

Cover plants inoculated with multifunctional microorganisms in the off-season in production systems of the Cerrado region

Plantas de cobertura inoculadas com microrganismos multifuncionais na entressafra em sistemas de produção da região do Cerrado

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Highlights

Microorganisms and cover crops are sustainable technologies.
Multifunctional microorganisms affected the accumulation of nutrients.
Plant covers reduced weeds.

Abstract

Agricultural practices such as the use of cover crops inoculated with microorganisms aim to transform productive systems into sustainable ecosystems, since it reduces negative impacts on the environment and production costs and at the same time, increases the productivity of the main crops by improving soil attributes. This study aimed to determine the effect of the application of beneficial rhizobacteria in cover crops grown in the off-season, the accumulation of nutrients by these plants and weed control. The experiments were conducted in an experimental area of Embrapa Arroz e Feijão, in the 2020, 2021 and 2022 seasons, with a randomized block design, in the 2x8 factorial scheme and four replications. The treatments consisted of the combination of 8 vegetable covers ((1. fallow/weeds), 2. Corn, 3. Mix 1 (White Lupin, Buckwheat, White Oats, Black Oats, *C. ochroleuca*, *C. Juncea*, Forage Turnip, Coram Cane); 4. 2 (Buckwheat, *C. spectabilis*, turnip, black oat); 5. Mix 3 (Millet, *C. ochroleuca*, black aveite, white oat, buckwheat, Coracan grass); 6. Mix 4 (*C. spectabilis*, buckwheat, millet and *C. breviflora*); 7. Mix (Oats, Buckwheat, Millet, Piatã and *C. Ochroleuca*); and 8. Mix 6 (Oats black, Turnip Forage, White Lupine, Coracan Grass, Buckwheat)) with or without microorganisms (rhizobacteria *Serratia marcescens* (BRM 32114) +

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Bacillus sp. (BRM 63573)). The evaluations consisted of the content of nutrients and dry biomass of the aerial part, using the LSD statistical test ($p < 0.05$). Vegetable toppings, especially corn, mix 4 and mix 5, treated with beneficial rhizobacteria increased the amount of phytomass at 60 days after planting, accumulation of macronutrients N, P, K, Ca and Mg and micronutrients Cu, Fe and Mn compared to the fallow treatment. Higher S content was found in the straws of mix 2 and mix 4 and Zn in corn straws, mix 4 and mix 5, regardless of microbial treatment. In addition, plant covers provided reductions in weed incidence. Therefore, plant cover and multifunctional microorganisms stand out as sustainable technologies, since the improvement of nutritional conditions and the amount of dry biomass of cover crops can directly reflect the availability of nutrients for the successor crop and greater protection and organic matter content for the soil.

Key words: Bioagents. Biomass. Plant cover. Beneficial effects. Nutrient.

Resumo

Práticas agrícolas, como o uso de plantas de cobertura inoculadas com microrganismos, objetivam transformar os sistemas produtivos em ecossistemas sustentáveis, uma vez que reduzem os impactos negativos ao ambiente e os custos de produção e, ao mesmo tempo, incrementam a produtividade das culturas principais por meio da melhoria nos atributos do solo. Este estudo objetivou determinar o efeito da aplicação de rizobactérias benéficas em plantas de cobertura cultivadas na entressafra, no acúmulo de nutrientes por essas plantas e no controle de plantas daninhas. Os experimentos foram conduzidos em área experimental em Santo Antônio de Goiás, Goiás, nas safras 2020, 2021 e 2022, com delineamento em blocos casualizados, no esquema fatorial 2x8 e quatro repetições. Os tratamentos consistiram na combinação de 8 coberturas vegetais ((1. pousio/plantas daninhas), 2. Milho, 3. Mix 1 (Tremoço Branco, Trigo Mourisco, Aveia Branca, Aveia Preta, *C. ochroleuca*, *C. Juncea*, Nabo Forrageiro, Capim coracana); 4. Mix 2 (trigo Mourisco, *C. spectabilis*, nabo forrageiro, aveia preta); 5. Mix 3 (Milheto, *C. ochroleuca*, aveia preta, aveia branca, trigo mourisco, Capim coracana); 6. Mix 4 (*C. spectabilis*, trigo mourisco, milheto e *C. breviflora*); 7. Mix 5 (Aveia, Trigo Mourisco, Milheto, Piatã e *C. Ochroleuca*); e 8. Mix 6 (Aveia preta, Nabo Forrageiro, Tremoço Branco, Capim coracana, Trigo Mourisco)) com ou sem microrganismos (rizobactérias *Serratia marcencens* (BRM 32114) + *Bacillus* sp. (BRM 63573)). As avaliações consistiram no teor de nutrientes e biomassa seca da parte aérea, utilizando o teste estatístico LSD ($p < 0,05$). Coberturas vegetais, com destaque para milho, mix 4 e mix 5, tratadas com rizobactérias benéficas aumentaram a quantidade de fitomassa, aos 60 dias após o plantio, acúmulo de macronutrientes N, P, K, Ca e Mg e micronutrientes Cu, Fe e Mn em comparação com o tratamento pousio. Maior teor de S foi encontrado nas palhadas do mix 2 e mix 4 e de Zn nas palhadas de milho, mix 4 e mix 5, independentemente do tratamento microbiano. Além disso, as coberturas vegetais proporcionaram reduções na incidência de plantas daninhas. Portanto, cobertura vegetal e microrganismos multifuncionais se destacam como tecnologias sustentáveis, uma vez que a melhoria das condições nutricionais e da quantidade de biomassa seca das plantas de cobertura podem refletir diretamente na disponibilidade de nutrientes para a cultura sucessora e maior proteção e conteúdo de matéria orgânica para o solo.

Palavras-chave: Bioagentes. Biomassa. Cobertura vegetal. Efeitos benéficos. Nutriente.

Introduction

Currently, the development of sustainable technologies in tropical agriculture is an indispensable condition for facing agricultural challenges and increasing productivity, as erosive processes and decomposition rates of organic matter are potentiated due to high temperatures (Michelon et al., 2019). In addition, the cultivation of reduced number of commodities such as soybean, cotton and corn results in serious agronomic problems such as soil exhaustion and incidence of pests and diseases, which can lead to the collapse of productive systems, since the soil becomes unproductive and production costs impractical (Adami et al., 2020).

Among the agricultural practices considered sustainable, it is mentioned the use of cover crops to protect the soil against erosion; besides improving both the physical properties of the soil, since it breaks down compacted layers and reduces water loss by evaporation due to the protection of the soil surface, as its chemical and biological properties by enriching the soil with higher organic carbon content (organic matter) cation exchange capacity and nutrient cycling due to biomass addition and degradation, as well as the ability to decrease weed seed bank (Aita & Giacomini, 2006; N. S. Pereira et al., 2016; M. A. Silva et al., 2021). In the agricultural production system of the Brazilian Cerrado, cover crops in the off-season (March to September) are widely used (Boer et al., 2007; Nascente & Crusciol, 2014).

The beneficial effect of the use of cover crops was reported by Adami et al.

(2020), in which they observed an increase in soybean and wheat productivity, at the same time as the suppression of Buva (*Conyza bonariensis*); L. S. Pereira et al. (2020) which reported increased grain yield of cowpea planted in succession; and Anschau et al. (2018), which showed improvements in soil physical properties, macroporosity and density, as well as high soybean productivity in succession to black oat ~~cultivation, vetch~~ and turnip, unmarried or intercropped. In recent decades, the use of more than one type of cover plant in the same area has been tested, since the diversity of plants with well contrasting characteristics in their root systems explores different soil depths. According to Anschau et al. (2018), this practice benefits the nutritional balance of the system, in addition to improving the physical and biological quality of the soil.

Another extremely important practice is the use of multifunctional microorganisms, more specifically rhizobacteria, which are able to promote plant growth since they act in the decomposition of biomass, availability of nutrients for plants such as phosphorus and iron, biological nitrogen fixation, production of phytohormones such as indole acetic acid (IAA), degradation of impurities, soil structure and increased plant tolerance to biotic and abiotic stress (M. A. Silva et al., 2020).

The beneficial effect of the use of multifunctional microorganisms was reported by Cruz et al. (2022), greater development of the root system in maize plants; Nascente et al. (2017), greater absorption of nutrients by upland rice plants and M. A. Silva et al. (2020) increase in soybean productivity. In addition, the use of *Bacillus subtilis* + *B. Megaterium* showed an increase in growth rates and leaf

contents N, Fe and Cu in oat culture (Santos et al., 2021); increase in root development and part of millet area (E. S. Borges et al., 2020), among others.

The hypothesis of this work is that the use of rhizobacteria *Serratia marcescens* (BRM 32114) + *Bacillus* spp. (BRM 63573) previously characterized as multifunctional and beneficial microorganisms for upland rice (Nascente et al., 2017; Sperandio et al., 2017; Nascente et al., 2019; Chagas et al., 2022) can also improve the development of cover crops and thus provide greater biomass production, greater nutrient absorption and greater weed control. Thus, this study aimed to determine the effect of the application of beneficial rhizobacteria on cover crops grown in the off-season, on biomass production, nutrient accumulation by these plants and weed control.

Material and Methods

Experimental conditions

The experiments were conducted in the field of the Embrapa Rice and Beans Experimental Station, located in Santo

Antônio de Goiás, GO, Brazil, 16°28'00" S and 49°17'00" W coordinated, and 823 m altitude, in the years 2020, 2021 and 2022. The climate is tropical savanna Aw (tropical with wet summer and dry winter) according to the Köppen classification (Köppen, 1918). The average annual rainfall is between 1500 and 1700 mm, and the average annual temperature is approximately 24.5 C, ranging from 4.2 to 34.8 C. During the cultivation of cover crops, temperature and rainfall data were recorded, with a total of 425 mm in the 2019/20 crop, 225mm in the 2020/21 crop and 145mm in the 2021/22 crop (Figure 1).

The cover crops were grown in the same area during the three years of experimentation, whose soil fertility analysis, performed before the installation of the experiment (2019/20), is described below: pH (H₂O) = 6,3; Ca²⁺ = 18,8 mmolc dm⁻³; Mg²⁺ = 7,2 mmolcdm⁻³; H + Al³⁺ = 11 mmoldm⁻³; Al³⁺ = 0 mmolcdm⁻³; P = 3,1 mg dm⁻³; K+ = 142 mg dm⁻³; Cu²⁺ = 0,6 mg dm⁻³; Zn²⁺ = 1,5 mg dm⁻³; Fe³⁺ = 6,7 mg dm⁻³; Mn²⁺ = 13,8 mg dm⁻³ and organic matter = 31,2 g kg¹. These values were determined following the methods proposed by Teixeira et al. (2017).

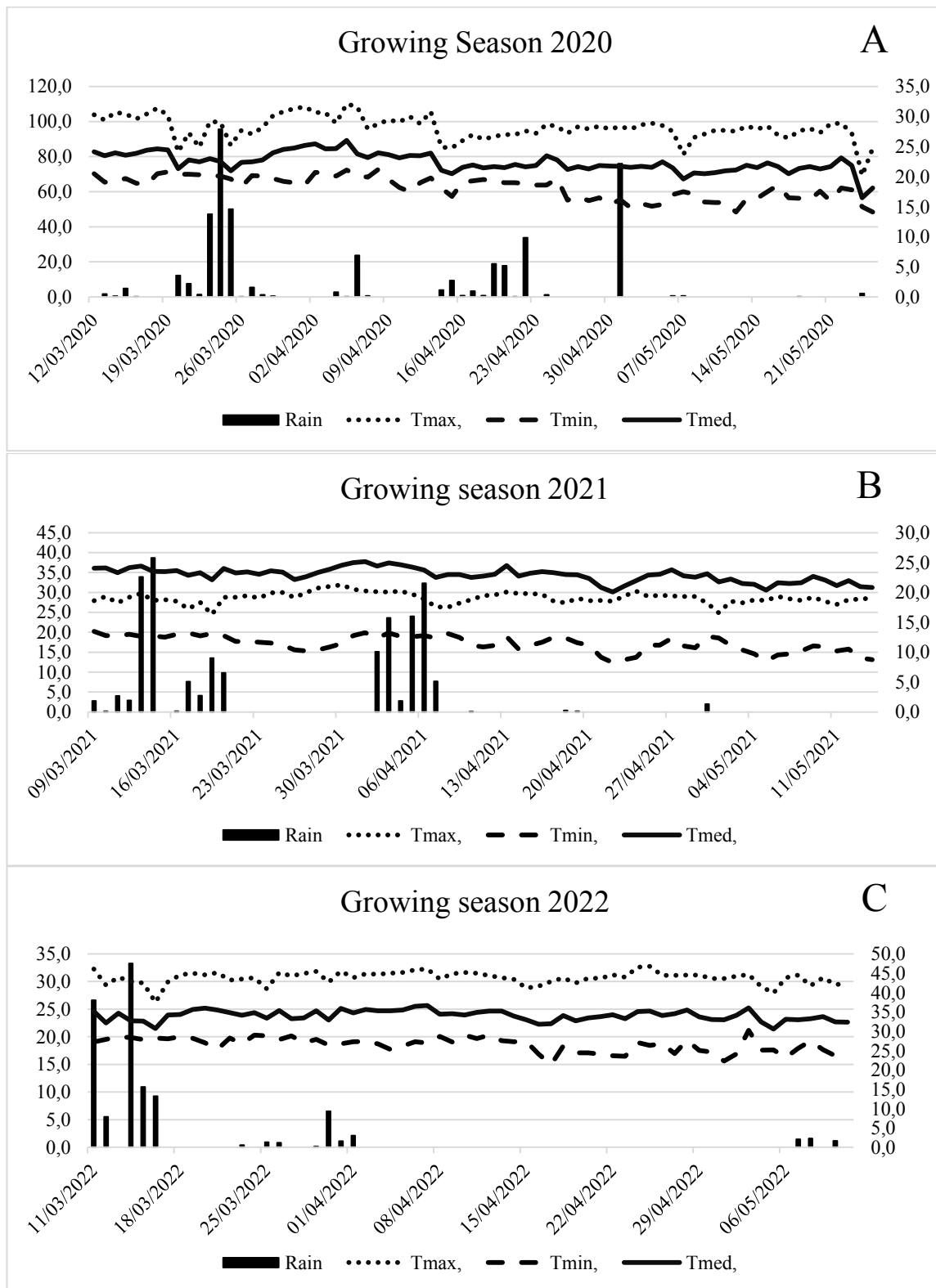


Figure 1. Minimum, maximum and average temperatures (C) and precipitation (mm) during the cultivation of cover crops, 2020 (A), 2021 (B) and 2022 (C). Santo Antonio de Goiás, Brazil.

Experimental design and treatments

The design was randomized blocks in factorial scheme 8x2, with four replications. The treatments consisted of a combination of 8 vegetable toppings (1. fallow/weeds, 2. Maize (*Zea mays* L.), 3. Mix 1 (White Lupin (*Lupinus Albus*), Buckwheat (*Fagopyrum esculentum*), White Oat and Black Oat (*Avena sativa*), Crotalaria (*C. ocheucrola* and *C. Juncea* (Brassica) Rapa forage) rapa) and coracana grass (*Eleusine coracana*); 4. Mix 2 (Buckwheat, Crotalaria (*C. spectabiliis*), turnip, black oat, white oat, buckwheat, Coraline, black oat); 5. Mix 3 (Millet (*Pennisetum glaucum*), *C. ochroleuca*, black oat, white oat, white oat, Coraccu, 6) (*C. spectabilis*, buckwheat, millet and *C. breviflora*); 7. Mix 5 (Oats, Buckwheat, Millet, Piatã (*Brachiaria brizantha*) and *C. Ochroleuca*); and 8. Mix 6 (Black Oat, Turnip Forage, White Millet, Buckthorn) with or without *Serratia marcescens* (BRM32114) + *Bacillus* sp. (BRM63573). The plots had dimensions of 5m x 10m and the useful area composed of the three central lines, disregarding 0.50 m on each side. Cover crops were sown after soybean harvest, cultivated in the summer 2019/20, 2020/21 and 2021/22, day 12/03/20 (first year), 09/03/2021 (second year) and 11/03/2022 (third year), using 150 kg ha⁻¹ of simple superphosphate. It was used the spacing of 0.45 m between rows, depth of 2 cm, with the use of 50 kg ha⁻¹ of seeds for each treatment of cover plants and 20 kg ha⁻¹ for corn. The harvest was carried out at 72 days after planting (DAP), in the first year, at 63 DAP (second year) and at 60 DAP (third year).

Preparation and application of microorganisms

The bacterial suspensions of *Serratia marcescens* (BRM 32114) and *Bacillus* sp. (BRM63573) were prepared separately with nutrient broth, grown for a period of 24 hours in liquid medium 523 (Kado & Heskett, 1970), at 28 °C, and the concentration fixed in spectrophotometer A540 = 0.5 (108 CFU per ml). The broth was stored in a cold chamber and the mixture of the suspensions of rhizobacteria was performed with a Micron-type furrow sprayer on the day of planting the cover plants, using 300 mL ha⁻¹ of each rhizobacteria suspension.

Reviews

Shoot dry biomass

The biomass production of the vegetation covers was determined in two moments: at 30 and 60 days after planting the cover plants (DBH). The biomass of cover crops and weeds were evaluated. Thus, it was used metallic square with an area of 1 m², randomly arranged in each plot. In the area of the square, the aerial part of the plants was collected, separated in cover plants and weeds. After that, they were dried in an oven with forced circulation of air, at 65 °C for 72 hours, until constant mass. Then, the mass (dry matter) of the shoot was determined, per plot, and the data were transformed into kg ha⁻¹.

Nutrient content

The dry matter of the aerial part of cover plants and weeds in the fallow area was ground in a Willey mill to determine the macronutrient content: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) and micronutrients: copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn), following the methodology proposed by Claessen (1997). To calculate the accumulation of macro and micronutrients, the nutritional contents multiplied by the biomass collected at 60 DAP were used.

Statistical analysis

The data presented in percentage increments in the results were calculated as a rule of 3 compared to fallow and control (without microorganisms). The data were submitted to analysis of variance and significant means compared by the LSD test for 5% probability, using the software Sisvar 5.6.

Results and Discussion

The application of the rhizobacteria *Serratia marcescens* (BRM 32114) + *Bacillus* sp. (BRM 63573) in cover crops resulted in increased contents of N (8.9%), P (7.5%), K (11.8%), Ca (17.3%) and Mg (12.6%), compared to cover plants not treated with beneficial microorganisms (Table 1). In addition, the macronutrient content, described above, was higher in the aerial part of the plant covers in relation to the treatment plants fallow/weeds.

The highlights were corn straw and mix 4 (*C. spectabilis*, buckwheat, millet and *C. breviflora*) among the vegetable covers evaluated, which showed an increase of 76.0 and 63.3% of N, 82.3 and 69.6% of P, 46.0 and 47.8% of K, 44.8 and 52.2% of Ca and 38.80 and 40.4% Mg, respectively, in relation to fallow/weeds. In relation to S, higher contents were observed in the straws of mix 2 and mix 4 of vegetation cover plants, regardless of microbial treatment.

The results allow to infer that the use of microorganisms provided greater absorption of nutrients by the vegetable toppings. Experiments of inoculation with *Serratia marcescens*, strain BRM32114, involving upland rice plants cultivated under different doses of N, showed its contribution to the increase of macronutrient content N, Ca and Mg (7, 11 and 9 %, respectively); shoot dry biomass (7.6%) and productivity (21.6%) (Nascente et al., 2019).

Table 1
Accumulation of macronutrients (kg ha⁻¹) in the aerial part of vegetable covers, cultivated between the months of March to May, and of weeds in an area under fallow, at 60 days after planting. Santo Antônio de Goiás, crops 2020, 2021 and 2022

Treatments	N	P	K	Ca	Mg	S
Microbial (M)*	(kg ha ⁻¹)					
BRM 32114 + BRM63573	112 a	13,57 a	144 a	35,51 a	20,38 a	13,19
Without microorganism	102 b	12,55 b	127 b	29,35 b	17,81 b	12,59
Cover plants (PC)						
Fallow	79 d	9,25 c	113 d	27,14 c	15,73 c	9,30 d
Corn	139 a	16,86 a	165 a	39,31 a	21,71 a	13,73 cd
Mix 1	105 bc	12,00 b	113 cd	31,12 bc	18,18 bc	12,24 bcd
Mix 2	97 bc	12,64 b	139 bc	36,30 a	19,94 ab	17,73 a
Mix 3	101 bc	12,56 b	113 d	26,74 c	16,29 c	10,39 c
Mix 4	129 a	15,69 a	167 a	41,30 a	22,08 a	14,66 a
Mix 5	106 b	12,23 b	153 ab	30,53 bc	20,85 ab	10,78 cd
Mix 6	91 cd	13,25 b	114 d	26,94 c	17,99 bc	14,29 ab
Factors ANOVA (p value)						
Microbial (M)	0,010	0,083	0,003	0,001	0,003	0,469
Cover plants (PC)	0,000	0,000	0,000	0,001	0,007	0,000
M * PC	0,000	0,025	0,001	0,000	0,001	0,004
CV (%)	23,44	30,87	28,75	40,24	31,59	44,10

*Means followed by the same letter do not differ from each other by the LSD test (P<0.05). 1 Tratamentos: Fallow/weeds; corn, Mix 1: White lupine, Buckwheat, White oats, Black oats, *Crotalaria ochroleuca*, *Crotalaria juncea*, Turnip forage and Coracana grass; Mix 2: Buckwheat, *Crotalaria spectabilis*, Nabo forrageiro, Averia preta; Mix 3: Millet, *C. ochroleuca*, Black oats, White oats, buckwheat and coracana grass; Mix 4: *C. spectabilis*, buckwheat, millet and *crotalaria breviflora*; Mix 5: Oats, buckwheat, Piatã grass and *C. ochroleuca*. Mix 6: Black Oats, Turnip, White Lupine, Coracan Grass, Buckwheat.

In this sense, microorganisms are being used in agriculture because they have the ability to convert unavailable nutrients to the available form through biological processes (Vessey, 2003; Kennedy et al., 2004). Among the rhizobacteria used in the present work, *Serratia marcescens*, strain BRM 32114, is a diazotrophic endophytic bacterium (Gyaneshwar et al., 2001), characterized as a producer of indole-3-acetic acid, cellulase, phosphatase, siderophores

and ACC deaminase (Martins et al., 2020) and the *Bacillus* spp. are rhizobacteria considered essential for nutrient cycling and great potential as biopesticide for the main rice disease, brusone (Chagas et al., 2022; Bettiol & Kimati, 1990).

In tropical areas low productivity soils prevail, in which the management of their fertility is a necessary practice to maintain the production system economically viable. The use of cover crops and their residues

(straw), as in the present experiment, the main objective is to provide essential nutrients to successive crops through the decomposition of their residues (Valadares et al., 2016). Species such as corn, millet and buckwheat (grasses) stand out for their high phytomass production, slow decomposition of their residues due to the high C/N ratio and the availability of nutrients, especially N, P and K, that help in the structural and nutritional quality of the soil and in the yield of the successor annual crops (Frasca et al., 2021a).

On the other hand, species such as crotalaria (leguminous) are characterized by the control of nematodes in the areas of annual crop production, rapid decomposition of their residues due to low C/N ratio, nutrient cycling and high biomass production (Pacheco et al., 2015). Naibo et al. (2018) described that the intercropping of leguminous species with grasses provides a wide soil cover corroborating with benefits to grain yield and no-tillage system. These intercropped systems, as shown in this study, can establish an efficient soil cover and, consequently, greater cycling of macro and micronutrients with reflex in the nutrition of the successor culture and in the healthier environment. Thus, it is found that the vegetable covers used in the present work accumulated nutrients and during the process of straw degradation can release them to be approved by the crop in succession.

Corroborating with the results obtained in this study, Moreira et al. (2014) reported that the use of sunflower, millet and turnip forage, as cover crops in corn production area, promoted nutrient enrichment in the soil and consequently,

accumulation on average 234% of P, 216% of K 274% of Ca, 237% of Mg and 261% of S in maize plants in succession. According to the authors, the results were evidenced by the absorption capacity and accumulation of these nutrients in the cover plants, and the residual capacity in the straw may favor the productive contents of the successor crop.

Scavazza et al. (2018) reported higher macronutrient content, N, P, K, Ca, Mg and S, in the soil under millet straw than under straw from other cover plants, *Crotalaria bilis*, *Cajanus cajan* L., *Canavalia ensis* L. and *Stilatorozum rimum*. As a result, higher contents of N (185.1 kg ha⁻¹), P (106.0 kg ha⁻¹), K (42.4 kg ha⁻¹), Ca (17.8 kg ha⁻¹) and Mg (7.9 kg ha⁻¹) were found in the aerial part of corn plants in succession. According to the authors, millet is characterized by its ability to produce a large amount of phytomass and, consequently, accumulate more macronutrients; in addition to its straw having a faster decomposition/ mineralization. As observed in the work, the increments in the accumulation of nutrients in the soil and the effect in interaction, may be associated with the multifunctional microorganisms that produce and excrete both organic acids and enzymes, which promote the release of phosphorus adsorbed on the surface of mineral particles or organic phosphorus originating from plant residues and microbial tissues, respectively, promoting their mobilization to the soil solution (Oliveira et al., 2021).

However, it is observed that the use of multifunctional microorganisms showed similar yield between cultivation with and without and the use of cover crops in macronutrients N, P, K and S (Table 2). In

addition, the use of corn as cover plant stood out among the others for the accumulation of N, P and K while mix 2 in the accumulation of S. Similar results were observed by Nascente et al. (2020) using *Serratia* and phosphate fertilization in upland rice plants obtaining similar results between the managements.

The authors report that the presence of rhizobacteria can provide mineralization of nutrients, especially non-labile P, making available in the soil solution for absorption of plants, because such microorganisms solubilize due to the secretion of organic acids and enzymes.

Table 2
Interaction between cover crops with or without *Serratia marcescens* (BRM32114) + *Bacillus* spp. (BRM63573) in the accumulation of N, P, K and S in cover crops in Santo Antônio de Goiás, GO. Crops 2019/2020, 2020/2021 and 2021/2022

Cover plants	With	Without	With	Without	With	Without	With	Without
	N		P		K		S	
kg ha ⁻¹								
Fallow	89 Ab	70 Bbc	10,8 Ab	7,6 Ac	134 Ab	92 Bb	11,2 Ab	7,4 Ab
Corn	168 Aa	110 Bbc	20,4 Aa	13,2 Bbc	210 Aa	119 Bab	17,9 Aab	9,5 Bb
Mix 1	112 Ab	99 Abc	12,7 Ab	11,2 Abc	126 Ab	119 Aab	12,0 Ab	12,0 Aabc
Mix 2	97 Ab	97 Abc	12,7 Ab	12,5 Aabc	131 Ab	145 Aab	18,5 Aa	16,2 Aab
Mix 3	103 Ab	98 Abc	12,6 Ab	12,5 Aabc	116 Ab	109 Aab	11,4 Ab	9,3 Ab
Mix 4	121 Ab	137 Aa	16,6 Ab	15,5 a	156 Aab	149 Aab	16,3 Aabc	12,2 Aab
Mix 5	108 Ab	105 Aab	12,2 Ab	14,0 Aabc	168 Aab	165 Aa	11,0 Ab	10,5 Abc
Mix 6	95 Ab	87 Bbc	12,2 Ab	12,2 Aab	116 Ab	111Aab	11,4 Bb	18,1 Aab

*Means followed by the same letter do not differ from each other by the LSD test (P<0.05). 1Tratamentos: Fallow/weeds; corn, Mix 1: White lupine, Buckwheat, White oats, Black oats, *Crotalaria ochroleuca*, *Crotalaria juncea*, Turnip forage and Coracana grass; Mix 2: Buckwheat, *Crotalaria spectabilis*, Nabo forrageiro, *Averia preta*; Mix 3: Millet, *C. ochroleuca*, Black oats, White oats, buckwheat and coracana grass; Mix 4: *C. spectabilis*, buckwheat, millet and *crotalaria breviflora*; Mix 5: Oats, buckwheat, Piatã grass and *C. ochroleuca*. Mix 6: Black Oats, Turnip, White Lupine, Coracan Grass, Buckwheat.

The application of the microorganisms *Serratia marcescens* (BRM 32114) + *Bacillus* sp. (BRM 63573) plants resulted in an increase in copper (Cu, 14.7%), iron (Fe, 25.9%) and manganese (Mn, 13.7%), compared to untreated cover crops (Table 3). According to Rezende et al. (2021), beneficial microorganisms are able to act from the outside of their plasma membrane, excreting

metabolites as siderophores capture micronutrients such as iron.

In general, the micronutrient content, Cu, Fe, Mn and Zn, was higher in the aerial part of cover plants in relation to weeds in fallow area (Table 3). The highlights were the corn straw, mix 4 (*C. spectabilis*, buckwheat, millet and *C. breviflora*) and mix 5 (Oat, Buckwheat, Capim Piatã and *C. ochroleuca*),

in which showed an increase of 75.6, 102.5 and 91.0% of Cu, 94.8, 9.73 and 104.89% of Fe, 78.5 and 75.9% of Zn, respectively, in relation to fallow/weed plants. In relation to

Zn, higher levels were observed in corn straw and mix 4 and 5 of cover crops, regardless of the microb treatment microbial.

Table 3

Accumulation of micronutrients (g ha⁻¹) in the aerial part of cover plants, cultivated between the months of March to May, and weeds in fallow area, at 60 days after planting (DBH). Santo Antônio de Goiás, years 2020, 2021 and 2022

Treatments	Cu	Fe	Mn	Zn
Microbial (M)*	(kg ha ⁻¹)			
BRM 32114 + BRM63573	30,29 a	2705 a	208 a	175
Without microorganisms	26,41 b	2148b	183 b	166
Fallow	19,53 c	1566 d	137 d	116 d
Corn	34,30 a	3050 ab	250 a	219 a
Mix 1	23,76 bc	2262 bcd	159 cd	158 cd
Mix 2	19,77 c	2540 bc	155 cd	145 c
Mix 3	25,67 bc	1901 cd	174 cd	175 b
Mix 4	39,55 a	3209 a	237 ab	207 a
Mix 5	37,30 a	3200 a	259 a	204 a
Mix 6	26,96 b	1685 cd	196 bc	143 c
Factors ANOVA (p value)				
Microbial (M)	0,003	0,018	0,016	0,136
Cover Plants (PC)	0,000	0,003	0,000	0,000
M * PC	0,141	0,058	0,202	0,014
CV (%)	43,88	36,59	36,52	25,83

*Means followed by the same letter do not differ from each other by the LSD test (P<0.05). 1 Tratamentos: Fallow/weeds; corn, Mix 1: White lupine, Buckwheat, White oats, Black oats, *Crotalaria ochroleuca*, *Crotalaria juncea*, Turnip forage and Coracana grass; Mix 2: Buckwheat, *Crotalaria spectabilis*, Nabo forrageiro, Azeria preta; Mix 3: Millet, *C. ochroleuca*, Black oats, White oats, buckwheat and coracana grass; Mix 4: *C. spectabilis*, buckwheat, millet and *crotalaria breviflora*; Mix 5: Oats, buckwheat, Piatã grass and *C. ochroleuca*. Mix 6: Black Oats, Turnip, White Lupine, Coracan Grass, Buckwheat.

The leguminous species have great tolerance to acidity and have the ability to fix atmospheric nitrogen in symbiosis with diazotrophic bacteria of the genera *Rhizobium* and *Bradyrhizobium*, promoting the increase

of total N content in the soil. In addition, they are promising in the eradication of soil pathogens, such as nematodes (Imbana et al., 2021).

In addition to the increase in macro and micronutrient contents, cover plants inoculated with the microorganisms *Serratia marcescens* (BRM 32114) + *Bacillus* sp. (BRM 63573) showed higher dry biomass of aerial part. The increase was 14.2 and 11.3% in plants with 30 and 60 days after planting (DBH), respectively, compared to cover plants not treated with beneficial

microorganisms (Table 4). Microorganisms are responsible for promoting plant growth due to the ability to provide nutrients such as phosphorus, produce siderophores for iron availability, synthesize phytohormones such as indole-3-acetic acid (IAA) and produce lytic enzymes such as chitinases and β -1,3-glucanases, in this case, acting in biological control (Frasca et al., 2021b).

Table 4
Dry biomass (kg ha⁻¹) of the aerial part of cover plants, cultivated between the months of March to May, and of weeds in an area under fallow (control treatment), at 30 and 60 days after planting (DAP). Santo Antônio de Goiás, harvest 2019/20, 2020/21 and 2021/22

Treatments	Biomass Cover Plant Material		Biomass Weeds	
	30 DAP	60 DAP	30 DAP	60 DAP
Microbial (M)*	(kg ha ⁻¹)			
<i>BRM 32114 + BRM63573</i>	628 a	5879 a	175	923 a
Without microorganisms	550 b	5280 b	183	653 b
Cover Plants (PC)	112 Ab	99 Abc	12,7 Ab	11,2 Abc
Fallow	444 d	4027 e	444 a	4027 a
Corn	646 ab	7570 a	187 b	266 b
Mix 1	646 ab	5527 cd	139 bcd	325 b
Mix 2	551 c	4760 d	92 d	222 b
Mix 3	582 bc	5137 cd	133 cd	335 b
Mix 4	655 a	6568 b	164 bc	211 b
Mix 5	653 ab	6037 bc	171 bc	302 b
Mix 6	541 c	5010 cd	105 d	316 b
Factors ANOVA (p value)				
Microbial (M)	0,001	0,002	0,558	0,010
Cover Plants (PC)	0,000	0,000	0,000	0,000
M * PC	0,000	0,009	0,000	0,552
CV (%)	22,09	19,75	43,19	42,17

*Means followed by the same letter do not differ from each other by the LSD test (P<0.05). 1 Tratamentos: Fallow/weeds; corn, Mix 1: White lupine, Buckwheat, White oats, Black oats, *Crotalaria ochroleuca*, *Crotalaria juncea*, Turnip forage and Coracana grass; Mix 2: Buckwheat, *Crotalaria spectabilis*, Nabo forrageiro, *Averia preta*; Mix 3: Millet, *C. ochroleuca*, Black oats, White oats, buckwheat and coracana grass; Mix 4: *C. spectabilis*, buckwheat, millet and *crotalaria breviflora*; Mix 5: Oats, buckwheat, Piatã grass and *C. ochroleuca*. Mix 6: Black Oats, Turnip, White Lupine, Coracan Grass, Buckwheat.

The biomass accumulation was higher in the aerial part of all cover plants, compared to weeds in fallow area (Table 4). Additionally, corn straws, mix 1 (white lupine, buckwheat, white oats, black oats, *crotalaria ochroleuca*, *crotalaria juncea*, turnip forage and coracana grass), mix 4 (*C. spectabilis*, buckwheat, millet and *C. breviflora*) and mix 5 (Oats, Buckwheat, Piatã Grass and *C. ochroleuca*), at 30 DAP, showed an average increase of 46.4% in the dry biomass of aerial part among the vegetation cover plants evaluated.

At 60 DAP, corn straw was highlighted, with an increase of 88.0% of dry biomass in relation to weeds in an area under fallow. At 60 DAP, the average accumulation of dry biomass of aerial part of the cover plants was higher at 5.8 t ha⁻¹, totaling 44% increase of phytomass in relation to weeds in an area under fallow. The application of *Serratia marcescens* (BRM 32114) + *Bacillus* sp. (BRM 63573) increased accumulation of dry biomass (41.3%) of weed shoots in the fallow area, at 60 DAP, compared to the area where the microorganisms were not applied. Based on these results, it is found that microorganisms provide increases in the development of several plant species.

Similar results of high dry biomass production by cover crops, as obtained in the present study, were found by Koenfender et al. (2016) when studying the potential use of intercropped systems of black oat, which white oat and vetch to obtain adequate levels of dry matter as a soil cover in winter, whose dry mass production was 4.2.4.5 and 2.9 t ha⁻¹, respectively. Pacheco et al. (2008), when evaluating the performance of cover crops on soybean seeding, reported that there was a significant difference between cover plants, brachiaria and maize, and weeds in

fallow area, where cover crops stood out with yields greater than 1.2 kg ha⁻¹ of dry mass at the beginning of the off-season. According to these authors, *Brachiaria* and corn straw (high C/N ratio), in quantity, is sufficient to compensate for the high rate of decomposition that occurs in tropical regions, which make them sustainable and viable straw alternatives in the Cerrado (Pacheco et al., 2008).

The reduction in the amount of weeds in the areas cultivated with cover crops is a result of biomass production, rapid growth which ensures competitive advantage over other species belonging to the weed community and tolerance to water deficiency (Table 4). Again, the highlight was corn that produced 7.5 t ha⁻¹ of straw, about 87% increase in phytomass in relation to fallow, as well as reduced the accumulation of phytomass of weeds by 93.4%. The use of corn in the off-season is a common practice in the Cerrados region, in addition to profitability to the producer, the crop can mitigate the loss of N, by cycling and immobilization in its phytomass, while its low rate of decomposition, high C/N ratio, establishes a larger volume of coverage for a prolonged period in the soil (E. C. Silva et al., 2008).

There was a mean reduction of 54.7 and 93.0% of the dry biomass of weeds at 30 and 60 DAP, respectively, in the areas cultivated with cover crops (Table 4). Cover crops using resources of the medium for their growth reduce the development and production of weed seeds, contributing to the exhaustion of seed banks in the soil (W. L. B. Borges et al., 2014). In the experimental area the weeds with the highest incidence were beldroega (*Portulaca oleracea*), bitter

grass (*Digitaria insularis*), black picão (*Bidens pilosa*), dairy (*Euphorbia heterophylla*), viola string (*Ipomoea purpurea*), santa Luzia herb (*Commelina erecta*) weeds (*Cenchrus echinatus*) and sawdust (*Sonchus oleraceus*). All these species have competitive potential with commercial crops and can reduce their yield and grain quality if no control method is adopted (Araújo et al., 2019). Based on these weed infestation reduction results in areas with cover crops, this is an important strategy to reduce weed infestation in agricultural areas, as well as reducing the use of herbicides and the cost of production.

As observed in the present experiment, in which cover crops provided reduction in weed biomass, Araújo et al. (2019) also showed that the cultivation of cover crops during the off-harvest period, in the pre-sowing of upland rice, reduced in 98.9% the production of dry biomass of weed shoots, at 75 DAP, in relation to the control treatment and 92.6% at 225 DAP. While, W. L. B. Borges et al. (2014) reported that the use of *U. ruziziensis* and *S. sudanense* in the off-season presented high suppressive effect on weeds, reduction in dry biomass productivity and weed density above 90%, and good soil cover (above 80%) until soybean flowering as a successor crop.

Therefore, the combination of technologies such as cover crops and multifunctional microorganisms, already in use in Brazilian agriculture, showed promise to establish more sustainable agricultural practices, since they provided greater production of biomass that protects the soil more, increase in the accumulation of nutrients that can be made available for the subsequent crop and reduce the biomass production of cover crops that provides

reduction in the use of herbicides and production costs, and reduce environmental pollution.

Conclusion

Multifunctional microorganisms provided significant increases in the accumulation of nutrients by the vegetable covers studied.

Vegetable toppings, especially corn, mix 4 and mix 5, treated with beneficial rhizobacteria increased the amount of phytomass at 60 days after planting, accumulation of macronutrients N, P, K, Ca and Mg and micronutrients Cu, Fe and Mn compared to the fallow treatment.

Higher S content was found in the straws of mix 2 and mix 4 and Zn in corn straws, mix 4 and mix 5, regardless of microbial treatment.

The vegetation covers provided reductions in the incidence of weeds.

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