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Sustainable agricultural practices to improve soil quality and productivity of soybean and upland rice

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Abstract

Combining cover crops with multifunctional microorganisms provides significant increments in the grain productivity of soybean and upland rice crops. However, there are still few studies that aimed to evaluate the effect of this combination on the productivity of agricultural systems. The objective of this study was to determine the effect of the mix of cover crops and the co-inoculation of multifunctional rhizobacteria on the quality of the soil, yield components, and on the grain yield of soybean and upland rice. The experimental design was in random blocks with 24 treatments in a 6x2x2 factorial scheme. Treatments were the combination of 6 (5 mix of cover crops (mixture of seeds of different cover crops species) and the control (fallow), 2 (upland rice and soybeans), 2 (co-inoculated (inoculation with two microorganisms) or not (no microorganisms), with four replications. Combination of cover crops provided an increase in Ca and Mn levels in the soil, increased activity of soil quality indicator enzymes (phosphatase and arylsulfatase), higher soybean yield, and higher number of upland rice panicles. The co-inoculation with multifunctional rhizobacteria led to an increase in phosphorus levels in the soil, the number of pods per plant, the mass of 100 grains for soybean cultivation, the number of grains per panicle, and productivity of upland rice. Therefore, a mix of cover crops, combined with the co-inoculation with multifunctional rhizobacteria are sustainable agricultural practices that can contribute to the improvement of soil quality and the increase of the productivity of soybean and upland rice crops.

Keywords: Glycine max; Oryza sativa; cover crop mix; multifunctional rhizobacteria; Bacillus sp; Serratia sp.; Azozpirillum brasilense; co-inoculation.

Abbreviations: PGPR_Plant Growt-Promoting Bacteria; NB_ without bacteria; WB_ with bacteria; MO_organic matter; N_Nitrogen; P_Phosphorus; K_Potassium; Ca_Calcium; Mg_Magnesium; Cu_Copper; Fe_ iron; Mn_Manganese; Zn_Zinc; H + Al_potential acidity**;** NPP_pod number per plant; NGP_grain number per pod; M100_mass of 100 grains; NP_number of panicle per meter; NGP_number of grains per panicle; M1000_mass of 1000 grains.

Introduction

The Cerrado region is responsible for about 60% of Brazil's agricultural production (Andrade et al., 2017), and soybean crops (*Glycine max*) and upland rice (*Oryza sativa)* are important crops for a sustainable production system in the region. Soybean is the main crop produced in the Cerrado. However, most farmers in the region carry out the monoculture of this crop and/or the continuous system of succession of soybean type (summer crop) /corn (crop) (Hosono e Caruso, 2016). This continuous succession tends to cause the physical, chemical and biological degradation of the soil, the increased incidence of pest insects, diseases and weeds, and consequently the increase in production costs and the decrease in crop productivity (Kappes and Zancanaro, 2014). The Cerrado is also the main biome for the cultivation of upland rice. However, the monoculture of this crop, for successive years in the same area is not the most sustainable way to maintain this crop in the field and leads to a decrease in productivity (Lanna et al., 2003).

Both soybean and upland rice crops still face other challenges in the region, such as low quality and high price inputs such as seeds and fertilizers, climatic factors, fluctuation of the selling price due to the influence of the national and international market; pest, disease and weed proliferation, soil compaction and nutrient deficiency (Inoue, 2019). However, some of these problems can be mitigated by the adoption of sustainable principles and practices, such as the No-till System (NTS) that involves crop rotation and the use of cover crops, and also with the use of multifunctional microorganisms (Cordeiro Junior et al., 2017).

One option for the inclusion of cover crops in the system would be cultivation during the crop, with the formation of straw on the soil surface, in order to ensure, or improve chemical, physical and biological quality, and the productive potential of the soil, in addition to providing significant increases in the productivity of successor crops (Nascente et al., 2013a). The cover crops accumulate nutrients in the plant material and release them during their decomposition, enabling the maintenance and improvement of soil fertility, as well as contributing to the increase of soil biological activity (Favarato et al., 2015). In addition, it can assist in the control of erosion, in the reduction of degradation, among others, being fundamental for the realization of sustainable soil management (Costa et al., 2013).

In addition, practices that minimize and/or optimize the use of inputs should be used in sustainable agricultural systems (Galindo et al., 2016). The inoculation of cultures with multifunctional microorganisms, in particular rhizobacteria, is one of these practices, acting directly in the promotion of plant growth. Multifunctional rhizobacteria are bacteria that favor the growth of plants by different mechanisms of action, such as: production of phytohormones, biological nitrogen fixation, solubilization of phosphorus and iron, production of siderophores, biological control of herbivores, insects and pathogenic microorganisms, among others (Glick, 2012; Santoyo et al., 2016). The greater root development provided by them contributes to a more efficient utilization of nutrients and fertilizers, resulting in an increase in the productivity of the system's component commercial crops (Galindo et al., 2018).

The efficiency of cover crops and multifunctional rhizobacteria can be further enhanced by the combination of species, called the cover plant mix (mixture of cover plant species) (Ziech et al., 2015) and co-inoculation (mixture of two or more multifunctional rhizobacteria) (Dalolio et al., 2018). However, there are still few studies that have evaluated the combination of mix of cover crops, cultivated in the pre-harvest, soybean and high-land rice co-inoculated by multifunctional microorganisms in agricultural systems. Therefore, the objective of this study was to determine the effects of the use of a mix of cover crops, cultivated in the off-season of soybean and upland rice (cultivated in summer), and the co-inoculation with multifunctional rhizobacteria for the chemical quality of the soil, the production components and productivity of soybeans and upland rice.

Results and discussion

Soil chemical properties and soil quality indicator enzymes before soybean cultivation

Significant differences were observed between cover plant mix and fallow only for pH, Ca content and the enzyme arylsulfatase in the area prior to soybean cultivation (Table 1). Mixes 4 and 5 provided significantly higher pH than the control (fallow) and mixes 1 and 3. The other mixes provided the soil with a pH similar to fallow. The covering plants can modify the pH of the rhizosphere by releasing organic acids (Grayston et al., 1997). The influence of cover crops on soil acidity depends on the species and the time of management of their residues, which can either increase or decrease the pH value of the soils, as can be observed in the results presented (Table 1). Although they generally provide greater acidification of the soil, some species may have higher concentration of basic cations in the phytomass, which favor an increase in pH, decreasing acidity (Lima Filho et al., 2014), as occurred with mixes 4 and 5.

Mix 4 (C. spectabilis, buckwheat, millet and *crotalaria breviflora*) deserves attention because it provided the highest Ca content and the highest activity of the enzyme arylsulfatase, being significantly higher than fallow land (Table 1). Tempesta (2020) also found significant Ca content in cultivated areas with a mix of cover crops (*Crotalaria ochroleuca* + forage turnip) (60.36 kg ha^{-1}) when compared to fallow land (26.72 kg ha^{-1}).

According to Mendes et al. (2018) for the enzyme arylsulfatase 30 values are considered low; between 31-70 considered moderate; and >71 adequate. In the present study, it can be observed that all treatments presented moderate value of the enzyme arylsulfatase (> 30), demonstrating that the soil in these areas has median quality, except for the control treatment (<30). However, the other treatments (mixes 1, 2, 3 and 5) were statistically similar to control. Research shows that the activity of some enzymes is important indicator of the sustainability of the cultivated soil (Evangelista et al. 2012), which is why the enzyme arylsulfatase is often used as an indicator of soil quality (Bouranis et al., 2019) like in this study.

On the other hand, the treatment with co-inoculation of the multifunctional rhizobacteria BRM 32114 (*Serratia* spp.) + BRM 63573 (*Bacillus* spp.) differed from the control treatment (without rhizobacteria) only for the phosphorus content (P), which was 95% higher (Table 1). The multifunctional rhizobacteria have several mechanisms of action to promote the growth of plants. Among them, solubilization capacity of inorganic phosphate is a characteristic of certain species of bacteria, independent of being in interaction with the plant; thus, improving the availability of this nutrient directly in the soil (Awais et al., 2017), as might have occurred in this study. They play a fundamental role in the solubilization of non-labile phosphorus, presenting themselves as bioinput, an alternative to the use of synthetic phosphate fertilizers (Awais et al., 2017), benefiting crops, decreasing production costs and increasing fertilization efficiency (Fabiańska et al., 2019). Machado (2015), detected endophytic bacterial lineages of the genus *Bacillus spp.* isolated from the pine nut culture with the potential to solubilise phosphate.

Soil chemical properties and soil quality indicator enzymes before upland rice cultivation

In the experimental area, where upland rice was cultivated, differences were observed for pH, H + Al (potential acidity), Mn content, and for the activity of phosphatase and arysulfatase enzymes and for treatments with a mix of cover crops (Table 2). There were no significant differences for any of the variables with the co-inoculation of the Ab-V5 and BRM 63573 multifunctional rhizobacteria isolates.

Regarding pH and potential acidity (H+Al), mixes 1 and 2 differed from mix 3, providing greater soil acidification, which is common to happen as mentioned above. However, there were no significant differences with treatments, including control (fallow land). Consistency is observed in the results when comparing the pH values with those of H + Al, since they are related variables. Similar to mixes 1 and 2, Franchini et al. (2000) also observed soil acidification, characterized by a decrease in pH and in the content of exchangeable Ca, and an increase in potential acidity (H + Al), after the cultivation of a cover plant.

Mix 3 provided with the highest value of manganese (Mn) in the soil being significantly higher than fallow (Table 2). Cover crops can produce high amounts of biomass, which during the period of degradation of the straw and after chemical desiccation (application of herbicide may release nutrients to the soil (Nascente et al., 2013). This is a fact that may explain the higher content of Mn in the area, where mix 3 was planted, compared to fallow.

Differences were found for the activity of phosphatase and arylsulfatase enzymes (Table 2). Mix 2 provided the highest value of phosphatase enzyme activity, and mix 3 for arylsulfatase activity, both being significantly higher than fallow. Kunze et al. (2011) reported high levels of acid phosphatase activity in cultivated areas with a consortium of cover crops. Freitas (2020) also verified a higher activity of the enzyme arylsulfatase in treatments with a mix of cover crops, probably due to the composition having coverage of different plant families. In all treatments, the values of arylsulfatase were low (30), which may indicate that the quality of the soil in this area (Table 2) was lower than in the area where soybean was planted (Table 1).

The straw of the cover crops may cause different carbon sources to contribute to the systems, which may have promoted microbial communities that provided greater activity of these enzymes (Bhat et al., 2017). In addition, the diversification of cover crops, with the use of mix (as carried out in this study), tends to cause greater dynamics of these microbial communities in the soil, which may lead to an increase in biological activity in relation to single cultivation (Sousa et al., 2020). Therefore, the lack of vegetation in fallow land tends to cause lower microbial activity, which was probably determinant for lower phosphatase and arylsulfatase activity compared to other treatments (mix 2 and 3, respectively).

According to Favarato (2015), among the chemical properties influenced by the use of roofing plants, we highlight the promotion of nutrient cycling in the soil, soil pH modification, mainly near the surface, increased organic matter in soil and humic compounds and increased base saturation among others. In the present study, a modification of pH and potential acidity was observed, a higher Ca and Mn content, and a higher value of phosphatase and arylsulfatase enzymes with the use of mixes of cover crops.

The few modifications found in the soil's chemical properties can be explained by conducting only one crop. According to Rosa et al. (2017), the increase in organic matter content and soil nutrients is often slow, and can take years to occur gradually. Although there have been no differences in these variables, the cultivation of cover plant mix protects the soil, because the soil is not exposed during the off-season or absent vegetation cover and over the years more expressive results can be obtained (Cunha et al., 2005).

Upland soybean yield components and grain yield

For soybean cultivation, there were no significant differences in treatments with cover plant mix, when compared to fallow for any of the production components (number of pods per plant (NPP), number of grains per pod (NGP) and mass of 100 grains (M100) (Table 3). On the other hand, for soybean productivity almost all treatments with a mix of cover crops were significantly higher than fallow land, with the exception of mix 2 (Table 3). Mix 5 provided an increase in productivity of 22%, followed by mix 4 with 17%, mix 3 of 15% and mix 1 of 13%, when compared to set-aside. Corroborating with our results, Fialho (2020) also verified higher yield of soybeans (3763 kg ha^{-1}) on the straw of Mix of cover crops (millet + *Crotalaria spectabilis* + *Urochloa ruziziensis*).

On the other hand, the treatment with co-inoculation of the rhizobacterial isolates BRM 32114 and BRM 63573 provided significant increments for the NPP (80 pods) and mass of 100 M100 grains (20.16 grams), when compared to the treatment without co-inoculation (67 pods and 19.58 grams, respectively) (Table 3). Silva et al. (2020) also found a higher number of pods per plant and a mass of 100 grains in co-inoculated soybeans with different isolates of multifunctional rhizobacteria, under controlled conditions. No significant increments were observed for productivity.

Upland rice yield components and grain yield

In relation to the cultivation of upland rice, the inverse of the soybean crop was occurred. No mix of cover crops had significant effect on productivity, providing a significant difference only for the panicle number component per meter (NP) (Table 4). In contrast, the co-inoculation with the Ab-V5 (*Azozpirillum brasilense*) + BRM 63573 (*Bacillus* spp.) rhizobacteria provided a significant increase in productivity when compared to treatment without co-inoculation.

Mix 1 provided a number of panicles per meter (121 panicles) significantly higher than fallow (105 panicles) (Table 4). Nascente and Stone (2018) also found a higher number of panicles per meter in upland rice grown on the straw of the corn + crotalaria mix (187 panicles per meter) compared to fallow (145 panicles per meter). In general, upland rice, when cultivated in the no-till system, has been having low productivity, not comparable to conventionally prepared soil (Nascente et al., 2013b). However, it was observed that the productivity obtained was much higher than the national average of 2,420 kg ha^{-1} (CONAB, 2022). These results indicate that the new rice cultivars and the correct management of the soil provided better development of the plants. The absence of significant differences between the plant covers tested may be an indicator that the soil presented good characteristics (Table 2) by providing a full development of the plants in all treatments.

Co-inoculation treatment with Ab-V5 and BRM 63573 isolates had a higher number of grains per panicle and higher productivity than treatment without co-inoculation. Fernandes et al. (2020) in home vegetation study evaluating inoculation and co-inoculation of upland rice by different multifunctional microorganisms. They found productivity significantly higher than control (no microorganisms) co-inoculation with the same test isolates (Ab-V5 + BRM 63573). Oliveira et al. (2018) evaluated two forms of inoculation with *Azospirillum brasilense* (seed and furrow) in maize associated with nitrogenous fertilization and reported that the use of inoculation, independent of nitrogenous fertilization, reflects increased grain production. These rhizobacteria (*Azospirillum* spp. and *Bacillus* spp.) may assist in increasing the production of grains from different crops, as observed with upland rice, due to different mechanisms of action, such as the production of growth-promoting compounds such as auxin, indolacetic acid, cytokinins and gibberellic acid, by nitrogen fixation, phosphorus solubilization, improvement of soil conditions, biological control, among others, that will positively influence plant productivity (Ribeiro et al., 2011).

Final considerations

Our results showed that the use of mixed cover crops in off-season agricultural systems involving soybean or high-land rice (grown in the summer) and co-inoculation of these crops with multifunctional rhizobacteria may provide increments in the soil and crop production factors.

Table 1. Soil chemical properties and soil quality indicator enzymes (0.0-0.10 m deep) after drying of the cover crops in October 2019, prior to soybean cultivation.

Treatment1	MO	pH	P	K	Ca	Mg	H +Al
Mix	g kg^{-1}			mg dm^{-3}	mmol$_c$ dm^{-3}		
Mix 1	34.01	5.96 b	19.15	142.25	31.56 ab	13.65	12.12
Mix 2	32.16	6.00 ab	18.86	168.62	32.05 ab	13.24	11.50
Mix 3	34.32	5.95 b	20.82	146.87	32.23 ab	14.21	12.75
Mix 4	33.85	6.11a	19.71	173.25	34.15 a	14.72	9.50
Mix 5	32.96	6.10 a	21.45	167.62	30.66 ab	13.31	9.87
Fallow	31.52	5.93 b	17.97	149.50	29.40 b	12.77	12.75
Co-inoculation	g kg^{-1}			mg dm^{-3}	mmol$_c$ dm^{-3}		
With	34.53	5.93	18 a	170.50	33.32	14.47	10.96
Without	32.58	6.08	9,2 c	145.54	30.05	12.83	11.88
Treatment1	Cu	Fe	Mn	Zn	β-glicosidase	Fosfatase	Arylsulfatase
Mix	mg dm^{-3}				[p-Nitrophenol] mg g^{-1} h^{-1}		
Mix 1	1.36	15.50	34.68	5.05	34.01	145.57	33.30 ab
Mix 2	1.36	15.02	30.55	4.82	32.16	146.31	34.92 ab
Mix 3	1.37	14.87	32.81	5.06	34.32	169.68	37.48 ab
Mix 4	1.36	15.31	30.68	4.90	33.85	152.92	38.65 a
Mix 5	1.50	15.75	31.53	4.95	32.96	135.66	34.17 ab
Fallow	1.23	14.46	29.72	4.48	32.05	126.50	29.46 b
Co-inoculation	mg dm^{-3}				[p-Nitrophenol] mg g^{-1} h^{-1}		
With	1.41	15.38	34.04	5.44	42.45	151.58	34.57
Without	1.31	14.93	34.70	4.31	42.05	140.63	34.75

Means followed by the same letter in the column do not differ by Fisher's LSD test at 5% significance, through the statistical program R. Columns without letters, had no significant difference between treatments. MO= organic matter; H + Al: potential acidity. [1]**Mix 1:** (Ultra Mix) White lupin, Buckwheat, White oat, Black oat, Crotalaria ochroleuca, Crotalaria juncea, Forage turnip and Coracana grass; **Mix 2:** (Vitale Mix) Buckwheat, Crotalaria spectabilis, Forage turnip, Black oat; **Mix 3:** (Forage Mix) Millet, C. ochroleuca, Black oats, White oats, Buckwheat and Coracana grass; **Mix 4:** (Reduct Mix) C. spectabilis, Buckwheat, Millet and Crotalaria breviflora; **Mix 5:** Oats, Millet, Buckwheat, Piata grass and *C. ochroleuca*. Co-inoculation: With (BRM 32114 (*Serratia* spp.) + BRM 63573 (*Bacillus* spp.); Without (no microorganisms).

Table 2. Soil chemical properties and soil quality enzyme activity (0.0-0.10 m deep) after drying out of the cover crops in October 2019, prior to the upland rice crop.

Treatment1	MO	pH	P	K	Ca	Mg	H +Al
Mix	g kg^{-1}		mg dm-^{3}		mmolc dm-^{3}		
Mix 1	33.20	5.93 b	16.43	114.62	29.16	12.44	17.12 a
Mix 2	30.47	5.91 b	13.18	102.57	28.18	12.32	17.37 a
Mix 3	32.95	6.13 a	16.05	111.87	31.50	13.56	14.25 b
Mix 4	31.96	6.00 ab	13.88	128.37	29.38	12.87	16.25 ab
Mix 5	31.70	6.00 ab	15.75	125.12	29.38	12.42	15.25 ab
Fallow	31.08	6.05 ab	14.08	114.87	29.04	12.30	15.87 ab
Co-inoculation	g kg^{-1}		mg dm-^{3}		mmolc dm-^{3}		
With	34.54	6.00	17.80	122.54	30.39	12.64	15.42
Without	32.59	6.00	11.46	111.79	29.07	13.06	16.63
Treatments	Cu	Fe	Mn	Zn	β-glicosidase	Fosfatase	Arylsulfatase
Mix	mg/dm^{3}				[p-Nitrophenol] mg.g^{-1}h^{-1}		
Mix 1	1.41	15.27	28.58 ab	4.15	30.73	106.50 ab	24.65 ab
Mix 2	1.48	15.80	27.88 ab	4.55	27.61	129.83 a	22.38 b
Mix 3	1.46	16.22	30.06 a	5.62	33.65	120.37 ab	28.92 a
Mix 4	1.46	15.55	27.53 ab	4.62	33.03	117.50 ab	23.66 ab
Mix 5	1.52	16.90	28.10 ab	5.18	33.17	104.61 ab	26.51 ab
Fallow	1.48	16.11	25.12 b	4.50	30.27	100.20 b	22.11 b
Co-inoculation	mg dm^{-3}				[p-Nitrophenol] mg.g^{-1}h^{-1}		
With	1.47	15.63	27.72	5.10	32.49	114.23	25.75
Without	1.45	16.19	28.43	4.42	31.74	112.110	24.61

Means followed by the same letter in the column do not differ by Fisher's LSD test at 5% significance, through the statistical program R. Columns without letters, had no significant difference between treatments. MO= organic matter; H + Al: potential acidity. [1]**Mix 1:** (Ultra Mix) White lupin, Buckwheat, White oat, Black oat, Crotalaria ochroleuca, Crotalaria juncea, Forage turnip and Coracana grass; **Mix 2:** (Vitale Mix) Buckwheat, Crotalaria spectabilis, Forage turnip, Black oat; **Mix 3:** (Forage Mix) Millet, C. ochroleuca, Black oats, White oats, Buckwheat and Coracana grass; **Mix 4:** (Reduct Mix) C. spectabilis, Buckwheat, Millet and Crotalaria breviflora; **Mix 5:** Oats, Millet, Buckwheat, Piata grass and *C. ochroleuca*. Co-inoculation: With (Ab-V5 + BRM 63573); Without (no microorganisms).

Table 3. Number of pods per plant (NPP), number of grains per pod (NGP) and mass of 100 grains (M100) and soybean productivity, cultivated after a mix of cover crops, 2019/2020 harvest.

Treatments	NPP	NGP	M100	Productivity
Mix	unit	unit	g	kg ha^{-1}
Mix 1	77	1.89	19.57	3603 ab
Mix 2	80	1.78	19.75	3415 bc
Mix 3	69	1.85	20.12	3671 ab
Mix 4	72	1.97	19.75	3723 ab
Mix 5	75	2.01	19.87	3891 a
Fallow	69	1.87	19.17	3182 c
Co-inoculation			g^{-1}	kg ha^{-1}
With	80 a	1.91	20.16 a	3759
Without	67 b	1.87	19.58 b	3582

Means followed by the same letter in the column do not differ by Fisher's LSD test at 5% significance, through the statistical program R. Columns without letters, had no significant difference between treatments. NPP= pod number per plant; NGP= grain number per pod; M100= mass of 100 grains. [1]**Mix 1:** (Ultra Mix) White lupin, Buckwheat, White oat, Black oat, Crotalaria ochroleuca, Crotalaria juncea, Forage turnip and Coracana grass; **Mix 2:** (Vitale Mix) Buckwheat, Crotalaria spectabilis, Forage turnip, Black oat; **Mix 3:** (Forage Mix) Millet, C. ochroleuca, Black oats, White oats, Buckwheat and Coracana grass; **Mix 4:** (Reduct Mix) *C. spectabilis*, Buckwheat, Millet and *Crotalaria breviflora*; **Mix 5:** Oats, Millet, Buckwheat, Piata grass and *C. ochroleuca*. Co-inoculation: With (BRM 32114 (*Serratia* spp.) + BRM 63573 (*Bacillus* spp.); Without (no microorganisms).

Table 4. Production components (number of panicles per meter, number of grains per panicle and mass of 1000 grains) and productivity of upland rice, cultivated after a mix of cover crops, 2019/2020 harvest.

Treatments	NP	NGP	M1000	Productivity
Mix	m^{-1}	unit	g	kg ha^{-1}
Mix 1	121 a	85	21.38	3883
Mix 2	108 b	86	21.42	3906
Mix 3	110 ab	86	22.11	3676
Mix 4	115 ab	87	20.36	4140
Mix 5	116 ab	94	21.91	3913
Fallow	105 b	92	20.89	3426
Co-inoculation	m^{-1}		g^{-1}	kg ha^{-1}
With	115	95 a	21.71	4143 a
Without	110	82 b	20.97	3504 b

Means followed by the same letter in the column do not differ by Fisher's LSD test at 5% significance, through the statistical program R. Columns without letters, had no significant difference between treatments. . NP= number of panicle per metre; NGP= number of grains per panicle; M1000= mass of 1000 grains. [1]**mix 1:** (Ultra Mix) white lupin, buckwheat, white oat, black oat, crotalaria ochroleuca, crotalaria juncea, forage turnip and coracana grass; **mix 2:** (Vitale Mix) buckwheat, crotalaria spectabilis, forage turnip, black oat; **mix 3:** (forage Mix) millet, c. ochroleuca, black oats, white oats, buckwheat and coracana grass; **mix 4:** (Reduct Mix) c. spectabilis, buckwheat, millet and crotalaria breviflora; **mix 5:** oats, Millet, buckwheat, piata grass and c. ochroleuca. Co-inoculation: With: Ab-V5 (*Azospirillum brasilense*) + brm 63573 (*Bacillus* spp.); Without (no microorganisms).

The use of cover crops mix increased the levels of Ca and Mn and provided higher value of enzymes indicating the quality of the soil (phosphatase and arylsulfatase), increased the productivity of the soybean crop and the number of panicles of the upland rice crop. The co-inoculation with multifunctional rhizobacteria provided higher phosphorus content in the soil, higher number of pods per plant and mass of 100 grains for soybean cultivation and higher number of grains per panicle and productivity of upland rice. The results may be even more promising over the years, since the evaluations were made only in one crop, taking into account that these practices have cumulative effect on the soil.

In addition, cover crops and multifunctional rhizobacterias are environmentally sound practices that can contribute to making agricultural systems more efficient, productive and sustainable at low cost. In addition, the use of cover crops allows to reduce soil erosion and is better than fallow, because continuous fallow increases the number of weeds in agricultural areas, impairs soil quality and can negatively influence successor crops (Castro et al., 2011; Nascente et al., 2013). The multifunctional rhizobacteria can provide several other benefits for the plants and the soil, cited throughout the text, such as nitrogen fixation, production of phytormonies, production of siderophores, biological control of pathogens, among others (Glick, 2012; Santoyo et al., 2016).

Materials and methods

Area description

The experiments were conducted at Capivara Farm of the Embrapa Rice and Beans, in the municipality of Santo Antônio de Goiás-GO, (16º28'00"S, 49º17 '00"W and altitude 823 m) in the 2019/2020 harvest. The region has a climate Köppen, Tropical AW with an average temperature of 23.3 °C and an average of 1428 mm in rainfall. The minimum, mean and maximum temperature and precipitation were monitored during the experiment, from March 2019 to March 2020 (Figure SI).

The predominant soil in the region is classified as Latossolo Vermelho Ácrico (Santos et al., 2018). Before the installation of experiments, soil chemical analyses were performed according to the methodology described by Donagema et al. (2011), where: pH (H_2O) = 5.90; Ca mmolc dm^{-3} = 25.2; Mg mmolc dm^{-3} = 10.5; P (Mehlich) mg dm^{-3} = 9.0; K mg dm^{-3} = 137; Organic Matter g dm^{-3} = 30.95; sand g kg^{-1} = 342; silt g kg^{-1} = 164, and clay g kg^{-1} = 494, with a clayey texture.

Experimental design and treatments

The experimental design was random blocks with 24 treatments in a 6x2x2 factorial scheme, with four repetitions (Table SI). The treatments consisted of 5 Mix of cover crops and fallow, cultivated in the 2019 crop, after the cultivation of soybeans and upland rice (summer crop). The seeds of both soybean and upland rice were co- or not inoculated, with multifunctional rhizobacteria. The plots were 6 m x 10 m in length. The useful area of the plot was composed of the 3 central lines with 0.50 m on each side for both crops.

Management of cover crops

The following cover plant mixes were used: 1. Mix 1 (Ultra Mix) - White lupin (*Lupinus Albus), Buckwheat (*Fagopyrum esculentum*),* White oat *(Avena sativa*), Black oat (*Avena strigosa*), *Crotalaria ochroleuca*, *Crotalaria juncea*, Forage turnip (*Raphanus sativus) and* Coracana grass (*Eleusine coracana*); *2. Mix 2 (Vitale Mix)* - buckwheat, *crotalaria spectabilis,* forage turnip and black oats*; 3. Mix 3 (Forage* Mix*)* - Millet *(Pennisetum glaucum* cv. ADR 300), *C. ochroleuca*, Black oats, White oats, Buckwheat and Coracana grass; 4. Mix 4 (Reduct Mix) *C. spectabilis,* buckwheat, millet and crotalaria breviflora; 5. Mix 5 - Oats, Millet, Buckwheat, Piatã grass (Urochloa brizantha vc. Piatã) and C. ochroleuca, and 6. Fallow (control). There were no cultivars released for the majority of the cover crops used in the mix. Besides, in each mix we had the same number of seeds for each species. These mixes were sown without the use of fertilizer in March 2019, in the same area before the main crop, off-season, and the crops of soybean and upland rice were cultivated in the following summer. Spacing of 0.45 m between lines at a depth of 2 cm was used with the use of 10 kg ha^{-1} of seeds from each cover plant mix. The sowing was carried out with no-till fertilizer sower (Semeato, model Personale Drill 13, Passo Fundo, RS, Brazil) at a depth of 5 cm and without the use of fertilizers. The cover crops were not irrigated. The area was dried 20 days before the sowing of soybean and upland rice crops with spraying of glyphosate 4 L ha^{-1} (Roundup Original®, 1440 g of acid equivalent per ha^{-1}). Fifteen days after drying the cover crops were crushed with a straw crusher (Triton®), leaving the straw in the area.

Soybean management

Soybean cultivar BRS 6970 IPRO was sown mechanically, using a no-till fertilizer sower (Semeato, model Personale Drill 13, Passo Fundo, RS, Brazil) in the 0.45 m spacing with 20 viable seeds per meter, in October 2019, 20 days after the drying of the cover crops. Sowing fertilization was calculated based on soil analysis and crop needs (Souza and Lobato, 2004). Therefore, the amount of fertilizer placed at the time of sowing was 90 kg ha^{-1} of P_2O_5, as triple superfosphate, and 48 kg ha^{-1} of K_2O, as potassium chloride. For the supply of nitrogen for soybean culture, all seeds were inoculated before seeding with liquid inoculant (*Bradyrhizobium japonicum*), as recommended by the manufacturer. The cultural tracts were also performed according to the standard recommendations of the soybean crop to keep the area free of weeds, diseases and insects.

Upland rice management

The upland rice BRS 501 CL was sown mechanically, using a no-till seeder (Semeato, model Personale Drill 13, Passo Fundo, RS, Brazil), in the 0.35 m spacing with 80 viable seeds per meter, in December 2019, 20 days after the drying of the cover crops. Seedling emergence occurred at 5 days after sowing. The basic fertilization to be applied in the sowing grooves was calculated according to the chemical characteristics of the soil and the recommendations of Sousa and Lobato (2003). Therefore, seeding was 15 kg ha^{-1} of N as urea, 90 kg ha^{-1} of P_2O_5 as triple superfosphate and 45 kg ha^{-1} of K_2O as potassium chloride. Nitrogen coating with 60 kg ha^{-1} of N (such as urea) was carried out 40 days after rice emergence. The cultural practices were carried out according to the standard recommendations for a rice crop to keep the area free of weeds, diseases and insects.

Preparation of multifunctional rhizobacteria and coinoculation of seeds

We used the combination of the BRM *63573 (Bacillus sp.)* and BRM 32114 (*Serratia marcensces)* multifunctional rhizobacteria isolates for soybean cultivation, and BRM 63573 and Ab-V5 (*Azospirillum brasilense*) for upland rice. These rhizobacteria were identified and selected in upland rice fields, stored and preserved in the collection of Multifunctional Microorganisms from Embrapa Arroz e Feijão. The bacterial suspensions were prepared with nutrient broth and water from crops grown for 24 hours in a solid nutrient agar medium at 28 °C, and the concentration was fixed in spectrophotometer for A540 = 0.5 (108 CFU, Colony Formation Unit) (Filippi et al., 2011). Soybean seeds were inoculated with microbes for 4 hours and high-lying rice for 24 hours, as recommended by the Agricultural Microbiology Laboratory of Embrapa Arroz e Feijão.

Soil chemical analysis

For the chemical analyses, deformed samples were collected randomly in each plot, in the 0-10 layers. Thus, eight soil subsamples were collected per plot to form a composite sample, soon after drying the cover crops, being in September 2019 in the area where the soybean was planted and in November 2019 in the area where the upland rice was planted. The deformed samples were taken to the Agroenvironmental Analysis Laboratory of Embrapa Arroz e Feijão, dried in greenhouses with forced air circulation and passed through sieves (2 mm mesh). The soil chemical characteristics (pH, MO, P, H + Al, Al, K, Ca, Mg, Cu, Fe, Zn and Mn) were determined according to the methodology of Donagema et al. (2011). Soil pH was determined in a 0.01 mol L^{-1} suspension of $Cacl_2$ (1: 2.5 soil/solution). Exchangeable Ca and Mg were extracted with neutral KCl at 1 mol/L ratio 1:10 soil/solution and determined by titration with a 0.025 mol/L NaOH solution. Phosphorus and exchangeable K were extracted with a Mehlich 1 extraction solution (0.05 mol/L Hcl at H_2SO^4 0.0125 mol/L). The extracts were colorimetrically analyzed for P and flame photometry was used to analyze K. Soil organic matter was determined by the Walkley and Black method (1934).

Analysis of soil quality bioindicators

Soil quality was assessed by determining three enzymes: β-glycosidase, phosphatase acid and arylsulfatase. The same samples collected for the chemical analyses were used, at a depth of 0-10 cm. The activity of the β-glucosidase enzyme was estimated according to the method proposed by Tabatabai (1994), using the colorimetric determination of p-nitrophenol after incubation of the soil with specific substrate (p-nitrophenol-β-D-glycopyranoside). The phosphatase activity was determined according to the methodologies proposed by Ivani and Tabatabai (1970), by spectrophotometry, quantifying the released pnitrophenol after incubation of 1.0 g of soil in 0.2 mL of toluene, 4 mL of modified universal buffer (MUB). The activity of arylsulfatase (Tabatabai and Bremner 1970) was evaluated by hydrolysis of potassium p-nitrophenyl sulfate, the substrate of the enzyme, to p-nitrophenol (PNP) by incubating the soil sample for 1 h at 37 °C. Based on the obtained enzyme results, these were interpreted as low, moderate or high contents according to recommendation by Mendes et al. (2018).

Evaluation of soybean production and productivity components

Soybeans were harvested in February 2020 in the useful area of each plot, using a mechanical harvester. Soybean grains were weighed and productivity was adjusted to a moisture content of 13% and converted to kg ha^{-1}. The production components, including the number of pods per plant (NPP), the number of grains per pod (NGP) and the mass of 100 grains (M100) (adjusted for a moisture content of 13%), were evaluated in 10 randomly chosen plants per plot.

Evaluation of the production and productivity components of upland rice

The rice was harvested in March 2020 in the useful area of the plots, using a mechanical harvester. The grains were weighed, adjusted to a moisture content of 13% and converted to kg ha^{-1} to determine productivity. The plots were also evaluated regarding the number of panicles (NP), number of grains per panicle (NGP) and the mass of 1000 grains (M1000). The number of panicles was determined by counting them within 1.0 m of one of the lines in the useful area of each plot. The number of grains per panicle and the weight of 1000 grains (humidity content adjusted to 13%) were randomly evaluated in each plot.

Statistical analysis

The data were submitted to analysis of variance and the averages compared by means of the LSD test ("Least significant difference"), through the statistical program R.

Conclusion

The use of cover plant mix led to an increase in soil Ca and Mn levels, a higher value of soil quality enzymes (phosphatase and arylsulfatase), a higher productivity of soybean cultivation and the number of panicles in the upland rice crop. The co-inoculation with multifunctional rhizobacteria provided higher phosphorus content in the soil, higher number of pods per plant and mass of 100 grains for soybean cultivation and higher number of grains per panicle and productivity of upland rice. Therefore, a mix of cover crops and co-inoculation with multifunctional rhizobacteria are sustainable agricultural practices that can contribute to the improvement of soil quality and the development of soybean and upland rice crops.

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