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MULTIFUNCTIONAL MICROORGANISMS AND PHOSPHORUS DOSAGES IN SOYBEAN-MAIZE AND SOYBEAN-RICE SUCCESSIONS UNDER NO-TILL SYSTEMS IN THE CERRADO

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Abstract
Multifunctional phosphate solubilizing microorganisms can contribute to reducing phosphorus doses without affecting the grain yield of crops. The aim of this work was to evaluate agricultural production systems involving soybean-maize and soybean-rice successions, inoculation of beneficial microorganisms and application of phosphorus doses with a view to sustainable intensification of agriculture and soil health and fertility in the Cerrados region. The experimental design was a randomized block design in a 2x4 factorial scheme with four replications. The treatments were composed of the combination of two phosphorus doses, 50% (45 kg ha^{-1} of P_2O_5) and 100% (90 kg ha^{-1} of P_2O_5) of the recommended dose with four uses of multifunctional microorganisms: 1. BRM 32111 (*Burkholderia* sp.), 2. BRM 32114 (*Serratia marcescens*), 3. co-inoculation (BRM 32111 + BRM 32114), and 4. control (no application of microorganisms). The microorganisms provided significant increases in the 100-grain weight and grain yield of soybeans, dry matter and nutrient accumulation of rice and maize, reduction of phytopathogenic fungus propagules, and increased accumulation of nutrients and activity of the enzymes Betaglicosidase and Arilsulfatase in the soil. Applying 50% phosphorus reduced the 100-grain weight and grain yield of soybean, dry matter and nutrient accumulation of rice, propagules of *Trichoderma* spp., and the nutrients in the soil. The soybean-maize succession showed higher levels of Arylsulfatase than the soybean-rice succession. The highest soybean yields were obtained by applying BRM 32114 with 50% and co-inoculation with 100% phosphorus.

Keywords: Bacteria. Co-inoculation. *Glycine max*. *Oryza sativa*, Solubilization, *Zea mays*.

1. Introduction

Brazil plays a key role in food production, especially in the Cerrado region, for its reference in the country's agribusiness scenario, corresponding to about 40% of the Brazilian agricultural production (Bolfe et al. 2020). Agricultural practices such as crop rotation and no-till systems are important management techniques farmers can use to help improve soil health and reduce soil degradation. However, many farmers in this region cultivate soybeans yearly in the summer (first crop season) and maize in the second crop (Hosono and Caruso 2016). Always using the same crops is not sustainable and can bring problems such as increased pests, diseases, and weeds. The upland rice, for example, could be an option in the second crop instead of maize and help break the cycles of pests, diseases, and weeds, contributing to the sustainability of agriculture in the Cerrado.

The use of multifunctional microorganisms in rice, maize, and soybean in the Cerrado has proven to be an excellent alternative to improve plant performance (Frasca et al. 2021). These microorganisms act directly or indirectly in promoting plant growth and provide increased grain yields of crops and reduced use of agricultural inputs and production costs (Nascente et al. 2017a; Sperandio et al. 2017). This management has been consolidated through co-inoculation, a technology characterized by adding two or more microorganisms to the target plant, aiming to maximize the various beneficial effects of their interactions (Chibeba et al. 2015).

Phosphorus (P), a plant nutrient, tends to bind to soil mineral colloids through electrostatic or covalent bonds, forming insoluble phosphates (Parra et al. 2022). Thus, soil P deficiency often limits plant growth, especially in strongly P-fixing soils, such as oxisol (Huang et al. 2021). There are soil microorganisms capable of solubilizing unavailable forms of P through the production of enzymes or organic acids (Jarosch et al. 2019; Bolo et al. 2021).

Despite the benefits of adopting different types of crop succession and the use of multifunctional microorganisms in agriculture, few studies have evaluated these two technologies. The aim of this work was to evaluate agricultural production systems involving soybean-maize and soybean-rice successions, inoculation of beneficial microorganisms and application of phosphorus doses with a view to sustainable intensification of agriculture and soil health and fertility in the Cerrados region.

2. Material and Methods

The no-till experiment was conducted at Embrapa Rice and Beans, in Santo Antônio de Goiás-GO (16º28’00”S, 49º17’00”W, and an altitude of 823 m) during the 2021/2022 crop season. The region has a climate, according to the Köppen classification, Tropical AW with an average temperature of 23.3 °C, whose average annual rainfall is 1428 mm. During the experiment, the climate data were monitored, and when necessary, pivot irrigation was used to meet the water demand of the crops. Before the installation of the experiment, the fertility of the soil, classified as Latossolo Vermelho-Escuro ácrico of clayey texture, was determined in the 0-0.20 m layers according to the methodology proposed by Teixeira et al. (2011). The results were: pH (water) 5,8; O.M. = 33,8 g kg^{-1}; P-Mehlich = 12,9 mg dm^{-3}; K =78,2 mg dm^{-3}; Ca^{2+} = 14,2 $mmol_c$ dm^{-3}; Mg^{2+} = 6,1 $mmol_c$ dm^{-3}; Al^{3+} = 1 $mmol_c$ dm^{-3}; H + Al = 27 $mmol_c$ dm^{-3}; Sum of bases = 22,3 $mmol_c$ dm^{-3}; Cu^{2+} = 1,4 mg dm^{-3}; Zn^{2+} = 4,3 mg dm^{-3}; Fe^{3+} = 21 mg dm^{-3}; Mn^{2+} = 13,7 mg dm^{-3}; and according to the methodology proposed by Mendes et al. (2017), Betaglicosidase = 19,6 mg g^{-1} h^{-1}; Arilssulfatase = 13,9 mg g^{-1} h^{-1}.

The randomized block design was used in a 2x4 factorial scheme with four replications. Thus, the treatments were composed of a combination of two phosphorus doses, 50% phosphorus (45 kg ha^{-1} of P_2O_5) and 100% (90 kg ha^{-1} of P_2O_5) of the recommended dose, with two multifunctional microorganisms and their co-inoculation (1. BRM 32111 (*Burkholderia* sp.), 2. BRM 32114 (*Serratia marcescens*), 3. co-inoculation (BRM 32111 + BRM 32114), and 4. control (without application of microorganisms). The size of the plots was 10 x 4.5 meters with a spacing of 0.45 meters between rows. A planting density of 16, 5 and 100 viable seeds per linear meter was used for soybeans, corn, and rice, respectively. For the evaluations, we used the six central rows of each crop, disregarding 0.90 meters on either side of the ends of the plots.

The soybean cultivar NS 6906 was sown in October 2021 and harvested in February 2022. Maize cultivar AG 8088 and upland rice cultivar BRS A 501 CL were sown in March 2022. In June, the chemical/physical management of the crops was carried out with the application of Diquat, a total-action herbicide, and the use of a knife roller. These crops were planted mechanically and cultivated according to the technical recommendations for each plant species. Because the area is also used to plant common beans in winter and the sowing period ends in the first half of July, it was not possible to harvest maize and rice grains, only the biomass. Sowing fertilization, except for phosphorus, of the crops was calculated based on soil analysis and crop requirements (Souza and Lobato 2004). All the crops received a top dressing of 90 kg ha^{-1} of KCl. For maize and rice, in addition to KCl, 100 kg ha^{-1} of urea was top-dressed.

Two isolates of rhizobacteria from the Embrapa Rice and Beans Microorganism Collection were selected and characterized as phosphate solubilizers: *Burkholderia* sp. (BRM 32111) and *Serratia marcescens* (BRM 32114). Bacterial suspensions were prepared with nutrient broth and water from cultures grown for 24 hours on a solid nutrient agar medium at 28 ºC, and the concentration was set in a spectrophotometer to A540 = 0.5 (10^8 CFU, Colony Forming Unit). The suspensions were applied directly in the furrow at the moment of soybean, maize, and upland rice sowing, with the help of a furrow sprayer (Micron®), at a dose of 300 mL ha^{-1}. In the co-inoculation, a dose of each isolate was applied, and in the control treatment, no microorganism was applied except *Bradyrhizobium japonicum* at soybean planting.

After physiological maturity, the soybeans were harvested mechanically in the useful area of each plot, and the grain yield was evaluated and adjusted to 13% humidity. Additionally, the yield components of each crop were evaluated, determined on 10 plants always within one of the three central rows, avoiding borders, determining the number of pods per plant, number of grains per pod, and 100-grain weight (adjusted for 13% humidity).

To evaluate rice and maize dry matter (DM), one square meter of the maize and rice plants were sampled at 100 days after sowing (DAS), with the maize plants at stage R5 and the rice plants at stage R4, according to the methodology used by Nascente et al. (2013). After determining the DM of maize and rice, samples were taken for analysis of the N, P, K, Ca, Mg, S, B, Cu, Zn, and Mn contents in the straw of the plants, according to the methodology described in Claessen (1997). The accumulation of nutrients was calculated by multiplying the contents of each nutrient by the DM of the rice and maize.

To assess the density of soil fungi, eight sub-samples of soil were taken from each experimental plot to form a composite sample, totaling four composite samples for each treatment. site sample. This collection was performed three weeks after the desiccation of the plants in the second crop, between the rows of common beans, at a depth of 0.00-0.10 m. The samples were taken to the laboratory, and the densities of the fungi *Fusarium solani*, *F. oxysporum*, *Trichoderma* spp., *Rhizoctonia solani* were estimated according to the methodology described in Komada (1975), Martin (1950), Napoleão et al. (2005), Nash and Snyder (1962), respectively.

Along with the removal of samples for the evaluation of fungal density, samples were also collected from each experimental plot to form deformed samples for macro and micronutrients, pH, and organic matter contents at a depth of 0.00-0.10 m, following the methodology proposed by Claessen (1997).

The evaluation of soil quality was carried out through the determination of two enzymes, β-glucosidase and arylsulfatase. Eight soil sub-samples were collected per plot to form the composite sample. This collection was performed together with the removal of samples to evaluate the fungal density. The β-glucosidase activity was estimated according to the method proposed by Tabatabai (1994) and the arylsulfatase activity by Tabatabai and Bremner (1970) recommendation. These enzymes serve as biological indicators of soil sustainability and quality, the higher the levels the better the biological and sustainable attributes of the soil. Based on the enzymatic results obtained, they were interpreted as low, moderate, or high according to the recommendation of Mendes et al. (2018).

Data were subjected to analysis of variance, and when the F test detected significance, the means were compared by the LSD test at $p<0.05$. The statistical package SISVAR 5.6 was used.

3. Results

The analysis of variance revealed that the use of microorganisms significantly affected the number of pods per plant (NPP) (Table 1). The soybean plants in the control treatment had a higher number of pods than the plants inoculated with BRM 32114. No differences were observed between treatments with P doses. Thus, applying only half of the recommended dose of P was sufficient to provide similar results in NPP. Regarding the number of grains per pod (NGP), no statistical differences were observed between treatments in the evaluation of microorganisms and doses of phosphorus. Regarding the 100-grain weight (100W), the co-inoculation and the isolate BRM 32111 provided statistically superior values, with increases of 5.42 and 3.61%, respectively, compared to the control treatment. There was also a statistical difference when comparing the doses of phosphorus, in which the application of the complete dose promoted a 2.38% increase in 100W compared to the application of only half the dose.

Table 1. Number of pods per plant (NPP), number of grains per pod (NGP), 100-grain weight (100W), and grain yield (YIELD) of soybean plants treated with multifunctional microorganisms *Serratia marcescens* (BRM 32114), *Burkholderia cepacea* (BRM 32111), Co-inoculation (BRM 32114 + BRM 32111) and Control (without microorganisms); and two phosphorus doses, 50% (45 kg ha^{-1} of P_2O_5) and 100% (90 kg ha^{-1} of P_2O_5) of the recommended dose.

Microorganism	NPP	NGP	100W	YIELD
	Unit	unit	g	kg ha^{-1}
BRM 32111	49.00^{ab}	2.07^{a}	17.21^{ab}	4188
BRM 32114	45.41^{b}	2.03^{a}	16.82^{bc}	4552
Co-inoculation	48.10^{ab}	2.06^{a}	17.52^{a}	4574
Control	52.20^{a}	1.98^{a}	16.61^{c}	4335
Samples per treatment	16	16	16	16
Average	48.72	2.04	17.04	4412
CV (%)	14.28	8.21	4.22	6.91
Phosphorus dose				
50%	49.5^{a}	2.01^{a}	16.81^{b}	4320
100%	47.9^{a}	2.11^{a}	17.22^{a}	4511
Samples per treatment	32	32	32	32
Average	48.7	2.06	17.02	4416
CV (%)	14.28	8.21	4.22	6.91

*Means followed by the same letter do not differ by the LSD test ($p < 0.05$).
** Significant interaction between factors.

There was an influence of the interaction between microorganisms and phosphorus doses on grain yield (Table 2). The highest grain yields were found with the application of BRM 32114 combined with 50% of the recommended dose of P and in the co-inoculation combined with 100% of the recommended dose of P.

The isolate BRM 32111 provided higher maize dry matter production than the other treatments, with an increase of 22.38% compared to the control treatment (Table 3). There was no significant difference when comparing the two phosphorus doses applied to the maize plant dry matter variable. The treatments with microorganisms and the dose of phosphorus did not influence the accumulation of N, P, Ca, and S in maize plants. The BRM 32111 provided a greater K accumulation in maize plants, statistically different from the control treatment (Table 3). There was no influence of the phosphorus doses on the K accumulation. The BRM 32111, being higher than the control treatment, with 48.13% higher accumulation, also affected the Mg accumulation in maize plants. Phosphorus doses did not influence the Mg accumulation.

The BRM 32114 and coinoculation promoted statistically higher dry matter of rice plants than the control treatment, with increments of 80.98% and 49.63%, respectively (Table 4). Regarding the phosphorus doses, the full dose promoted an increase of 36.61% compared to the 50% of the dose, being statistically superior (Table 4). In addition, it may be that the microorganisms were not efficient in providing the phosphorus needed for plant growth.

The treatment with BRM 32111 promoted a greater N accumulation in rice plants compared to the control treatment, with an increase of 46.59% (Table 4). In contrast, no effects were observed in the application of phosphorus doses. The microorganism BRM 32114 and the co-inoculation provided higher P accumulations in the upland rice plants, with 72.58% and 65.32% increases compared to the control treatment. The rice plants showed a higher P accumulation with the recommended dose, with an increase of 21.13%.

Table 2. Soybean grain yield according to the microorganism type, *Serratia marcescens* (BRM 32114), *Burkholderia cepacea* (BRM 32111), Co-inoculation (BRM 32114 + BRM 32111) and Control (without microorganisms); and two phosphorus doses, 50% (45 kg ha^{-1} of P_2O_5) and 100% (90 kg ha^{-1} of P_2O_5) of the recommended dose.

Microorganism	Grain Yield (kg ha^{-1})	
	------------ Phosphorus dose ------------	
	50%	100%
BRM 32111	3979^{cB}	4397^{bA}
BRM 32114	4647^{aA}	4458^{bA}
Co-inoculation	4459^{abA}	4717^{aA}
Control	4196^{bcB}	4474^{bA}
Samples per treatment	4	4
Average	4320	4512
CV (%)	6.21	

*Means followed by the same lowercase letter in the column and uppercase letter in the rows do not differ by the LSD test ($p < 0.05$).

Just as in maize, BRM 32111 promoted greater accumulations of K in rice plants, and together with co-inoculation, they presented respective increases of 66.09% and 61.27% compared to not applying microorganisms (Table 4). The full dose of phosphorus was the one that promoted the highest K accumulation, being 31.05% higher than the application of half the dose. The accumulation of Ca, Mg, and S was positively affected by applying the BRM 32114 and Co-inoculation, which promoted a greater accumulation of these nutrients in rice plants compared to the control treatment. The P dose influenced Ca and Mg accumulations in rice plants, with the full dose providing greater accumulation. The phosphorus dose did not affect the accumulation of S in rice plants.

Table 3. Dry matter production (DM) and accumulation of N, P, K, Ca, Mg, and S of maize plants grown in the second crop season according to the microorganism type, *Serratia marcescens* (BRM 32114), *Burkholderia cepacea* (BRM 32111), Co-inoculation (BRM 32114 + BRM 32111) and Control (without microorganisms); and two phosphorus doses, 50% (45 kg ha^{-1} of P_2O_5) and 100% (90 kg ha^{-1} of P_2O_5) of the recommended dose.

Factor	DM	N	P	K	Ca	Mg	S
		----------------------------- kg ha^{-1} -----------------------------					
Microorganism							
BRM 32111	11217^{a}	225^{a}	31.13^{a}	229^{a}	31.12^{a}	33.61^{a}	21.37^{a}
BRM 32114	9462^{b}	195^{a}	23.80^{a}	170^{b}	28.75^{a}	28.84^{ab}	19.36^{a}
Co-inoculation	9658^{b}	244^{a}	28.59^{a}	184^{ab}	23.00^{a}	25.96^{ab}	17.86^{a}
Control	9166^{b}	197^{a}	25.09^{a}	166^{b}	25.12^{a}	22.69^{b}	18.44^{a}
Samples per treatment	16	16	16	16	16	16	16
Average	9876	215	27.15	187	27.00	27.78	19.26
CV (%)	10.23	11.45	15.41	16.21	7.41	7.10	12.31
Phosphorus dose							
50%	9605^{a}	212^{a}	26.38^{a}	175^{a}	26.21^{a}	27.36^{a}	18.74^{a}
100%	10147^{a}	219^{a}	27.93^{a}	200^{a}	27.78^{a}	28.19^{a}	19.95^{a}
Samples per treatment	32	32	32	32	32	32	32
Average	9876	216	27.16	188	27.00	27.78	19.35
CV (%)	10.23	11.45	15.41	16.21	7.41	7.10	12.31

*Means followed by the same letter do not differ by the LSD test ($p < 0.05$).

Table 4. Dry matter (DM) production and accumulation of N, P, K, Ca, Mg, and S of rice plants grown in the second crop season according to the type of microorganism, *Serratia marcescens* (BRM 32114), *Burkholderia cepacea* (BRM 32111), Co-inoculation (BRM 32114 + BRM 32111) and Control (without microorganisms); and two phosphorus doses, 50% (45 kg ha^{-1} of P_2O_5) and 100% (90 kg ha^{-1} of P_2O_5) of the recommended dose.

Factor	DM	N	P	K	Ca	Mg	S
		---------------------------------- kg ha^{-1} ----------------------------------					
Microorganism							
BRM 32111	1374^{b}	88^{a}	2.60^{bc}	46.14^{a}	3.99^{b}	4.16^{ab}	4.27^{b}
BRM 32114	2465^{a}	47^{b}	4.28 ^{a}	29.06^{b}	7.20^{a}	6.20^{a}	8.12^{a}
Co-inoculation	2038^{a}	72^{ab}	4.10^{ab}	44.80^{a}	5.84^{ab}	6.02^{ab}	6.65^{ab}
Control	1362^{b}	47^{b}	2.48^{c}	27.78^{b}	4.47^{b}	3.98^{b}	4.83^{b}
Samples per treatment	16	16	16	16	16	16	16
Average	1810	64	3.37	36.95	5.38	5.09	5.97
CV (%)	27.33	13.45	17.23	11.37	15.98	14.78	14.32
Phosphorus dose							
50%	1590^{b}	56^{a}	2.85^{b}	31.98^{b}	4.66^{b}	4.43^{b}	5.30^{a}
100%	2031^{a}	71^{a}	3.88^{a}	41.91^{a}	6.09^{a}	5.74^{a}	6.63^{a}
Samples per treatment	32	32	32	32	32	32	32
Average	1811	64	3.37	36.95	5.38	5.09	5.97
CV (%)	27.33	13.45	17.23	11.37	15.98	14.78	14.32

*Means followed by the same letter do not differ by the LSD test ($p < 0.05$).

In the biological soil analysis of the soybean-maize and soybean-rice areas, the areas treated with BRM 32111 and BRM 32114 showed lower mean numbers of *Fuarium solani* propagules than the other treatments with microorganisms (Table 5). The control treatment showed 17.65% more propagules than isolate BRM 32111 and 23.76% than BRM 32114. Regarding *F. oxysporum*, isolate BRM 32111 provided the lowest average number of propagules, statistically different from the control treatment, which showed increases of 40.27%. The isolates BRM 32111 and BRM 32114 differed statistically from the control treatment in the average number of *M. phaseolina* propagules, which showed very high levels compared to the two rhizobacteria treatments.

The control treatment was the one that presented the highest amount of *Trichoderma* spp. propagules (Table 5). The addition of the microbial treatments may have reduced the level of these fungi by increasing competition in the rhizosphere; however, they are not below the amount normally found in tropical soils, since according to Bononi et al. (2020), the estimated amount is on average between 10 (10^1) and 1000 (10^3) viable propagules per gram of soil in soils from temperate and tropical regions. The microbial treatments did not affect *R. solani*. The phosphorus doses did not statistically interfere with the number of propagules of any of the fungi evaluated, except for *Trichoderma* spp., in which the application of the full dose presented 34.06% more propagules than the application of half the dose. The soybean-maize succession showed a lower mean number of *M. phaseolina* propagules than the soybean-rice succession, which may indicate greater antagonistic action to the maize crop fungus than upland rice. The areas with soybean-maize succession presented the greatest amount of *Trichoderma* spp. propagules, with an increase of 24.51% compared to the areas of soybean-maize succession. The succession factor did not influence any of the other fungi evaluated.

The co-inoculation was the treatment that provided the highest levels of Ca, Mg, and K in the soil but did not differ statistically from the control treatment (Table 6). The BRM 32114 treatment was the one that provided the highest level of P in the soil but did not differ statistically from the control treatment. Concerning the P dose, applying the full dose promoted higher levels of Ca, Mg, P, and K in the soil. The succession used did not influence the levels of Ca, Mg, and P in the soil but did influence the levels of K. The soil in the soybean-rice succession areas showed statistically higher levels of K, with increases of 18.13% in the nutrient compared to the soybean-maize succession areas.

Table 5. Soil biological analysis for detection of *F. solani*, *F. oxysporum*, *M. phaseolina*, *Trichoderma* spp., and *R. solani* from areas with soybean-maize and soybean-rice succession treated withmultifunctional microorganisms, *Serratia marcescens* (BRM 32114), *Burkholderia cepacea* (BRM 32111), Co-inoculation (BRM 32114 + BRM 32111) and Control (without microorganisms); and two phosphorus doses, 50% (45 kg ha^{-1} of P_2O_5) and 100% (90 kg ha^{-1} of P_2O_5) of the recommended dose.

Factor	*F. solani*	*F. oxysporum*	*M. phaseolina*	*Trichoderma* spp.	*R. solani*
Microorganism	propagule g^{-1} soil				%OMC
BRM 32111	6508^{b}	4449^{b}	101^{b}	2466^{b}	13.66^{a}
BRM 32114	6025^{b}	6584^{a}	127^{b}	2847^{b}	15.51^{a}
Co-inoculation	8695^{a}	5924^{ab}	661^{a}	2479^{b}	11.11^{a}
Control	7903^{a}	7449^{a}	583^{a}	5771^{a}	11.11^{a}
Samples per treatment	16	16	16	16	16
Average	7233	6102	368	3391	12.85
CV (%)	35.82	38.67	41.97	45.97	36.21
Phosphorus dose					
50%	7601^{a}	5987^{a}	318^{a}	4087^{a}	13.89^{a}
100%	6864^{a}	6216^{a}	369^{a}	2695^{b}	11.80^{a}
Samples per treatment	32	32	32	32	32
Average	7233	6102	344	3391	12.85
CV (%)	35.82	38.67	41.97	45.97	36.21
Succession					
Rice	7525^{a}	6470^{a}	432^{a}	2917^{b}	11.69^{a}
Maize	6940^{a}	5732^{a}	254^{b}	3864^{a}	14.00^{a}
Samples per treatment	32	32	32	32	32
Average	7233	6101	343	3391	12.85
CV (%)	35.82	38.67	41.97	45.97	36.21

*Means followed by the same letter do not differ by the LSD test ($p < 0.05$).

**%OMC = percentage of organic matter colonized.

The microorganisms applied influenced the activity of the enzyme Betaglicosidase in the soil, with the highest activity in the treatments with BRM 32111 and in the co-inoculation, which differed statistically from the control treatment (Table 6), showing increases of 8.27% and 12.38%, respectively. The activity of Betaglycosidase in these areas is more than double that observed in the soil analysis performed before the implementation of the experiment (19.6 mg g^{-1} h^{-1}), demonstrating how sustainable agricultural systems can improve soil health. Phosphorus doses and successions did not influence the Betaglycosidase activity in the soil. The co-inoculation showed the highest activity of Arylsulfatase, differing statistically from the control treatment, with a superiority of 31.74% in the enzyme activity. The phosphorus dose did not affect the Arylsulfatase activity in the soil. The soybean-maize succession areas presented 15.54% more Arylsulfatase activity than the soybean-rice succession areas. The average activity of arylsulfatase in these areas were considerably higher than those found in the soil analysis initially performed before the implementation of the experiment, which showed values of 13.9 mg g^{-1} h^{-1}.

4. Discussion

The use of beneficial microorganisms used alone or in co-inoculation provided significant increases in the 100-grain weight and yield of soybean, the dry matter and nutrient accumulation of rice and maize, reduction of phytopathogenic fungus propagules, Ca, Mg, P, and K levels, and activity of Betaglycosidase, and Arylsulfatase in the soil.

Similar NPP results were observed by Silva et al. (2020), who observed little NPP impact in untreated soybean plants compared to treatment with isolates BRM 32111 and BRM 32114 (Table 1).

Frasca et al. (2022) observed higher 100-grain weight using rhizobacteria co-inoculation than the no application of microorganisms. Adequate phosphorus supply is important for good soybean yields (França Neto et al. 2016). As phosphate fertilizers have low efficiency and only around 10% to 25% of the phosphorus applied to the soil is available to the plants (Baliah 2016), the application of only half the dose did not meet the need of the soybean plants in all treatments with microorganisms and negatively impacted 100W.

Table 6. Contents of Ca, Mg, P, and K and activity of Betaglicosidase (Betag) and Arilsulfatase (Arils) in the soil of areas with soybean-maize and soybean-rice succession treated with multifunctional microorganisms, *Serratia marcescens* (BRM 32114), *Burkholderia cepacea* (BRM 32111), Co-inoculation (BRM 32114 + BRM 32111) and Control (without microorganisms); and two phosphorus doses, 50% (45 kg ha^{-1} of P_2O_5) and 100% (90 kg ha^{-1} of P_2O_5) of the recommended dose.

Factor	Ca	Mg	P	K	Betag	Arils
	mmolc dm-3		mg dm^{-3}		mg g^{-1} h^{-1}	
Microorganism						
BRM 32111	21.09^{b}	8.82^{b}	14.26^{b}	69.13^{b}	41.38^{a}	15.97^{ab}
BRM 32114	21.17^{b}	8.94^{b}	21.21^{a}	70.19^{ab}	32.92^{b}	15.23^{b}
Co-inoculation	25.06^{a}	10.96^{a}	13.71^{b}	80.94^{a}	42.95^{a}	18.22^{a}
Control	22.85^{ab}	9.23^{ab}	17.56^{ab}	70.13^{ab}	38.22^{b}	13.83^{b}
Samples per treatment	16	16	16	16	16	16
Average	22.54	9.49	16.69	72.60	38.87	15.81
CV (%)	19.73	22.43	36.41	23.94	18.14	23.78
Phosphorus dose						
50%	21.18^{b}	8.75^{b}	15.14^{b}	68.19^{b}	38.85^{a}	15.69^{a}
100%	23.90^{a}	10.23^{a}	18.23^{a}	77.00^{a}	38.89^{a}	15.93^{a}
Samples per treatment	32	32	32	32	32	32
Average	22.54	9.49	16.69	72.60	38.87	15.81
CV (%)	19.73	22.43	36.41	23.94	18.14	23.78
Succession						
Rice	22.87^{a}	9.51^{a}	17.22^{a}	78.63^{a}	39.00^{a}	14.67^{b}
Maize	22.21^{a}	9.46^{a}	16.15^{a}	66.56^{b}	38.73^{a}	16.95^{a}
Samples per treatment	32	32	32	32	32	32
Average	22.54	9.49	16.69	72.60	38.87	15.81
CV (%)	19.73	22.43	36.41	23.94	18.14	23.78

*Means followed by the same letter do not differ by the LSD test ($p < 0.05$).
** Significant interaction between factors.

The fact that the microorganism BRM 32114 provided superior grain yield with the application of only half the dose may be directly linked to the plant-rizobacterium relationship (Table 2). Oliveira-Paiva et al. (2022), working with the commercial inoculant BiomaPhos® along with the application of 50% of the recommended phosphate fertilization, obtained superior results to the application and non-application of the product along with 100% of the recommended dose. The authors reported the efficiency of phosphorus solubilizing rhizobacteria in increasing soybean yield and that larger doses of phosphorus can reduce the activity of these microorganisms since they reduce the need for the plant-rhizobacteria association to obtain the nutrient. Reducing phosphate fertilization while maintaining high soybean productivity is a promising outcome for sustainable agricultural yield.

Kalayu (2019) reports that the genus *Burkholderia* sp., as the isolate BRM 32111, promotes increased yield and plant development in crops of agricultural interest through different mechanisms, particularly phosphorus solubilization (Table 3). No significant differences were found when compared to the two doses of phosphorus applied in the dry matter variable of the corn plant. In other words, half the P dose and the microorganisms were sufficient to provide the same biomass production as the full dose (100% P). The increase in K accumulation with the use of multifunctional microorganisms is explained according to Kour et al. (2020), due to the fact that in addition to P solubilization, there are also microorganisms capable of increasing the availability and absorption of K by plants.

BRM 32114 and coinoculation promoted significant increases in the dry matter of rice plants compared to the other treatments (Table 4). Upland rice shows promising results in the literature regarding its use combined with multifunctional microorganisms, with studies where significant increases in gas exchange, nutrient accumulation, and biomass production have been observed (Nascente et al. 2017a; Sperandio et al. 2017). Responses were also noted in relation to the applied phosphorus doses, with greater biomass yield with the application of 100% of the recommended dose. According to Fidelis et al. (2014), upland rice is a generally efficient and responsive crop regarding the use of phosphorus, a fact that can be observed with the effects of the doses applied in the experiment.

The greater absorption and accumulation of N, K and Mg in rice plants treated with BRM 32111 (Table 4) may have occurred because it is an isolate capable of generating greater root development, thus exploring a larger soil volume (Fernandes et al. 2020). The higher accumulation of P may be directly linked to the increased uptake by P solubilization performed by the bacteria of the genus *Serratia*, a growth promotion mechanism in which the higher accumulation of the nutrient in plants is reported in several studies (Awais et al. 2017; Nascente et al. 2017b; Frasca et al. 2023; Cruz et al. 2023). Rice plants showed greater P accumulation at 100% of the recommended dose. This result confirms the responsiveness of co-inoculated upland rice to phosphorus application in yield and the accumulation of nutrients in plant tissues, even when not using a reduced dose of phosphorus. Overall, the application of multifunctional microorganisms suggests that, through various methods of action, these organisms have impacted the ability of rice plants to absorb nutrients.

Multifunctional microorganisms have been effective in reducing propagules of pathogenic fungi. Banerjee et al. (2020) found the ability of *Serratia marcescens* isolates to antagonize *F. solani*. The result is in line with what was obtained in this work (Table 5). Several studies in the literature suggest the genus *Burkholderia* as a promising biological agent against *F. solani* (Elshafie et al. 2012, Heo et al. 2022) and *F. oxysporum* (Araújo et al. 2017; Xu et al. 2020; Ahmad et al. 2021). Zaman et al. (2021) observed the ability of isolates of the genus *Burkholderia* to perform effective biological control of *M. phaseolina* through the mechanism of antagonism. Like *Burkholderia* genus, *Serratia* genus is also known for its great potential in the biological control of phytopathogens (Kshetri et al. 2019; Abreo et al. 2021). The control treatment was the one with the highest amount of *Trichoderma* spp. propagules (Table 5), unlike the other fungi evaluated here, is considered a beneficial bioagent for plants (Silva et al. 2020; Cruz et al. 2023).

Based on the results, it can be inferred that using half of the phosphorus dose with the BRM 32114 and co-inoculation provided higher soybean yields and did not differ from using the full dose of P. With this, the cost of crop production can be reduced by reducing nutrient use. For biomass production of maize, it was also observed that half the dose of P with the microorganisms was sufficient to obtain similar production as using the full dose, with isolate BRM 32111 standing out the most. In rice, the bacteria could not provide all the P that the crop needed, so the treatments with the full dose of P provided better results.

The application of these sustainable systems has significantly improved soil health indicators of beta-glucosities and arylsulfatase (Table 6) but were still interpreted as low for both enzymes according to the recommendation of Mendes et al. (2018). It can also be noted that for both Betaglycosidase and Arylsulfatase, the best systems included using multifunctional microorganisms regardless of the phosphorus dose, rectifying the importance of using bioagents in sustainable systems. The enzymes Betaglicosidase and Arylsulfatase are BioAS indicators that allow monitoring of the health of the soil through the functioning of the biological machinery of the soil (Mendes et al. 2022). According to Mendes et al. (2019), they are sensitive indicators that demonstrate changes in soil health, depending on the management system, better management the greater the presence of these enzymes in the soil, reflecting on the yield of crops and economic aspect.

5. Conclusions

The use of beneficial microorganisms used alone or in co-inoculation provided significant increases in the 100-grain weight and yield of soybean, the dry matter and nutrient accumulation of rice and maize, reduction of phytopathogenic fungus propagules, Ca, Mg, P, and K levels, and activity of Betaglycosidase, and Arylsulfatase in the soil. The application of only half the dose of phosphorus promoted reductions in the 100-grain weight and yield of soybean, dry matter and accumulation of P, K, Ca, and Mg of rice plants, number of propagules of *Trichoderma* spp., and the contents of Ca, Mg, P, and K in the soil. The soybean-maize succession had higher activity of Arylsulfatase in the soil than the soybean-rice succession, in addition to a reduction in *M. phaseolina* propagules and an increase in *Trichoderma*.

In the interaction between microorganisms and P doses, the highest soybean yields were observed with the application of BRM 32114 with half of the recommended dose and the co-inoculation with the application of the full dose. For maize plants, the application of isolate BRM 32111 showed higher biomass production and nutrient accumulation. For rice plants, isolate BRM 32114 and co-inoculation had a positive impact on biomass and nutrient accumulation. The use of rhizobacteria was beneficial for maize and rice crops.

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