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Effect of bioagents and cover crops on soil attributes and common bean plant development[1]

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ABSTRACT

The search for cultivation techniques that provide productive, social and environmental benefits to the agroecosystem is of great seriousness for the sustainable intensification of agriculture. This study aimed to determine the effect of crop cover crops on the soil attributes and development of bean plants treated with the consortium of bioagents *Serratia marcencens* + *Trichoderma koningiopsis* grown in the winter. The experiments were conducted for three crop seasons, in a randomized blocks design arranged in a 2 x 8 factorial scheme, with four replications. The treatments consisted of the combination of eight cover crops with two microbial treatments. The bioagents and the mix of cover crops, especially the treatment 2 (corn) and the mixes 3 (millet, *Crotalaria ochroleuca*, black oat, white oat, buckwheat and coracana grass) and 5 (black oat, buckwheat, millet, Piatã grass and *C. Ocholeuca*), provided significant increases in the soil chemical and biological quality, with increases in the contents of Ca, Mg, K, H + Al and organic matter, as well as in the main soil pathogens that affect the bean crop, concerning fallow. In addition, there was an increase in the number of pods per plant and grains per pod. The use of these technologies provided savings, if compared to the use of chemical fertilization.

KEYWORDS: *Phaseolus vulgaris* L., multifunctional microorganisms, sustainable agriculture.

RESUMO

Efeito de bioagentes e coberturas vegetais
em atributos do solo e no desenvolvimento de feijoeiro

A busca por técnicas de cultivo que proporcionem benefícios produtivos, sociais e ambientais ao agroecossistema é de grande seriedade para a intensificação sustentável da agricultura. Objetivou-se determinar o efeito de coberturas vegetais nos atributos do solo e no desenvolvimento de feijoeiro tratado com o consórcio de bioagentes *Serratia marcencens* + *Trichoderma koningiopsis*, em cultivo de inverno. Os experimentos foram conduzidos por três safras agrícolas, com delineamento em blocos casualizados, em esquema fatorial 2 x 8, com quatro repetições. Os tratamentos foram compostos pela combinação de oito coberturas vegetais e dois tratamentos microbianos. Os bioagentes e a mistura de plantas de cobertura, especialmente o tratamento 2 (milho) e as misturas 3 (milheto, *Crotalaria ochroleuca*, aveia preta, aveia branca, trigo mourisco e capim coracana) e 5 (aveia preta, trigo mourisco, milheto, capim Piatã e *C. ochroleuca*), proporcionaram aumentos significativos na qualidade química e biológica do solo, com aumento nos teores de Ca, Mg, K, H + Al e matéria orgânica, bem como dos principais patógenos do solo que afetam a cultura do feijoeiro, em relação ao pousio. Além disso, houve aumento no número de vagens por planta e de grãos por vagem. O uso dessas tecnologias proporcionou economia, em relação ao uso da adubação química.

PALAVRAS-CHAVES: *Phaseoulus vulgaris* L., micro-organismos multifuncionais, agricultura sustentável.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) exhibits a high sociocultural and nutritional importance in Latin America and Africa, because it is a source of protein for low-income populations (Rezende et al. 2021b). The common bean production in Brazil, in the 2021/2022 crop season, was 1.7 million tons in about 1.4 million hectares, with an average grain yield of 1,010 kg ha^{-1} (Conab 2022). In the current agricultural context, management practices such as the use of bioagents and cover crops, which aim at the sustainability of agroecosystems, have been increasingly adopted, since they ensure the increase of grain yield, reduction of production costs and conservation of the environment (Rocha et al. 2021).

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Bioagents are beneficial multifunctional microorganisms that can be associated with the rhizosphere of plants and play a role in promoting growth by the composition of rhizopods and root exudates, causing several modifications directed to the processes of cycling and distribution of nutrients to the soil, release of solubilizing substances of phosphates and iron chelators, nitrogen biological fixation, production of enzymes such as lipases and ACC deaminase (1-aminocycloprane-1-carboxylate), synthesis of phytohormones and biological control (Frasca et al. 2021).

Recent studies have shown that the application of bioagents promotes a higher shoot and root biomass production of upland rice (Silva et al. 2023); higher grain yield and its components in soybean (Chagas Junior et al. 2022); higher leaf contents of N, Fe and Cu in oat plants (Santos et al. 2021); and Ca, P, S, Zn and Cu in *C. juncea*, *C. spectabilis* and *C. ochroleuca* (Lanna et al. 2021). Therefore, for the diversification of multifunctional microorganisms in a controlled environment, the co-inoculation of *Serratia marcencens* + *Trichoderma koningiopsis* provided significant increases in gas rates and yield of common bean (Rezende et al. 2021a).

Another extremely important practice is using cover crops in the off-season, acting as soil protectors and preceding the cultivation of the winter irrigated crop. These plants can improve the soil chemical and biological quality and increase the sustainability/ economic viability of the agricultural business (Oliveira et al. 2022). Cover crops can be used in monoculture or mixtures of species (mix), the latter being characterized as different intercropped species with agronomic characteristics of interest and aiming to improve the efficiency of production systems (Klein et al. 2022).

Andrade et al. (2021) described beneficial effects such as higher water retention in the soil and high phytomass production in common bean plants after cultivating *Urochloa* hybrids and *Panicum Maximum* cv. Aries. Klein et al. (2022) showed that using a mix of cover crops (black and white oats, forage pea, vetch, rye and turnip) suppressed weed incidences in the black bean crop.

Despite the benefits provided to production systems, few studies have assessed the effect of combining cover crops and bioagents. The hypothesis of this study is based on the application of the bioagents *Serratia marcencens* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736) in association with cover crops to stimulate the improvement of soil quality and performance of common bean plants. Thus, this study aimed to determine the effect of cover crops on soil attributes and the development of bean plants treated with the consortium of bioagents *Serratia marcencens* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736) grown in the winter.

MATERIAL AND METHODS

The experiments were conducted in a no-tillage system, at the experimental area of the Embrapa Arroz e Feijão (16º28'00"S, 49º17'00"W and 823 m of altitude), under irrigated conditions, in the 2020, 2021 and 2022 winter seasons.

The climate in the area is tropical Aw-type (tropical with wet summer and dry winter), according to the Köppen classification. In the three years of experimentation, the area was cultivated with soybean in the summer, cover crops in the off-season and common bean in the winter. The soil fertility analysis (layer 0-0.20 m) was performed before the beginning of the experimental period, with the following results: pH (H_2O) = 6.3; Ca^{2+} = 18.8 mmol$_c$ dm^{-3}; Mg^{2+} = 7.2 mmol$_c$ dm^{-3}; H + Al^{3+} = 11 mmol$_c$ dm^{-3}; Al^{3+} = 0 mmol$_c$ dm^{-3}; P = 3.1 mg dm^{-3}; K^+ = 142 mg dm^{-3}; Cu^{2+} = 0.6 mg dm^{-3}; Zn^{2+} = 1.5 mg dm^{-3}; Fe^{3+} = 6.7 mg dm^{-3}; Mn^{2+} = 13.8 mg dm^{-3}; organic matter = 31.2 g kg^{-1}. These values were determined following the methods by Teixeira et al. (2017). Three tons of limestone were applied in 2018; subsequently, there was no liming.

The rainfall and temperature during the bean cultivation in the three years of experimentation are shown in Figure 1.

The experimental design was randomized blocks arranged in a 2 x 8 factorial scheme, with four replications. The treatments consisted of a combination of eight cover crops {fallow (control treatment); corn (*Zea mays*); mix 1 [white lupin (*Lupinus albus*), buckwheat (*Fagopyrum esculentum*), white oat (*Avena sativa*), black oat, *Crotalaria ochroleuca*, *C. juncea*, fodder radish (*Raphanus sativus*) and coracana grass (*Eleusine coracana*)]; mix 2 (buckwheat, *C. spectabiliis*, fodder radish, black oat); mix 3 [millet (*Pennisetum glacum*), *C. ochroleuca*, black oat, white oat, buckwheat and coracana grass]; mix 4 (*C. spectabilis*,

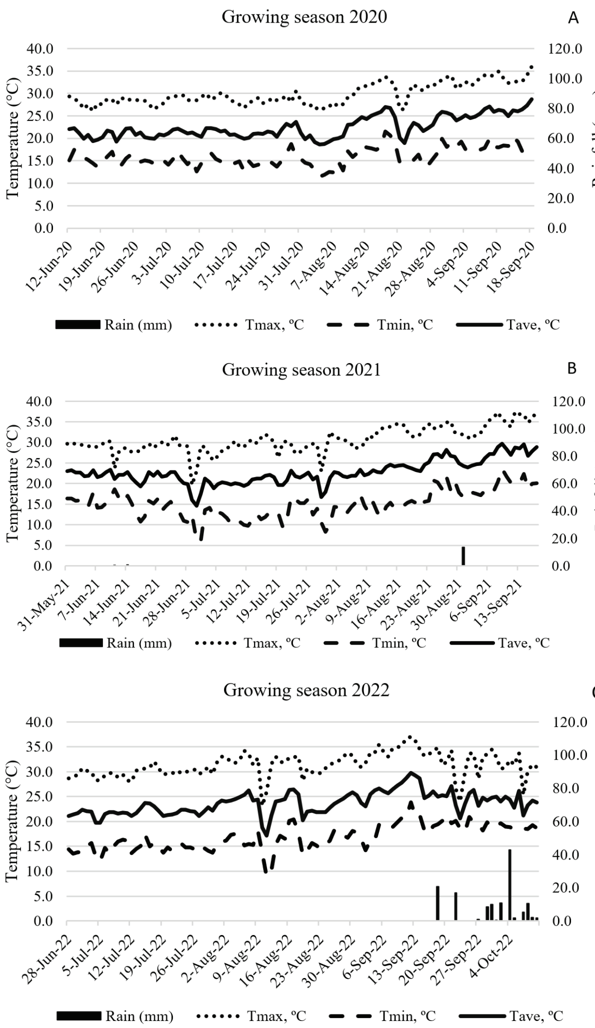

Figure 1. Temperature and rainfall during the bean cycle in the 2020, 2021 and 2022 winter seasons, in Santo Antônio de Goiás, Goiás, Brazil. Tmax: maximum temperature; Tmin: minimum temperature; Tave: average temperature.

C. breviflora, buckwheat and millet); mix 5 [black oat, buckwheat, millet, Piatã grass (*Brachiaria brizantha*) and *C. Ocholeuca*]; and mix 6 (black oat, fodder radish, white lupine, coracana grass and buckwheat)}, with two microbial treatments: consortium of *Serratia marcencens* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736) and control (no microorganism). The plots had dimensions of 5 x 10 m, and the useful area comprised the three central rows, disregarding 0.50 m on each side.

The consortium of the bioagents *Serratia marcenses* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736) was applied to the bean plants via sowing furrow, with the aid of a Micron equipment, at the dose of 300 mL ha^{-1} of bacterial suspension and 600 mL ha^{-1} of fungal suspension, and mixed immediately before sowing. *Serratia marcenses* (BRM 32114) is a rhizobacteria and *Trichoderma koningiopsis* (BRM 53736) is a fungus belonging to the collection of pathogenic and multifunctional microorganisms of the Embrapa Arroz e Feijão.

The *Serratia marcenses* (BRM 32114) suspension was prepared in nutrient broth, grown for 24 hours in solid medium 523 (Kado & Heskett 1970) at 28 ºC, and the concentration fixed in a spectrophotometer A540 = 0.5 (108 CFU). The *Trichoderma koningiopsis* (BRM 53736) was cultivated in a PDA culture medium, then multiplied in a substrate (rice grains). The suspension was prepared in distilled water, and the concentration was established in 1 x 10^8 conidia mL^{-1}. Before combining the isolates in the consortium, the compatibility test was made between them, thus ensuring that one isolate did not negatively influence the growth of the other.

The sowing of the cover crops occurred on March 15, 2020, March 12, 2021, and March 9, 2022, at a density of 30 kg ha^{-1} for the mix, 20 kg ha^{-1} for corn, and, for the control treatment (fallow), spontaneous plants grew in the area. No fertilization and irrigation were used during the development of the cover crops, remaining for 75 days in the first harvest and 70 days in the second and third harvests. The cover crops were desiccated with one application of glyphosate (1.8 kg ha^{-1} of acid equivalent) at 30 days before the common bean sowing.

The common bean sowing took place on June 12, 2020, May 31, 2021 and June 23, 2022, using 12 seeds m^{-2}. The BRS FC402 cultivar, characterized by a medium cycle, high yield potential, high commercial grain quality and resistance to anthracnose and fusarium wilt (Embrapa 2017), was used. In the fertilization applied to the sowing furrows, 200 kg ha^{-1} of MAP were used. In topdressing, 90 kg ha^{-1} of urea were applied, 50 % at the V4 stage and 50 % at the R6 stage (flowering). The doses were calculated according to Sousa & Lobato (2004), based on the results of the soil chemical analysis conducted before the experimental period. The crop management to keep the area free of weeds, diseases and insects followed the technical recommendations for the bean crop.

The following evaluations were carried out:

a) Density of pathogenic and beneficial fungi in the soil under cultivation of common bean: samples composed of soil (eight subsamples per plot) were collected at 15 days after sowing (DAS) of the common bean (0-10 cm soil layer) in the 2021 and 2022 crop seasons. After collection, the samples were sent to the laboratory to determine the density of the fungi *Fusarium solani* (Komada 1975), *F. oxysporum* (Martin 1950), *Sclerotinia sclerotiorum* (Napoleão et al. 2005), *Rhizoctonia solani* (Nash & Snyder 1962) and *Trichoderma* spp. (Weinhold 1977);

b) soil quality bioindicators: the activities of the soil enzymes β-glucosidase, phosphatase and arylsulfatase were considered bioindicators (Mendes et al. 2018). Thus, samples composed of soil (eight subsamples per plot) were collected at 15 DAS (0-10 cm soil layer) in the 2021 and 2022 crop seasons. The activity of the enzyme β-glucosidase was estimated according to Tabatabai (1994), using the substrate ρ-nitrophenyl-β-D-glucopyranoside. The activity of arylsulfatase was determined according to Tabatabai & Bremner (1970), by hydrolysis of the substrate ρ-nitrophenyl potassium sulfate. The quantification of phosphatase activity followed the methodology by Eivazi & Tabatabai (1977), using the substrate ρ-nitrophenyl phosphate. For the determination of β-glucosidase, phosphatase and arylsulfatase, the soil was incubated for 1 h at 37 ºC, and the activities were expressed as ρ-nitrophenol in mg g^{-1} h^{-1};

c) Soil physical-chemical characteristics: undisturbed samples were collected to determine the physical characteristics: density, macro, micro and total porosity (Araújo 2022). Deformed samples were collected to determine the chemical characteristics: macronutrient contents (P, K, Ca and Mg), pH and organic matter (Claessen 1997). The samples were collected at 10 DAS, at a depth of 0-10 cm, in the three years of experimentation for the chemical characteristics and in 2021 and 2022 for the physical characteristics;

d) Yield and production components: grain yield was determined mechanically after grain maturation at 96, 115 and 103 days after planting, in the 2020, 2021 and 2022 crop seasons, respectively. To determine the number of pods per plant, number of grains per pod and 100-grain mass, ten plants were manually collected within the useful area of each plot. The grain yield was determined in the harvest of each plot's useful area, with the grains drying (moisture correction to 13 %) and expressed in kg ha^{-1};

e) Estimation of nutrient input: the analysis was based on determining nutrients in the cover crops and fallow plants. Thus, the accumulation of nitrogen in the cover crops ranged from 79 to 139 kg ha^{-1}; phosphorus from 9.25 to 16.86 kg ha^{-1}; potassium from 113 to 167 kg ha^{-1}; calcium from 26.94 to 41.30 kg ha^{-1}; magnesium from 15.73 to 22.08 kg^{-1}; and sulfur from 9.30 to 17.33 kg ha^{-1}. The accumulation of micronutrients in the straw varied from 19.53 to 39.55 g ha^{-1} for copper; 1,566 to 3,209 g ha^{-1} for iron; 137 to 259 g ha^{-1} for manganese; and 116 to 219 g ha^{-1} for zinc.

The chemical fertilizers, correctives and soil conditioners most commonly used in the common bean cultivation (urea, MAP, potassium chloride, gypsum, iron sulfate, manganese, copper and zinc) were considered in the calculation. The potential savings in acquiring fertilizers were calculated considering the total amount of fertilizers equivalent to the accumulation of nutrients in the vegetable covers (Araújo 2022).

The data were submitted to analysis of variance and, when the F test detected significance, the means were compared by the LSD test ($p < 0.05$). The blocks and all the interactions were considered as random effects. The Sisvar 5.6 statistical package was used.

RESULTS AND DISCUSSION

The consortium of the bioagents *Serratia marcescens* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736) in common bean plants significantly altered the soil fertility (Table 1). The contents of Ca, Mg, K, H + Al and organic matter increased by 9.5, 5.2, 16.1, 9.9 and 8.6 %, respectively, if compared to the soil not treated with bioagents. Additionally, it was observed a soil fertility improvement after the insertion of the bioagents and cover crops in the experimental area, when compared to the soil fertility before the adoption of the management practices, except for the pH and K contents, which reduced by 6.5 and 14.2 %, respectively.

Araújo & Monteiro (2007) reported that bioagents, due to their metabolic potentialities, in which microorganisms degrade organic matter, cause the release of their mineral components that constitute the essential nutrients and chemical quality of soil cultivated with corn and pasture, since they promote a higher rate of availability of these nutrients. Tomelero et al. (2006) showed that the adoption of the organic production system with the use of cover

Table 1. Chemical attributes of soil cultivated with common bean in succession to cover crops and treated with the bioagents *Serratia marcenses* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736).

Treatments	pH H_2O	Ca	Mg	Al	H + Al	P	K	OM
		mmol$_c$ dm^{-3}				mg dm^{-3}		g kg^{-1}
Microbial microorganisms (MM)								
BRM 32114 + BRM 53736	6.03 a*	29.98 a	16.02 a	0.17	14.62 a	34.63	135.27 a	35.35 a
Control (without bioagents)	5.89 b	27.12 b	15.18 b	0.15	12.26 b	32.12	121.83 b	32.30 b
Cover crops (CC)								
Fallow (control)	5.95	28.25	15.03	0.08	14.00	35.40	132.58 ab	30.77 b
Corn	6.01	29.37	15.92	0.08	13.04	34.28	126.83 ab	34.05 a
Mix 1	5.93	28.01	15.48	0.20	14.20	34.80	117.08 b	34.99 a
Mix 2	5.97	29.12	15.82	0.12	12.79	39.72	147.75 a	34.67 a
Mix 3	5.91	28.22	15.32	0.33	13.70	37.11	150.00 a	34.02 a
Mix 4	5.99	28.94	16.17	0.08	12.51	33.40	118.20 b	33.42 a
Mix 5	5.97	28.01	15.63	0.29	13.08	36.30	116.04 b	33.77 a
Mix 6	5.92	28.50	15.42	0.12	14.16	35.67	119.66 b	33.94 a
Crop season (CS)								
2020	6.06 a	28.16	15.32	0.10	12.69 b	29.98 b	149.73 a	32.76 b
2021	6.06 a	28.25	15.46	0.12	12.73 b	30.02 b	143.92 a	32.99 b
2022	5.73 b	29.32	16.06	0.28	15.05 a	40.79 a	88.53 b	35.91 a
Anova factors (p-value)								
MM	0.000	0.000	0.027	0.726	0.000	0.268	0.038	0.000
CC	0.818	0.953	0.861	0.207	0.732	0.757	0.030	0.020
CS	0.000	0.318	0.257	0.310	0.001	0.000	0.000	0.000
MM * CC	0.070	0.069	0.059	0.214	0.107	0.516	0.554	0.132
Standard deviation	0.220	4.850	2.490	0.380	4.240	11.300	52.560	3.040
CV (%)	3.840	16.500	16.860	47.080	30.200	36.930	34.770	12.280

* Means followed by the same letter do not differ by the LSD test ($p < 0.05$). Mix 1: white lupine, buckwheat, white oat, black oat, *Crotalaria ochroleuca*, *C. juncea*, fodder radish and coracana grass; mix 2: buckwheat, *C. spectabilis*, fodder radish and black oat; mix 3: millet, *C. ochroleuca*, black oat, white oat, buckwheat and coracana grass; mix 4: *C. spectabilis*, buckwheat, millet and *C. breviflora*; mix 5: black oat, buckwheat, millet, Piatã grass and *C. ochroleuca*; mix 6: black oat, fodder radish, white lupine, coracana grass and buckwheat.

crops also increased the content of organic matter and microbial biomass in the soil, when compared to the soil under a conventional production system. According to these authors, the organic production system stimulated the improvement of biological quality and soil productivity.

Concerning the effect of cover crops on the fertility of the soil cultivated with common bean, there was an increase of 10.8 % in the organic matter content in the areas with cover crops, in comparison to the fallow area (Table 1). However, the macronutrient contents did not differ from each other, except for the K content, to which a reduction was observed in the soil cultivated with common bean in succession to the mixes 1, 4, 5 and 6. It is emphasized that the corn and the mixes 2 and 3 contributed to a higher K content in the soil cultivated with common bean; however, they did not differ significantly from the soil under fallow (control treatment).

Similar results were found by Michelon et al. (2019), who observed an increase in the contents of K and organic matter in the soil cultivated with winter cover crops (black oat, vetch, turnip, blue lupine, forage pea and ryegrass), single and intercropped, maybe due to the greater availability and low decomposition of straw, and insertion of crops such as oat, which has a high content of lignin, favoring the increase of organic matter. These authors also reported that the non-observance of increased levels of other macronutrients, in general, was associated with the short time of cover crops cultivation and the fact that that action is only effective when the practice becomes commonplace. This may also have occurred in the experiments, since the cover crops were established only in the years of experimentation (2020, 2021 and 2022).

According to Araújo & Monteiro (2007), soil quality is defined as the ability of the ecosystem to support biological productivity and promote plant health. Thus, if considering only microbial biomass, few changes in soil quality are observed; however, the use of bioagents associated with higher contents of organic matter, food for microbial biomass, can increase the availability of nutrients in the soil under different vegetable toppings. The biochemical processes associated with the decomposition of organic matter and consequent immobilization and mineralization of nutrients by the soil microbiota (Sobucki et al. 2019), influenced by temperature, pH, humidity and carbon/nitrogen ratio, are closely related to each other. Therefore, the relationship among bioagents, organic matter and exudate production in the root system of crops contributes to the entry of organic compounds in the soil-plant system and cementing agents in the soil profile, maintaining and increasing the quality of the agroecosystem (Sobucki et al. 2019).

The presence of the bioagents *Serratia marcescens* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736) significantly altered the activity of the enzymes β-glycosidase, phosphatase and arylsulfatase in the soil under common bean cultivation (soil quality bioindicators) (Table 2). The increase reached 10.8 % for the β-glucosidase activity, 14.8 % for phosphatase and 29.9 % for arylsulfatase, when compared to the soil that was not treated with bioagents. In addition, there was a greater activity of phosphatase in the soil under cultivation of common bean in succession to the mix 2 and arylsulfatase in the soil under cultivation of common bean in succession to the mixes 1, 2 and 5; however, they did not differ significantly from the soil under fallow. The action of microorganisms can alter the bioindicators of soil quality by their enzymatic activity, which can secrete extracellular enzymes such as cellulose and lignin, and the amount of nutrients in the soil, which, when there is a consumption of essential nutrients (nitrogen and phosphorus), indirectly affect the enzymatic activity (Casali et al. 2016).

The soil cultivated with the mix 2 showed an increase of 9.5 % in the phosphatase activity, while the soils cultivated with the mixes 1, 2 and 5 showed increases of 2.8, 4.0 and 4.0 %, respectively, in arylsulfatase activity, when compared to the soil under fallow. The cover crops used alone or in the mix in this study were able to provide several benefits to the plant, since their root systems may have improved the soil structure, favoring the root growth of common bean plants; its roots may have formed continuous channels, allowing water and nutrients absorption in subsurface layers and along with microbiota; silt micro aggregates, clay microstructures and matter particles may have promoted good conditions for the action of soil enzymes, which, consequently, increased nutrient cycling (Casali et al. 2016).

Table 2. Quality bioindicators of soil cultivated with common bean in succession to cover crops and treated with the bioagents *Serratia marcenses* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736).

Treatments	β-Glicosidase	Fosfatase	Arylsulfatase
	ρ-nitrophenol (mg g^{-1} h^{-1})		
Microbial microorganisms (MM)			
BRM 32114 + BRM 53736	47.22 a*	163.76 a	75.44 a
Control (without bioagents)	39.96 b	142.58 b	58.08 b
Cover crops (CC)			
Fallow (control)	45.95	151.67 abc	68.04 ab
Corn	44.32	141.68 c	57.33 b
Mix 1	43.74	160.69 ab	70.00 a
Mix 2	45.75	167.67 a	70.87 a
Mix 3	42.62	147.28 bc	64.97 ab
Mix 4	40.79	150.78 abc	67.85 ab
Mix 5	41.05	156.38 abc	70.85 a
Mix 6	44.56	149.20 bc	64.18 ab
Crop season (CS)			
2020	38.93 b	152.69	78.12 a
2021	39.97 b	152.25	76.05 a
2022	52.66 a	154.73	44.06 b
Anova factors (p-value)			
MM	0.000	0.000	0.000
CC	0.375	0.118	0.215
CS	0.000	1.000	0.000
MM * CC	0.823	0.000	0.262
Standard deviation	9.460	23.820	23.210
CV (%)	21.120	20.190	28.480

* Means followed by the same letter do not differ by the LSD test ($p < 0.05$). Mix 1: white lupine, buckwheat, white oat, black oat, *Crotalaria ochroleuca*, *C. juncea*, fodder radish and coracana grass; mix 2: buckwheat, *C. spectabilis*, fodder radish and black oat; mix 3: millet, *C. ochroleuca*, black oat, white oat, buckwheat and coracana grass; mix 4: *C. spectabilis*, buckwheat, millet and *C. breviflora*; mix 5: black oat, buckwheat, millet, Piatã grass and *C. ochroleuca*; mix 6: black oat, fodder radish, white lupine, coracana grass and buckwheat.

Rossetto et al. (2023) described that, during wheat cultivation in succession to different cover crops (millet, sunn hemp, velvet bean, pigeon pea, fodder radish and buckwheat), isolated and in the mix, the soil showed an increased activity of the enzymes β-glicose and arylsulfatase. According to the authors, the increase in the yield of the successor crop (wheat) was correlated with the increase in the activity of these enzymes and the good condition of the soil due to the use of cover crops.

There was interaction between bioagents and cover plants in the activity of the phosphatase enzyme of the soil under bean cultivation (Table 3). The soil

Table 3. Interaction between bioagents and cover crops on phosphatase enzyme activity in soil cultivated with common bean in succession to cover crops and treated with *Serratia marcenses* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736).

Cover crops	With bioagents	Without bioagents
	Phosphatase	
	ρ-nitrophenol (mg g^{-1} h^{-1})	
Fallow	169.71 Ab*	133.64 Bbc
Corn	148.11 Ac	135.25 Aabc
Mix 1	162.99 Abc	158.39 Aab
Mix 2	205.37 Aa	129.96 Bc
Mix 3	159.66 Abc	134.90 Babc
Mix 4	166.95 Abc	134.61 Babc
Mix 5	153.39 Abc	159.37 Aa
Mix 6	143.91 Abc	154.49 Aabc

* Means followed by the same letter do not differ by the LSD test ($p < 0.05$). Mix 1: white lupine, buckwheat, white oat, black oat, *Crotalaria ochroleuca*, *C. juncea*, fodder radish and coracana grass; mix 2: buckwheat, *C. spectabilis*, fodder radish and black oat; mix 3: millet, *C. ochroleuca*, black oat, white oat, buckwheat and coracana grass; mix 4: *C. spectabilis*, buckwheat, millet and *C. breviflora*; mix 5: black oat, buckwheat, millet, Piatã grass and *C. ochroleuca*; mix 6: black oat, fodder radish, white lupine, coracana grass and buckwheat.

with common bean showed a higher phosphatase activity when treated with bioagents and cultivated in succession to cover crops, except when the soils were cultivated in advance with the mixes 5 and 6. The biological diversity of the soil greatly impacts the biological processes that occur there and, consequently, the dynamics of the activity of enzymes that influence the cycling of nutrients essential to plants (Araújo et al. 2015). On the other hand, good soil chemical conditions also correlate with this environment's carbon and nitrogen contents (Matos et al. 2020).

The presence of the bioagents *Serratia marcenses* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736) decreased in 69, 63 and 83 % the population of the pathogenic fungi *F. solani, F. oxysporum* and *Rhizoctonia solani* in the soil cultivated with common bean. In contrast, no significant differences were observed in the propagules of *Trichoderma* spp. (beneficial microorganism) and *Macrophomina phaseolina* (pathogenic fungus), concerning the soil not treated with bioagents (Table 4).

Bioagents have direct and indirect mechanisms of action when they interact with plants. Indirectly, they act as antagonists of pathogens and are therefore considered biopesticides (Lopes et al.

Table 4. Soil biological analysis in common bean cultivation using *Serratia marcenses* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736) after cover crops mixes.

Treatments	*Fusarium oxysporum*	*Macrophomina phaseolina*	*Rhizoctonia solani*	*Trichoderma* spp.
	Propagule g^{-1} of soil			
Microbial microorganisms (MM)				
BRM 32114 + BRM 53736	3,978 b*	26.45	12.26 b	3,849
Control (without bioagents)	6,310 a	26.45	14.62 a	3,666
Cover crops (CC)				
Fallow (control)	5,922 a	52.91 b	11.16	3,314
Corn	5,548 ab	158.73 a	15.61	3,481
Mix 1	6,011 a	0.00 b	15.95	2,385
Mix 2	3,871 b	0.00 b	14.51	4,785
Mix 3	4,257 ab	0.00 b	13.65	2,883
Mix 4	5,833 a	0.00 b	19.88	4,176
Mix 5	5,975 a	0.00 b	22.66	3,932
Mix 6	3,734 b	0.00 b	16.18	3,103
Crop season (CS)				
2020	5,754 a	39.68 a	12.69 a	15.32
2021	4,534 b	13.22 b	12.73 b	15.46
Anova factors (p-value)	0.000	0.997	0.329	0.797
MM	0.016	0.000	0.351	0.194
CC	0.006	0.054	0.873	0.836
MM * CC	0.764	0.085	0.577	0.142
CV (%)	38.500	47.080	33.570	37.230

* Means followed by the same letter do not differ by the LSD test ($p < 0.05$). Mix 1: white lupine, buckwheat, white oat, black oat, *Crotalaria ochroleuca*, *C. juncea*, fodder radish and coracana grass; mix 2: buckwheat, *C. spectabilis*, fodder radish and black oat; mix 3: millet, *C. ochroleuca*, black oat, white oat, buckwheat and coracana grass; mix 4: *C. spectabilis*, buckwheat, millet and *C. breviflora*; mix 5: black oat, buckwheat, millet, Piatã grass and *C. ochroleuca*; mix 6: black oat, fodder radish, white lupine, coracana grass and buckwheat.

2021). Rhizobacteria such as the *Serratia* genus induce the plant's defense system against pathogens through competition for nutrients or by producing phytoalexins, exopolysaccharides and antioxidants, while fungi of the *Trichoderma* genus produce toxic secondary metabolites, antibiotics and enzymes of the plant's defense system such as chitinase, glucanase and peroxides (Lopes et al. 2021).

The cover crops affected the propagules of *F. oxysporum* and *Macrophomina phaseolina* in the soil cultivated with common bean (Table 5). The soils cultivated with the mixes 2 and 6 presented an average reduction of 35.8 % in the propagules of *F. oxysporum*, concerning the soil under fallow. On the other hand, the soil cultivated with corn showed an increase of 200 % in the propagules of *Macrophomina phaseolina*, concerning the soil under fallow.

Oliveira et al. (2009) reported a 13.9 % reduction in the percentage of organic residues colonized by the pathogen in the population of *F. oxysporum* and an increase of 194 % for propagule per gram in the soil of the *Trichoderma* spp. population, in succession to common bean. The authors reported that *F. oxysporum* exhibits a greater sensitivity to environmental changes, in addition to the common bean being a host and increasing the incidence of bean/bean rotation. The increase of the *Trichoderma* spp. population, a beneficial microorganism, may be related to its antagonism to other soil microorganisms. Moura et al. (2020) had a 28 % increase in the incidence of *Fusarium* spp. in soil under sugarcane and corn cultivation, after inoculating demi chromosomes from the forest. The authors justified that *Fusarium* spp. associated with other microorganisms can increase their abundance to enable their competition for space and nutrients.

The use of bioagents and cover crops did not alter the physical characteristics of the soil cultivated with common bean (Table 5). It is known that changes in the soil physical properties occur after a longer experimental time (Imbana et al. 2021). Imbana et al. (2021) also did not obtain improvement in the physical quality of the soil cultivated with legumes in succession to velvet bean, sunn hemp, pigeon pea and common bean. Soil physical properties are associated with the time and amount of mineral and organic components in the soil (Imbana et al. 2021).

The use of bioagents positively impacted the number of pods per plant and number of grains

Table 5. Physical characteristics of the soil cultivated with common bean in succession to cover crops and treated with the bioagents *Serratia marcenses* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736).

Treatments	Porosity (m^3 m^{-3})			Density
	Macro	Micro	Total	mg m^{-3}
Microbial microorganisms (MM) BRM 32114 + BRM 53736				
Control (without bioagents)	0.061	38.947	45.053	1.443
Cover crops (CC)				
Fallow (control)	0.046	28.382	39.391	1.456
Corn	0.059	33.819	42.289	1.432
Mix 1	0.047	34.742	41.869	1.442
Mix 2	0.058	33.662	41.970	1.459
Mix 3	0.056	33.776	42.169	1.441
Mix 4	0.040	34.609	43.401	1.449
Mix 5	0.060	33.255	41.775	1.451
Mix 6	0.055	32.710	42.226	1.464
Crop season (CS)	0.059	32.744	40.041	1.456
2021	0.006	0.007	0.006	0.362
2022	0.493	0.213	0.556	0.384
Anova factors (p-value)	0.462	0.827	0.218	0.774
Standard deviation	0.020	1.370	1.730	0.040
CV (%)	27.950	5.310	3.720	3.910

* Means followed by the same letter do not differ by the LSD test ($p < 0.05$). Mix 1: white lupine, buckwheat, white oat, black oat, *Crotalaria ochroleuca*, *C. juncea*, fodder radish and coracana grass; mix 2: buckwheat, *C. spectabilis*, fodder radish and black oat; mix 3: millet, *C. ochroleuca*, black oat, white oat, buckwheat and coracana grass; mix 4: *C. spectabilis*, buckwheat, millet and *C. breviflora*; mix 5: black oat, buckwheat, millet, Piatã grass and *C. ochroleuca*; mix 6: black oat, fodder radish, white lupine, coracana grass and buckwheat.

per pod in the bean plants, while the yield and 100-grain weight were not significantly altered (Table 6). The bioagents promoted an increase of 20 % in the number of pods per plant and 16 % in the number of grains per pod, concerning the bean plants not inoculated with bioagents. Similar results were found by Rocha et al. (2021), who noticed that black bean plants inoculated with *Azospirillum brasiliense* showed an average increase of 25 % in the number of pods per plant and grains per pod, but without increased yield and 100-grain weight, while Santos et al. (2022), in soybean plants, increased yield by 10.1 %, concerning the control, inoculating endophytic *Trichoderma*.

The use of cover crops promoted differences in the evaluated parameters of common bean, except for the number of pods per plant and 100-grain weight (Table 6). However, it was observed that the mixes 1 (white lupine, buckwheat, white oat, black oat, *Crotalaria ochroleuca*, *C. juncea*, fodder radish and coracana grass) and 5 (black oat, buckwheat, millet, Piatã grass and *C. ochroleuca*) obtained higher results than the other cover crops and fallow. It was also observed that common bean plants in succession to the mix 1 obtained the highest mean (20 %) for number of grains per pod and yield, in comparison to fallow and the other cover plants.

According to Rocha et al. (2021), constant drought conditions after the flowering of common bean plants and non-healthy seed lots can greatly influence the results of grain yield and its components. This fact was also observed in this study, since water deficit interference occurred in the reproductive phase of the common bean plants in the three years of experiment.

Table 6. Grain yield and yield components [number of pods per plant (NPP), number of grains per pod (NGP) and 100-grain weight (W100)] of common bean plants treated with *Serratia marcenses* (BRM 32114) + *Trichoderma koningiopsis* (BRM 53736) and cultivated after using a mix of cover crops.

Treatments	NPP unit	NGP unit	W100 g	Grain yield kg ha^{-1}
Microbial microorganisms (MM)				
BRM 32114 + BRM 53736	18 a*	3.24 a	22.93	992
Control	15 b	2.79 b	20.46	990
Cover crops (CC)				
Fallow	16	3.17 b	20.24	997 bc
Corn	18	2.54 c	23.22	943 c
Mix 1	16	3.78 a	19.81	1,197 a
Mix 2	17	2.40 c	20.04	877 c
Mix 3	16	2.94 bc	18.76	975 bc
Mix 4	16	2.86 bc	20.92	944 c
Mix 5	18	3.20 b	29.56	1,130 ab
Mix 6	16	3.24 ab	20.99	868 c
Crop seasons (CS)				
2020	15 b	2.37 b	20.00 b	935 b
2021	24 a	3.04 ab	30.14 a	1680 a
2022	11 c	3.34 a	14.94 b	359 c
Anova factors (p-value)				
MM	0.004	0.002	0.319	0.798
CC	0.843	0.000	0.454	0.563
MM * CC	0.199	0.007	0.494	0.901
Standard deviation	7.020	1.150	17.040	36.200
CV (%)	29.13	23.260	29.130	22.550

* Means followed by the same letter do not differ by the LSD test ($p < 0.05$). Mix 1: white lupine, buckwheat, white oat, black oat, *Crotalaria ochroleuca*, *C. juncea*, fodder radish and coracana grass; mix 2: buckwheat, *C. spectabilis*, fodder radish and black oat; mix 3: millet, *C. ochroleuca*, black oat, white oat, buckwheat and coracana grass; mix 4: *C. spectabilis*, buckwheat, millet and *C. breviflora*; mix 5: black oat, buckwheat, millet, Piatã grass and *C. ochroleuca*; mix 6: black oat, fodder radish, white lupine, coracana grass and buckwheat.

Different results were reported by Nunes et al. (2006), who observed increased grain yield and 100-grain mass in common bean plants grown in succession to the mix *P. Maximum* cv. Mombasa, *B. brizantha*, *B. Decumbens* and *P. Maximum* cv. Tanzania. They did not observe an increase in the number of pods per plant and number of grains per pod, but emphasized the importance of the correct choice of the species that precedes the main crop and the favorable climatic conditions, since the planting of vegetable covers in times of low moisture in the soil can provide a faster decomposition of the straw, as well as a greater evaporation of the water retained in the soil, consequently negatively impacting the development of the main crop. According to Klein et al. (2022), cover crops of the Fabaceae family, as sunn hemp and white lupine, and of the Poaceae family, as black and white oat and coracana grass, contribute additionally to improving the system's quality, as the Fabaceae plants fix the atmospheric nitrogen and the Poaceae plants have biomass with a lower decomposition rate.

As for the estimate of nutrient intake in the straw, it is observed that the use of bioagents promoted a reduction in the fertilization cost, concerning the control (fallow), for all nutrients (Table 7). The total amount of nutrients in the cover crops using *Serratia marcescens* + *Trichoderma koningiopsis* can generate an estimated reduction of up to USD 441.79 per hectare, when compared to the supply of quantities equivalent to the use of chemical

Table 7. Estimated value of nutrients provided by cover crop straw using equivalent amounts for chemical fertilizers, correctives and soil conditioners.

Treatments	N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn	Total
	USD ha^{-1}										
Microbial microorganisms (MM)											
BRM32114 + BRM53756	140.00	10.86	172.80	44.39	5.50	18.47	0.42	40.58	5.72	3.06	441.79
Without microorganisms	127.50	10.04	152.40	36.69	4.81	17.63	0.37	32.22	5.03	2.91	389.59
Cover crops (CC)											
Fallow (control)	98.75	7.40	135.60	33.93	4.25	13.02	0.27	23.49	3.77	2.03	322.50
Corn	173.75	13.49	198.00	49.14	5.86	19.22	0.48	45.75	6.88	3.83	516.40
Mix 1	131.25	9.60	135.60	38.90	4.91	17.14	0.33	33.93	4.37	2.77	378.79
Mix 2	121.25	10.11	166.80	45.38	5.38	24.82	0.28	38.10	4.26	2.54	418.92
Mix 3	126.25	10.05	135.60	33.43	4.40	14.55	0.36	28.52	4.79	3.06	360.99
Mix 4	161.25	12.55	200.40	51.63	5.96	20.52	0.55	48.14	6.52	3.62	511.14
Mix 5	132.50	9.78	183.60	38.16	5.63	15.09	0.52	48.00	7.12	3.57	443.98
Mix 6	113.75	10.60	136.80	33.68	4.86	20.01	0.38	25.28	5.39	2.50	353.23

Mix 1: white lupine, buckwheat, white oat, black oat, *Crotalaria ochroleuca*, *C. juncea*, fodder radish and coracana grass; mix 2: buckwheat, *C. spectabilis*, fodder radish and black oat; mix 3: millet, *C. ochroleuca*, black oat, white oat, buckwheat and coracana grass; mix 4: *C. spectabilis*, buckwheat, millet and *C. breviflora*; mix 5: black oat, buckwheat, millet, Piatã grass and *C. ochroleuca*; mix 6: black oat, fodder radish, white lupine, coracana grass and buckwheat. [1] Dollar quote held on January 31, 2023 (USD 12.5).

fertilizers, correctives and soil conditioners. Among the cover crops, the use of corn stood out, with an estimated reduction of USD 516.40 per hectare, a reduction of about USD 193.90, concerning fallow.

Araújo (2022), using cover crops in soybean cultivation, highlighted that the use of millet, *U. ruziziensis*, millet + *U. ruziziensis* and pigeon pea could generate savings of up to USD 658.79 and USD 760.77 per hectare, when considering the supply of equivalent amounts through chemical fertilizers, correctives and soil conditioners, especially potassium and nitrogen. Due to the complex dynamics of nutrients in the soil, it is not possible to specify the exact value of savings in money. Still, such technologies can promote the supply of nutrients, especially nitrogen, in the area by biological fixation in leguminous plant species. In addition, the mechanisms of action of multifunctional microorganisms enable the recycling of other nutrients from different soil profiles, by the ability of penetrating the roots of cover crops (Araújo 2022).

In summary, the combination of technological strategies such as multifunctional microorganisms (bioagents) and cover crops, already in use in the Brazilian agriculture, showed a promise to establish more sustainable agricultural practices, since they provide a better soil physicochemical quality and reduction of pathogenic fungi, increase the nutrients that can be made available for the subsequent crop and provide a reduction in the use of fertilizers and production costs, increasing the profitability and environmental contamination. In addition, they provide significant increases in the common bean grain yield and activity of the enzymes β-glicosidase and arylsulfatase, which are sustainability indicators.

CONCLUSIONS

1. Bioagents and mixes of cover plants, especially the corn treatment and the mix 4 (*Crotalaria spectabilis*, *C. breviflora*, buckwheat and millet), provide significant increases in the soil chemical and biological quality, with an increase in the contents of Ca, Mg, K, H + Al and organic matter, in addition to a decrease in the density of the main soil pathogens that affect the bean crop, concerning fallow, as well as an increase in the number of pods per plant and number of grains per pod in common bean plants in these mixes, concerning fallow;
2. The use of fallow (control treatment) and the mixes 1 (white lupin, buckwheat, white oat, black oat, *C. ochroleuca*, *C. juncea*, fodder radish and coracana grass) and 5 (black oat, buckwheat, millet, Piatã grass and *C. Ocholeuca*) provided increases in the common bean grain yield;
3. The use of these technologies provides a reduction in the contribution of fertilizers necessary for chemical fertilization by accumulating nutrients in the straw, concerning the fallow treatment.

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