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Research Article
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Residual phosphate fertiliser and *Azospirillum brasilense* inoculation on soil quality and soybean productivity[1]

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ABSTRACT - Maintaining adequate levels of labile phosphorus (P) in weathered soils is challenging due to its constant fixation. Mineral fertilisation is therefore often employed to ensure adequate nutrient levels in the soil. Such techniques as the use of phosphate-solubilising bacteria, are gaining recognition for minimising production costs. Genus *Azospirillum brasilense* demonstrates this ability, increasing nutrient availability and reducing the amount of P applied through mineral fertilisation. The aim of this study was to evaluate the residual effect of phosphate fertiliser in soil under a no-till system, verifying whether inoculating the preceding crop with *A. brasilense* increased soybean production and productivity, and the bioindicators of soil quality. The experiment was conducted in a typical clayey dystrophic Red Latosol, employing a randomised block design with four replications, in a 5 x 2 factorial scheme, comprising five doses of P_2O_5 in the form of MAP applied during 2013 and 2020, with and without inoculating the grasses preceding the soybean with *A. brasilense.* There was a positive effect on the soybean population during the 2019/20 crop season due to the inoculation of the preceding crop, as well as responses in microbiological activity, such as total carbon, microbial respiration, biomass carbon, total organic carbon, acid phosphatase, arylsulfatase and β-glucosidase. The results show that cropping systems which maintain adequate soil health, together with the inoculation of grasses with *A. brasilense*, can be a low-cost management option for sustaining crops and contributing to system maintenance.

Keywords: System fertilisation. Enzyme activity. Diazotrophic bacteria. Residual effect. Soil health.

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INTRODUCTION

The world population has now surpassed 8.2 billion inhabitants (United Nations, 2024), requiring greater agricultural food production. However, soils of low fertility and with advanced levels of erosion lead to losses in crop productivity and increased soil acidity, with a consequent reduction in nutrient levels and restrictions on total food production (Legaz *et al.,* 2017). The adoption of no-till systems in tropical countries is an indispensable technique for improving soil conditions and maintaining soil health, benefiting microbiological activity, minimising erosion, supplying nutrients through the decomposition of crop residue, favouring the equilibrium and self-regulation of the environment, and promoting improvements in the genetic performance of crops, which reflects in greater productivity (Hernani, 2021; Ribeiro *et al.,* 2025).

Soils in areas of tropical climate exhibit a high degree of weathering, with a predominantly acidic pH, aluminium (Al) and manganese (Mn) toxicity, low cation exchange capacity (CEC), low base saturation (V%), nutrient deficiency—especially phosphorus (P), and aerobic bacteria that are highly active in terms of humus degradation. No-till systems are therefore essential, as the increase in organic matter (OM) content can benefit the thermal regulation of the environment and aid in soil production and conservation (Martins *et al.* 2023; Ren *et al.,* 2023). At the same time, monitoring soil fertility, together with an understanding and knowledge of the microbiological factors, is essential to assess the health and quality of the soil (Ribeiro *et al.,* 2025).

Phosphorus (P) is an essential nutrient for plant dynamics and metabolism (Taiz *et al.,* 2017); however, in tropical soils, P may become unavailable to plants due to reacting with the Fe and Al ions that are present in high concentrations (Braos *et al.,* 2020). One option for improving the management of phosphate fertilisation is the use of phosphate-solubilising bacteria that are able to convert the insoluble P adsorbed to Fe and Al oxyhydroxides, thereby breaking the strong bond and making the P available to plants (Kour *et al.,* 2021).

The bacterium, *Azospirillum brasilense*, acts to solubilise soil phosphorus (Pereira et *al.,* 2020; Sun *et al.,* 2022). Furthermore, it promotes greater root development, improving exploitation of the soil profile, which can increase phosphorus absorption and enzyme activity due to the increase in the microbial community stimulated by the long-term no-till system (Cassán; Vanderleyden; Spaepen, 2014; Spaepen *et al.,* 2014). Another benefit attributed to the use of *A. brasilense* is the production of phytohormones, such as indole-3-acetic acid (IAA), that aid in the development of lateral roots, bud production and increased root hairs (Cohen *et al.,* 2014; Ucea-Herrera; Quiroz-Velasquez; Hernandez-Mendoza, 2020). *A. brasilense* demonstrates this ability, increasing phosphorus availability, which can considerably reduce the amount required through mineral fertilisation in long-term no-till systems and promote improvements in the microbiology of the soil. This effect can be prolonged in grasses that precede the soybean crop, affording increased grain yield.

The aim of this study was to evaluate the residual effect of phosphate fertilisation in soil under long-term no-till management, verifying any interaction between productivity and the inoculation of crops preceding the soybean with the bacterium *A. brasilense* to increase production, and its effect on the bioindicators of soil quality.

MATERIAL AND METHODS

Location, Characterisation and Installation of the Experiment

The research was conducted under field conditions in the district of Selvíria, Mato Grosso do Sul (20°20' S and 51°24' W, altitude 335 m). The relief in the area is characterised as moderately flat. According to the Köppen classification (1928), the climate is described as Aw, humid tropical with a rainy season during the summer and dry season during the winter. Monthly data on the average, maximum and minimum air temperature and rainfall were collected throughout the experiment (Figure 1).

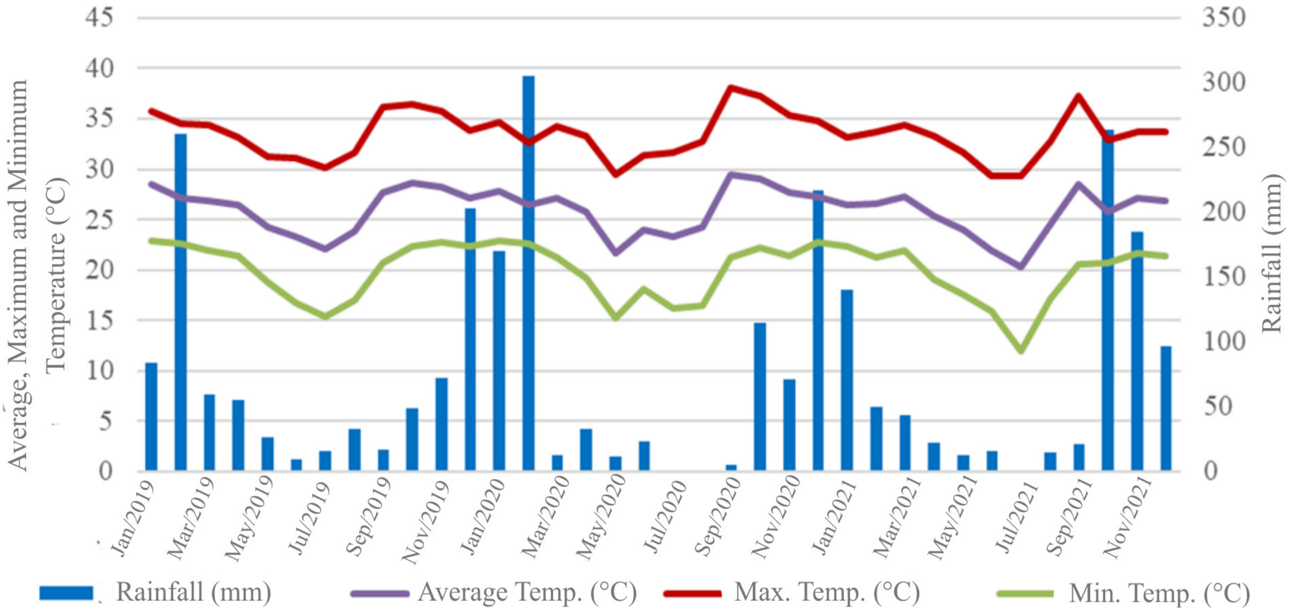
The soil in the experimental area was classified as a typical clayey dystrophic Red Latosol (52% clay) (Santos *et al.,* 2018). The experimental area had a history of cultivation with annual crops under a no-till system for 18 years up to the time of the study. Prior to the experiment, there had been a succession of crops in the area to provide mulch for the system and improve the physical, chemical and biological aspects of the soil (Figure 2).

When setting up the experiment (14 April 2013), an initial chemical characterisation of the soil was carried out (Raij *et al.,* 2001) for the 0.00 - 0.20 m layer (Table 1), with a further chemical characterisation during the 2019/20 season. Based on the results of the chemical analysis, and in order to raise the base saturation to 70%, surface liming with dolomitic limestone (PRNT of 87%) was carried out at a dose of 1.7 t ha^{-1} with no incorporation.

The experimental design was of randomised blocks with four replications in a 5 x 2 factorial scheme (five doses of P_2O_5 and two treatments, with and without inoculation), giving a total of 40 experimental units. Each experimental unit (plot) was 4.4 m in width and 10 m in length, for a total area of 44 m². All the crops were grown in the same area, which including a working border with a length and width of 1 m, totalling 1 m².

The doses of P_2O_5 were based on Mascarenhas and Tanaka (1997) and applied as monoammonium phosphate - MAP (0, 30, 60, 120 and 240 kg ha^{-1} P_2O_5), broadcast at the time of sowing the black oats (cv. IAPAR 61) in 2013. For the second treatment factor (with or without seed inoculation with *Azospirillum brasilense),* the Ab-V5 and Ab-V6 strains (guaranteed 2x10^8 CFU/mL) were inoculated into the rotated grasses from 2013-2020. A liquid inoculant was used at a dose of 100 mL per 25 kg of seeds. Inoculation was carried out in the shade immediately before sowing.

Figure 1 - Climate data on average, maximum, and minimum temperature and rainfall, in the district of Selvíria, Mato Grosso do Sul, during the experimental period



Note: Data collected at the weather station of the Irrigation and Drainage Laboratory of the Faculty of Engineering at Ilha Solteira, São Paulo, Brazil

Figure 2 - History of the experimental area under long-term no-till management in the district of Selvíria, Mato Grosso do Sul, crop seasons 2013/14 to 2020/21

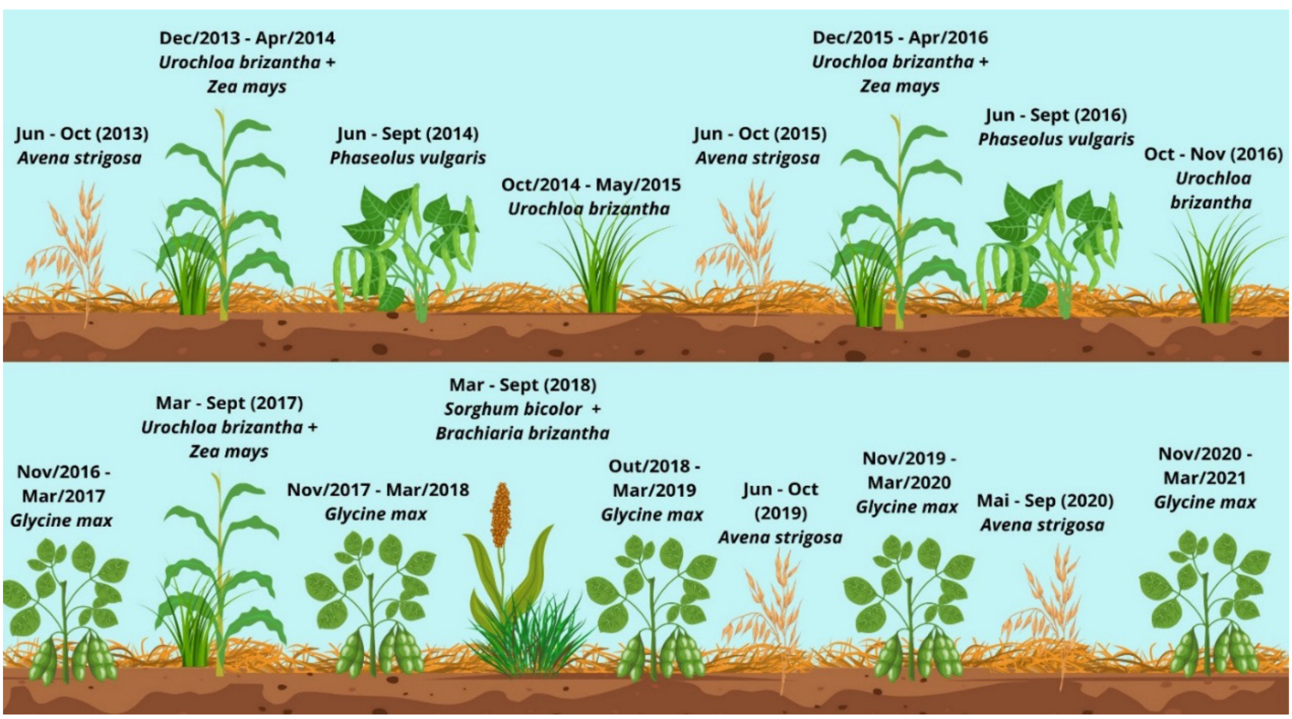


Table 1 - Chemical characterisation of the soil in the 0.00 - 0.20 m layer for the long-term no-till experiment, for the 2013/14 and 2019/20 crop seasons, respectively. Selvíria, Mato Grosso do Sul, Brazil

Layer m	P_{resin}	OM	pH	K	Ca	Mg	H + Al	Al	SB	CEC	V
	mg dm^{-3}	g dm^{-3}	$CaCl_2$	---------- mmol$_c$ dm^{-3} ----------							%
2013/14											
0.00 - 0.20	16	25	5.0	2.2	19	14	36	2.5	35.2	71.2	49
2019/20											
0.00 - 0.20	5	26	4.8	2.1	17	13	36	2	32.1	67	45.3

Phosphorus (P_{resin}); organic matter (OM); hydrogen ion potential (pH); potassium (K); calcium (Ca); magnesium (Mg); potential acidity (H + Al); aluminum (Al); sum of bases (SB); cation exchange capacity (CEC); base saturation (V)

In 2013, the initial levels of P in the soil were average (Raij *et al*., 1996). It was therefore decided to broadcast the phosphate fertiliser with no incorporation. One day before sowing the black oats, the doses of P_2O_5 were applied in each experimental plot using monoammonium phosphate (MAP) as the source (52% P_2O_5 and 12% N). At the time of sowing, the nitrogen (N) equivalent was also applied based on the doses studied in the research; this was supplied by the MAP containing 12% N and complemented by the application of 115 kg ha^{-1} urea for the 30 kg ha^{-1} dose of P_2O_5, 100 kg ha^{-1} urea for the 60 kg ha^{-1} dose, 60 kg ha^{-1} urea for the 120 kg ha^{-1} dose and 10 kg ha^{-1} urea for the 240 kg ha^{-1} dose. Topdressing was applied at the start of grass tillering, on 7 March 2013, with the application of 60 kg ha^{-1} K_2O using potassium chloride (KCl) as the source, and 60 kg ha^{-1} N (urea), as per the recommendations for black oats (Cantarella; Raij; Camargo, 1997).

From the soil analysis carried out each cycle (first and second crops), phosphate fertiliser was not necessary until 2019. From 2013 to 2019, only the residual effect of the P applied in 2013 was evaluated, applying only N and/or KCl as topdressing, according to each rotated crop. After the soybean harvest in the 2019/20 season, a chemical soil analysis was carried out (Table 1) at 20 different points within each plot at depths of 0.00 - 0.20 m. After air-drying, the samples were homogenised, ground, passed through a 100-mesh sieve and analysed as per the methodology proposed by Raij. *et al*. (2001).

After the 2020 analysis, P levels were found to be low (Table 1), so it was decided to reapply the P. MAP was used to apply the doses (0, 30, 60, 120 and 240 kg ha^{-1} P_2O_5) which were broadcast when sowing the black oats (cv. EMBRAPA 29). Nitrogen fertilisation was carried out using 115 kg ha^{-1} urea for the 30 kg ha^{-1} dose of P_2O_5, 100 kg ha^{-1} urea for the 60 kg ha^{-1} dose, 60 kg ha^{-1} urea for the 120 kg ha^{-1} dose and 10 kg ha-1 urea for the 240 kg ha^{-1} dose, in addition to the MAP.

To form the mulch for the no-till system, the plants in the area were desiccated with 4 L ha^{-1} Glyphosate. Three experiments (crops) were carried out: soybean (November/2019), black oats (May/2020) and soybean (November/2020), when the production components and productivity of the soybean crop were evaluated.

The soybean (cv. TMG 7063 IPRO) were sown on 5 November 2019 and 5 November 2020 using a seed and fertiliser drill with a shank type (knife) furrow opener for no-till farming, at a spacing of 0.45 metres between rows and approximately 14.9 seeds per metre of furrow. The seeds were treated with a product based on strobilurin, benzimidazole and pyrazole (2 mL kg^{-1} seeds) and inoculated with *Bradyrhizobium. japonicum* (100 g of commercial product per 40 kg of seeds) in a peat-based solid formulation. Inoculation was carried out using a concrete mixer shortly before sowing. For both years, the base fertiliser used for the soybean was 136 kg ha^{-1} KCl, with no additional fertilisation. Cropping practices were carried out for the preventative control of pests and diseases, including the application of herbicide for weeds in the early stages, until the spaces between the rows had completely closed.

Microbiological and enzymatic evaluations of the soil

For the microbial biomass and enzyme analyses, soil samples were collected from the 0.00 - 0.10 m layer of each plot at the end of both soybean crop cycles (2020/21), with an average of 15 subsamples per plot. The samples were passed through a 2-mm mesh sieve and stored in a refrigerator for later microbial analysis. Microbial respiration (MR) was measured as per Rezende, Assis and Nahas (2004), using two controls with no soil for better standardisation. Microbial biomass carbon (MBC) was determined based on the methodology of Jenkinson and Ladd (1976), modified by Oliveira, Mendes and Vivaldi (2001). Soil microbial biomass carbon (MBC) was determined as the difference between the CO_2 released from the F (fumigated) and NF (non-fumigated) samples over a 10-day period following fumigation, using a correction factor (Kc) of 0.41 (Anderson; Domsch, 1978).

The metabolic quotient (qCO_2) was estimated from the ratio between microbial respiration (MR) and the soil microbial biomass carbon. Total organic carbon (TOC) was determined following the methodology proposed by Walkley and Black (1934), with final titration of the excess dichromate using ferrous ammonium sulphate. A substrate of 0.05 M p-nitrophenyl-β-D-glucopyranoside (PNG 0.05 M) was used in the reaction to determine the activity of the enzymes β-glycosidase and acid phosphatase, while for arylsulfatase, the substrate was 0.05 M p-nitrophenyl (PN 0.05 M).

Evaluation of the production and productivity components of the soybean

Soybean production and productivity were evaluated one day before the harvest. The plant population (POP) was determined by counting the plants in 4 m of the four central rows of each plot, the final value was then extrapolated to the number of plants per hectare. Plant height (PH) was measured in the working area of each plot using ten consecutive plants per row, that were measured from the ground to the top of the plant, with the values expressed in metres (m). The height of the first pod insertion (PIH) was measured on the same plants used for measuring height, from the ground to the first pod. The number of pods per plant (NPP) was determined as the average of 10 plants. The number of grains per plant (NGP) was obtained from the average number of grains on 10 plants per plot. The 100-grain weight (W100) was determined by separating four grain samples, which were sent to the laboratory to be counted on an electronic counter, these were then weighed using a precision balance (0.01 g) with the grain moisture corrected to 13% (wet basis). Grain productivity (PROD) was measured in the central plants of each plot (4 m from 4 rows). Following manual collection, the material was threshed and the grains weighed to obtain the productivity per plot, with the moisture corrected to 13% and values expressed in kg ha^{-1}.

Statistical analysis

The normality of the data was assessed using the Shapiro-Wilk test. The results underwent analysis of variance by F-test ($P < 0.05$). The mean values for the effect of inoculation or non-inoculation with *A. brasilense* were compared using Student's t-test ($P < 0.05$). The residual effect of the phosphate fertiliser was evaluated by regression analysis, adopting the equation with the highest coefficient of determination (R^2) whenever the models were significant ($P < 0.05$). All the statistical analyses were carried out using the SISVAR® software (Ferreira, 2011), while the SIGMAPLOT v15.0 software (Systat Software Inc., 2023) was used to produce the graphs.

RESULTS AND DISCUSSION

Microbiological and enzymatic evaluations of the soil

The results for MR, MBC, and TOC showed significant mean values for inoculation with *A. brasilense* ($P \leq 0.05$) (Figure 3). The plant/microorganism interactions give rise to differing responses, and may contribute significantly to enzyme activity, improving the environmental conditions (Cassán; Díaz- Zorita, 2016; Di Salvo *et al.,* 2018; Silva *et al.,* 2024).

The higher MR rate with inoculation shows that the metabolism of the microorganisms was accelerated, favouring residue degradation and nutrient cycling. Bacterial inoculation increased the microbiological activity indices of the colony, which, in turn, started to release higher rates of CO_2. However, MBC and TOC also showed significant mean values ($P \leq 0.05$), proving that even with the increase in microbial metabolism, inoculation aided the processes of CO_2 fixation and nutrient cycling, given the greater incorporation of organic matter. The results indicate low values for the release of C-CO_2 regardless of whether the area was inoculated with *A. brasilense* or not (Table 2). The greater MBC in the inoculated areas resulted in a higher TOC content, suggesting a reduction in emissions.

The area being under a no-till system for several years is also one of the important factors contributing to the results. No significant differences were seen for qCO_2, which is a direct indicator of CO_2 use efficiency in the soil, showing that the years under a no-till system have resulted in a balanced soil, since high qCO_2 values typically represent areas that are more stressed (Santos *et al.,* 2018).

A large amount of mulch, irrigation and crop rotation can favour microbial biomass and allow for greater equilibrium of the soil/microorganism system. Mulch acts as a barrier to high temperatures and favours the maintenance of soil moisture, crucial for the microbial colony in tropical soils (Li; Li; Pan, 2020; Salomão *et al.,* 2020). The values in question (Figure 3) represent the historical data for the area where black oats, inoculated with *Azospirillum*, were grown during the 2019/20 winter crop season (Figure 2). Later, when the soybean were sown, they were inoculated with *Bradyrhizobium* and, since the area already had a history of inoculation and/or co-inoculation over the years, presented an increase in all the indices under study.

The residual effect of the P_2O_5 doses was significant for MR and qCO_2 ($P \leq 0.05$) (Table 2). The microbial colony showed higher MR rates as the P dose increased, with an optimum value of 290 kg ha^{-1} P_2O_5. For qCO_2, the values decreased with the increasing P doses, reaching an optimum value at 94 kg ha^{-1} P_2O_5. The 94 kg ha^{-1} dose of P_2O_5 was able to nourish the microbiota, which depend on P to carry out their vital functions, resulting in lower qCO_2 values and helping to balance the soil system.

Figure 3 - Soil microbial respiration (MR), microbial biomass carbon (MBC), metabolic quotient (qCO_2) and total organic carbon (TOC) in the 0.00–0.10 m layer following soybean cultivation, as a function of inoculation with *A. brasilense* (2020/21). Selvíria, Mato Grosso do Sul, Brazil. Control: control treatment (with no inoculation); Azos: *Azospirillum brasilense.* Mean values followed by the same letter do not differ statistically by Student's t-test ($P \leq 0.05$)

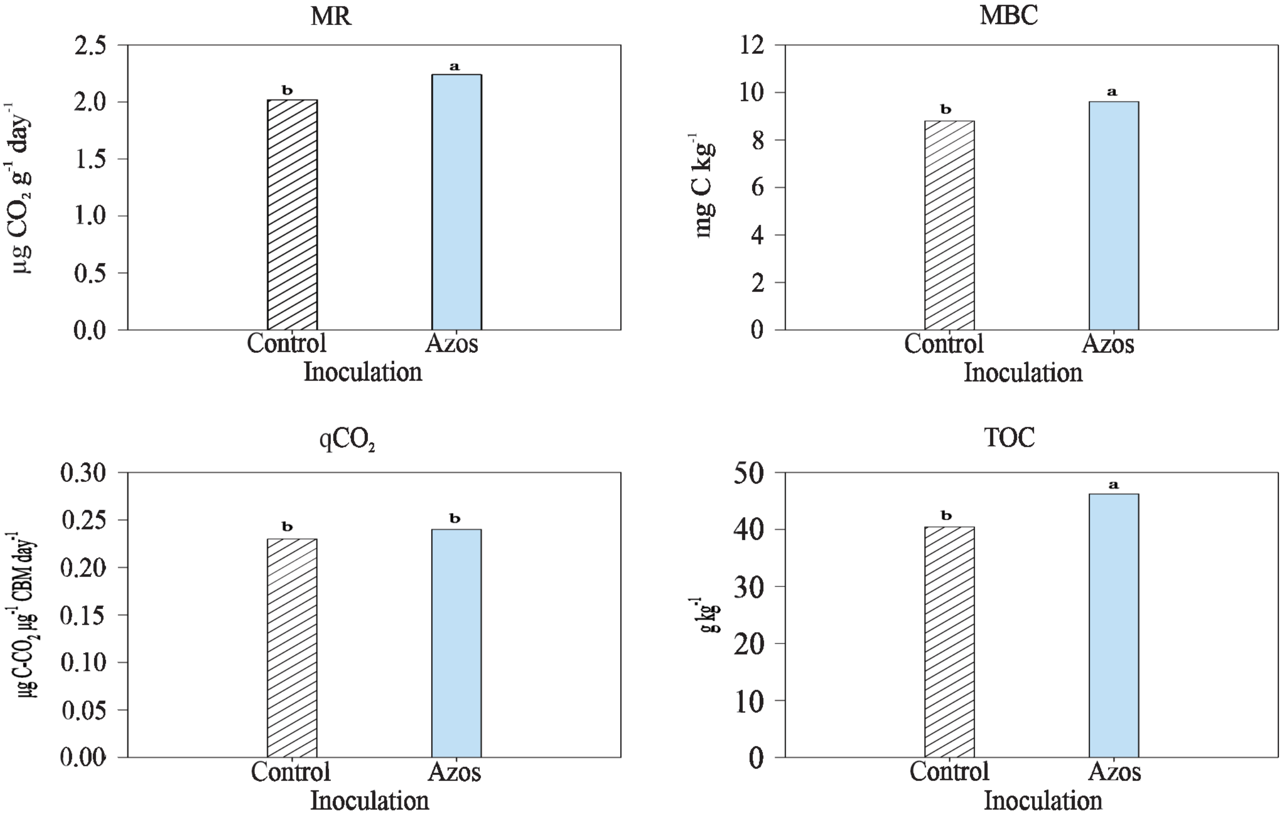


Table 2 - Soil microbial respiration (MR), microbial biomass carbon (MBC), metabolic quotient (qCO_2) and total organic carbon (TOC) in the 0.00–0.10 m layer following soybean cultivation, as a function of the residual effect of P_2O_5 doses (2020/21). Selvíria, Mato Grosso do Sul, Brazil

Residual doses of P_2O_5 (kg ha^{-1}) (D)	MR	MBC	qCO_2	TOC
0	2.42	9.04	0.26	40.92
30	2.23	8.63	0.25	39.50
60	2.20	8.87	0.24	43.38
120	1.90	10.13	0.23	44.59
240	1.89	9.42	0.20	43.77
Pr > Fc	0.25	0.017*	0.0002*	0.681
LSD	0.232	0.561	0.013	3.408
Equation model – E	Q	-	Q	-
R^2 (%)	96.68	-	99.17	-
F-test – E	0.153	-	0.172	-
Pr > F – I x D	0.130^{ns}	0.121^{ns}	0.074^{ns}	0.140^{ns}
CV (%)	16.86	9.38	5.73	12.13

*: significant at 5% probability by F-test; ^{ns}: not significant; LSD: least significant difference; CV: coefficient of variation; I: inoculation with *Azospirillum brasilense*; $MR = 1E\text{-}05x^2 + 0.0058x + 2.4251$ (P.Max. = 290 kg ha^{-1} P_2O_5); $qCO_2 = -0.0002x^2 + 0.0375x + 5.7085$ (P.Min. = 94 kg ha^{-1} P_2O_5)

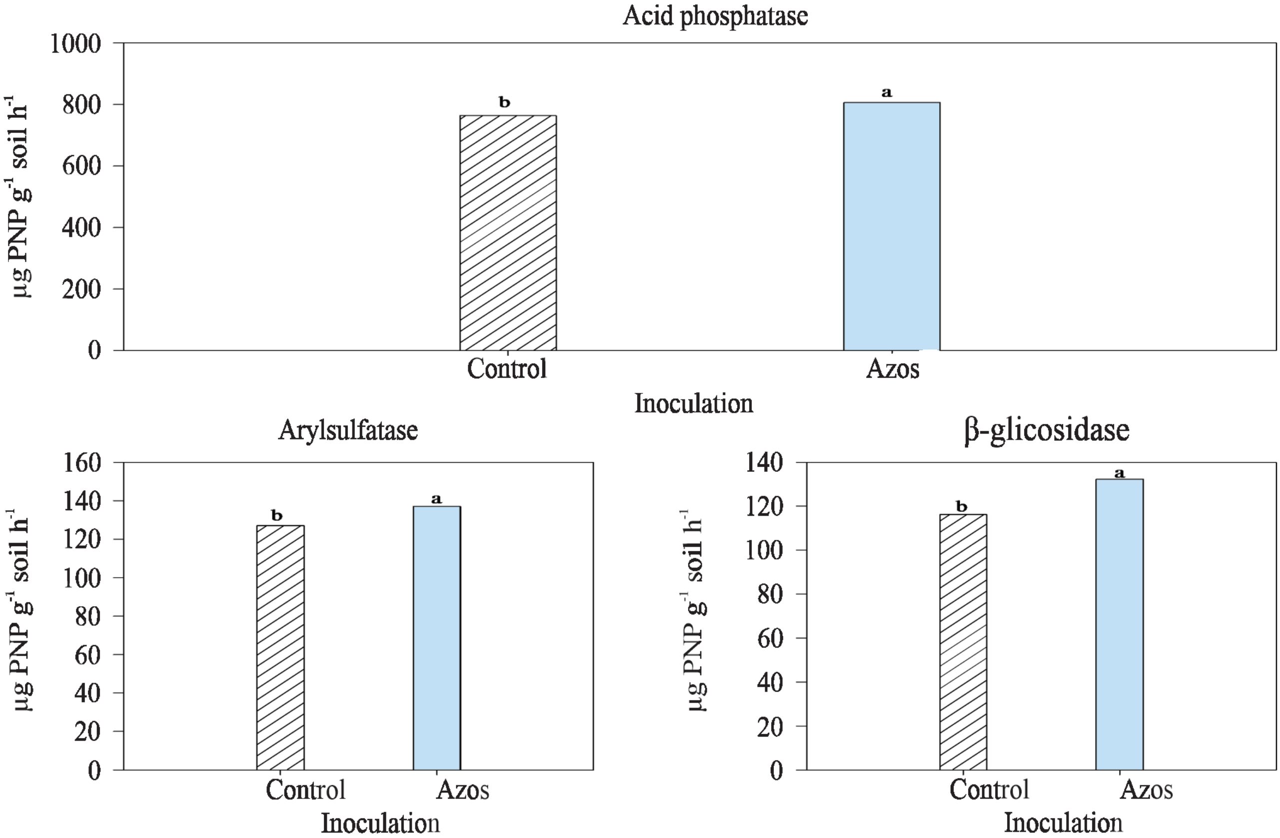
In terms of inoculating the rotated grasses with *A. brasilense,* there were significant differences between the enzymes β-glucosidase, acid phosphatase and arylsulfatase by t-test ($P \leq 0.05$), with higher enzyme values following inoculation (Figure 4). In the case of β-glucosidase, which is a direct bioindicator of cellulose degradation, soil organic matter (SOM) formation and microorganism activity, inoculation was found to increase enzyme activity, showing that inoculation is effective and able to maintain efficiency during the first/second crop cycle. The bacteria of genus *A. brasilense* are described as capable of producing phytohormones, which can stimulate the development of plant roots (Cássan; Vanderleyden; Spaepen, 2014; Spaepen *et al.,* 2014). Furthermore, the genus can act as a phosphate solubiliser, aiding P availability in tropical environments, as well as improving biological N fixation (Freitas *et al.,* 2019; Pereira *et al.,* 2020; Sun *et al.,* 2022).

Inoculating these bacteria into rotated crops can significantly increase the levels of MR, MBC and TOC, showing that periodic inoculation is essential for increasing the availability/activity of the microorganisms in the soil, thereby favouring both their presence and multiplication, especially in soils under long-term no-till systems that include the accumulation of plant material that tends to maintain the equilibrium between fungal hyphae and bacterial colonies in the soil due to maintaining the temperature and humid environment (Bettiol *et al.,* 2023; Köberl *et al*., 2020).

In tropical soils, thermal fluctuations, the occurrence of dry spells and highly weathered soils are factors that can have a negative effect on the presence and maintenance of organic matter and microorganisms in the soil. Such microorganisms are fundamental, and act as bioindicators of soil quality (Dubey *et al.,* 2019; Jansson; Hofmockel, 2020).

Agricultural management is essential for maintaining and/or increasing soil health. In this study, several years under a no-till system led to good results for RM, MBC and TOC, even with no inoculation. This suggests that soils managed using the appropriate agricultural practices can help mitigate the effects of CO_2 in the atmosphere. The low qCO_2 values indicate good system management during this period, leaving a balanced system that acts as a mitigator of greenhouse gases and maintains the quality and fertility of the soil, and a suitable environment for the microbial colony to develop (Alves *et al.,* 2011).

Figure 4 - Soil enzyme activity in the 0.00–0.10 m layer following soybean cultivation, as a function of residual phosphate fertilisation and inoculation with *A. brasilense* (2020/21). Selvíria, Mato Grosso do Sul, Brazil. Control: control treatment (with no inoculation); Azos: *Azospirillum brasilense.* Mean values followed by the same letter do not differ statistically by Student's t-test ($P \leq 0.05$)



The mean levels observed for arylsulfatase and acid phosphatase can be explained by the intrinsic characteristics of the biology of the microorganisms, since *A. brasilense* is able to help solubilise non-labile P in addition to producing phytohormones, helping N fixation and improving the water balance and exploitation of the soil by the roots (Hungria; Rondina; Nunes, 2021). This helps to increase the activity of these enzymes, since under a no-till system and the inoculation of rotation crops, there is an increase in beneficial microorganisms, which allows for better biosynthesis (Prando *et al.,* 2022).

In terms of the P_2O_5 doses, significant mean values ($P \leq 0.05$) were seen for the enzymes arylsulfatase and β-glucosidase (Table 3). For arylsulfatase, the optimal dose was 190 kg P_2O_5 ha^{-1}, affording better nutrition for the soil microbiota, which contributed to the increased enzyme activity. For β-glucosidase, the optimal dose was 125 kg ha^{-1}, since with the supply of P, microorganisms can focus their metabolism on the quality of cellulose degradation. However, it is important to note the reduction in acid phosphatase, which was due to greater P availability in the environment from the use of fertilisers and the reduced need for the mulch to degrade for the microbiota to obtain phosphorus.

The zero-dose presented high levels of both enzymes, showing that soil managed under a no-till system is healthy and metabolically balanced (Table 3). Acid phosphatase activity decreased with the increasing P doses, with the zero-dose showing the highest average. With the lower P availability, the microbiota work harder for their nutrition, solubilising P from organic matter and non-labile P. Once again, the proper management of the area under the no-till system is evident, where the use of bacteria on the rotation plants is sufficient to increase enzyme activity without the need for phosphate fertiliser, provided there is an adequate supply of good-quality mulch.

The enzyme β-glucosidase is used as a measure of soil quality as it is able to evaluate the final stage of cellulose degradation and the release of glucose for microbiological activity. The enzyme detects the degradation of organic matter, identifying the effects of the management and nutrients from the glucose, an essential source of carbon for microorganisms (Mendes; Sousa; Reis, 2021). Higher values of this enzyme therefore indicate more organic matter being metabolised by the microorganisms, which may lead to a considerable increase in arylsulfatase and acid phosphatase levels.

Table 3 - Soil enzyme activity in the 0.00–0.10 m layer following soybean cultivation, as a function of residual phosphate fertilisation and inoculation with *A. brasilense* (2020/21). Selvíria, Mato Grosso do Sul, Brazil

Residual doses of P_2O_5 (kg ha^{-1}) (D)	Acid Phosphatase	Arylsulfatase	β-glucosidase
0	803.55	131.26	115.66
30	788.74	107.77	116.68
60	793.37	161.99	123.75
120	768.38	116.71	137.94
240	772.32	142.64	127.60
Pr > Fc	0.154	0.0003*	0.0001*
DMS	20.13	14.39	5.50
Equation model - E	Q	-	Q
R^2 (%)	86.37	-	85.36
F-test – E	0.239	-	0.0003*
Pr > F – I x D	0.0105^{ns}	0.134^{ns}	0.0106^{ns}
CV (%)	3.95	16.87	6.86

LSD: least significant difference; CV: coefficient of variation; *: significant at 5% probability by F-test; ns: not significant; I: inoculation with *Azospirillum brasilense*; Arylsulfatase: $y = 0.001x^2 + 0.3811x + 803.82$ (P.M = 190 kg ha^{-1} P_2O_5); β-glucosidase: $y = -0.0012x^2 + 0.2992x + 112.19$ (P.Max = 125 kg ha^{-1} P_2O_5)

Inoculating the rotation grasses with *A. brasilense* increased enzyme activity. This is possible, as soil bacteria are free-living, meaning their efficiency can be affected by fluctuations in the system, such as temperature, a lack of water, the quality of the inoculated root tissue, the intrinsic genetic factors of the inoculated plant (which can affect bacterial multiplication), the presence of mulch in the area, the type of soil, and the local climate conditions (Domeignoz-Horta *et al.,* 2020; Silva-Sánchez; Soares; Rousk, 2019), conditions which differed from those in the study area, where there was a large amount of mulch on the ground, which may have favoured high humidity and milder temperatures.

The increased enzyme activity seen in the inoculated areas can be attributed to the increased number of individuals in the colony, and allowed the larger bacterial population to be metabolically active, acting specifically to improve the environmental conditions and solubilise P. Inoculating the black oat seeds increased dry matter production, improved soil fertility by adding high-quality mulch, and resulted in an increase in the production of the main crop (Correa Filho *et al.,* 2017; Ribeiro *et al.,* 2025). There are reports of a 15% increase in the levels of N, 26% in the levels of K and 10% in those of P, as well as increases in Ca and Mg, in addition to contributing to increased micronutrient levels in plants inoculated with *A. brasilense* (Brum *et al.,* 2021; Carmo *et al.,* 2023). P levels in inoculated plants increased, due to the ability of the bacteria to solubilise P and improve the activity of the soil microbiota, favouring the cycling of organic and non-labile P (Cassán; Vanderleyden; Spaepen, 2014; Spaepen *et al.,* 2014).

In general, microorganisms require phosphorous for their metabolism to carry out their vital functions; however, increasing the supply of P in the system by fertilisation can affect the metabolism of the microbiota, reducing the solubilisation of non-labile P and the degradation of mulch (Carmo *et al.,* 2023).

The doses of phosphate fertiliser contributed to an increase in arylsulfatase and β-glucosidase activity, with increases also seen in qCO_2 and MBC, albeit with a reduction in acid phosphatase activity (Table 3). The recommended doses for the phosphate fertiliser should therefore follow not only soil fertility parameters, but also include microbiological characteristics to balance the system and favour nutrient cycling and biodiversity in the soil. Areas under long-term no-till management tend to have a greater supply of P from the mineralisation of mulch, which can reach 60% of all the available P in the soil (Guilbeaut-Mayers; Turner; Laliberté, 2020). As the years go by and the no-till system consolidates, it tends to balance itself, with organic P playing an essential role, as the biological processes carried out by soil microorganisms tend to become key players in making P available to plants and to other soil organisms (Li; Hallama; Romanyà, 2024; Torres *et al.,* 2023).

Evaluation of the production and productivity components of the soybean

Plant height (PH) and plant population (POP) in the soybean during the 2019/20 crop season differed significantly by t-test ($P \leq 0.05$) for inoculation (Table 4). Without inoculation, PH was greater than in the treatment with inoculation, a fact explained by the isolated action of *Bradyrhizobium*, with no competition from *A. brasilense.* Inoculation with *A. brasilense* resulted in an 11% increase in POP compared to the treatment with no inoculation (Table 3). According to the regression test, there were significant differences in the mean values for the residual effect of the phosphate fertiliser from the first application of P_2O_5, carried out in 2013.

In the 2020/21 crop season, there was a linear adjustment of residual phosphate fertilisation for plant height (PH) (Table 4): as the P doses increased, so did growth, from 1.13 m with no phosphate fertiliser to 1.19 m with the dose of 240 kg ha^{-1}. In general, the POP was only affected during the first year of soybean cultivation (2019/20) (Table 4), when the stand in the inoculated area was higher. However, as the cultivar is of indeterminate growth, there were more pods (Table 5) and a greater number of grains in the uninoculated area, even with fewer plants.

Table 4 - Average plant height (PH), height of the first pod insertion (PIH) and plant population (POP) in the soybean, as a function of the inoculation of preceding crops with *Azospirillum brasilense* and residual phosphate fertilisation during the 2019/20 and 2020/21 crop seasons. Selvíria, Mato Grosso do Sul, Brazil

Treatment	PH		PIH		POP	
	cm				Plants ha^{-1}	
Inoculation – I	2019/20	2020/21	2019/20	2020/21	2019/20	2020/21
No inoculation	114 a	116	14	18	238.148 b	224.938
With inoculation	107 b	114	13	17	265.185 a	238.395
Pr > Fc	0.008*	0.220^{ns}	0.111^{ns}	0.389^{ns}	0.0001*	0.163^{ns}

Continuation Table 4

P_2O_5 dose (kg ha^{-1}) – D						
0	112	113(1)	14	18	250.926	228.703
30	115	114	15	17	245.833	220.062
60	109	117	13	18	260.185	247.839
120	109	115	12	18	244.444	231.173
240	109	119	15	17	256.944	230.556
Pr > Fc	0.538^{ns}	0.588^{ns}	0.127^{ns}	0.893^{ns}	0.441^{ns}	0.467^{ns}
LSD	4.94	4.94	1.55	1.36	12.721	19.239
Equation model – E	-	L	-	-	-	-
R^2 (%)	-	72.6	-	-	-	-
F-test – E	-	0.0001	-	-	-	-
Pr > F – I x D	0.290^{ns}	0.880^{ns}	0.133^{ns}	0.830^{ns}	0.099^{ns}	0.331^{ns}
CV (%)	6.9	6.6	17.3	11.9	7.8	12.9

Mean values followed by the same letter in a column do not differ by t-test at 5% probability. *: significant at 5% probability by F-test; ns: not significant; LSD: least significant difference; CV: coefficient of variation. (1) $y = 113.5 + 0.0206x$ ($R^2 = 72.6\%$)

The results for inoculation and the residual effect of the phosphate fertiliser in the rotation crops showed no significant average values for PH, PIH or POP (Table 4) in soybean sown in succession during the 2020/21 crop season. Soils in consolidated no-till systems tend to reach equilibrium over the years, where most nutrients are made available by the mineralisation of organic matter and later by nutrient cycling in the organic matter. In the present case, the effect of inoculation is more pronounced in these soils due to the metabolic influence of microorganisms on the soil dynamics (Hernani, 2021).

During the 2019/20 crop season, the number of pods per plant (NPP) and the number of grains per plant (NGP) differed significantly in response to the treatments ($P \leq 0.05$). In the treatment with no inoculation, NPP and NGP values were higher than in the treatment with inoculation. Once again, the fact the soil was under no-till management for a long period meant it was able to sustain soybean development. The lower values for the treatment with inoculation can also be explained by the heat waves that occurred from mid-November to January (Figure 1) and which are common in the study area, in addition to the period of drought. This high fluctuation in temperature may have impaired the efficiency of the inoculation. There was a quadratic fit between W100 and the P doses during the 2019/20 crop season, albeit with no increase in grain productivity (GP).

There were no significant differences in production parameters during the 2020/21 crop season in terms of inoculation (Table 5); however, there was an obvious increase in GP from one crop season to the other, once again explained by the temperature and rainfall indices (Figure 1) that were responsible for the increase in average value seen each year. In 2020/21, the temperatures from mid-November to January were milder and the rainfall was constant, which favoured grain production in the soybean. In relation to the residual effect of the phosphate fertiliser, there were no significant differences in either 2019/20, with the residual effect of the 2013 fertilisation, or in 2020/21, with the effect of the 2020 fertilisation (Table 5). Once again, the differences in average GP for each season can be attributed to fluctuations in temperature, drought and the soil being under no-till management for years, together with a good supply of mulch and a high level of nutrient cycling (Figures 1 and 2).

Regarding inoculation with *A. brasilense* and its effects on soybean production and productivity, the uninoculated areas showed significant average values for PH, NPP, and NGP. Under high-mulch conditions, organic matter (OM) is crucial for agricultural development in tropical soils since, due to temperature, rainfall and the characteristics of the clayey soils, plant material is quickly mineralised. In addition to helping replenish nutrients, the OM can retain high volumes of water, assisting crops during periods of water deficit and improving the density of the soil, which favours soil exploitation by the plant roots (Bettiol *et al.,* 2023; Hungria; Rondina; Nunes, 2021).

In terms of phosphorus application, increasing the dose significantly increased the productive parameters of brachiaria and maize, respectively (Cabral *et al.,* 2020; Oliveira *et al.,* 2019). There were positive responses in W100 for the soybean as a function of increasing phosphorus doses in soils of

Table 5 - Average number of pods per plant (NPP), number of grains per plant (NGP), 100-grain weight (W100), and grain productivity (GP) in the soybean, as a function of the inoculation of preceding crops with *Azospirillum brasilense* and residual phosphate fertilisation during the 2019/20 and 2020/21 crop seasons. Selvíria. Mato Grosso do Sul, Brazil

Treatment	NPP		NGP		W100		GP	
	-		-		g		kg ha^{-1}	
Inoculation – I	2019/20	2020/21	2019/20	2020/21	2019/20	2020/21	2019/20	2020/21
No inoculation	41 a	46	81 a	111	18.4	15.2	2965	3956
With inoculation	36 b	44	65 b	105	18.7	15.2	2483	3895
Pr > Fc	0.033*	0.552^{ns}	0.007*	0.421^{ns}	0.272^{ns}	0.919^{ns}	0.053^{ns}	0.567^{ns}
P_2O_5 dose (kg ha^{-1}) – D								
0	42	41	77	97	18.3(1)	15.6	2875	3909
30	35	49	66	118	18.3	15.2	2722	4015
60	39	45	81	102	18.6	15.0	2810	3893
120	38	48	74	119	18.4	15.3	2653	3833
240	39	44	67	103	19.0	15.0	2560	4008
Pr > Fc	0.511^{ns}	0.331^{ns}	0.381^{ns}	0.233^{ns}	0.560ns	0.720^{ns}	0.927^{ns}	0.745^{ns}
LSD	4.66	5.5	11.2	15.3	0.61	0.63	489	
Equation model – E	-	-	-	-	L	-	-	-
R^2 (%)	-	-	-	-	78.6	-	-	-
F-test – E	-	-	-	-	0.0001*	-	-	-
Pr > F – I x D	0.748^{ns}	0.946^{ns}	0.591^{ns}	0.933^{ns}	0.732^{ns}	0.599^{ns}	0.996^{ns}	0.098^{ns}
CV (%)	18.7	18.8	23.7	22.0	5.1	6.5	27.8	8.5

Mean values followed by the same letter in a column do not differ by t-test at 5% probability. *: significant at 5% probability by F-test; ns: not significant; LSD: least significant difference; CV: coefficient of variation. (1) $y = 113.5 + 0.0206x$ ($R^2 = 72.6\%$)

the Cerrado (Silva *et al.,* 2015); however, it should be noted that this does not include the 18-year history under no-till management evaluated in the present study. In areas of consolidated no-till management, organic matter is primarily responsible for soil fertility and, consequently, plant nutrition (Hernani, 2021). Soils with a long history of no-till management tend to be balanced in terms of fertility and microbiology, and heavy fertilisation often fails to achieve good results, potentially resulting in microbiological imbalance without any visible response in plant production to fertilisation (Li; Li; Pan, 2020). For soils in tropical climates, maintaining mulch on the ground and effective management of the no-till system are essential, as they allow for improvements in the condition of the soil, good yields and lower costs in relation to the use of mineral fertilisers.

CONCLUSIONS

1. The areas inoculated with *Azospirillum brasilense* showed increased microbial and enzyme activity in the soil, and played a fundamental role in nutrient cycling and carbon sequestration. In general, under the soil and climate conditions of this study, the no-till system, together with *A. brasilense* inoculation, has proved to be an advantageous management practice that offers improvements in microbial respiration (MR), microbial biomass carbon (MBC), total organic carbon (TOC), and the enzymes acid phosphatase, arylsulfatase and β-glucosidase. In consolidated no-till systems, the highest concentrations of P are supplied by the soil organic matter (OM), where mineral fertilisation with P_2O_5 can reduce acid phosphatase activity. Therefore, when opting for mineral fertilisation, the fertility of the soil, number of years under a no-till system and the soil microbiology should all be taken into account;
2. In terms of grain productivity, the history of long-term no-till management was found to have contributed to the high average productivity of the area, including the P_2O_5 doses and co-inoculation with *A. brasilense*. This demonstrates that the soybean responds well to soil management, especially to a no-till system. The use of plant growth-promoting bacteria (PGPB) is a viable option for production; however, in long-term no-till systems, there are no significant effects on the average

production components or on soybean productivity, as the soil tends to become balanced, favouring nutrient availability and nutrient dynamics in the system. It is therefore clear that over the years the no-till system of soil management, together with a high input of mulch, led to the zero-dose of phosphorus (control) producing the same amount of phosphorus as the highest dose, a result of the soil fertility in this system being in equilibrium over the years, which helps to reduce the use of mineral fertiliser.

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DATA AVAILABILITY STATEMENT

The research data is available in the repository (http://hdl.handle.net/11449/235708).

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