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Research Article

Mixes of cover crops and *Trichoderma asperellum* for enhancing soybean crop yield and sustainability[1]

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ABSTRACT

Cover crops during the off-season and multifunctional microorganisms represent strategic technologies with potential to enhance the sustainability of soybean production. This study aimed to investigate the effects of cover crop mixes and the application of multifunctional microorganisms on the economic analysis, gas exchange, yield components and grain yield of soybean plants. The experimental design followed a randomized block pattern, in a 6 x 2 factorial arrangement, with four replications. The treatments consisted of six cover crop combinations [fallow (control); millet (*Pennisetum glaucum*) with crotalarias (*Crotalaria juncea*, *C. spectabilis* and *C. ochroleuca*); millet and pigeon pea (*Cajanus cajanus*); millet and *Urochloa ruziziensis*; millet, *U. ruziziensis* and pigeon pea; millet and buckwheat (*Fagopyrum esculentum*)], with or without the application of a *Trichoderma asperellum* pool. The combination of millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea showed the highest dry matter production and yielded the greatest nutrients content in the straw, which could provide a reduction in fertilization for the following crop. The soybean plants cultivated after millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea demonstrated elevated photosynthetic rates and improved the instantaneous water-use efficiency. The application of multifunctional microorganisms led to a 16 % increase in the photosynthetic rate of the soybean plants. The highest yield was achieved by the soybean plants cultivated in areas with millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea. Moreover, the application of multifunctional microorganisms contributed to increase the pod count per meter, grains per pod, mass of 100 seeds and overall soybean grain yield.

KEYWORDS: *Glycine max*, *Trichoderma asperellum*, multifunctional microorganisms, crop rotation, millet.

RESUMO

Mixes de plantas de cobertura e *Trichoderma asperellum* na melhoria da sustentabilidade e produtividade da cultura da soja

As plantas de cobertura na entressafra e os micro-organismos multifuncionais representam tecnologias estratégicas com potencial para aumentar a sustentabilidade na produção de soja. Objetivou-se investigar os efeitos de misturas de plantas de cobertura e da aplicação de micro-organismos multifuncionais na análise econômica, trocas gasosas, componentes de produtividade e produtividade de grãos de soja. O delineamento experimental foi em blocos casualizados, em arranjo fatorial 6 x 2, com quatro repetições. Os tratamentos consistiram de seis combinações de plantas de cobertura [pousio (controle); milheto (*Pennisetum glaucum*) com crotalárias (*Crotalaria juncea*, *C. spectabilis* e *C. ochroleuca*); milheto e feijão-guandu (*Cajanus cajanus*); milheto e *Urochloa ruziziensis*; milheto, *U. ruziziensis* e feijão-guandu; milheto e trigo mourisco (*Fagopyrum esculentum*)], com ou sem aplicação de pool de *Trichoderma asperellum*. A combinação milheto + *U. ruziziensis* e milheto + *U. ruziziensis* + feijão-guandu apresentou maior produção de matéria seca e maior teor de nutrientes na palhada, o que poderia proporcionar redução na adubação da cultura seguinte. As plantas de soja cultivadas após milheto + *U. ruziziensis* e milheto + *U. ruziziensis* + feijão-guandu apresentaram taxas fotossintéticas elevadas e melhoraram a eficiência instantânea do uso da água. A aplicação de micro-organismos multifuncionais levou a um aumento de 16 % na taxa fotossintética das plantas de soja. A maior produtividade foi alcançada pelas plantas de soja cultivadas em áreas com milheto + *U. ruziziensis* e milheto + *U. ruziziensis* + feijão-guandu. Além disso, a aplicação de micro-organismos multifuncionais contribuiu para o aumento da contagem de vagens por metro, grãos por vagem, massa de 100 sementes e produtividade geral global de grãos da soja.

PALAVRAS-CHAVE: *Glycine max*, *Trichoderma asperellum*, micro-organismos multifuncionais, rotação de culturas, milheto.

INTRODUCTION

The soybean crop [*Glycine max* (L.) Merril] holds significant relevance within the context of Brazilian agribusiness, not only to the domestic market, but also to the trade balance, being regarded as a key agricultural commodity (FAO 2020).

Globally, it stands as the primary oilseed crop, and, in the 2019/2020 harvesting season, the cultivated area reached 122.647 million hectares,

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yielding 341.76 million tons (FAO 2020). Of this, Brazil contributed with 126 million tons from a cultivated area of 36.9 million hectares (USDA 2020). Impressively, this output surpassed that of the second-largest global producer, the United States, which managed 96.841 million tons from a cultivated area of 30.36 million hectares (USDA 2020).

Given the critical importance of this worldwide crop and the global demand for sustenance, the pursuit of technologies that enhance development, increase soybean grain yield, mitigate abiotic stress-induced damages and confer heightened resistance to phytopathogens becomes paramount. Such endeavors not only curtail the use of pesticides and synthetic fertilizers, but also fortify production sustainability while fostering social, environmental and economic benefits (Bononi et al. 2020).

In this vein, the adoption of multifunctional microorganisms emerges as a promising tool toward sustainable technological solutions. These microorganisms engender favorable effects on plant growth, with discernible positive repercussions on grain yield (Barazani & Friedman 2000).

Brazilian farmers have a long tradition of inoculating nitrogen-fixing bacteria in soybean crops. Using this technology, Brazil has saved billions of dollars and reduced greenhouse gas emissions (Hungria et al. 2007). Nowadays, there is an increasing trend in the use of coinoculation of nitrogen-fixing bacteria with other microorganisms, such as *Azospirillum* sp. or *Trichoderma* sp. (Silva et al. 2020a). Chagas Junior et al. (2019) reported significant increases in soybean grain yield when coinoculated with *Bradyrhizobium japonicum* and *Trichoderma asperellum*.

The *Trichoderma* fungi genus exhibits the capacity to positively influence seed germination, crop development and overall yield, due to its propensity to stimulate growth, enhance plant nutrition, primarily via phosphorus solubilization (Silva et al. 2012), control phytopathogens (Bononi et al. 2020) and synthesize indoleacetic acid (Chagas et al. 2016). The economic significance of these fungi within agriculture remains substantial.

Research conducted by the Embrapa Arroz e Feijão and Universidade Federal Rural da Amazônia has led to the identification of four isolates of the *T. asperellum* fungus (UFRA.T06, UFRA.T09, UFRA.T12 and UFRA.T52), all of which exhibit favorable effects in promoting plant growth (França et al. 2015). Silva et al. (2020a, 2020b) observed a heightened photosynthetic capacity, increased dry matter, elevated nutrient content and enhanced yield in soybean plants treated with a consortium of *T. asperellum* isolates, either alone or in synergy with other microorganisms. In this way, four strains of *Trichoderma* sp. (UFRA.T06, UFRA.T09, UFRA.T12 and UFRA.T52) were sequenced and identified as *T. asperellum* and characterized as producers of phosphatase and cellulase. During the growth promotion of rice plants, the best results were achieved when *T. asperellum* was in a mix, instead of isolated (Silva et al. 2012). When co-cultivated with *Magnaporthe oryzae*, the same isolates produced the lytic enzymes chitinase (CHI), glucanase (GLU) and protease (PRO) (Silva et al. 2012, Sousa et al. 2021).

Simultaneously, the role of isolated or blended cover crops, which serve as precursors to the main crops, assumes relevance within agricultural systems. Their employment in a no-tillage system proves indispensable for improving soil quality and fostering the optimal development of subsequent crops. These cover crops impart enhancements in physical attributes, chemical aspects (Favarato et al. 2015) and biological traits (Almeida et al. 2016). Lima et al. (2012) demonstrated that soybean cultivation following *Avena strigosa* Schereb, *Raphanus sativus* L. and *Vicia sativa* L. facilitates a more robust seedling emergence. Moreover, Torres et al. (2015) revealed that soybean grain yield faces an increase when cultivated after diverse cover crops. For instance, when soybean succeeds *Pisum sativum* L., *Raphanus sativus* L. and *Avena strigosa* S., its yield reaches an average escalation of 12.2 %, when compared to fallow sowing (Deimling et al. 2019). Nascente et al. (2013a) highlighted that employing millet as a cover crop preceding upland rice cultivation results in the highest grain yield, when compared to other cover crop choices. Nevertheless, investigations involving the blending of millet with other cover crops and its consequent impact on agricultural crops such as soybean remain scarce.

Hence, the use of cover crops and multifunctional microorganisms within soybean cultivation stands out as a prospective strategy to ensure the sustainability of soybean production within the Cerrado (Brazilian Savanna) region. However, there is a lack of scientific studies that concurrently incorporate cover crops and multifunctional microorganisms within soybean cultivation.

The hypothesis of the present study is that a combination of cover crops cultivated in the off-season with *Trichoderma asperellum* provides better conditions for soybean crops, resulting in a significant effect on grain yield. Accordingly, this study aimed to investigate the effects of cover crop mixes and the application of multifunctional microorganisms on economic analysis, gas exchange, yield components and grain yield of soybean plants.

MATERIAL AND METHODS

The study was carried out within a rainfed no-tillage area at the Embrapa Arroz e Feijão, in Santo Antônio de Goiás, Goiás state, Brazil (16º29’47”S, 49º17’20”W and altitude of 805 m), during the 2018/2019 harvesting season.

The soil in this area is classified as Acrylic Red Latosol (Santos et al. 2018), a sandy clay loam, kaolinitic, thermic Typic Haplorthox (FAO 2006) with the following chemical and physical attributes recorded at the beginning of the experiment: pH (H_2O) = 6.0; Ca = 25.2 $mmol_c$ dm^{-3}; Mg = 10.5 $mmol_c$ dm^{-3}; P (Mehlich) = 9.0 mg dm^{-3}; K = 137 mg dm^{-3}; organic matter = 33.85 g dm^{-3}; sand = 342 g kg^{-1}; silt = 164 g kg^{-1}; clay = 494 g kg^{-1}. The soil physical and chemical attributes analysis was conducted in accordance with Donagema et al. (2011).

The region experiences a tropical Savannah climate, as per the Köppen classification (Alvares et al. 2013). This results in two distinct seasons: a dry period extending from May to September (fall/winter), followed by a rainy season from October to April (spring/summer). The average annual rainfall is 1,500-1,700 mm, while the annual mean temperature is 22.7 ºC, fluctuating between 14.2 and 34.8 ºC.

The experimental design followed a pattern of randomized blocks, with a 6 x 2 factorial arrangement, featuring four replications. The treatments comprised a combination of six cover crops (either in isolation or mix), as it follows: fallow (control); millet (*Pennisetum glaucum*) and crotalarias (*Crotalaria juncea*, *C. spectabilis* and *C. ochroleuca*); millet and pigeon pea (*Cajanus cajan*); millet and *Urochloa ruziziensis*; millet, *U. ruziziensis* and pigeon pea; millet and buckwheat (*Fagopyrum esculentum*), both with and without the application of multifunctional microorganisms - in this case, a consortium of *Trichoderma asperellum* isolates (UFRA.T06, UFRA.T09, UFRA.T12 and UFRA.T52) introduced during the soybean cultivation. Each plot measured 5.40 x 10 m in length, with a useful area defined by excluding a soybean line from each side and disregarding 0.50 m from each end.

The desiccation of the area was conducted at 15 days prior to the cover crop sowing and accomplished through the application of 4 L ha^{-1} [1,440 g of acid equivalent (a.e.) ha^{-1}] of glyphosate. The sowing of the cover crops took place on March 12, 2018, within a no-tillage system following the initial soybean cultivation. The fallow area was supplemented with 100 kg ha^{-1} of simple superphosphate. Mechanized sowing was conducted with a spacing of 0.45 m between rows and a sowing depth of 2 cm. A combination of 10 kg ha^{-1} of millet and 20 kg ha^{-1} of seeds from another plant species (*U. ruziziensis*, pigeon pea, buckwheat or crotalarias - 1/3 of seeds for each species of crotalaria) was used, with the crop component comprising 80 % of the mixed seed distribution. Following sowing, the cover crops were cultivated in line with agronomic recommendations until their desiccation (9 months after sowing) at 20 days prior to the soybean sowing, achieved through the application of glyphosate (1,800 g a.e. ha^{-1}). To enhance the effectiveness of *T. asperellum* in the soybean crops, it was applied three times during the growing season, following the methods suggested by Silva et al. (2020a, 2020b). Therefore, the application of the *Trichoderma asperellum* consortium occurred during the soybean crop at three stages: seed inoculation; spraying of the consortium suspension onto the soil at 7 days after sowing (DAS); and spraying of the consortium suspension onto the soybean plants at 21 DAS. For the control treatments without microorganism application, solely water was employed.

The *Trichoderma asperellum* consortium (consisting of UFRA.T06, UFRA.T09, UFRA.T12 and UFRA.T52 isolates) was isolated and selected from rhizosphere soils of reforestation areas and native Amazon forests, akin to Silva et al. (2012) findings. The consortium was preserved and stored at the fungal culture collection of the Universidade Federal Rural da Amazônia.

Two studies were conducted with numerous isolated microorganisms and pairs of microorganisms in soybean crops under controlled conditions, and one of the most promising results was achieved with the *T. asperellum* mix (Silva et al. 2020a, 2020b). Consequently, this combination was used under field conditions. The four strains of *Trichoderma* sp. (UFRA.T06, UFRA.T09, UFRA.T12 and UFRA.T52) were sequenced and identified as *Trichoderma asperellum*, characterized as producers of phosphatase and cellulase. During the growth promotion of rice plants, the best results were achieved when *T. asperellum* was in a mix rather than isolated (Silva et al. 2012, Sousa et al. 2021).

The formulation of the *T. asperellum* consortium involved cultivating each isolate in Petri dishes containing potato-dextrose-agar (PDA) under continuous light, at 25 ºC, for five days. This was then bioformulated following the Silva et al. (2012) protocol. For seed treatment, a concentration of 10 g (6×10^9 conidia) of powder derived from grinding rice colonized by *T. asperellum* was applied per 1 kg of soybean seeds (Silva et al. 2012, França et al. 2015). The conidia suspension for spraying was prepared at a concentration of 10^8 conidia mL^{-1} by mixing equal proportions of each *T. asperellum* isolate.

The soybean BRS 6970IPRO cultivar was sown in January 2019, with spacing of 0.45 m and density of 22 seeds m^{-1}. Fertilization involved the use of 200 kg ha^{-1} of monoammonium phosphate (MAP), with *Bradyrhizobium japonicum* (strain SEMIA 5079 and SEMIA 5080 with concentration of 5×10^9 CFU mL^{-1}) solution being sprayed within the planting furrow. Although there is no incompatibility between *Bradyrhizobium* and *Trichoderma*, to avoid any potential effects on nitrogen fixation, these microorganisms were inoculated at different times, following the methods suggested by Silva et al. (2020a, 2020b). Additionally, *Bradyrhizobium japonicum* was used in both treatments, with and without *T. asperellum*. Therefore, the use of *B. japonicum* in the soil served to standardize this application across treatments. Plant emergence occurred at 6 DAS. At 21 DAS, potassium fertilization was carried out with 150 kg ha^{-1} of potassium chloride. The crop was subjected to cultural control measures, adhering to agronomic guidelines, to safeguard against insect pests, diseases and weeds.

The assessment of dry matter production and chemical composition of cover crop shoots was undertaken on the day of the soybean sowing's desiccation. For this, a metal square measuring 1.0 m^2 was positioned at random within each plot. Entire plant shoots were harvested and subsequently dried in a forced-air circulation oven at 65 ºC, for 72 hours, until a constant mass was achieved to ascertain the dry matter. Following this, the samples were used to analyze the nutrient content (N, P, K, Ca, Mg, Fe, Zn, Cu and Mn) following Malavolta et al. (1997) guidelines. The total nutrient quantities per hectare were determined by multiplying the dry matter production per hectare with the respective nutrient content.

Gas exchanges were evaluated during the soybean plants' flowering stage. Photosynthetic rate (A; µmol CO_2 m^{-2} s^{-1}), transpiration rate (E; mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs; mol H_2O m^{-2} s^{-1}) and instantaneous water-use efficiency (A/E) were determined using a portable gas meter employing infrared IRGA (LCpro, ADC BioScientific) between 08:00 a.m. and 10:00 a.m. The measurements were taken on the central region of the leaf blade of fully expanded leaves within the upper third of the plant. The equipment was configured to replicate air concentrations of 370-400 mol mol^{-1} of CO_2, which represented the reference condition for the IRGA photosynthesis chamber. An active photosynthetic photon flux density of 1,200 µmol m^{-2} s^{-1} was used. A minimum equilibrium time of 2 min was established before readings were taken.

At 105 days after emergence (DAE), subsequent to the physiological maturation of the soybean, mechanized harvesting was performed within the useful area of each plot. Prior to this, a sample of plants was collected at a randomly determined meter within the useful area of each plot. The total pod count determined the number of pods per meter. The aggregate grain count was divided by the pod count to calculate the number of grains per pod. Through proportional transformation, the sample's grain mass was determined, considering a water content correction of 13 %, which led to the calculation of the mass of 100 grains, ultimately converted to kg ha^{-1} to determine the grain yield.

The economic analysis was rooted in the assessment of the nutrients' value present within the straw, which could substitute or lessen chemical fertilization in subsequent harvests. To accomplish this, considerations encompassed chemical fertilizers, correctives, soil conditioners and the minimum nutrient concentrations they offer, juxtaposed against current prices. This approach facilitated the quantification of the total fertilizer amount equivalent to the nutrients' supply from cover crop straw, alongside determining the comprehensive cost of agricultural inputs.

The acquired data were subjected to analysis using the Anova function from the "stats" package, which is part of the R statistical software, version 3.5.0 (R Core Team 2017). This involved performing an analysis of variance (Anova), with subsequent application of the LSD mean comparison test at a 0.05 probability level for statistically significant outcomes.

RESULTS AND DISCUSSION

Significant differences ($p < 0.05$) were observed in both the dry matter production and chemical composition of the cover crops (Table 1). The combination of millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea resulted in the highest quantities of straw dry matter: 10.15 and 10.93 Mg ha^{-1}, respectively. The pronounced regrowth capability following the initial spring rains, occurring from October onwards, facilitated substantially the dry matter accumulation due to the presence of vegetative buds in the clumps and stems of the *U. ruziziensis* species (Pacheco et al. 2011). These cover crops are recognized for their elevated biomass production under various conditions, which is crucial for maintaining the sustainability of the no-tillage system, safeguarding soil integrity and releasing nutrients to the subsequent crop. This is especially valuable in the Cerrado region, characterized by naturally low soil fertility (Pacheco et al. 2011, Moro et al. 2013, Nascente et al. 2013a, Nascente et al. 2013b). Notably, the fallow treatment exhibited the lowest straw dry matter production, comparable to the millet + buckwheat and millet + crotalaria mixtures.

Areas cultivated with millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea displayed a significantly higher nutrient availability for the subsequent crop, except for iron (Table 1). The extensive biomass production observed translates to a heightened nutrient cycling rate, particularly noteworthy for the abundant availability of potassium (132 and 159 kg ha^{-1}, respectively). The overall nutrient content in the straw from areas featuring millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea could potentially lead to savings of up to R$ 3,326.90 and R$ 3,841.92 per hectare, when contrasted with supplying equivalent nutrient amounts through chemical fertilizers, correctives and soil conditioners. Notably, potassium (R$ 1,722.42 and R$ 2,074.73 per ha, respectively) and nitrogen (R$ 997.23 and R$ 1,123.64 per ha, respectively) stood out as the primary contributors to these savings (Table 2). Given the intricate nutrient dynamics within the soil, it is challenging to pinpoint exact figures for these savings. However, these cover crops hold the potential to supply nitrogen to the area through the biological fixation made by leguminous species and to recycle other nutrients by absorbing them from varying depths in the soil profile. This includes deeper layers that remain unutilized by the root systems of agricultural crops, which are subsequently made available on the soil surface through straw decomposition and nutrient mineralization.

Pearl millet, according to Nascente et al. (2016), decomposes rapidly, releasing nutrients, especially potassium, thus conferring benefits to the subsequent crop production. *U. ruziziensis* has the capability to extend its roots to considerable depths, absorbing nutrients from the deeper soil profile and redistributing them upon straw decomposition (Pacheco et al. 2011). Conversely, pigeon pea, as a leguminous plant, offers the added advantage of biological nitrogen fixation (Hungria et al. 2007).

Table 1. Dry matter and nutrient content in cover crop straw prior to soybean sowing.

Factors	DM	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Cover plants	Mg ha^{-1}			kg ha^{-1}				g ha^{-1}		
Fallow	4.38 c*	53 bc	6.40 bc	54 b	11.3 d	8.0 b	16.2 b	1,529 b	228 b	78 b
Millet + crotalarias	5.90 bc	69 abc	6.00 bc	47 bc	23.3 bc	10.3 b	20.2 ab	4,271 ab	162 b	59 b
Millet + pigeon pea	6.58 b	90 a	10.64 ab	56 b	27.0 abc	10.3 b	29.1 a	4,544 ab	170 b	77 b
Millet + *Urochloa ruziziensis*	10.15 a	71 abc	10.00 ab	132 a	31.3 ab	17.6 a	21.8 ab	4,235 ab	358 a	141 a
Millet + *U. ruziziensis* + pigeon pea	10.93 a	80 ab	11.80 a	159 a	35.6 a	19.1 a	22.4 ab	3,344 b	423 a	161 a
Millet + buckwheat	5.25 bc	38 c	2.20 c	9 c	20.5 cd	8.5 b	17.9 b	11,543 a	223 b	52 b
Anova (F probability test)	0.001	0.067	0.017	0.001	0.004	0.001	0.164	0.192	0.001	0.001

* Means followed by the same letter in the column do not differ by the t test (LSD) at $p \leq 0.05$. DM: dry matter. Crotalarias: mixture of *Crotalaria ochroleuca*, *C. spectabilis* and *C. juncea*.

Table 2. Estimated nutrient value supplied by cover crop straw, when compared to equivalent amounts of chemical fertilizers, correctives and soil conditioners.

Factors	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn	Total
Cover plants	R\$ ha^{-1}									
Fallow	744	214	705	16.24	16.00	1.35	62	8.13	3.42	1,770
Millet + crotalarias	969	200	613	33.49	20.60	1.68	174	5.78	2.58	2,020
Millet + pigeon pea	1,264	354	731	38.81	20.60	2.42	185	6.06	3.37	2,605
Millet + *U. ruziziensis*	997	334	1,722	44.99	35.20	1.81	172	12.76	6.18	3,327
Millet + *U. ruziziensis* + pigeon pea	1,124	394	2,075	51.18	38.20	1.86	136	15.08	7.05	3,842
Millet + buckwheat	534	73	117	29.47	17.00	1.49	470	7.95	2.28	1,253

Crotalarias: mixture of *Crotalaria ochroleuca*, *C. spectabilis* and *C. juncea*.

The findings underscore the fact that cover crops such as pearl millet, *U. ruziziensis* and pigeon pea, as practiced in this study during the off-season, offer a sustainable approach to enhance the soil physical, chemical and biological attributes. This aligns with Nascente & Stone (2018), which demonstrated improvements in soil physical parameters and elevated soybean yield following the cultivation of upland rice, grasses and legumes as cover crops. The enhancement of soil attributes, coupled with potential savings in subsequent crop fertilization, validates the investment in cover crop sowing expenses, which can serve as an alternative to the second crop in a crop rotation plan. This approach contributes to agricultural sustainability in times of fertilizer scarcity, promoting a responsible production.

The soybean net photosynthesis (A) increased significantly (16 %; $p < 0.05$) when the plants were treated with multifunctional microorganisms, in comparison to untreated plants (Table 3). Concerning cover crops, the highest photosynthetic rates were observed in soybean plants cultivated after the millet + *U. ruziziensis* + pigeon pea and millet + *U. ruziziensis* combinations. On the other hand, soybean plants cultivated after fallow treatment exhibited the lowest photosynthetic rates, with no significant difference from the millet + crotalaria, millet + pigeon pea, and millet + buckwheat areas. It is widely acknowledged that photosynthesis constitutes the fundamental process for biomass production and, ultimately, agricultural yield (Taiz et al. 2014). Furthermore, multifunctional microorganisms positively influence the photosynthetic capacity of soybean plants (Souza et al. 2015, Nascente et al. 2016). The use of cover crops prior to the main crop did not impact gas exchange parameters in the cultivated soybean plants. Conversely, the use of

Table 3. Influence of cover crops and multifunctional microorganisms on photosynthesis (A), transpiration (E), stomatal conductance (gs) and instantaneous water-use efficiency (A/E) of soybean plants.

Factor	A	E	gs	A/E
Cover plants	µmol CO_2 m^{-1} s^{-1}	mmol H_2O m^{-1} s^{-1}		
Fallow	11.62 c*	3.43 a	0.57 a	4.03 a
Millet + crotalarias	14.16 bc	3.60 a	0.64 a	4.09 a
Millet + pigeon pea	14.87 bc	3.45 a	0.60 a	4.44 a
Millet + *U. ruziziensis*	16.31 ab	3.64 a	0.62 a	4.75 a
Millet + *U. ruziziensis* + pigeon pea	18.44 a	3.54 a	0.57 a	5.77 a
Millet + buckwheat	13.68 bc	3.68 a	0.62 a	4.34 a
Use of microorganisms				
With	15.97 a	3.48 a	0.62 a	5.13 a
Without	13.72 b	3.63 a	0.59 a	4.01 b
Factor	Anova (F probability test)			
Cover plants (CP)	0.0045	0.9828	0.9264	0.2580
Microorganisms (M)	0.0214	0.5103	0.5205	0.0167
CP * M	0.8647	0.6792	0.9296	0.6357

* Means followed by the same letter in the column do not differ by the t test (LSD) at $p \leq 0.05$. Crotalarias: mixture of *Crotalaria ochroleuca*, *C. spectabilis* and *C. juncea*.

multifunctional microorganisms resulted in a greater water-use efficiency (A/E), without altering the transpiration parameters and stomatal conductance of plants.

The yield of the soybean treated with multifunctional microorganisms, as well as the production components such as pods per meter, grains per pod and mass of 100 grains, exhibited higher values, if compared to control plants without microorganisms (Table 4). The increase in the number of pods per meter, grains per pod and mass of 100 grains directly correlates with productivity gains (Bordin et al. 2014). This enhancement is an important outcome resulting from the application of beneficial microorganisms in soybean cultivation. Fungi of the *Trichoderma* genus can positively impact seed germination, development and grain yield due to their production of growth-promoting substances (Silva et al. 2012). These microorganisms also contribute to improved plant nutrition, particularly through phosphorus solubilization and indole-acetic acid synthesis (Chagas et al. 2016). Photosynthesis serves as the foundation of plant production, being one of the primary factors responsible for biomass accumulation and plant yield (Taiz et al. 2014). The observed increase in photosynthetic rates, coupled with improved instantaneous water-use efficiency through *Trichoderma* fungi, likely played a role in the positive enhancements observed in the soybean production parameters.

In the context of cover crops, the combinations of millet + *U. ruziziensis* + pigeon pea and millet + *U. ruziziensis* yielded the highest soybean productivity, followed by the millet + pigeon pea, millet + buckwheat and fallow treatments. Conversely, millet + crotalarias (*C. ochroleuca*, *C. spectabilis* and *C. juncea*) displayed the lowest yield (Table 4). The enhanced grain yield in soybean plants resulting from millet + *U. ruziziensis* + pigeon pea and millet + *U. ruziziensis* can be attributed to the rapid decomposition of these cover crops, which efficiently released nutrients in the soil through straw degradation. The considerable availability of potassium (Table 3) could have contributed to improved photosynthetic efficiency in soybean plants, particularly during periods of reduced rainfall.

Pearl millet and *U. ruziziensis*, both grass species, possess the ability to enrich soil nutrient levels due to their robust biomass production, nutrient recycling and rapid decomposition and mineralization of nutrients (Fageria 2014). As highlighted by Pacheco et al. (2011) and Nascente et al. (2013a), cover crops generate substantial biomass and release nutrients into the soil during the straw degradation phase. Consistently with these findings, Nascente & Stone (2018) documented improvements in soil chemistry and physics, as well as increased soybean yield over two successive cycles following the cultivation of pearl millet + *U. ruziziensis* + pigeon pea in conjunction with an upland rice crop. Furthermore, Pacheco et al. (2013) underscored that soybean following different cover crops exhibited a heightened yield, when compared to those cultivated in fallow areas. However, the millet + crotalarias

Table 4. Impact of cover crops and multifunctional microorganisms on soybean yield and production components.

Factor	Pods	Seeds	Mass of	Yield
Cover plants	per meter	per pod	100 seeds	kg ha^{-1}
Fallow	411 c*	2.44 a	16.82 a	4,022 b
Millet + crotalarias	423 c	2.50 a	17.80 a	3,642 c
Millet + pigeon pea	446 bc	2.49 a	17.18 a	4,050 b
Millet + *U. ruziziensis*	512 a	2.55 a	16.66 a	4,680 a
Millet + *U. ruziziensis* + pigeon pea	506 ab	2.55 a	16.86 a	4,980 a
Millet + buckwheat	460 abc	2.48 a	16.11 a	4,102 b
Use of microorganisms				
With	492 a	2.56 a	17.31 a	4,448 a
Without	427 b	2.44 b	16.50 b	4,044 b
Factor	Anova (F probability test)			
Cover plants (CP)	0.0137	0.4935	0.2674	0.0000
Microorganisms (M)	0.0014	0.0036	0.0499	0.0002
CP * M	0.6241	0.4467	0.9631	0.6603

* Means followed by the same letter in the column do not differ by the t test (LSD) at $p \leq 0.05$. Crotalarias: mixture of *Crotalaria ochroleuca*, *C. spectabilis* and *C. juncea*.

(*C. ochroleuca*, *C. spectabilis* and *C. juncea*) treatment demonstrated a suboptimal soybean yield (Table 4), possibly due to the limited straw production and nutrient availability, which could have adversely impacted the soybean production.

Thus, the implementation of cover crop mixtures preceding soybean sowing is highly recommended due to the manifold benefits they bestow upon the subsequent crop, such as enhanced nutrient cycling, as exemplified by the millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea treatments. These cover crops not only preserve the soil integrity, but also facilitate improved water utilization across the cultivated area, thanks to their substantial dry matter production, thereby providing noteworthy ecological advantages. Furthermore, given the positive results achieved in soybean crops, it is likely that using *Trichoderma asperellum* in the cover crops would result in better plant development and increased nutrient availability for subsequent crops. Moreover, the integration of multifunctional microorganisms into soybean cultivation proves pivotal, as it enhances both physiological and agronomic aspects of the plant, culminating in higher-quality production and potential for increased producer income. Hence, the combination of cover crop mixtures and multifunctional microorganisms presents a viable strategy to foster a sustainable soybean agricultural production in the Cerrado region. It is important to note the three inoculation steps (seed, soil and plant); however, good results were obtained with this practice. For farmers, it should be integrated with other activities, such as topdressing fertilization or irrigation, in order to reduce costs.

CONCLUSIONS

1. The combination of cover crops, specifically millet + *Urochloa ruziziensis* and millet + *U. ruziziensis* + pigeon pea, reached the highest straw dry matter production and provided optimal nutrient levels for the subsequent soybean crop. Additionally, it could lead to a reduction in subsequent soybean fertilization requirements;
2. Soybean plants cultivated after millet + *U. ruziziensis* + pigeon pea and millet + *U. ruziziensis*, treated with a consortium of *Trichoderma asperellum*, exhibited the highest photosynthetic rates and instantaneous water-use efficiency;
3. The combinations of millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea yielded an increased number of pods per meter and a higher soybean grain yield. The use of *Trichoderma asperellum* isolates (pool) further enhanced the quantity of pods per meter, grains per pod, mass of 100 grains and overall soybean grain yield;
4. Cultivating a blend of cover crops in tandem with the application of the multifunctional microorganism *Trichoderma asperellum* culminates in augmented soybean production and contributes to the sustainable advancement of this crop within the Cerrado region.

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