at 10 months of age from commercial hybrid RB867515, harvested in the experimental field of the State University of Mato Grosso do Sul – UEMS, in the municipality of Cassilândia, MS, Brazil. The sprouting process of one node is called Pre-Sprouted Seedlings (PSS) and is commonly used in the sugarcane cropping system in Brazil. This genotype was used because it is a cultivar responsive to the inoculation of diazotrophic bacteria (Gírio *et al*., 2015; Gonçalves *et al*., 2020; Rodrigues *et al*., 2023), and because it is the main sugarcane cultivar grown in the Cerrado region, representing around 25% of the sugarcane growing area in Brazil (Santos and Borém, 2013).

Pre-sprouted sugarcane seedlings produced in plastic boxes (53 × 32 × 7.5 mm), filled with Carolina Soil® commercial substrate, were transplanted 25 days after sprouting into 15-L plastic pots. The mini-setts did not receive any heat treatment or the use of fungicides and insecticides. After transplanting, all sugarcane seedlings were grown in a protected environment and daily irrigated with a micro-sprinkler irrigation system, using an 8 to 12 mm irrigation depth.

Inoculation of pre-sprouted seedlings with *Bacillus* sp. was carried out using the commercial liquid inoculant BiomaPhos®, containing the strains BRM 119 (*Bacillus megaterium*) and BMF 2484 (*Bacillus subtilis*) at minimum concentrations of 4 × 10^9 colony forming units (CFU) per mL of product, at a dosage of 3.0 mL of inoculant per pre-sprouted seedling. Inoculation with *Azospirillum* sp. was performed using a mixture of the liquid inoculant AzoKop® containing the Ab-V5 and Ab-V6 strains of *Azospirillum brasilense*, with a minimum concentration of 2.0 × 10^8 CFU per mL, and the inoculant Aprinza® containing the BR11145 strain of *Azospirillum amazonense* (Syn. *Nitrospirillum amazonense*), with a minimum concentration of 1.0 × 10^8 CFU per mL, both inoculants were applied at a rate of 2.0 mL of inoculant per pre-sprouted seedling. The inoculants were applied on the roots when the pre-sprouted seedlings were transplanted to the pots, using a pipette graduated in milliliters.

Quantification of morphological traits

At 120 days after sugarcane transplanting, the plants were harvested and the plant height (PH), stalk diameter (SD), number of tillers (NT), leaf area (LA), and dry matter accumulation of the plant organs were measured. Plants were separated into leaves, stalks, and roots, oven-dried at 65 °C for three days, and then weighed. The shoot dry matter (SDM) was obtained from dry matter production of the leaves and stalks. Total dry matter (TDM) was obtained from the sum of all the seedling parts (leaves, stalks, and roots). The root/shoot ratio (RSR) was calculated from the dry matter production of shoots and roots.

The PH was determined using a metal tape measure, considering the distance from the soil surface to the +1 leaf (Kuijper's leaf numbering system). The SD was measured at 5 cm from the base of the primary stalk using a digital caliper. NT determination corresponded to the total number of plants in each pot. LA was estimated using the equation proposed by Hermann and Câmara (1999): LA = L×W×0.75×(N+2), where, LA is the leaf area, L is the +3 leaf length, W is the largest width of the +3 leaf; 0.75 is shape factor; N is the number of fully expanded leaves and with at least 20% green area (0 to +7 leaf) and 2 is a correction factor.

Determination of phosphorus concentration

Samples of dry plant material from shoots (leaves + stalks) and roots were finely ground in a Willey mill and subjected to digestion with nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 3:1 (v:v) ratio, and then the P concentration was determined by the colorimetry method at 725 nm wavelength using a UV-visible spectrophotometer (Perkin Elmer-Lambda 3B) as described by Malavolta *et al*. (1997). Based on these results, the amount of accumulated P (g/pot) in the shoot biomass was determined.

Phosphorus use efficiency

The PUE was separated into P uptake efficiency (PUpE, in mg P mg^{-1} P) and P utilization efficiency (PUtE, in g TDM mg^{-1} P) as proposed by Neto *et al*. (2016). Equations 1–3 were used to calculate PUpE at each level of residual soil P availability, which represents the ratio between the plant P uptake (mg pot^{-1}) and the amount of residual P available in the soil (mg pot^{-1}). The PUtE represents the amount of total dry matter (g/pot) produced per unit of P accumulated in the plant (mg pot^{-1}) and was calculated for each level of residual soil P availability using Equations 4–6:

$$PUpE_{Low\ P} = \frac{([P]_{Low\ residual\ P} \times TDM_{Low\ residual\ P})}{P\ available_{Low\ residual\ level}} \qquad [Eq.1]$$

$$PUpE_{Medium\ P} = \frac{([P]_{Medium\ residual\ P} \times TDM_{Medium\ residual\ P})}{P\ available_{Medium\ residual\ level}} \qquad [Eq.2]$$

$$PUpE_{High\ P} = \frac{([P]_{High\ residual\ P} \times TDM_{High\ residual\ P})}{P\ available_{High\ residual\ level}} \qquad [Eq.3]$$

$$PUtE_{Low\ P} = \frac{TDM_{Low\ residual\ P}}{([P]_{Low\ residual\ P} \times TDM_{Low\ residual\ level})} \qquad [Eq.4]$$

$$PUtE_{Medium\ P} = \frac{TDM_{Medium\ residual\ P}}{([P]_{Medium\ residual\ P} \times TDM_{Medium\ residual\ level})} \qquad [Eq.5]$$

$$PUtE_{High\ P} = \frac{TDM_{High\ residual\ P}}{([P]_{High\ residual\ P} \times TDM_{High\ residual\ level})} \qquad [Eq.6]$$

Physiological P use efficiency (PPUE, in g^2 TDM mg^{-1} P) corresponds to the amount of TDM produced for a given plant P concentration, and was calculated for each level of residual soil P availability according to Equations 7–9 as proposed by Hammond *et al*. (2009):

$$PPUE_{Low\ P} = \frac{TDM_{Low\ residual\ P}}{[P]_{Low\ resisual\ P}} \qquad [Eq.7]$$

$$PPUE_{Medium\ P} = \frac{TDM_{Medium\ residual\ P}}{[P]_{Medium\ residual\ P}} \qquad [Eq.8]$$

$$PPUE_{High\ P} = \frac{TDM_{High\ residual\ P}}{[P]_{High\ residual\ P}} \qquad [Eq.9]$$

Statistical analysis

The data were previously tested for homoscedasticity of variances (Levene test; $P > 0.05$) and normality of residues (Shapiro-Wilk test; $P > 0.05$). Data were submitted to analysis of variance (ANOVA), and the means of morphological traits, plant P concentration and P use efficiency indices for the effects of residual phosphate fertilization and inoculation of phosphate-solubilizing bacteria were compared by the Tukey test, at the 0.05 level of confidence. The analyses were performed using Sisvar® software (version 5.9) for Windows (Statistical Analysis Software, UFLA, Lavras, MG, BRA).

Statistical correlations based on Pearson's correlation networks (threshold set at 0.60, $P < 0.05$) were performed between the morphological traits and P use efficiency indices for the sugarcane plants grown under residual phosphate fertilization levels and inoculated with phosphate-solubilizing bacteria. A correlation network was used to graphically illustrate Pearson's correlation analyses, in which the proximity between the nodes is proportional to the absolute correlation values between the plant morphological traits. The bands' relative thickness and color density indicate the strength of Pearson's correlation coefficients, and the color of each band indicates a positive or negative correlation (red for negative and green for positive).

RESULTS

The analysis of variance reported significant effects ($p \leq 0.05$) of the interaction between residual P levels and inoculation of phosphate-solubilizing bacteria (*Bacillus* sp. or *Azospirillum* sp.) for most morphological traits and P use efficiency indices of sugarcane plants, except for stalk diameter, plant P concentration and P utilization efficiency (PUtE) (Table 2). The significant interaction between these factors indicates that sugarcane plants inoculated with *Bacillus* sp. or *Azospirillum* sp. have distinct responses to residual soil P availability when compared to non-inoculated plants.

The coefficient of variation (CV) values obtained in the experimental trial were less than 15% for all morphological traits and P use efficiency indices of sugarcane plants, a value considered low for agricultural experiments (Table 2). The CV is a measure of dispersion used to estimate the precision of the experimental trial, and when the values are less than 20% it indicates that the results of the experiment have excellent precision.

Residual P availability levels and inoculation of *Bacillus* sp. and *Azospirillum* sp. significantly affected ($p \leq 0.05$) the plant height, stalk diameter, number of tillers and leaf area of sugarcane plants (Figure 1). The increase in residual soil P availability resulted in greater shoot growth of sugarcane plants, especially plant height, number of tillers and leaf area (Figure 1). Under low residual P availability, plant height was significantly higher in plants inoculated with *Bacillus* sp., followed by plants inoculated with *Azospirillum* sp., and significantly lower in non-inoculated plants (Figure 1A). When plants were inoculated with *Bacillus* sp. and *Azospirillum* sp. there was an increase of 23 cm (27%) and 12 cm (14%) in plant height compared to non-inoculated plants, respectively. Under medium residual P availability, the plant height was significantly greater in plants inoculated with *Bacillus* sp., and significantly lower in plants inoculated with *Azospirillum* sp. or uninoculated plants (Figure 1A). The inoculation of phosphate-solubilizing bacteria under high residual P availability had no significant effect ($p > 0.05$) on shoot morphological traits of sugarcane plants (Figure 1).

Stalk diameter under low residual P availability was significantly higher in plants inoculated with *Bacillus* sp. and *Azospirillum* sp. compared to non-inoculated plants. However, under medium

Table 2 - Summary of analysis of variance for the effects of residual P level and inoculation of phosphate-solubilizing bacteria (*Bacillus* sp. or *Azospirillum* sp.) on the morphological traits and P use efficiency indices of sugarcane plants (*Saccharum* sp., var. RB867515) grown in a sandy clay loam Oxisol

Sources of variation	Probability > F						
	PH	SD	NT	LA	SDM	RDM	TDM
Block	0.128	0.215	0.098	0.413	0.214	0.384	0.296
Residual phosphorus (P)	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Inoculation (I)	0.001	0.006	0.001	0.001	0.012	0.001	0.001
Interaction P × I	0.004	0.128	0.008	0.025	0.035	0.044	0.047
CV (%)	8.78	7.31	12.83	5.84	10.79	14.82	10.05
Sources of variation		P	PA_c	PUpE		PUtE	PPUE
Block		0.645	0.421	0.274		0.356	0.218
Residual phosphorus (P)		0.041	0.001	0.001		0.038	0.001
Inoculation (I)		0.043	0.001	0.001		0.044	0.001
Interaction P × I		0.085	0.001	0.001		0.137	0.001
CV (%)		9.14	10.49	9.71		6.37	8.58

PH: plant height; SD: stalk diameter; NT: number of tillers; LA: leaf area; SDM: shoot dry matter; RDM: root dry matter; TDM: total dry matter. P: plant phosphorus concentration; PAc: phosphorus accumulated in the plant biomass; PUpE: phosphorus uptake efficiency; PUtE: phosphorus utilization efficiency; PPUE: physiological phosphorus use efficiency.

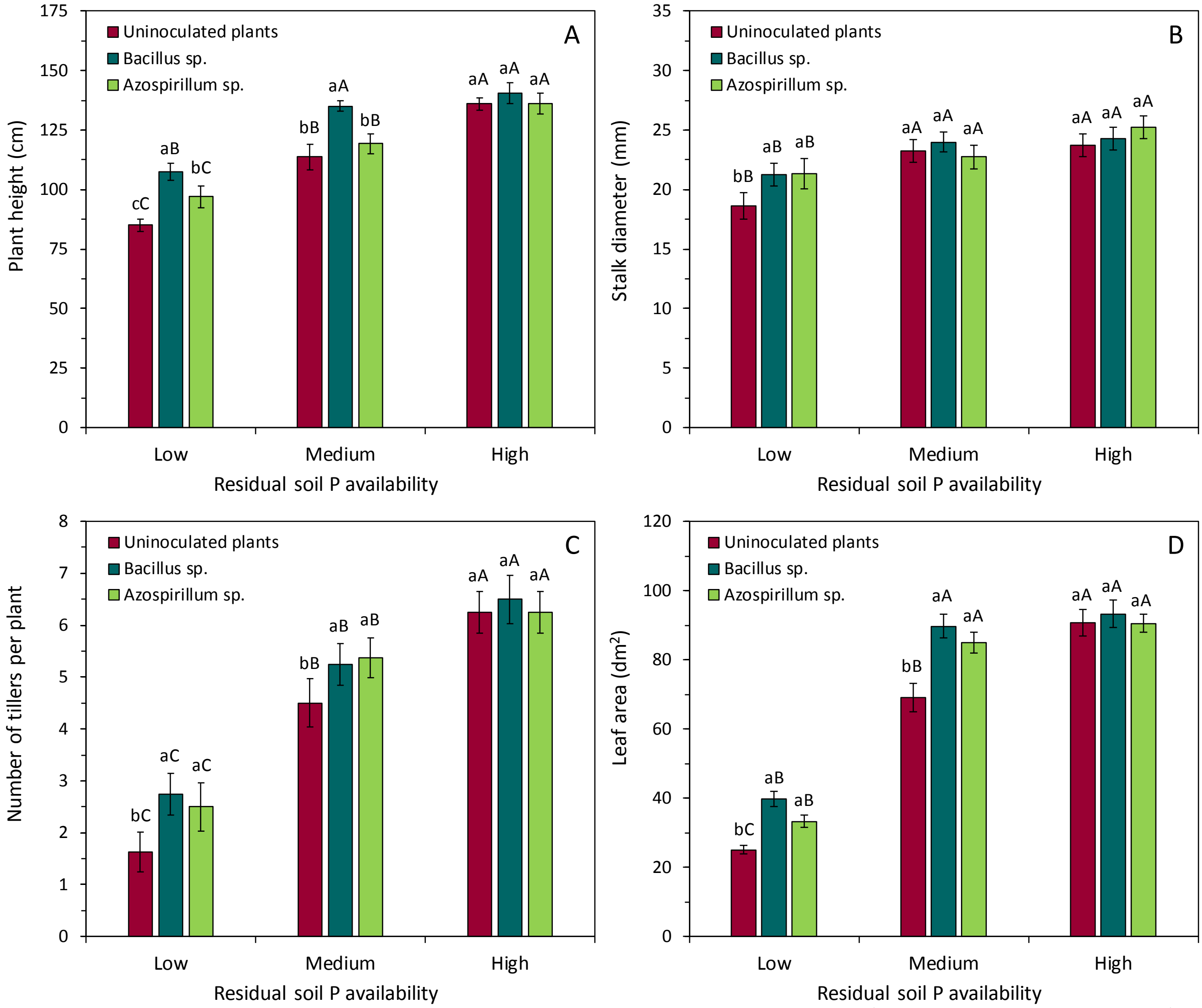

Figure 1 - Effect of soil residual P level and inoculation of phosphate-solubilizing bacteria (*Bacillus* sp. or *Azospirillum* sp.) on plant height (A), stalk diameter (B), number of tillers per plant (C) and leaf area (D) of sugarcane plants (*Saccharum* sp., var. RB867515) grown in a sandy clay loam Oxisol. Bars followed by distinct lowercase letters for the inoculation of phosphate-solubilizing bacteria, and distinct uppercase letters for soil residual P levels show significant differences (Tukey test; $p < 0.05$). Data refers to mean values (n = 4) ± standard error.

or high residual P availability, the inoculation of phosphate-solubilizing bacteria did not change the stalk diameter of sugarcane plants (Figure 1B). Under low or medium residual P availability, the number of tillers and leaf area was significantly higher in plants inoculated with *Bacillus* sp. and *Azospirillum* sp. when compared to non-inoculated plants (Figure 1C and 1D). These results show that inoculation with phosphate-solubilizing bacteria improved the initial growth of sugarcane plants under low and medium residual P availability.

Residual P availability levels and inoculation of *Bacillus* sp. and *Azospirillum* sp. significantly affected ($p \leq 0.05$) the shoot, root, and total dry matter accumulation and ratio root:shoot dry matter of sugarcane plants (Figure 2). Increasing residual soil P availability resulted in higher shoot, root, and total dry matter accumulation in sugarcane plants (Figure 2A, 2B and 2C). However, residual P levels had little effect on the root: shoot ratio of sugarcane plants (Figure 2D).

Under low or medium residual P availability, shoot, root and total dry matter accumulation was significantly higher in plants inoculated with *Bacillus* sp. and *Azospirillum* sp. when compared to non-inoculated plants (Figure 2A, 2B and 2C).

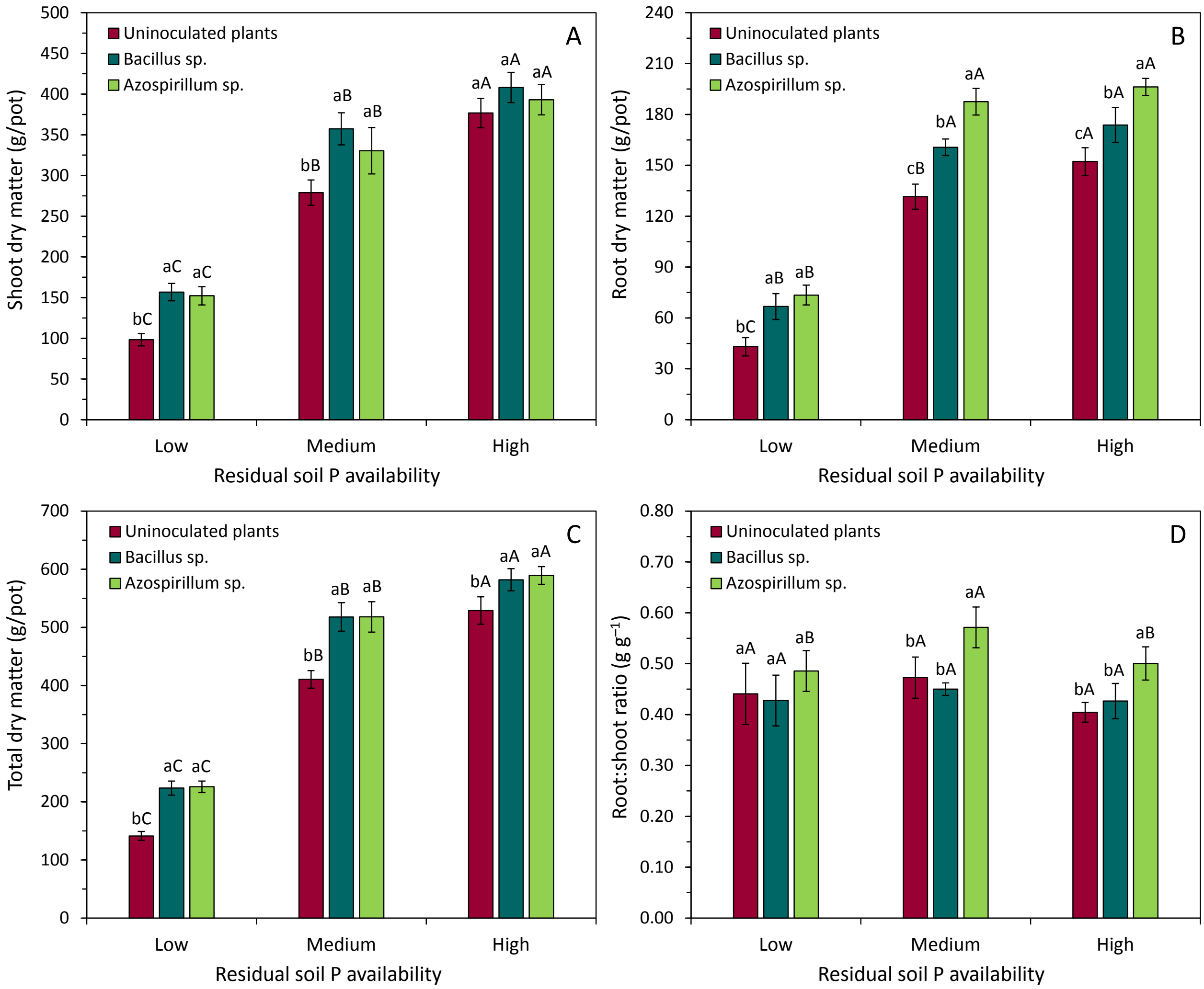

Figure 2 - Effect of soil residual P level and inoculation of phosphate-solubilizing bacteria (*Bacillus* sp. or *Azospirillum* sp.) on shoot dry matter (A), root dry matter (B), total dry matter (C) and root: shoot dry matter ratio (D) of sugarcane plants (*Saccharum* sp., var. RB867515) grown in a sandy clay loam Oxisol. Bars followed by distinct lowercase letters for the inoculation of phosphate-solubilizing bacteria, and distinct uppercase letters for soil residual P levels show significant differences (Tukey test; $p < 0.05$). Data refers to mean values ($n = 4$) ± standard error.

Under high residual P availability, the inoculation of phosphate-solubilizing bacteria had no significant effect ($p > 0.05$) on shoot dry matter accumulation of sugarcane plants (Figure 2A).

Root dry matter under high residual P availability was significantly higher in plants inoculated with *Azospirillum* sp., followed by plants inoculated with *Bacillus* sp., and significantly lower in non-inoculated plants (Figure 2B). Total dry matter accumulation under high residual P availability was significantly higher in plants inoculated with *Bacillus* sp. and *Azospirillum* sp. compared to non-inoculated plants (Figure 2D).

Under low residual P availability, the root:shoot dry matter ratio of sugarcane plants was not significantly affected by the inoculation of phosphate-solubilizing bacteria. Under medium or high residual P availability, root:shoot ratio was significantly higher in plants inoculated with *Bacillus* sp. and *Azospirillum* sp. when compared to non-inoculated plants (Figure 2D).

Residual P levels and inoculation of *Bacillus* sp. and *Azospirillum* sp. significantly affected ($p \leq 0.05$) the P concentration and P accumulation in the sugarcane plants (Figure 3). Medium and high residual P availability resulted in higher P concentration in sugarcane plants compared to non-inoculated plants, except when plants were inoculated with *Bacillus* sp. (Figure 3A). In turn, the increase in residual soil P availability resulted in progressive accumulation of P in the dry biomass of sugarcane plants (Figure 3B).

Under low or medium residual P availability, the amount of P accumulated in the biomass was significantly higher in plants inoculated with *Bacillus* sp. and *Azospirillum* sp. when compared to non-inoculated plants. However, under high residual P availability, the inoculation of phosphate-solubilizing bacteria had no significant effect ($p > 0.05$) on P accumulation of sugarcane plants (Figure 3B).

Residual P levels and inoculation of *Bacillus* sp. and *Azospirillum* sp. significantly affected ($p \leq 0.05$) the P uptake efficiency (PUpE), P utilization efficiency (PUtE) and physiological P use efficiency (PPUE) of the sugarcane plants (Figure 4). The PUpE under low or medium residual P availability was significantly higher in plants inoculated with *Bacillus* sp. and *Azospirillum* sp. when compared to non-inoculated plants. However, under high residual P availability, the inoculation of phosphate-solubilizing bacteria had no significant effect ($p > 0.05$) on PUpE of sugarcane plants (Figure 4A).

The PUtE was significantly higher under low residual P availability compared to medium and high residual P availability (Figure 4B). Under low residual P availability, the PUtE was significantly higher in plants inoculated with *Bacillus* sp. and non-inoculated plants when compared to plants inoculated with *Azospirillum* sp. However, under medium or high residual P availability, the inoculation of phosphate-solubilizing bacteria had no significant effect ($p > 0.05$) on PUtE of sugarcane plants (Figure 4B).

The PPUE of sugarcane plants increased progressively with increasing residual soil P availability (Figure 4C). Under low or medium residual P availability, the PPUE was significantly higher in plants inoculated with *Bacillus* sp. and *Azospirillum* sp. when compared to non-inoculated plants. In turn, under high residual P availability, the PPUE was significantly higher in plants inoculated with *Azospirillum* sp. compared to non-inoculated plants (Figure 4C).

A network diagram was constructed from all plant morphological traits and P use efficiency indices and their respective correlations (Figure 5). All interactions between morphological traits and P use efficiency indices can be observed from the

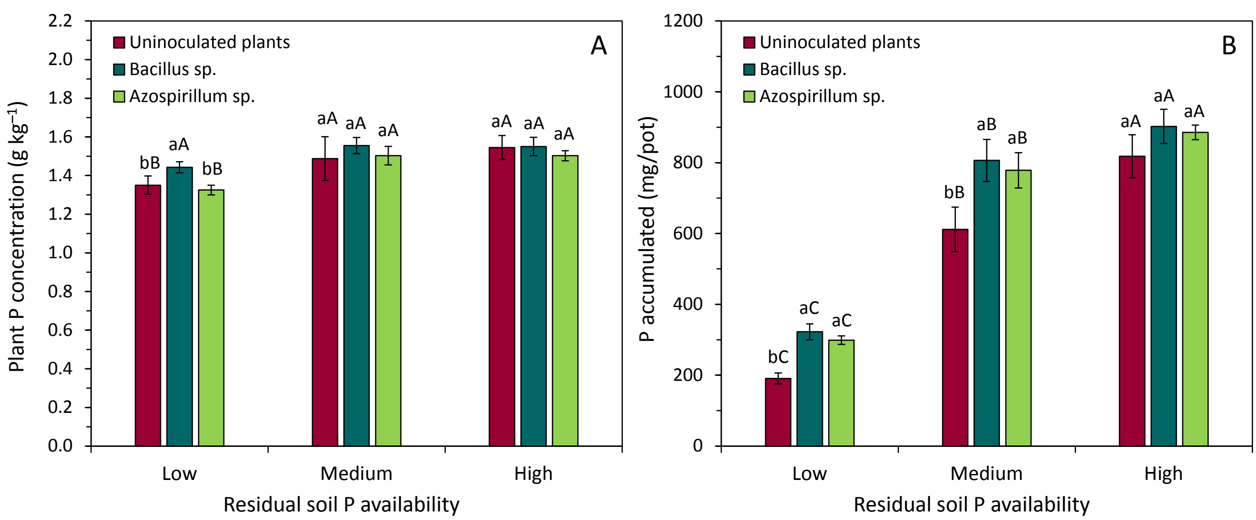

Figure 3 - Effect of soil residual P level and inoculation of phosphate-solubilizing bacteria (*Bacillus* sp. or *Azospirillum* sp.) on plant P concentration (A) and amount of P accumulated (B) in the sugarcane plants (*Saccharum* sp., var. RB867515) grown in a sandy clay loam Oxisol. Bars followed by distinct lowercase letters for the inoculation of phosphate-solubilizing bacteria, and distinct uppercase letters for soil residual P levels show significant differences (Tukey test; $p < 0.05$). Data refers to mean values ($n = 4$) ± standard error.

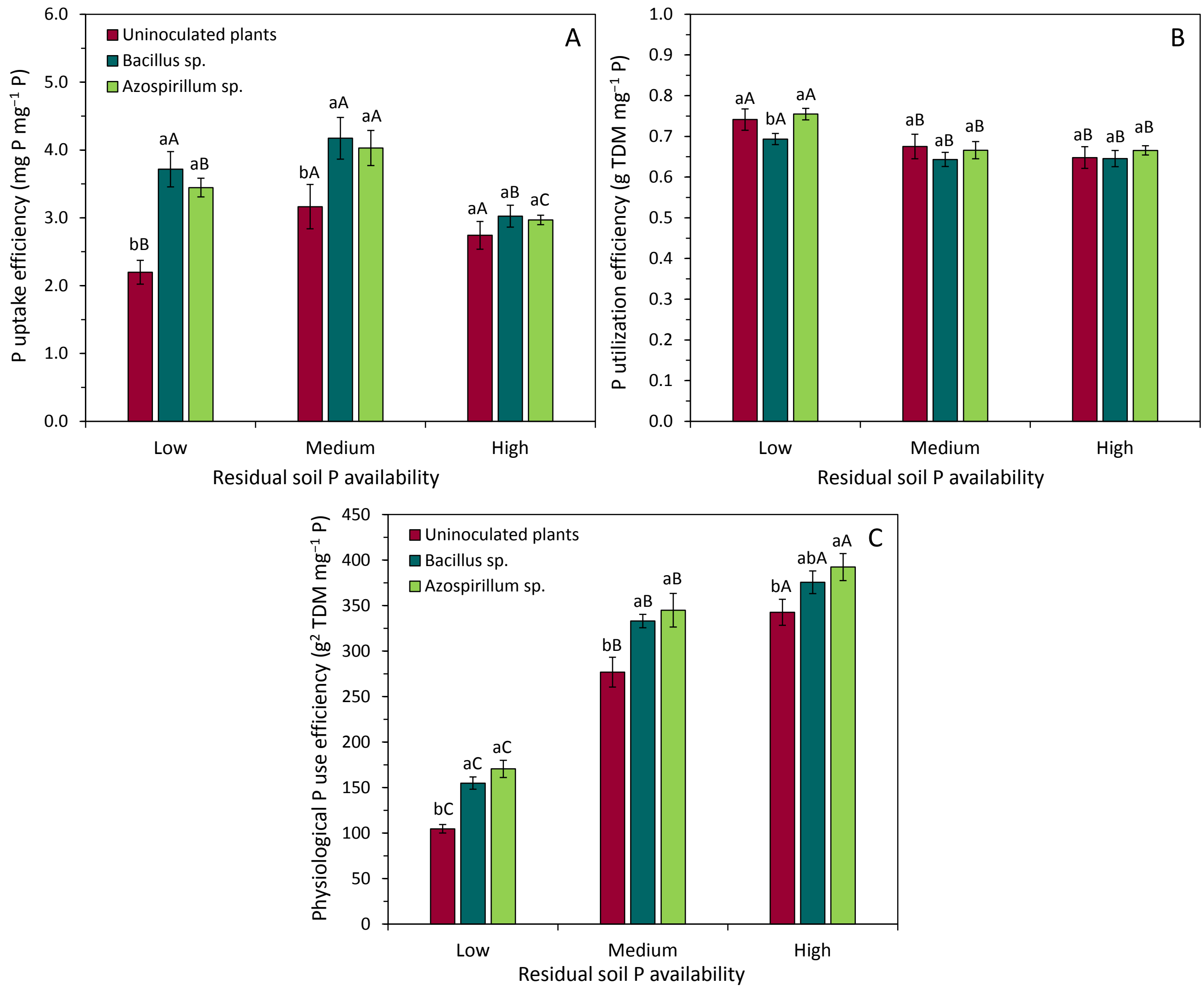

Figure 4 - Effect of soil residual P level and inoculation of phosphate-solubilizing bacteria (*Bacillus* sp. or *Azospirillum* sp.) on P uptake efficiency – PUpE (A), P utilization efficiency – PUtE (B) and physiological P use efficiency – PPUE (C) in the sugarcane plants (*Saccharum* sp., var. RB867515) grown in a sandy clay loam Oxisol. Bars followed by distinct lowercase letters for the inoculation of phosphate-solubilizing bacteria, and distinct uppercase letters for soil residual P levels show significant differences (Tukey test; $p < 0.05$). Data refers to mean values ($n = 4$) ± standard error.

correlation network. Pearson's linear correlation coefficient was used to determine the interrelationship between morphological traits and P use efficiency of sugarcane plants grown under residual P fertilization levels and inoculated with phosphate-solubilizing bacteria (Figure 5). Correlation analysis reported a positive and highly significant association between all morphological traits of sugarcane plants, except for the root:shoot ratio (RSR). There was also a significant and positive correlation between most morphological traits and P concentration (P), amount of P accumulated in the biomass (PAc) and physiological P use efficiency (PPUE). Significant and negative correlations were reported between P utilization efficiency (PUtE) and most plant morphological traits. PUtE also had a significant and negative correlation with P concentration (P), amount of P accumulated in the biomass (PAc) and physiological P use efficiency (PPUE). In turn, the RSR and P uptake efficiency (PUpE) do not have significant correlations with any other characteristic measured in sugarcane plants (Figure 5).

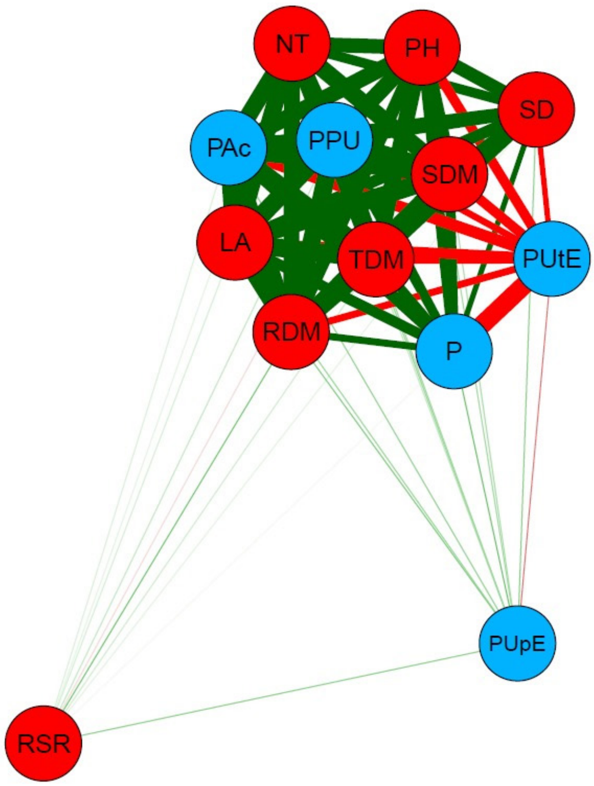

Figure 5 - Correlation networks illustrate the most significant Pearson correlations between morphological traits and P use efficiency indices of the sugarcane plants (*Saccharum* sp., var. RB867515) grown under soil residual P levels and inoculated with phosphate-solubilizing bacteria (*Bacillus* sp. or *Azospirillum* sp.). Thicker green lines represent the highest positive correlations (threshold set at 0.6 and *P* values < 0.05). Thicker and red lines represent the highest negative correlations (threshold set at 0.6 and *P* values < 0.05).

Abbreviations: PH: plant height; SD: stalk diameter; NT: number of tillers; LA: leaf area; SDM: shoot dry matter; RDM: root dry matter; TDM: total dry matter. RSR: root: shoot ratio. P: plant phosphorus concentration; PAc: phosphorus accumulated in the plant biomass; PUpE: phosphorus uptake efficiency; PUtE: phosphorus utilization efficiency; PPUE: physiological phosphorus use efficiency.

DISCUSSION

Annually applied phosphate fertilization rates of 30, 60, and 120 kg ha^{-1} P_2O_5 resulted in distinct levels of residual P availability in a sandy clay loam Oxisol of the Brazilian Cerrado. Soil samples from this field experiment were collected and used in the present pot study to investigate the interaction between residual phosphate fertilization and inoculation of phosphate-solubilizing bacteria in sugarcane plants. Our results showed that increased soil residual P availability improved growth (shoots and roots), plant phosphorus accumulation, and physiological P use efficiency in sugarcane plants.

Plants grown under high residual P availability had an average increase in plant height (43%), number of tillers (176%), leaf area (180%), shoot dry matter (185%), root dry matter (188%), plant P accumulation (221%) and physiological P use efficiency (158%) when compared to plants under low residual soil P availability (Figures 1, 2, 3, and 4). These results demonstrate the importance of adequate phosphorus supply for early development and metabolism of sugarcane plants in Brazilian tropical soils.

The higher plant growth and development under high residual soil P availability reflects the higher initial concentration of available P in the soil (21.3 mg dm^{-3} P) when compared to the initial concentration of available P in the treatment under low residual P availability (6.2 mg dm^{-3} P) (Table 1). Therefore, the low P availability of tropical soils caused by the high adsorption capacity of phosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) to soil colloids, especially to iron (Fe) and aluminum (Al) oxides and hydroxides associated with 1:1 clays (kaolinite) and 2:1 clays (montmorillonite) (Lizcano-Toledo *et al*., 2021; Soares *et al*., 2021), has been a limiting factor for stalk development and productivity in sugarcane crops (Schiavo *et al*., 2018; Withers *et al*., 2018; Rodrigues *et al*., 2023).

Plant height (Figure 1A), number of tillers (Figure 1C), leaf area (Figure 1D), shoot dry matter (Figure 2A) and root dry matter (Figure 2B) were the morphological traits of sugarcane plants most impacted by soil residual P availability levels. Rodrigues *et al*. (2023) reported greater increases in plant height, number of tillers, leaf area, and shoot and root dry matter of sugarcane plants fertilized with high P levels.

Phosphorus plays an important role in sugarcane plant's growth and physiological metabolism, acting in the processes of photosynthesis, respiration, signal transduction, plant tillering, root development, and water uptake (Zhang *et al*., 2018; Jiaying *et al*., 2022; Khan *et al*., 2023). Phosphorus is also a structural component of many biomolecules, including phospholipids, nucleic acids, and adenosine triphosphate (ATP), which guide plant development and protein synthesis (Jiaying *et al*., 2022). Therefore, P deficiency results in sugarcane growth retardation, young plants are dwarfed and thin, with reduced leaf area, and the plant tillering potential is drastically inhibited, as observed in the present study.

The greater initial growth of sugarcane plants under low or medium residual P availability was dependent on the inoculation of phosphate-solubilizing bacteria (Figures 1 and 2). Other studies have also proven the effectiveness of inoculating bacteria of the genus *Azospirillum* sp. and *Bacillus* sp. in sugarcane crops, resulting in greater development and stalk yield (Santos *et al*., 2018; Moura *et al*., 2019; Gonçalves *et al*., 2020; Rodrigues *et al*., 2023).

Gírio *et al*. (2015) reported that coinoculation with *A. amazonense*, *Burkholderia tropica*, *Herbaspirillum seropedicae* and *Gluconacetobacter diazotrophicus* improved sprouting and growth of roots and shoots of sugarcane plants. Santos *et al*. (2018) showed that the application of *B. pumilus* increased total dry matter (13%), number of tillers (37%), stalk diameter (48%) and root growth (23%) when associated with mineral fertilization. Costa *et al*. (2021) found that inoculation with *A. brasilense* resulted in increased plant height, shoot dry matter, root dry matter, and seedling quality of the sugarcane varieties IACSP95-5000, RB855035 and RB867515. Rodrigues *et al*. (2023) reported that inoculation with *B. megaterium* and *B. subtilus* improved tillering and shoot dry matter accumulation of sugarcane plants, indicating increased P solubilization and/or increased P uptake from the soil. Therefore, these results highlight the importance of inoculating phosphate-solubilizing bacteria, especially *Bacillus* sp. and *Azospirillum* sp., to improve soil P availability and increase the initial development of sugarcane plants.

The potential of these bacteria to solubilize phosphate ions from fractions not available in the soil (non-labile P) and stimulate plant growth are associated with the mechanisms of excretion of organic acids and production of siderophores (Sharma *et al*., 2013; Kumar *et al*., 2018; Kalayu, 2019). Indeed, studies by Velloso *et al*. (2020) and Silva *et al*. (2023) showed that some *Bacillus* species, including *B. megaterium*, *B. subtilis* and *B cereus*, have the ability to solubilize phosphate (via release of organic and inorganic acid and proton extrusion), mineralize phosphate (via phosphatase production) and produce chelating agents with high affinity for aluminum (Al^{3+}) and iron (Fe^{2+}) metals present in insoluble Al and Fe oxides in tropical soils (via siderophore production). Similarly, Oliveira-Paiva *et al*. (2024) reported that *B. megaterium* B119 and *B. subtilis B2084* exhibited plant growth-promoting properties relevant to improving soil P dynamics, including phosphate solubilization and mineralization, production of indole-3-acetic acid (IAA)-like molecules, siderophores, exopolysaccharides (EPS), biofilms, and phosphatases. In turn, *Azospirillum* species, including *A. brasilense* and *A. amazonense*, can stimulate plant development through several biological mechanisms, including biological nitrogen fixation (Gonçalves *et al*., 2020), plant hormone synthesis (Fukami *et al*., 2018), phosphate solubilization (Turan *et al*., 2012), and induction of tolerance to abiotic and biotic stresses (Fukami *et al*., 2018; Silva *et al*., 2019).

Furthermore, the greater development of the root system of sugarcane plants, evidenced by the greater accumulation of root dry matter (Figure 2B) and the higher root: shoot dry matter ratio (Figure 2D) with the inoculation of *Azospirillum* sp. under medium and high residual soil P availability, may be associated with the ability of some *Azospirillum* species, especially *A. brasilense*, to produce and secrete plant hormones, such as auxin, into the rhizosphere. Indeed, Fukami *et al*. (2018) showed that *A. brasilense* strains Ab-V5 and Ab-V6 have a high capacity to synthesize indole-3-acetic acid - IAA (an auxin), gibberellic acid (GA3) and salicylic acid (SA). The production of these plant hormones, especially IAA, in the rhizosphere region stimulates meristematic activity and promotes the elongation and development of lateral roots (Santos *et al*., 2021). In turn, the greater development of the root system allows the plant to absorb more water and nutrients, favoring its development and productivity (Gonçalves *et al*., 2020; Costa *et al*., 2021).

The higher P accumulation in the biomass of sugarcane plants inoculated with *Bacillus* sp. and *Azospirillum* sp. under low or medium residual P availability (Figure 3B) indicates that the inoculation of phosphate-solubilizing bacteria improved P acquisition from the soil. Similar results were reported by Pereira *et al*. (2020), who showed that the inoculation of *Bacillus subtilis* or *Azospirillum brasilense* resulted in higher P uptake by corn plants. Oliveira-Paiva *et al*. (2024) also reported that inoculation with *B. megaterium* and *B. subtilis* improved P acquisition by corn plants. These results show that both *B. subtilis* and *B. megaterium* as well as *A. brasilense* and *A. amazonense* are bacteria with excellent capacity to solubilize phosphate from the soil and, therefore, have potential for use as biofertilizers in tropical soils with deficient and moderate P levels. Malic, oxalic, and glutamic acids, for instance, are organic acids that these bacteria can produce and excrete into the soil. These acids can complex the Al^{3+} (accompanying ion) of aluminum phosphate ($AlPO_4$), releasing the phosphate ion (PO_4^{-3}) into the soil solution that plant roots can absorb (Sharma *et al*., 2013; Fukami *et al*., 2018; Oliveira-Paiva *et al*., 2024). Therefore, the use of phosphate-solubilizing bacteria (*Bacillus* sp. or *Azospirillum* sp.) allows Brazilian farmers to adopt a sustainable and low-cost technology and thus obtain an increase in the development and acquisition of P by sugarcane plants in soils with low or medium residual P availability.

Phosphorus uptake efficiency (PUpE) and physiological P use efficiency (PPUE) were also improved by inoculation of *Bacillus* sp. or *Azospirillum* sp. under conditions of low and medium residual P availability (Figures 4A and 4C). Our results showed that the PUpE of plants inoculated with *Bacillus* sp. and *Azospirillum* sp. was increased by 69% and 57% under low residual P availability and by 31% and 27% under medium residual P availability, respectively, when compared to non-inoculated plants (Figure 4A). On the other hand, the PPUE of sugarcane plants inoculated with *Bacillus* sp. and *Azospirillum* sp. was increased by 48% and 63% under low residual P availability and by 20% and 25% under medium residual soil P availability (Figure 4C). Pereira *et al*. (2020) also reported that inoculation of *B. subtilis* and *A. brasilense* improved PUpE in corn plants, which resulted in greater P acquisition, crop development and grain yield. The greater PUpE and greater PPUE of plants inoculated with *Bacillus* sp. and *Azospirillum* sp. strains is associated with the multifunctional mechanisms of these bacteria, especially those related to soil phosphate solubilization and mineralization (Fukami *et al*., 2018; Oliveira-Paiva *et al*., 2024), which play a crucial role in increasing P uptake and use efficiency, especially in soils with high P adsorption capacity (Kumar *et al*., 20118; Kalayu, 2019). Indeed, many studies have demonstrated the effectiveness of several mechanisms of phosphate solubilization and mineralization exhibited by strains of *Bacillus* sp. and *Azospirillum* sp. in mitigating P adsorption in tropical soils, where soluble P reacts with clay minerals and metal ions (Ca, Fe, Al) to form poorly soluble complexes that are unavailable to plants (Fukami *et al*., 2018; Kalayu, 2019; Pavinato *et al*., 2021; Oliveira-Paiva *et al*., 2024).

However, our results showed that the mechanisms of phosphate solubilization and/or mineralization by strains BRM 119 (*B. megaterium*), BMF 2484 (*B. subtilus*), Ab-V5 and Ab-V6 (*A. brasilense*) and BR11145 (*A. amazonense*) under conditions of high soil P availability are still inefficient. This inference is justified by the absence of positive response on most morphological traits, P acquisition and P use efficiency of sugarcane plants grown under high residual soil P availability (Figures 1, 2, 3 and 4). However, the physiological basis of how high soil P availability influences phosphate solubilization by bacteria of the genus *Bacillus* sp. and *Azospirillum* sp. remains unknown. Therefore, future research should be conducted to elucidate how the various physicochemical and molecular mechanisms (e.g., root exudates, extracellular polysaccharides, organic acids, phosphatases, and phosphate- specific transport systems) utilized by phosphate-solubilizing bacteria, especially *Bacillus* sp. and *Azospirillum* sp., are impacted when applied to soils with high available P concentration.

Overall, our results reinforce the importance of using *Bacillus* sp. (B119 and B2084) and *Azospirillum* sp. (Ab-V5, Ab-V6 and BR11145) strains to stimulate plant development and improve P use efficiency of sugarcane crops grown in tropical soils with low and medium P availability, which reduces the costs associated with mineral P fertilization. In conclusion, we firmly believe that phosphate-solubilizing bacteria represents a highly significant biotechnological tool to improve sugarcane development and increase stalk productivity, as well as promoting the development of modern and sustainable agriculture for future generations.

CONCLUSIONS

In tropical soils with low or medium residual P availability, inoculation of *Bacillus* sp. and *Azospirillum* sp. may be a sustainable agricultural practice to be recommended for the cultivation of sugarcane crops, especially because it improves plant development, P acquisition and P use efficiency, which reduces the costs associated with fertilization with mineral phosphorus, a non-renewable fertilizer source.

The phosphate solubilization/mineralization efficiency and potential of *Bacillus* sp. (BRM 119 and BMF 2484) and *Azospirillum* sp. (Ab-V5, Ab-V6 and BR11145) strains are reduced when these bacteria are applied to tropical soils with high available P concentration.

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CONTRIBUTIONS

Gilciany Ribeiro Soares: Investigation, Methodology, Data curation, Formal data analysis, Software, Visualization, Writing– original draft & editing; **Fábio Steiner**: Methodology, Software, Conceptualization, Visualization, Validation, Data curation, Formal data analysis, Writing– review & editing, Fund acquisition and Supervision; **Jiovana Kamila Vilas Boas**: Investigation, Methodology, Data curation, Formal data analysis, Software, Visualization, and Writing– original draft & editing; **Jorge González Aguilera**: Methodology, Software, Conceptualization, Visualization, Validation, Data curation, Formal data analysis, and Writing– review & editing; **Alan Mario Zuffo**: Methodology, Software, Conceptualization, Visualization, Validation, Data curation, Formal data analysis, and Writing– review & editing; **Wellingthon da Silva Guimarães Júnnyor**: Methodology, Software, Conceptualization, Visualization, Validation, Data curation, Formal data analysis, Writing– review & editing, Fund acquisition and Supervision; **José Vitor Marçal do Prado**: Methodology, Software, Conceptualization, Visualization, Validation, Data curation, Formal data analysis, Writing– review & editing; **Gustavo Castoldi:** Investigation, Methodology, Data curation, Formal data analysis, Software, Visualization, Writing– original draft & editing and Supervision. **Luciano Façanha Marques:** Data curation, Formal data analysis, and Writing– review & editing.

Ethical Approval
Not applicable.

Consent to Participate
Not applicable.

Consent for Publication
The final version of the manuscript was reviewed and approved by all authors.

Competing Interests
The authors declare no competing interests.

Data Availability Statement
The data are available in the paper and if necessary the original data can be made available.

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