



# Crop rotation, cover crop mix, and multifunctional rhizobacteria for the sustainable development of soybeans

Mariana Aguiar Silva<sup>1</sup>, Dennis Ricardo Cabral Cruz<sup>2\*</sup>, Laylla Luanna de Mello Frasca<sup>3</sup>, Amanda Lopes Ferreira<sup>3</sup>, Cássia Cristina Rezende<sup>3</sup>, Anna Cristina Lanna<sup>3</sup>, Marta Cristina Corsi de Filippi<sup>3</sup> and Adriano Stephan Nascente<sup>3</sup>

<sup>1</sup>Faculdade de Agronomia, Universidade Federal de Goiás, Av. Esperança s/n, Campus Samambaia, 74690-900, Goiânia, Goiás, Brazil. <sup>2</sup>Universidade Estadual de Goiás, Unidade Universitária Posse, Posse, Goiás, Brazil. <sup>3</sup>Empresa Brasileira de Pesquisa Agropecuária, Embrapa Arroz e Feijão, Santo Antônio de Goiás, Goiás, Brazil. \*Author for correspondence. E-mail: denisribral@gmail.com

**ABSTRACT.** In the Cerrado, soybean monoculture (*Glycine max*) and second-crop corn succession reduce soil quality and compromise long-term productivity. The application of conservation practices such as crop rotation, cover crops, and multifunctional rhizobacteria is essential for sustainable soybean cultivation in the region. This study aimed to evaluate how cover crop mixtures cultivated during the second crop season, in rotation with upland rice, and the co-inoculation of soybeans with multifunctional rhizobacteria affect soil quality (physical, chemical, and biological), as well as soybean nutrient uptake, biomass production, yield components, and final yield. The experiment was conducted in a randomized block design, arranged in a  $7 \times 2 = 14$  factorial scheme with four replications. The treatments included five cover crop mixtures and two fallow systems: fallow 1 – rice/fallow/soybean (rotation between upland rice and soybean, without cover crops during the off-season) and fallow 2 – soybean/fallow/soybean (no rotation with upland rice and no cover crops in the off-season). All treatments with cover crop mixtures were also conducted under a rotational system, with soybean cultivated after upland rice in the summer season. Soybean seeds were either co-inoculated or not with *Bacillus* sp. (BRM 63573) and *Serratia marcescens* (BRM 32114). Rotation with upland rice and cover crop mixes provided higher P and Zn content in the soil, higher activity of the  $\beta$ -Glycosidase enzyme, lower density, higher total porosity, lower incidence of phytopathogenic fungi (*Fusarium solani* and *Rhizoctonia solani*), greater biomass production, greater macro and micronutrient content in the aerial part, greater number of pods per plant and grain yield of the soybean crop concerning the areas that remained fallow. Fallow 2 (soybean/fallow/soybean) provided lower results for most of the analyzed variables (soil attributes, nutrient uptake, biomass production, grain yield and soybean yield components). Co-inoculation provided higher soil microporosity, lower density of the phytopathogenic fungus (*Fusarium oxysporum*) in the soil, greater biomass production and nutrient accumulation in the shoot of soybean plants, compared to treatment without co-inoculation.

**Keywords:** *Glycine max*; no-tillage system; coinoculation; *Bacillus* spp.; *Serratia marcescens*.

Received on February 1, 2025.

Accepted on July 23, 2025.

## Introduction

Soybean (*Glycine max* (L.) Merrill) stands out economically as one of the world's main crops, being the main oilseed cultivated in the world, and its consumption has several benefits (Zhang et al., 2016). It is the main crop of Brazilian agribusiness, and the most cultivated in Brazil in the last three decades. Currently, Brazil is the world's largest producer and exporter of soybeans, with an estimated production of 167.4 million tons and exports worth 104.7 million tons in the 2024/2025 crop season (Companhia Nacional de Abastecimento. [CONAB], 2025).

Soybean is also the main crop in the Cerrado region, with predominant cultivation in a no-tillage system (NTS), whose pillars are crop rotation, minimal soil disturbance, and keeping the soil covered. However, the erroneous practice of NTS in the region has limited its sustainability because in most cases, the adoption of monocropping and/or succession of soybeans and second-crop corn has been observed (Hosono & Caruso, 2016). These management practices tend to cause physical, chemical, and biological soil degradation, in addition to increasing the incidence and severity of pests, diseases, and weeds in production systems, thereby affecting their long term sustainability (Bertollo & Levien, 2019).

Thus, the challenge of producing soybeans in a sustainable way arises. In this context, the soil must be managed with agricultural practices that maintain its capacity to absorb and make water and nutrients available to plants, as well as improve biological activity and prevent soil compaction (Plaza-Bonilla et al., 2016). Agricultural practices that provide adequate chemical, physical, and biological soil conditions are essential to achieve satisfactory yields in agricultural systems (Veloso et al., 2022).

Among soil conservation practices, the adoption of crop rotation and cover crops within cropping systems has been widely disseminated as an alternative to improve soil management systems and sustainability (Veloso et al., 2022). Upland rice is a particularly suitable option for soybean rotation systems, as it is a grass species characterized by high biomass production and does not serve as a host for most microorganisms and insects capable of harming soybean plants (Cordeiro Junior et al., 2017). The utilization of cover crops improved soil quality, enabled better use of water and environmental resources, reduced damage caused by insect pests, lowered incidence of pathogens, greater weed control, reduced compaction, increased biological activity and yield of crops (Nascente et al., 2013; Silva et al., 2022). Anschau et al. (2018) reported that soybean cultivated in succession to cover crop straw (*U. ruziziensis*) yielded 33.4% more compared to single maize succession.

In recent years, another conservationist agricultural practice that has gained greater recognition is the use of multifunctional rhizobacteria as alternative inputs, aiming to obtain crop yield gains in addition to being a practice that is ecofriendly (Wahab et al., 2025). Multifunctional rhizobacteria provide several benefits to plants through different mechanisms of action, such as biological nitrogen fixation, phosphate solubilization, phytohormone production, and biological control, among others (Rezende et al., 2021). The benefits include the higher germination rate of seeds, greater root development, higher concentration of nutrients in plants, increase in phytomass accumulation and plant height, reduction in the use of fertilizers, and higher grain yield (Ratz, 2017).

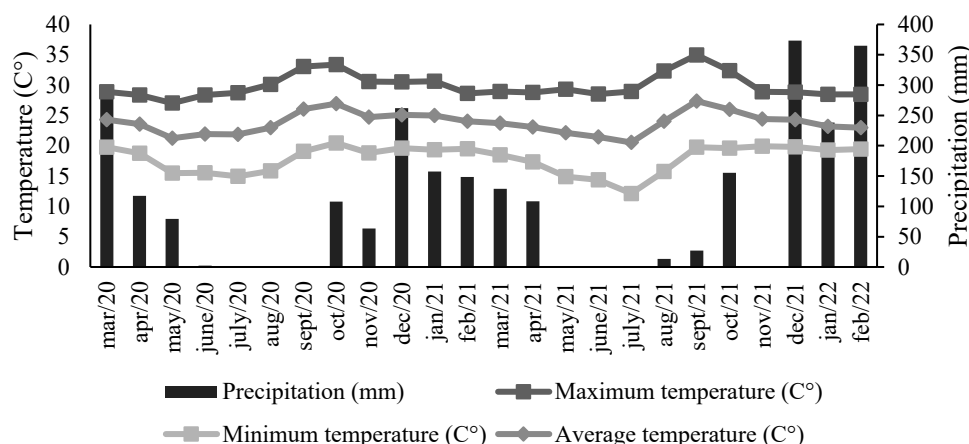
By combining different species, the benefits provided by cover crops and multifunctional rhizobacteria can be even greater, this practice is called the mix of cover crops (Ziech et al., 2015) and co-inoculation (Dalolio et al., 2018), respectively. Araújo et al. (2021) found higher grain yield of upland rice in an area previously cultivated with a mix of cover crops (*Pennisetum glaucum* + *Crotalaria juncea* + *C. spectabilis* + *C. ochroleuca*), using a fallow area as control. In a study with soybeans, a greater accumulation of shoot dry biomass was confirmed when the plants were co-inoculated with the isolates BRM 63573 (*Bacillus* sp.) and BRM 32114 (*Serratia marcescens*), compared to their isolated use and the control treatment (without microorganism) (Silva, 2020b).

This study is among the first to evaluate under field conditions in the Cerrado, the combined effects of cover crop mixtures, upland rice rotation, and co-inoculation with multifunctional rhizobacteria on soybean performance and soil quality. This integrated approach offers a novel perspective on reducing external inputs while promoting the sustainability of production systems. Despite growing interest in these practices, consistent information on their combined use and contributions to sustainable soybean production remains limited. Therefore, this study aimed to evaluate their impact on soil quality, nutrient accumulation, biomass production, yield components, and overall soybean yield.

## Material and methods

This study was conducted in a no-tillage area under rainfed conditions at Capivara Farm of Embrapa Rice and Beans, in Santo Antônio de Goiás, Goiás State, Brazil (16°28'00" S, 49°17'00" W, and altitude of 823 m) in the 2020/2021 and 2021/2022 crop seasons, after growing upland rice (summer) and mixes of cover crops (second-crop). In the 2019/2020 crop season, the area where the soybean in the present experiment was sown was cultivated with upland rice in the summer and cover crops in the second crop season. During the experiment, the minimum, average, and maximum temperature and precipitation were monitored (Figure 1).

According to the Brazilian Soil Classification System, the predominant soil in the region is the Red Acrisolic Latosol (Santos et al., 2018) which corresponds to Typic Acrustox in the USDA Soil Taxonomy. Before installing the experiments, soil chemical analyses were conducted according to the methodology described by Donagemma et al. (2011) and the results were: pH (H<sub>2</sub>O) = 6; Ca<sup>2+</sup> = 29.44 mmol<sub>c</sub> dm<sup>-3</sup>; Mg<sup>2+</sup> = 12.65 mmol<sub>c</sub> dm<sup>-3</sup>; H + Al<sup>3+</sup> = 16.01 mmol<sub>c</sub> dm<sup>-3</sup>; P = 14.89 mg dm<sup>-3</sup>; K<sup>+</sup> = 116.23 mg dm<sup>-3</sup>; Cu<sup>2+</sup> = 1.22 mg dm<sup>-3</sup>; Zn<sup>2+</sup> = 4.77 mg dm<sup>-3</sup>; Fe<sup>3+</sup> = 15.97 mg dm<sup>-3</sup>; Mn<sup>2+</sup> = 27.87 mg dm<sup>-3</sup>, and organic matter = 31.89 g kg<sup>-1</sup>, sand g kg<sup>-1</sup> = 342; silt g kg<sup>-1</sup> = 164, and clay g kg<sup>-1</sup> = 494, with a clayey texture. These baseline analyses were used to characterize the initial soil condition prior to soybean planting and to monitor changes resulting from the different treatments in subsequent crop seasons.



**Figure 1.** Temperature (°C) and precipitation (mm) during the experiment from March 2020 to February 2022. Santo Antônio de Goiás, Goiás State, Brazil.

The experiment was conducted in a randomized block design, arranged in a  $7 \times 2$  factorial scheme with four replications. Two factors were evaluated:

Factor 1 – Type of soil cover (7 levels): five cover crop mixtures (Mix 1, Mix 2, Mix 3, Mix 4, and Mix 5) and two fallow treatments (Fallow 1 and Fallow 2) were used as controls, as described in Table 1.

Factor 2 – Co-inoculation with multifunctional rhizobacteria (2 levels): with and without co-inoculation (control).

**Table 1.** Description of treatments.

Treatments	Rhizobacteria	Soil Cover System	Rotation type
1	NMR	Fallow 1	Upland rice/fallow/soybean
2	NMR	Fallow 2	Soybean/fallow/soybean
3	NMR	Mix 1	-
4	NMR	Mix 2	-
5	NMR	Mix 3	-
6	NMR	Mix 4	-
7	NMR	Mix 5	-
8	WMR	Fallow 1	Upland rice/fallow/soybean
9	WMR	Fallow 2	Soybean/fallow/soybean
10	WMR	Mix 1	-
11	WMR	Mix 2	-
12	WMR	Mix 3	-
13	WMR	Mix 4	-
14	WMR	Mix 5	-

NMR: No multifunctional rhizobacteria; WMR: With multifunctional rhizobacteria; Mix 1: (Ultra Mix) White lupine, Buckwheat, White oat, Black oat, *Crotalaria ochroleuca*, *Crotalaria juncea*, Fodder radish, and Coracana grass; Mix 2: (Mix Vitale) Buckwheat, *Crotalaria spectabilis*, Fodder radish, Black oat; Mix 3: (Forage mix) Millet, *C. ochroleuca*, Black oat, White oat, Buckwheat, and Coracana grass; Mix 4: (Mix Reduct) *C. spectabilis*, Buckwheat, Millet, and *Crotalaria breviflora*; Mix 5: (Mix Mega) Black oat, Buckwheat, Millet, Piatã grass and *C. ochroleuca*; Fallow 1: no mix of cover crops, with rice/soybean rotation (upland rice/fallow/soybean); Fallow 2: no mix of cover crops, no rice/soybean rotation (soybean/fallow/soybean).

All treatments with cover crop mixtures were performed in a crop rotation system in which upland rice was cultivated during the summer season, followed by cover crop mixtures in the second crop season, and then soybean. Fallow 1 also involved crop rotation with upland rice, but without cover crops during the off-season. Conversely, Fallow 2 was conducted without rotation (soybean/fallow/soybean), serving as a negative control.

To assess their effects on production system sustainability, cover crop mixtures were selected based on their distinct morphological and functional characteristics, as well as their agronomic benefits. The fallow control treatments were included to compare the effects of the absence of soil cover and crop rotation on soil management. The rhizobacteria used for co-inoculation were selected based on greenhouse experiments conducted by Silva et al. (2020a and b), which showed increased biomass production and soybean yield.

Each plot measured 6 m  $\times$  10 m, and the useful area consisted of three central rows, excluding 0.5 m at each end. Evaluations were carried out during the second crop seasons of 2020 and 2021, after the cultivation of upland rice, cultivar BRS 501 CL (summer season). Soybean seeds were co-inoculated or not with multifunctional rhizobacteria, based on the second factor levels.

These mixes were sown in the second crop on March 9, 2020, and April 2021, after the upland rice harvest in the same area before the main crop (soybean). A spacing of 0.45 m between rows was used with 10 kg ha<sup>-1</sup> of seeds from each mix of cover crops. Sowing was carried out with a no-till seed drill at a depth of 5 cm and without the use of fertilizers. The cover crops were not irrigated and developed from March 15 to October 16 in 2020 and from March 15 to October 20 in 2021. Due to low rainfall in the 2021/2022 crop season, plant establishment and development were impaired (Figure 1). The area was desiccated 20 days before sowing the soybean crop with 4 L ha<sup>-1</sup> glyphosate (Roundup Original®, 1440 g of acid equivalent per ha<sup>-1</sup>). Fifteen days after desiccation, the cover crops were crushed with a straw shredder (Triton®), and straws were left in the area.

The soybean cultivar NS 6906 IPRO was selected for its early maturity, broad adaptation to Cerrado conditions, high yield potential, and resistance to major pests and diseases such as *Helicoverpa armigera*, which is a critical factor for the region. The crop was sown mechanically, using a no-till seed drill at a spacing of 0.45 m with 20 viable seeds per meter, on November 5, 2020, and November 10, 2021, 20 days after desiccation of cover crops. The fertilization was calculated based on soil analysis and crop needs (Souza & Lobato, 2004). Therefore, the amount of fertilizer applied at sowing was 90 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> (triple superphosphate) and 48 kg ha<sup>-1</sup> of K<sub>2</sub>O (potassium chloride). Prior to sowing, all seeds were inoculated with liquid inoculant (*Bradyrhizobium japonicum*), following the manufacturer's recommendation to supply nitrogen to the soybean crop. According to standard soybean crop recommendations, cultural practices were carried out to keep the area free of weeds, diseases, and insects.

The isolates were chosen based on previous studies which utilized multifunctional microorganisms in soybean cultivation in a greenhouse (Silva et al., 2020a). The combination of multifunctional rhizobacteria isolates BRM 63573 (*Bacillus* sp.) and BRM 32114 (*Serratia marcescens*) was used for soybean co-inoculation. These rhizobacteria are stored and preserved in the collection of Multifunctional Microorganisms at Embrapa Rice and Beans. Bacterial suspensions were prepared with nutrient broth and water from cultures grown for 24 hours in a solid nutrient agar medium at 28°C, and the concentration was fixed in a spectrophotometer at A<sub>540</sub> = 0.5 (10<sup>8</sup> CFU, Colony Formation Unit) (Filippi et al., 2011). Prior to soybean sowing, the suspension was applied to the sowing furrow (600 mL ha<sup>-1</sup>, with 300 mL of each microorganism mixed at the time of sowing) with the aid of a furrow sprayer (Micron®), as recommended by the Microbiology laboratory at Embrapa Rice and Beans.

For chemical analyses, deformed soil samples were randomly collected from each plot in the 0 - 10 cm layer. Thus, eight soil subsamples were collected per plot to form a composite sample shortly after the desiccation of cover crops on November 9, 2021, in the area where soybeans were sown. Composite samples were taken to the Agro-environmental analysis laboratory at Embrapa Rice and Beans, dried in air-forced circulation ovens, and passed through sieves (2 mm mesh). Soil chemical characteristics (pH, OM, P, H + Al, Al, K, Ca, Mg, Cu, Fe, Zn, and Mn) were determined according to the methodology of Donagemma et al. (2011). Soil pH was determined in a 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> suspension (1:2.5 soil/solution). Exchangeable Ca and Mg were extracted with 1 mol L<sup>-1</sup> neutral KCl in a 1:10 soil/solution ratio and determined by atomic absorption spectroscopy. H + Al was extracted using 0.5 mol L<sup>-1</sup> Ca acetate and determined by titrimetry. Phosphorus and exchangeable K were extracted with a Mehlich 1 extraction solution (HCl 0.05 mol L<sup>-1</sup> in H<sub>2</sub>SO<sub>4</sub> 0.0125 mol L<sup>-1</sup>) and determined by atomic emission spectroscopy. Cu, Zn, Fe, and Mn were extracted using Melich 1 solution and determined by atomic absorption spectroscopy. Soil organic matter (OM) was determined by oxidation with sulfochromic solution, followed by determination with spectrophotometry.

Undeformed soil samples were collected with cylinders measuring 0.05 m in diameter and 0.05 m in height, in all treatments on the same day of collecting deformed samples in the 0 - 10 cm layer, with eight replications. Soil bulk density (Sd) and total porosity (TP) were determined. TP was calculated using the equation: TP = (1 - Sd/Pd), where Pd is particle density (Donagemma et al., 2011). Soil quality was assessed by evaluating two enzymes: β-glucosidase and arylsulfatase. The same soil samples collected at 0-10 cm depth were used for chemical analysis. The β-glucosidase enzyme activity was estimated according to the method proposed by Tabatabai (1994), using the colorimetric determination of p-nitrophenol, after incubation of the soil with a specific substrate (p-nitrophenol-β-D-glucopyranoside). The arylsulfatase activity (Tabatabai & Bremner, 1970) was evaluated by hydrolyzing potassium p-nitrophenyl sulfate, enzyme substrate, to p-nitrophenol (PNP), followed by soil sample incubation for one hour at 37°C. The results obtained from the enzymes were interpreted as low, moderate, or high levels, as recommended by Mendes et al. (2018). To evaluate the density of soil fungi, a composite sample was formed by collecting eight soil subsamples per plot. This collection was also performed on the day of sample collection for chemical and physical analyses. The samples were taken

to the Agricultural Microbiology laboratory at Embrapa Rice and Beans and the densities of phytopathogenic fungi were determined; *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, and the beneficial fungus *Trichoderma* spp. was estimated based on the methodology described in (Komada, 1975; Martin, 1950; Nash & Snyder, 1962; Napoleão et al., 2005), respectively.

When the soybean plants reached the reproductive phase (R7 stage - beginning of maturation) in the 2021/2022 crop season, the shoot of the plants was cut in one meter of each plot, then wrapped in duly identified envelopes. The material was dried in an air-forced circulation oven at 65°C, until constant mass. Thereafter, the material of each envelope was weighed to determine the dry biomass of shoots in each plot. After drying and weighing, the shoot of plants in each treatment was ground in a Wiley-type mill, and used for the analysis and determination of macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (B, Cu, Fe, Mn, Zn, and Mo), following the methodology of Donagemma et al. (2011).

In the usable area of each plot, a self-propelled harvester was used for soybean harvest at the R8 stage on February 24, 2021 (first crop season) and February 25, 2022 (second crop season). Soybeans were weighed, and yield was adjusted to a moisture content of 13% and converted into kg ha<sup>-1</sup>. The yield components, number of pods per plant (NPP), number of grains per pod (NGP), and 100-grain weight (100W) (adjusted to 13% moisture content) were evaluated in ten randomly chosen plants per plot.

A factorial design (7 × 2) was used, and the analysis of variance (ANOVA) was initially conducted using a model that included interaction terms between the evaluated factors, but aimed at testing for significant interactive effects. This procedure was applied to each response variable. After confirming that none of the interactions was statistically significant ( $p > 0.05$ ), data from the 2020/2021 and 2022/2023 growing seasons were pooled, and the ANOVA was re-run using an additive model without the interaction term. Residual normality was assessed using the Kolmogorov-Smirnov test, and the homogeneity of variances was verified with Levene's test. Once model assumptions were met, a parametric multiple comparison test (Fisher's LSD) was applied. All statistical analyses were performed using the R software (version 4.3.1 ucrt).

## Results and discussion

In the soil chemical analysis, significant differences were observed concerning cover crop mixes only for P and Zn contents (Table 2). Mix 3 (Millet, *C. ochroleuca*, Black oat, White oat, Buckwheat, and Coracana grass) provided significantly higher soil P content compared to fallows 1 and 2, and Zn in the soil was higher than fallows 1 and 2 and cover crop mixes 1 and 2. Regarding co-inoculation with multifunctional rhizobacteria, no significant differences were found for any analyzed variables.

Michelon et al. (2019) also found greater availability of P in soil cultivated with cover crops (lupines, vetch, fodder radish, ryegrass, oat, and mix (oat + fodder radish + vetch)). In this study, the treatment with mix 3 provided a quantity of P 48% higher than that verified in fallow 1 (without cover crops) and 54% higher than in fallow 2 (without cover crops and rotation with upland rice). These results align with Hallama et al. (2019), who reported that cover crops increase P availability for subsequent crops by accumulating P in biomass and enhancing its mineralization. It can be inferred that this mix favored greater phosphorus availability in the soil. Also, mix 3 provided Zn 50% higher than fallow 1 and 56% higher than fallow 2. This characteristic is particularly important for Cerrado soils, where the use of cover crops enhances the availability of essential nutrients for subsequent crops, including P, K, Ca, Mg, S, Zn, Fe, Cu, B, Mn, and Mo, by improving soil nutrient cycling and reducing nutrient losses through leaching and erosion (Tubana et al., 2020). Cover crops improve the soil content of these nutrients, and this benefits the subsequent crop (soybean).

Significant differences were also found in the activity of the  $\beta$ -Glycosidase enzyme (Table 2). Mixes 4 and 5 provided the highest enzyme activity values. These treatments differed significantly from fallow 2, being higher by 31 and 29%, respectively. In a study by Ferreira et al. (2017), high values of the  $\beta$ -Glycosidase enzyme were found in a no-tillage system/ a system that advocates the use of crop rotation and permanence of soil cover, especially using cover crops. Mendes et al. (2020) evaluated monocropping, succession, and rotation and found differences in soybean yield when obtaining the best results in biologically active soils with good enzymatic activity due to the insertion of cover crops. In agreement with these findings, Adetunji et al. (2020) demonstrated that the quantity and quality of cover crop residues, especially when terminated at flowering, significantly affects  $\beta$ -Glycosidase activity and soil fertility. Upland rice and cover crop residues left in the soil may cause different carbon sources to contribute to the systems, which could have promoted microbial communities that provided greater activity of these enzymes (Bhat et al., 2017).

**Table 2.** Soil chemical properties and soil quality bioindicators (glycosidase and arylsulfatase enzymes) in the 0 - 0.10 m layer after the desiccation of cover crops (November 9, 2021).

Treatments	pH	OM	P	K	Ca	Mg	H+Al
Soil cover system	H <sub>2</sub> O	g kg <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	Mmol L <sup>-1</sup>	Mmol L <sup>-1</sup>	mmol L <sup>-1</sup>
Mix 1	5.91	29.55	18.65 ab	105	23.01	11.28	15.75
Mix 2	5.81	29.67	15.52 ab	121	23.51	11.46	15.62
Mix 3	5.73	29.60	20.73 a	112	23.42	11.60	17.50
Mix 4	5.92	31.46	16.43 ab	118	25.26	12.61	14.00
Mix 5	5.86	30.25	15.07 ab	120	24.47	11.97	15.37
Fallow 1	5.91	29.60	14.02 b	120	23.22	11.67	15.00
Fallow 2	5.91	30.83	13.48 b	120	22.90	11.67	14.87
Co-inoculation	H <sub>2</sub> O	g kg <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mmol/L	mmol/L	mmol/L
With	5.90	29.97	14.46	122	23.07	11.65	14.78
Without	5.81	29.73	14.48	111	24.30	11.83	16.10
CV (%)	4.22	12.39	34.38	25.02	20.46	17.15	23.30
Treatments	Cu	Zn	Fe	Mn	Arylsulfatase	β-Glycosidase	
Mix	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	[p-Nitrophenol] mg kg <sup>-1</sup> h <sup>-1</sup>		
Mix 1	1.66	3.73 b	16.96	25.46	25.01	27.35 ab	
Mix 2	1.60	3.86 b	16.42	25.88	23.98	27.26 ab	
Mix 3	1.72	5.76 a	18.08	24.96	21.98	25.86 ab	
Mix 4	1.72	5.25 ab	17	27.26	27.53	30.52 a	
Mix 5	1.66	4.55 ab	17.03	26.35	26.60	30.12 a	
Fallow 1	1.65	3.85 b	21.72	27.12	24.18	24.26 ab	
Fallow 2	1.70	3.70 b	17.18	26.40	21.26	23.35 b	
Co-inoculation	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	[p-Nitrophenol] mg kg <sup>-1</sup> h <sup>-1</sup>		
With	1.69	4.12	17.25	24.97	24.88	27.43	
Without	1.65	4.14	18.28	27.42	23.84	26.49	
CV (%)	29.77	37.02	36.78	21.80	28.24	25.10	

Means followed by the same letter in the columns do not differ by Fisher's LSD test at 5% significance. Columns without letters had no significant difference between treatments. OM = organic matter; H + Al: potential acidity. <sup>1</sup>Mix 1 (Mix Ultra): White lupine, Buckwheat, White oat, Black oat, *Crotalaria ochroleuca*, *Crotalaria juncea*, Fodder radish, and Coracana grass; Mix 2 (Mix Vitale): Buckwheat, *Crotalaria spectabilis*, Fodder radish, and Black oat; Mix 3 (Mix Forrageiro): Millet, *C. ochroleuca*, Black oat, White oat, Buckwheat, and Coracana grass; Mix 4 (Mix Reduct): *C. spectabilis*, Buckwheat, Millet, and *Crotalaria breviflora*; Mix 5 (Mix Mega): Black oat, Buckwheat, Millet, Piatã grass, and *C. ochroleuca*; Fallow 1: no mix of cover crops, rice/fallow/soybean; Fallow 2: no mix of cover crops and no rotation with upland rice, soybean/fallow/soybean. Co-inoculation: With (BRM 32114 (*Serratia marcescens*) + BRM 63573 (*Bacillus spp.*); Without (without microorganisms).

According to Mendes et al. (2018), the classification intervals of the activity of this enzyme in the soil are  $\leq 90$ , low activity level; from 91 to 225, moderate activity level; and  $>225 \mu\text{g of PNG g}^{-1}$ , adequate activity level. Our results showed a very low activity level, such that none of the treatments exceeded  $31 \mu\text{g of PNG g}^{-1}$ . It is important to emphasize that this classification refers to Cerrado Oxisols; therefore, soils from different regions may not fit this classification (Mendes et al., 2018). These results reinforce the importance of maintaining conservation practices such as crop rotation and the adoption of cover crops and multifunctional microorganisms to improve soil quality.

Regarding the soil physical analysis, soils under fallow treatments (1 and 2) presented the highest values for density, being significantly higher than areas cultivated with mixes 1 and 2 (Table 3). These results are justified by the greater concentration of root volume in the soil, which promoted the use of cover crop mixes in the other treatments. Francziskowski et al. (2019) also found lower soil density when using different cover crop species together (mix), due to the different layers in which the roots acted, with thin roots acting more superficially and thicker roots acting in greater depth.

In treatments with cover crop mixtures, soil bulk density values were below the critical threshold for most grain crops (approximately  $1.40 \text{ Mg dm}^{-3}$ ), ranging from 1.25 to  $1.38 \text{ Mg dm}^{-3}$ . In contrast, fallow areas approached or exceeded this value. According to Haruna et al. (2020), cover crops reduce bulk density by contributing residues with lower mass per unit volume and promoting the formation of biopores through root activity, which improves porosity and structural quality. Values above  $1.40 \text{ Mg dm}^{-3}$  restrict root growth, water infiltration, and gas exchange, negatively impacting crop yield. Thus, the soybean crop sown afterward may have limited root development and impaired yield in fallow areas.

Soils under fallow (1 and 2) presented the lowest values for total porosity, significantly lower than all treatments with the mixes of cover crops. The soil with mix 2 recorded the highest value for both variables (Table 3). Corroborating our results, Anschau et al. (2018) also found greater total porosity in the soil after growing a cover crop (*Urochloa ruziziensis*) compared to fallow. According to Costa et al. (2015), the use of

plants with different root systems and C/N ratio can favor both the control and prevention of compaction, decrease density, and increase porosity in the surface (as observed in this study) and subsurface layers, which stimulates the root growth of subsequent crops. Since fallow 1 did not adopt mixes of cover crops and fallow 2 did not adopt mixes of cover crops and rotation with upland rice, there were no plants with different root systems and C/N ratio, thus favoring higher density and lower total soil porosity.

**Table 3.** Soil physical properties at 0 - 0.10 m depth, after the desiccation of cover crops (November 9, 2021).

Treatments	Soil bulk density	Total porosity
Soil Cover System	g cm <sup>-3</sup>	%
Mix 1	1.34 b	43.29 ab
Mix 2	1.35 b	44.11 a
Mix 3	1.36 ab	43.19 ab
Mix 4	1.36 ab	43.27 ab
Mix 5	1.38 ab	43.14 ab
Fallow 1	1.41 a	41.29 c
Fallow 2	1.40 a	41.30 c
Co-inoculation	g cm <sup>-3</sup>	%
With	1.38	43.39
Without	1.36	42.43
CV (%)	3.48	3.51

Means followed by the same letter in the columns do not differ by Fisher's LSD test at 5% significance. Columns without letters had no significant difference between treatments. <sup>1</sup>Mix 1 (Mix Ultra): White lupine, Buckwheat, White oat, Black oat, *Crotalaria ochroleuca*, *Crotalaria juncea*, Fodder radish, and Coracana grass; Mix 2 (Mix Vitale): Buckwheat, *Crotalaria spectabilis*, Fodder radish, and Black oat; Mix 3 (Mix Forrageiro): Millet, *C. ochroleuca*, Black oat, White oat, Buckwheat, and Coracana grass; Mix 4 (Mix Reduct): *C. spectabilis*, Buckwheat, Millet, and *Crotalaria breviflora*; Mix 5 (Mix Mega): Black oat, Buckwheat, Millet, Piatã grass, and *C. ochroleuca*; Fallow 1: no mix of cover crops, rice/fallow/soybean; Fallow 2: no mix of cover crops and no rotation with upland rice, soybean/fallow/soybean. Co-inoculation: With (BRM 32114 (*Serratia marcescens*) + BRM 63573 (*Bacillus* spp.)); Without (without microorganisms).

As regards biological analysis, it was found that areas under fallow (1 and 2) were the treatments that provided the highest number of *Fusarium solani* inoculums in the soil, being significantly superior to mixes of cover crops, except for mix 3 (Table 4). Similar results were reported by Sousa et al. (2025), who observed greater severity of root rot caused by *Fusarium solani* in areas with a history of fallow, reinforcing the importance of cover crops in managing this pathogen. As for *F. oxysporum*, there were no differences between treatments (Table 4).

**Table 4.** Inoculum density of soil fungi (propagule g<sup>-1</sup> soil) at 0 - 0.10 m depth after desiccation of cover crops (November 9, 2021).

Treatments	<i>F. solani</i>	<i>F. oxysporum</i>	<i>R. solani</i>	<i>Trichoderma</i> spp.
Soil Cover System				
Mix 1	1587 b	4127	23.13 ab	8043
Mix 2	1587 b	4657	18.50 b	6615
Mix 3	2064 ab	4074	18.50 b	5291
Mix 4	1482 b	4339	20.58 b	6403
Mix 5	1746 b	3757	26.37 ab	5874
Fallow 1	2805 a	4668	37.01 a	5980
Fallow 2	2810 a	5080	36.15 a	5982
Co-inoculation				
With	1710	3069 a	26.90	5574
Without	2046	5609 b	21.12	7161
CV (%)	49.93	60.18	66.70	72.21

Means followed by the same letter in the columns do not differ by Fisher's LSD test at 5% significance. Columns without letters had no significant difference between treatments. <sup>1</sup>Mix 1 (Mix Ultra): White lupine, Buckwheat, White oat, Black oat, *Crotalaria ochroleuca*, *Crotalaria juncea*, Fodder radish, and Coracana grass; Mix 2 (Mix Vitale): Buckwheat, *Crotalaria spectabilis*, Fodder radish, and Black oat; Mix 3 (Mix Forrageiro): Millet, *C. ochroleuca*, Black oat, White oat, Buckwheat, and Coracana grass; Mix 4 (Mix Reduct): *C. spectabilis*, Buckwheat, Millet, and *Crotalaria breviflora*; Mix 5 (Mix Mega): Black oat, Buckwheat, Millet, Piatã grass, and *C. ochroleuca*; Fallow 1: no mix of cover crops, rice/fallow/soybean; Fallow 2: no mix of cover crops and no rotation with upland rice, soybean/fallow/soybean. Co-inoculation: With (BRM 32114 (*Serratia marcescens*) + BRM 63573 (*Bacillus* spp.)); Without (without microorganisms).

Areas under fallow (1 and 2) recorded the highest inoculum population of *Rhizoctonia solani* in the soil, significantly higher than mixes 2, 3, and 4 (Table 4). Regarding *Trichoderma* spp. (beneficial fungus), there was no difference between the evaluated treatments. Soybean monocropping tends to increase the amount of phytopathogens that survive in the remains of the crop, such as the fungi addressed in this study, which remove nutrients from plants with living and senescent activity. This system provides ideal substrates for

these pathogens (Amorim et al., 2016). Thus, the use of crop rotation and cover crops as in this study, interferes with the survival and dissemination of pathogens by disrupting their life cycles and reducing their inoculum in the soil, thereby functioning as effective cultural control methods (Wang et al., 2022).

Considering co-inoculation, it was only for the fungus *Fusarium oxysporum*, that there was a difference between treatments with and without multifunctional rhizobacteria (Table 4). The treatment without co-inoculation was 83% superior to the treatment with co-inoculation. Apart from promoting plant growth, multifunctional rhizobacteria are also efficient in suppressing phytopathogens (Pascholati et al., 2019), such as *F. oxysporum*, through mechanisms like the production of antibiotics (iturin, surfactins, phenazines), hydrolytic enzymes (proteases, chitinases), and competition for nutrients (siderophores) (Ali et al., 2020). In a study by Andrade et al. (2021), different bacterial isolates of the genus *Bacillus* spp., which is one of the genera evaluated in this study, inhibited the growth of the fungal colony of *F. oxysporum* when compared with the control, indicating antagonism.

According to Zhang et al. (2024), diversified cropping systems that incorporate complementary root growth strategies improve soil chemical, physical, and biological properties by enabling the exploration of different soil depths. These systems also contribute to the suppression of pests, diseases, and weeds by disrupting their life cycles, making them an effective strategy for sustainable soil management. This explains the better results found using soybean rotation with upland rice and cover crop mixes, which influenced the soil chemical properties (higher P and Zn content by the mix 3), physical properties (soil bulk density and total porosity), biological quality (greater activity of the  $\beta$ -Glycosidase enzyme in mixes 4 and 5), in addition to reducing the inoculum of phytopathogenic fungi in the soil.

Compared to fallow treatments, the soybean plants sown in rotation with upland rice and cover crop mixes had higher shoot biomass production (Table 5). This may have occurred due to crop rotation and the cultivation of cover crop mixes. Even with low soil cover maintenance, proper crop rotation in the Cerrado region favors nutrient cycling and improves soil physical and biological conditions, which is capable of ultimately increasing biomass production and crop yield (Silva et al., 2022b). Cover crops also improve soil quality, reduce the incidence of weeds, insect pests, and diseases, provide more suitable conditions for better development and biomass production, and increase the yield of the subsequent crop, especially in the case of Cerrado soils, which are mostly acidic, with low fertility and low organic matter content (Souza et al., 2025; Schmidt et al., 2019).

**Table 5.** Shoot biomass and macronutrients accumulation in the shoot (which became available in the soil after harvest) of soybean plants at the R7 stage co-inoculated with multifunctional rhizobacteria (BRM 32114 (*Serratia marcescens*) + BRM 63573 (*Bacillus* spp.) grown in rotation with upland rice (first crop) and in succession to cover crop mixes or fallow (second crop) in the 2021/2022 crop season.

Treatments	Biomass	N	P	K	Ca	Mg	S
Soil Cover System	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>
Mix 1	9152 ab	350 a	28.21 ab	138 ab	107 ab	32.87 ab	25.45 ab
Mix 2	8953 ab	360 a	28.77 ab	139 ab	83 b	27.87 b	26.61 ab
Mix 3	8776 bc	350 a	31.71 a	137 ab	80 b	27.37 b	27.12 a
Mix 4	9670 ab	353 a	30.58 a	148 a	103 ab	41.75 a	27.36 a
Mix 5	9920 a	405 a	33.39 a	155 a	126 a	24 bc	27.87 a
Fallow 1	7899 cd	128 b	14.21 c	129 b	33 c	8.62 c	25.36 ab
Fallow 2	7512 d	191 b	23.81 b	112 b	110 ab	21.50 bc	20.43 b
Co-inoculation	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>
With	9186 a	341 a	29.31	141	92 a	35.51	28.49 a
Without	8494 b	270 b	25.34	132	74 b	32.24	22.97 b
CV (%)	10.59	22.35	22.91	13.86	27.03	16.48	18.31

Means followed by the same letter in the columns do not differ by Fisher's LSD test at 5% significance. Columns without letters had no significant difference between treatments. <sup>1</sup>Mix 1 (Mix Ultra): White lupine, Buckwheat, White oat, Black oat, *Crotalaria ochroleuca*, *Crotalaria juncea*, Fodder radish, and Coracana grass; Mix 2 (Mix Vitale): Buckwheat, *Crotalaria spectabilis*, Fodder radish, and Black oat; Mix 3 (Mix Forrageiro): Millet, *C. ochroleuca*, Black oat, White oat, Buckwheat, and Coracana grass; Mix 4 (Mix Reduct): *C. spectabilis*, Buckwheat, Millet, and *Crotalaria breviflora*; Mix 5 (Mix Mega): Black oat, Buckwheat, Millet, Piatã grass, and *C. ochroleuca*; Fallow 1: no mix of cover crops, rice/fallow/soybean; Fallow 2: no mix of cover crops and no rotation with upland rice, soybean/fallow/soybean. Co-inoculation: With (BRM 32114 (*Serratia marcescens*) + BRM 63573 (*Bacillus* spp.); Without (without microorganisms).

Soybean plants that grew after mixes 1, 2, 4, and 5 were significantly superior to those that grew after fallow 1 (upland rice/fallow/soybean) (Table 5). All mixes (1, 2, 3, 4, and 5) provided greater development of soybean plants (higher biomass production) compared to fallow 2 (soybean/fallow/soybean), where there was no crop rotation. Similarly, greater heights of soybean plants, which is a variable correlated with biomass production, were observed when cultivated in succession with *Urochloa ruziziensis* and *Avena strigosa*,

compared to fallow in the study conducted by Anschau et al. (2018). Our results can be explained by the improvements that the cultivation of upland rice and cover crop mixes, which preceded soybeans, provided for soil quality such as better conditions for the development of soybean plants compared to fallow areas. These results are even more relevant, as studies are scarce on how crop rotation and the adoption of cover crops can influence the development of crops of economic interest, such as soybeans. Despite benefiting production, the plant residues after soybean harvest are also beneficial to the soil after decomposition, helping to protect the soil, cycle nutrients, and provide better conditions for beneficial soil microorganisms.

Regarding the macronutrients available in the soil after the soybean harvest, all mixes provided significantly higher Nitrogen values in soybean plants, compared to the areas under fallow (Table 5). It is important to emphasize that all mixes of cover crops have leguminous species, which could have promoted the higher concentration of N in soybean plants, consequent upon the biological fixation of N. Kaye et al. (2019) and White et al. (2017), reported that mixes of cover crops containing at least one legume produced a higher N content of the biomass compared to when only non-legume species were used. Regarding P, soybean plants cultivated after all cover crop mixes showed values significantly higher than fallow 1, and those grown after mixes 3, 4, and 5 differed from fallow 2. Soybean plants cultivated after mixes 4 and 5 were higher than those in both areas under fallow in terms of K. Soybean plants grown after all cover crop mixes were superior to those cultivated after fallow 1 for Ca. For Mg, soybean plants cultivated after cover crop mixes 1, 2, 3, and 4 had significantly higher content than fallow 1, and only mix 4 differed statistically from fallow 2. For S, soybean plants cultivated after cover crop mixes 3, 4, and 5 were significantly superior to fallow 2.

Significant differences were also verified for micronutrients (Table 6). All soybean plants cultivated after the mixes showed values higher than fallow 1 for B and Cu contents. Soybean plants after mix 2 and mix 3 differed from fallow 2 for B content, and Cu content, respectively. However, soybean plants cultivated after fallow 1 were significantly superior to all other treatments for Fe and Mn content, which may indicate that weed species favored greater absorption of these micronutrients. For Zn, soybean plants cultivated after mixes 2, 3, 4, and 5 were significantly higher compared to those grown after fallow 2, and no treatment was statistically different from fallow 1. For Mo, soybean plants cultivated after mixes 3 and 5 were superior to fallow 1, and only soybean plants cultivated after mix 5 were superior to plants cultivated after fallow 2.

**Table 6.** Shoot biomass and accumulation of micronutrients in the shoot (which became available in the soil after harvest) of soybean plants at the R7 stage co-inoculated with multifunctional rhizobacteria (BRM 32114 (*Serratia marcescens*) + BRM 63573 (*Bacillus* spp.)) grown in rotation with upland rice (first crop) and in succession to cover crop mixes or fallow (second crop) in the 2021/2022 crop season.

Treatments	B	Cu	Fe	Mn	Zn	Mo
Soil Cover System	g ha <sup>-1</sup>	g ha <sup>-1</sup>	g ha <sup>-1</sup>	g ha <sup>-1</sup>	g ha <sup>-1</sup>	g ha <sup>-1</sup>
Mix 1	228 ab	50.11 ab	1537 b	334 b	218 ab	2.207 abc
Mix 2	230 a	47.88 ab	1884 b	354 b	234 a	1.592 bc
Mix 3	213 ab	56.96 a	1346 b	276 b	248 a	4.022 ab
Mix 4	227 ab	52.58 ab	1492 b	304 b	224 a	2.996 abc
Mix 5	227 ab	55.22 ab	1556 b	318 b	260 a	4.992 a
Fallow 1	87 c	32.79 c	3095 a	933 a	218 ab	0.425 c
Fallow 2	187 b	45.30 b	1425 b	248 b	177 b	1.813 bc
Co-inoculation	g ha <sup>-1</sup>	g ha <sup>-1</sup>	g ha <sup>-1</sup>	g ha <sup>-1</sup>	g ha <sup>-1</sup>	g ha <sup>-1</sup>
With	208	50.34	1728 a	438 a	244 a	2.665
Without	192	47.05	1796 b	353 b	207 b	2.491
CV (%)	21.00	21.42	38.74	54.97	17.53	106.62

Means followed by the same letter in the columns do not differ by Fisher's LSD test at 5% significance. Columns without letters had no significant difference between treatments. <sup>1</sup>Mix 1 (Mix Ultra): White lupine, Buckwheat, White oat, Black oat, *Crotalaria ochroleuca*, *Crotalaria juncea*, Fodder radish, and Coracana grass; Mix 2 (Mix Vitale): Buckwheat, *Crotalaria spectabilis*, Fodder radish, and Black oat; Mix 3 (Mix Forrageiro): Millet, *C. ochroleuca*, Black oat, White oat, Buckwheat, and Coracana grass; Mix 4 (Mix Reduct): *C. spectabilis*, Buckwheat, Millet, and *Crotalaria breviflora*; Mix 5 (Mix Mega): Black oat, Buckwheat, Millet, Piatã grass, and *C. ochroleuca*; Fallow 1: no mix of cover crops, rice/fallow/soybean; Fallow 2: no mix of cover crops and no rotation with upland rice, soybean/fallow/soybean. Co-inoculation: With (BRM 32114 (*Serratia marcescens*) + BRM 63573 (*Bacillus* spp.)); Without (without microorganisms).

No improvements were observed in the soil chemical properties, except for mix 3 regarding P and Zn (Table 2). The expressive results obtained about the nutrient content of soybeans cultivated in succession to the mixes of cover crops concerning both areas under fallow, can be explained by other improvements provided by these cover crops. Among these improvements, we can mention the changes in soil physical properties brought about by the mixes of cover crops, such as lower soil bulk density, better total porosity, and others that were not evaluated in this study, which probably provided greater root development of the

soybean crop and consequently greater access to soil nutrients. After the harvest, straws from the soybean plants will decompose and return these nutrients to the soil and benefit the entire system.

Regarding co-inoculation with multifunctional rhizobacteria, significant and relevant results were obtained (Tables 5 and 6). Co-inoculation with isolates BRM 32114 and BRM 63573 provided significantly higher values for biomass production, N, Ca, S, Fe, Mn, and Zn contents than treatment without co-inoculation. Corroborating our results, Silva et al. (2020a) found that soybean shoot biomass co-inoculated with isolates BRM 32114 and BRM 63573 was significantly higher than the treatment without co-inoculation. The authors also found a higher P content with co-inoculation (32114 + *Trichoderma asperellum* Pool and BRM 32110 + BRM 32114 + Ab-V5 + *Trichoderma asperellum* Pool + BRM 63573 + 1381) that contained the same isolates assessed in the present study.

Multifunctional rhizobacteria promote growth, increase the root system by producing phytohormones, facilitate the absorption of nutrients, biological nitrogen fixation, and phosphate (P) solubilization, that is not readily available in the soil for absorption/assimilation by plants, and in the production of siderophores, organic molecules, and iron chelators (Ahemad & Kibret, 2014; Glick, 2012). These mechanisms of action explain the good results obtained regarding the accumulation of biomass and nutrients by the soybean plants co-inoculated in this study.

Soybean plants grown after cover crop mixes had a higher number of pods per plant (NPP) and grain yield (YLD) compared to soybean plants cultivated after fallow (Table 7). Soybean plants grown after mixes 1 and 2 had a higher number of pods per plant than plants grown after fallow 1, and soybean plants grown after mixes 1, 2, 3, and 5 showed a higher number of pods than plants grown after fallow 2. Soybean plants cultivated after all cover crop mixes had higher grain yields than those cultivated in areas under both fallows (1 and 2). The soybean plants cultivated after mix 5 and fallow 2 recorded the highest and lowest grain yield, respectively.

**Table 7.** Grain yield and yield components of soybean grown in succession to mixes of cover crops or fallow and co-inoculated with multifunctional rhizobacteria (BRM 32114 (*Serratia marcescens*) + BRM 63573 (*Bacillus* spp.) 2020/2021 and 2021/2022 crop seasons.

Treatments	NPP	NGP	100W	YLD
Soil Cover System	n° plant <sup>-1</sup>		g	kg ha <sup>-1</sup>
Mix 1	49.38 a	2.05	17.81 ab	3844 b
Mix 2	50.28 a	1.97	17.94 ab	4092 ab
Mix 3	48.73 ab	2.04	17.93 ab	3940 ab
Mix 4	47.64 abc	1.99	17.98 ab	4046 ab
Mix 5	48.50 ab	2.03	17.99 ab	4204 a
Fallow 1	44.70 bc	2.06	16.31 b	3541 c
Fallow 2	42.22 c	1.93	16.89 b	2817 d
Co-inoculation	n° plant <sup>-1</sup>		g	kg ha <sup>-1</sup>
With	47.39	2.04	18.01	3930
Without	48.10	1.99	17.81	3786
CV (%)	13.57	10.87	7.66	10.99

Means followed by the same letter in the columns do not differ by Fisher's LSD test at 5% significance. Columns without letters had no significant difference between treatments. NPP – Number of pods per plant; NGP – Number of grains per pod; 100W – 100-grain weight; YLD – Grain yield. Mix 1 (Mix Ultra): White lupine, Buckwheat, White oat, Black oat, *Crotalaria ochroleuca*, *Crotalaria juncea*, Fodder radish, and Coracana grass; Mix 2 (Mix Vitale): Buckwheat, *Crotalaria spectabilis*, Fodder radish, and Black oat; Mix 3 (Mix Forrageiro): Millet, *C. ochroleuca*, Black oat, White oat, Buckwheat, and Coracana grass; Mix 4 (Mix Reduct): *C. spectabilis*, Buckwheat, Millet, and *Crotalaria breviflora*; Mix 5 (Mix Mega): Black oat, Buckwheat, Millet, Piata grass, and *C. ochroleuca*; Fallow 1: no mix of cover crops, rice/fallow/soybean; Fallow 2: no mix of cover crops and no rotation with upland rice, soybean/fallow/soybean. Co-inoculation: With (BRM 32114 (*Serratia marcescens*) + BRM 63573 (*Bacillus* spp.); Without (without microorganisms).

Compared to the area with fallow, the gain in grain yield when mixes of cover crops were used can be explained by the better soil conditions and the greater biomass production and nutrient content of soybean plants. All treatments had a great diversity of cover crop species, which may have favored the results found. According to Holmes et al. (2017), the success of combining different species (mix) of cover crops is closely linked to the morphological and functional characteristics of each species, since the more divergent the characteristics (root system, plant height, cycle, nitrogen fixation, phytomass production, etc.), the better for the system. These results corroborate those of Nascente and Stone (2018), who reported improvements in the soil chemical and physical attributes and in the grain yield of soybean cultivated for two cycles after a mix of millet + *U. ruziziensis* + pigeon pea and the cultivation of upland rice when compared to fallow.

There were no significant differences for any variables concerning co-inoculation with multifunctional rhizobacteria (Table 7). Silva et al. (2020b) also showed no increase in soybean grain yield when co-inoculated

with the isolates (BRM 32114 + BRM 63573) compared to the treatment without microorganisms. Despite many years of research, there is still a great variability of responses regarding the use of inoculation and co-inoculation with multifunctional microorganisms, especially under field conditions where they are subject to adversities that influence their efficiency (Silva et al., 2022b).

The use of cover crop mixtures may have improved the physical, chemical, and biological properties of the soil, thereby favoring the development of soybean plants. Microorganisms generally benefit plants under stress conditions, which did not occur in the experimental area because cover crops were used. Therefore, sustainable practices such as rotation with upland rice, cover crop mixtures, and co-inoculation with multifunctional rhizobacteria, are recommended for the improvement of soil quality and soybean development. Rotation with upland rice and cover crops increased soil phosphorus (P) and zinc (Zn) content,  $\beta$ -glucosidase enzyme activity, and total porosity, but reduced soil density and the incidence of *Fusarium solani* and *Rhizoctonia solani*. They also increased biomass production, nutrient content in the aerial part, number of pods per plant, and soybean yield compared to fallow areas. Furthermore, co-inoculation with isolates BRM 32114 and BRM 63573 reduced the density of *Fusarium oxysporum* in the soil and increased biomass production and nutrient accumulation in soybean compared to the treatment without co-inoculation.

The cover crops in the second crop season (2021/2022) were sown late due to the harvest of upland rice in April, which resulted in poor seedling establishment and insufficient soil coverage because of inadequate rainfall (Figure 1). This highlights the importance of using a super-early rice cultivar for efficient soybean rotation with upland rice and cover crop mixes. Additionally, more significant results from crop rotation, cover crops, and multifunctional rhizobacteria were observed in the long term. These factors suggest that rotation with rice, cultivation of cover crop mixes, and co-inoculation with rhizobacteria can contribute to sustainable development, which benefits soybean production in the Cerrado.

## Conclusion

The rotation of soybean with upland rice and cover crop mixtures, particularly Mix 5 (Mega) and Mix 4 (Reduct), improved soil physical and biological quality, increased nutrient accumulation in shoots, and enhanced soybean yield. Co-inoculation with multifunctional rhizobacteria further reduced soil pathogen density and improved biomass and nutrient uptake, but did not significantly affect yield. These findings support the recommendation of using diversified cover crop mixtures and microbial inoculants in rotation with upland rice, to promote sustainable soybean production in the Cerrado.

## Data availability

Data will be made available on request.

## References

- Adetunji, A. T., Ncube, B., Meyer, A. H., Mulidzi, R., & Lewu, F. B. (2020). Soil  $\beta$ -glucosidase activity, organic carbon and nutrients in plant tissue in response to cover crop species and management practices. *South African Journal of Plant and Soil*, 37(3), 202-210. <https://doi.org/10.1080/02571862.2020.1718786>
- Ahemad, M. & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King Saud University-Science*, 26(1), 1-20. <https://doi.org/10.1016/j.jksus.2013.05.001>
- Ali, S., Hameed, S., Shahid, M., Iqbal, M., Lazarovits, G., & Imran, A. (2020). Functional characterization of potential PGPR exhibiting broad-spectrum antifungal activity. *Microbiological Research*, 232, 1-17. <https://doi.org/10.1016/j.micres.2019.126389>
- Amorim, L., Rezende, J. A. M., Bergamin Filho, A., & Camargo, L. E. A. (2016). *Manual de fitopatologia. Doenças das plantas cultivadas* (5. ed., v. 2). Embrapa Arroz e Feijão; Embrapa Florestas.
- Andrade, B. R. D., Silva, M. L. R. B., Oliveira, L. G., Rosa, R. C. T., Gurgel, L. M. S., & Paula Oliveira, J. (2021). Ação antagonista à *Macrophomina phaseolina* e *Fusarium oxysporum* e diversidade genética de bactérias obtidas de solo cultivado com *Vigna unguiculata* (L.) Walp. *Pesquisa Agropecuária Pernambucana*, 26(2), 1-7. <https://doi.org/10.12661/pap.2021.004>

- Anschau, K. A., Seidel, E. P., Mottin, M. C., Lerner, K. L., Francziskowski, M. A., & Herrmann, D. R. (2018). Propriedades físicas do solo, características agronômicas e produtividade da soja em sucessão a plantas de cobertura. *Scientia Agraria Paranaensis*, 17(3), 293-299.
- Araujo, F. C., Nascente, A. S., Filippi, M. C. C., Silva, M. A., Sousa, V. S., & Lanna, A. C. (2021). Cover crops and multifunctional microorganisms can affect development of upland rice. *Australian Journal of Crop Science*, 15(1), 137-144. <https://doi.org/10.21475/ajcs.21.15.01.2963>
- Bertollo, A. M., & Levien, R. (2019). Compactação do solo em Sistema de Plantio Direto na palha. *Pesquisa Agropecuária Gaúcha*, 25(3), 208-218. <https://doi.org/10.36812/pag.2019253208-218>
- Bhat, N. A., Riar, A., Ramesh, A., Iqbal, S., Sharma, M. P., Sharma, S. K., & Bhullar, G. S. (2017). Soil biological activity contributing to phosphorus availability in vertisols under long-term organic and conventional agricultural management *Frontiers in Plant Science*, 8, 1-11. <https://doi.org/10.3389/fpls.2017.01523>
- Companhia Nacional de Abastecimento. (2025). *Acompanhamento da safra brasileira de grãos: Safra 2024/2025 – 6º levantamento*. CONAB. <https://www.conab.gov.br>
- Cordeiro Junior, P. S., Finoto, E. L., Bárbaro-Torneli, I. M., Martins, M. H., Soares, M. B. B., Bolonhezi, D., & Martins, A. L. M. (2017). Desempenho agronômico de cultivares de soja para a região centro norte paulista, safra 2016/17. *Nucleus*, 59-66. <https://doi.org/10.3738/1982.2278.2820>
- Costa, N. R., Andreotti, M., Lopes, K. S. M., Yokobatake, K. L., Ferreira, J. P., Pariz, C. M., Bonini, C. S. B., & Longhini, V. Z. (2015). Atributos do solo e acúmulo de carbono na integração lavoura-pecuária em sistema plantio direto. *Revista Brasileira de Ciência do Solo*, 39(3), 852-863. <https://doi.org/10.1590/01000683rbcs20140269>
- Dalolio, R. S., Borin, E., Cruz, R. M. S., & Alberton, O. (2018). Co-inoculação de soja com *Bradyrhizobium* e *Azospirillum*. *Journal of Agronomic Sciences*, 7(2), 1-7.
- Donagemma, G. K., Campos, D. V. B., Calderano, S. B., Teixeira, W. G., & Viana, J. H. (2011). *Manual de métodos de análise de solo*. Embrapa Solos. (Documentos, 132).
- Ferreira, E. P. B., Stone, L. F., & Martin-Didonet, C. C. G. (2017). População e atividade microbiana do solo em sistema agroecológico de produção. *Revista Ciência Agronômica*, 48(1), 22-31. <https://doi.org/10.5935/1806-6690.20170003>
- Filippi, M. C. C., Silva, G. B., Silva-Lobo, V. L., Côrtes, M. V. C. B., Moraes, A. J. G., & Prabhu, A. S. (2011). Leaf blast (*Magnaporthe oryzae*) suppression and growth promotion by rhizobacteria on aerobic rice in Brazil. *Biological Control*, 58(2), 160-166. <https://doi.org/10.1016/j.biocontrol.2011.04.016>
- Francziskowski, M. A., Seidel, E. P., Fey, E., Anschau, K. A., & Mottin, M. C. (2019). Propriedades físicas do solo nos sistemas de plantio direto e preparo reduzido com diferentes plantas de cobertura. *Revista Engenharia na Agricultura*, 27(6), 556-564. <https://doi.org/10.13083/reveng.v27i6.966>
- Glick, B. R. (2012). Plant growth-promoting bacteria: mechanisms and applications. *Científica*, 2012, 1-15. <https://doi.org/10.6064/2012/963401>
- Hallama, M., Pekrun, C., Lambers, H., & Kandeler, E. (2019). Hidden miners-the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant and Soil*, 434, 7-45. <https://doi.org/10.1007/s11104-018-3810-7>
- Haruna, S. I., Eke, D., Haque, M. A., & Ku, S. (2025). Cover crop effects on select soil physicochemical and biological properties. *Agriculture, Ecosystems & Environment*, 393, 109817. <https://doi.org/10.1016/j.agee.2025.109817>
- Holmes, A. A., Thompson, A. A., & Wortman, S. E. (2017). *Species-specific contributions to productivity and weed suppression in cover crop mixtures*. *Agronomy Journal*, 109(6), 2808-2819. <https://doi.org/10.2134/agronj2017.06.0309>
- Hosono, A., & Caruso, L. (2016). *Agricultural transformation in the Brazilian Cerrado: A model of development and prosperity*. <https://www.csis.org/events/agriculturaltransformation-brazilian-cerrado-model-developmentand-prosperity>
- Kaye, J., Finney, D., White, C., Bradley, B., Schipanski, M., Alonso-Ayuso, M., Hunter, M., Burgess, M., & Mejia, C. (2019). Managing nitrogen through cover crop species selection in the U.S. mid-Atlantic. *PLoS ONE*, 14(4), 1-23. <https://doi.org/10.1371/journal.pone.0215448>

- Komada, H. (1975). Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Review of Plant Protection Research*, 8, 114-124.
- Martin, J. P. (1950). Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Science*, 69(3), 215-232.
- Mendes, I. C., Ono, F. B., Oliveira, M. I., Silva, R. G., Kappes, C., Reis Junior, F. B., & Zancanaro, L. (2020). Rotação de culturas, bioindicadores e saúde do solo. In P. A. Silva, & L. V. Oliveira (Org.), *Boletim de Pesquisa 2019/2020* (pp. 102-110). Fundação MT.
- Mendes, I. C., Sousa, D. M. G., Reis Junior, F. B., & Lopes, A. A. C. (2018). *Bioanálise de solo: como acessar e interpretar a saúde do solo*. Embrapa Cerrados.
- Michelon, C. J., Junges, E., Casali, C. A., Pellegrini, J. B. R., Rosa Neto, L., Oliveira, Z. B., & Oliveira, M. B. (2019). Atributos do solo e produtividade do milho cultivado em sucessão a plantas de cobertura de inverno. *Revista de Ciências Agroveterinárias*, 18(2), 230-239. <https://doi.org/10.5965/223811711812019230>
- Napoleão, R., Café Filho, A. C., Nasser, L. C. B., Lopes, C. A., & Silva, H. R. (2005). Intensidade do mofo-branco do feijoeiro em plantio convencional e direto sob diferentes lâminas d'água. *Fitopatologia Brasileira*, 30(4), 374-379. <https://doi.org/10.1590/S0100-41582005000400006>
- Nascente, A. S., Crusciol, C. A. C., Stone, L. F., & Cobucci, T. (2013). Upland rice yield as affected by previous summer crop rotation (soybean or upland rice) and glyphosate management on cover crops. *Planta Daninha*, 31(3), 147-155. <https://doi.org/10.1590/S0100-83582013000100016>
- Nascente, A. S., & Stone, L. F. (2018). Cover crops as affecting soil chemical and physical properties and development of upland rice and soybean cultivated in rotation. *Rice Science*, 25(6), 340-349. <https://doi.org/10.1016/j.rsci.2018.10.004>
- Nash, S. M., & Snyder, W. C. (1962). Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathology*, 52(6), 567-572.
- Pascholati, S. F., Souza, V. H. M., & Cardoso Filho, J. A. (2019). Indução de resistência por *Trichoderma*: uso na agricultura. *Embrapa Soja-Livro Científico*, 7(1), 235-246.
- Plaza-Bonilla, D., Nolot, J.-M., Passot, S., Raffailac, D., & Justes, E. (2016). Grain legume-based rotations managed under conventional tillage need cover crops to mitigate soil organic matter losses. *Soil and Tillage Research*, 156, 33-43. <https://doi.org/10.1016/j.still.2015.09.021>
- Ratz, R. J., Palácio, S. M., Espinoza-Quiñones, F. R., Vicentino, R. C., Michelim, H. J., & Richter, L. M. (2017). Potencial biotecnológico de rizobactérias promotoras de crescimento de plantas no cultivo de milho e soja. *Engevista*, 19(4), 890-905. <https://doi.org/10.22409/engevista.v19i4.894>
- Rezende, C. C., Silva, M. A., Frasca, L. L. M., Faria, D. R., Filippi, M. C. C., Lanna, A. C., & Nascente, A. S. (2021). Microrganismos multifuncionais: Utilização na agricultura. *Research, Society and Development*, 10(2), 1-15. <https://doi.org/10.33448/rsd-v10i2.12725>
- Santos, H. G., Jacomine, P. K. T., Anjos, L. H. C. dos, Oliveira, V. A., Lumbrreras, J. F., Coelho, M. R., ... & Cunha, T. J. F. (2018). *Sistema brasileiro de classificação de solos* (5ª ed., rev. e ampl.). Brasília, DF: Embrapa.
- Silva, J. M., Almeida, C. X., Pena, L. K., Jorge, R. F., Silva, L. R., & Duarte, I. R. G. (2022a). Estimativa da macroporosidade e microporosidade em função de sistemas de manejo e plantas de cobertura em Latossolo Vermelho cultivado com soja. *Research, Society and Development*, 11(3), 1-8. <https://doi.org/10.33448/rsd-v11i3.26833>
- Silva, M. A., Nascente, A. S., Lanna, A. C., Rezende, C. C., Cruz, R. D. C., Frasca, L. L. M., Ferreira, A. L., Ferreira, I. V. L., Duarte, J. R. M., & Filippi, M. C. C. (2022b). Sistema de plantio direto e rotação de culturas no Cerrado. *Research, Society and Development*, 11(13), 1-10. <https://doi.org/10.33448/rsd-v11i13.35568>
- Silva, M. A., Nascente, A. S., Filippi, M. C. C., Lanna, A. C., Silva, G. B., & Silva, J. F. A. (2020a). Individual and combined growth-promoting microorganisms affect biomass production, gas exchange and nutrient content in soybean plants. *Revista Caatinga*, 33(3), 619-632. <https://doi.org/10.1590/1983-21252020v33n305rc>
- Silva, M. A., Nascente, A. S., Filippi, M. C. C., Lanna, A. C., Silva, G. B., Fernandes, J. P. T., & Elias, M. T. A. (2020b). Screening of beneficial microorganisms to improve soybean growth and yield. *Brazilian Archives of Biology and Technology*, 63, 1-13. <https://doi.org/10.1590/1678-4324-2020190463>

- Schmidt, J. H., Junge, S., & Finckh, M. R. (2019). Cover crops and compost prevent weed seed bank buildup in herbicide-free wheat–potato rotations under conservation tillage. *Ecology and evolution*, 9(5), 2715–2724. <https://doi.org/10.1002/ece3.4942>
- Sousa, J. M., Costa, M. M., Moreira, G. M., Menezes, A. S., Lima, C. S., & Pfenning, L. H. (2025). Members of the *Fusarium solani* species complex causing root rot of common bean and cowpea in Brazil. *Tropical Plant Pathology*, 50(23). <https://doi.org/10.1007/s40858-025-00705-4>
- Souza, D. M. G., & Lobato, E. (2004). *Cerrado: correção do solo e adubação* (2. ed.). Embrapa.
- Souza, V. S., Canisares, L. P., Schiebelbein, B. E., Santos, D. C., Menillo, R. B., Pinheiro Junior, C. R., & Cherubin, M. R. (2025). Cover crops enhance soil health, crop yield and resilience of tropical agroecosystem. *Field Crops Research*, 322, 109755. <https://doi.org/10.1016/j.fcr.2025.109755>
- Tabatabai, M. A. (1994). Soil enzymes. In R. W. Weaver, S. Angle, P. Bottomley, D. Bezdicek, S. Smith, A. Tabatabai, & A. Wollum (Eds.), *Methods of soil analysis: Part 2 Microbiological and biochemical properties*, 5.2. (pp. 775–833). SSSA Book Series. <https://doi.org/10.2136/sssabookser5.2.c37>
- Tabatabai, M. A., & Bremner, J. M. (1970). Arylsulfatase activity of soils. *Soil Science Society of America Journal*, 34(2), 225–229. <https://doi.org/10.2136/sssaj1970.03615995003400020016x>
- Tubana, B., Forestieri, D., Fultz, L. M., & Mite, J. (2020). Cover cropping enriches soil: many factors cause nutrient contributions to vary. *Louisiana Agriculture*, 63(4), 14–15.
- Veloso, F. R., Silva, L. F. V., Jacinto, A. C. P., Jorge, R. F., Almeida, C. X., & Carvalho, E. R. (2022). Sistemas de cultivo e plantas de cobertura para produção de soja no Cerrado. *Research, Society and Development*, 11(11), 1–12. <https://doi.org/10.33448/rsd-v11i11.33439>
- Wahab, A., Batool, F., Abdi, G., Muhammad, M., Ullah, S., & Zaman, W. (2025). Role of plant growth-promoting rhizobacteria in sustainable agriculture: Addressing environmental and biological challenges. *Journal of Plant Physiology*, 307(1), e154455. <https://doi.org/10.1016/j.jplph.2025.154455>
- Wang, G., Li, X., Xi, X., & Cong, W.-F. (2022). Crop diversification reinforces soil microbiome functions and soil health. *Plant and Soil*, 476, 375–383. <https://doi.org/10.1007/s11104-022-05436-y>
- White, C. M., DuPont, S. T., Hautau, M., Hartman, D., Finney, D. M., Bradley, B., LaChance, J. C., & Kaye, J. P. (2017). Managing the trade off between nitrogen supply and retention with cover crop mixtures. *Agriculture, Ecosystems & Environment*, 237, 121–133. <https://doi.org/10.1016/j.agee.2016.12.016>
- Zhang, J., Liu, J., Yang, C., Du, S., & Yang, W. (2016). Photosynthetic performance of soybean plants to water deficit under high and low light intensity. *South African Journal of Botany*, 105, 279–287. <https://doi.org/10.1016/j.sajb.2016.04.011>
- Zhang, W.-P., Surigaoge, S., Yang, H., Yu, R.-P., Wu, J.-P., Xing, Y., Chen, Y., & Li, L. (2024). Diversified cropping systems with complementary root growth strategies improve crop adaptation to and remediation of hostile soils. *Plant and Soil*, 502, 7–30. <https://doi.org/10.1007/s11104-023-06464-y>
- Ziech, A. R. D., Conceição, P. C., Luchese, A. V., Balin, N. M., Candiotto, G., & Garmus, T. G. (2015). Proteção do solo por plantas de cobertura de ciclo hibernar na região Sul do Brasil. *Pesquisa Agropecuária Brasileira*, 50(5), 374–382. <https://doi.org/10.1590/S0100-204X2015000500004>

**Associate Editor in charge:**

Alessandro Lucca Braccini

ORCID: <https://orcid.org/0000-0002-6915-4804>

Carlos Alberto Scapim

ORCID: <https://orcid.org/0000-0002-7047-9606>