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Full Length Article

Biological Attributes in Soils with Cover Crops in the Soybean Direct Seeding System in Southwest of Goiás, Brazil

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Abstract

With the introduction of brachiaria as a cover crop, the no-tillage system stands out due to its high dry matter yield and efficiency in nutrient recycling, promoting the improvement of the biological properties of the soil. However, as important as reporting crop yields, it is necessary to understand the biological response that causes this increase. Assuming that intercropping systems with brachiaria in the no-tillage system promote the improvement of biological attributes, this study aimed to evaluate biological attributes in soils under different intercropping systems after three years under the no-tillage system in the dry and rainy period in Rio Verde and Montividiu, Southwest of Goiás, Brazil. The study was conducted during three crop seasons following the soybean crop in Rio Verde and Montividiu in the Southwest of Goiás. The treatments included corn in monocropping, corn intercropped with *Urochloa ruziziensis*, *U. brizantha* cv. Marandu, and *U. brizantha* cv. BRS Paiaguás and sorghum intercropped with *U. ruziziensis*. The biological attributes evaluated were microbial biomass carbon and nitrogen, basal soil respiration, metabolic and microbial quotient, *β*-glucosidase, arylsulfatase, acid phosphatase, urease, and fluorescein diacetate. It was observed that management influences biological attributes and enzymatic activity. The intercropping influenced microbial biomass carbon, basal soil respiration, $q\text{CO}_2$, and *q*Mic. The *β*-glucosidase and arylsulfatase enzymes were the most sensitive to management. The arylsulfatase enzyme could not demonstrate the biological efficiency of brachiaria in the 3rd year in one area. © 2023 Friends Science Publishers

Keywords: *β*-glucosidase; Arylsulfatase; Enzymes; Brachiaria; Intercropping

Introduction

In Brazil, the direct seeding system is a production model that soybean producers widely accept due to the minimum soil disturbance and moderate use of pesticides and machinery. Thus, aiming to achieve maximum yield in the same production area, the use of intercropping in the no-tillage system in the crop rotation model has increased in the Cerrado, aiming to diversify crops and reduce input costs (Ryan *et al.* 2012; Quintino *et al.* 2016).

Thus, with the introduction of forage species, the no-tillage system stands out due to its high dry matter production, efficiency in recycling nutrients from the deeper layers, and nutrient availability in the superficial layers through the straw (Crusciol *et al.* 2012). Thus, intercropping under no-tillage has become a promising option for the model, as it influences the increase in the contribution of plant residues and, consequently, the increase in organic matter and the speed of water infiltration into the soil, as in the use of forages from the genera *Panicum* and *Urochloa* (Garcia *et al.* 2012).

To infer soil quality according to the presence or absence of vegetation cover and, consequently, conversion to straw, it is necessary to evaluate the biological attributes through microbial biomass, basal soil respiration, and soil enzymatic activity. Soil microbial biomass is the living fraction of organic matter responsible for soil biological processes and is highly sensitive to external factors (Balota *et al.* 1998; Dortzbach *et al.* 2013). Microbial respiration is the most commonly used method to determine an indirect estimate of the rate of decomposition of organic matter (Farias *et al.* 2018) and the enzymes *β*-glucosidase, arylsulfatase, acid phosphatase, and urease are linked to the cycle of carbon (C), sulfur (S), phosphorus (P), and nitrogen (N) and the fluorescein diacetate (FDA) demonstrate the potential of a group of enzymes, aiming to infer biologically more active soils (Mendes *et al.* 2021a).

Mendes *et al.* (2018) evaluated the management systems with soybean in rotation with corn, brachiaria, and corn intercropped with brachiaria and observed increases in the enzymes *β*-glucosidase and arylsulfatase in treatments with

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the presence of brachiaria; thus, the capacity of brachiaria was evident in maintaining a biologically healthier soil under Cerrado conditions. Benetis (2014) observed in the same experiment an increase in soybean yield in treatments with brachiaria, around 572 kg ha^{-1}, demonstrating the influence of biological attributes on crop yield.

Observing the sensitivity of biological attributes and their impact on crop yield, after 21 years of studies evaluating the state of the biological functioning of the soil, Embrapa launched the Soil Bioanalysis (BioAS) technology, which consists of activity analysis of the arylsulfatase and β-glucosidase enzymes associated with the S and C cycles, respectively, as they are linked to the potential yield and sustainability of land use (Mendes *et al.* 2021a).

Therefore, knowing the importance of adopting conservation management systems based on the assumptions that intercropping systems with brachiaria in no-tillage systems promote the improvement of soil biological attributes, the present study aimed to evaluate the biological attributes in soils under intercropping in two production areas in Southwest of Goiás, Brazil.

Materials and Methods

Study areas

The study was conducted during the 2017/2018, 2018/2019, and 2019/2020 crop seasons with soybean cultivation in no-tillage after the cultivation of corn in monocropping and corn and sorghum in intercropping in two locations in Southwest Goiano. One is in Rio Verde, GO (17° 47' 53" latitude and 50° 55' 41" longitude, altitude of 715 m), in the experimental farm of GAPES (Associated Research Group of Southwest Goiano), and the other in the Boa Esperança farm in Montividiu, GO (17° 26' 39" latitude and 51° 10' 29" longitude, altitude of 821 m).

Sowing, fertilization, handling with products (herbicides, insecticides, and fungicides), and harvesting were carried out when necessary, adopting the same criteria and conditions. Soil sample collections in both areas were carried out on April 15 (the end of the rainy period) and September 18 (the end of the dry period) in 2020.

Regarding the chemical characteristics of the soil at the beginning of the conduction in the experimental farm of GAPES, Ca^{2+}, Mg^{2+}, H+Al, and K^+ were 1.41, 0.54, 3.6, and 0.11 $cmoL_c$ dm^{-3}, respectively, P (mel) 3.2 mg dm^{-3}, pH ($CaCl_2$) 5.0, organic matter (OM) 18.7 g dm^{-3}, and base saturation of 36% and particle-size of 52.0% sand, 40.5% clay, and 7.5% silt. At Boa Esperança farm, the chemical characteristics of the soil at the beginning of the experiment were as follows: Ca^{2+}, Mg^{2+}, H+Al, and K^+ of 1.31, 0.85, 2.7; 0.09 $cmoL_c$ dm^{-3}, respectively, P (mel) 22.4 mg dm^{-3}, pH ($CaCl_2$) 5.4; organic matter (OM) 15.8 g dm^{-3}, and base saturation of 54% and particle-size of 75.5% sand, 19.5% clay, and 5.0% silt.

Adopting the criteria proposed by Köppen (1931), the climate is classified as tropical savanna with dry winters and rainy summers (A_w-type), with average annual precipitation above 1,000 mm in both areas (Fig. 1, 2).

Evaluated treatments

The 12 m x 37.5 m (450 m^2) strips were allocated in a randomized block design randomLy within the area. The evaluated treatments were 1) corn in monocropping, 2) corn intercropped with *Urochloa ruziziensis*, 3) corn intercropped with *U. brizantha* cv. Marandu, 4) corn intercropped with *U. brizantha* cv. BRS Paiaguás, and 5) sorghum intercropped with *U. ruziziensis*.

Soil samples (0–10 cm) were taken from each treatment, where four composite samples were collected. Each composite sample originated from three simple samples collected randomLy in each plot. The samples were air-dried, grounded, and sieved in a 2 mm mesh in the laboratory.

Laboratory analysis

Carbon (C-BM) and nitrogen (N-BM) from microbial biomass: The chloroform-fumigation-extraction (CFE) method proposed by Vance *et al.* (1987), with the soil extractor ratio 1:2.5 (Tate *et al.* 1988), was used to determine the carbon in the microbial biomass (C-BM). The analysis was performed with three replicates of 20 g for each sample collected, three fumigated with chloroform and three not fumigated, according to Brookes *et al.* (1982) and Witt *et al.* (2000). The moisture content of the samples was adjusted to 70% of field capacity. All replicates were subjected to extraction with 50 mL of potassium sulfate solution (K_2SO_4) 0.5 moL L^{-1}.

An aliquot of the extract (8 mL) was treated with a potassium dichromate solution ($K_2Cr_2O_7$) 0.4 N in an acidic medium. Residual dichromate was measured by titration with an ammoniacal ferrous sulfate solution $[(NH_4)^2Fe(SO_4)_2.6H_2O]$ 0.04 N using diphenylamine as an indicator. The extraction and quantification were based on the Walkley and Black (1934) methodology modified according to Tedesco *et al.* (1995). The amount of C-BM was determined by the difference between the organic carbon extracted from the fumigated and non-fumigated soil samples, considering the correction factor (Kc) of 0.41 (Sparling and West 1988). The results of C-BM were expressed in mg C kg^{-1} soil.

The fumigation-extraction, the procedure described by Brookes *et al.* (1985), was used to determine microbial biomass nitrogen (N-BM). The extracts obtained using the CFE method of C-BM (Brookes *et al.* 1982; Witt *et al.* 2000) were used to quantify N-BM. The extract (10 mL) was removed and transferred to tubes with 2 g of catalyst mixture and 5 mL of sulfuric acid. The digestion was carried out in a digester block at 350ºC for two hours, with steam distillation for N analysis (Kjeldahl) followed by neutralization by acid-base volumetry (Alves *et al.* 1994). The amount of BM-N was determined by the difference between the N extracted from fumigated and non-fumigated soil samples, considering a Kc of 0.54 (Brookes *et al.* 1985). The N-BM results were expressed in mg N kg^{-1} soil.

Basal soil respiration (BSR)

The assessment of microbial respiration was based on the methodology of Jenkinson and Powlson (1976), starting with the weighing of two replicates of 20 g of soil and transferred together with a flask with 10 mL of 1 M sodium hydroxide (NaOH) to a 2 L hermetically closed flask, so that there is no entry of CO_2 from outside air and leakage of internally produced CO_2. After seven days of incubation, the flask containing NaOH was removed, and barium chloride ($BaCl_2$) 10% (m/v) was added for total CO_2 precipitation. Titration was carried out with two drops of 1% phenolphthalein (m/v) and titrated under stirring with 0.5 M hydrochloric acid (HCl). The color will go from pink to colorless, estimating the amount of CO_2 released from the unfumigated soil. The results of microbial respiration were expressed in mg C-CO_2 kg^{-1} soil h^{-1}.

Metabolic (*q*CO_2) and microbial (*q*Mic) quotient

The *q*CO_2 was calculated by the ratio between the respiration rate and the C-BM (Anderson and Domsch 1993), expressed in mg C-CO_2 g^{-1} BMS-C h^{-1}. The *q*Mic was calculated by the ratio between C-BM and organic carbon (OC), expressed as a percentage.

Sample soil (1 g) was weighed and transferred to a polyethylene beaker (blank) to carry out the OC determination. Sodium dichromate digester solution (10 mL) ($Na_2Cr_2O_7$).$2H_2O$ 4N + sulfuric acid (H_2SO_4) 10 N was added. Then, it was shaken on a horizontal shaker for 10 min. After stirring, it was left to stand for one hour. After, 50 mL of distilled water was added and left to settle overnight. For determination, reading was performed in a moLecular absorption spectrophotometer at a wavelength of 650 nm (transmittance), hitting zero with the blank test.

β-glucosidase

The *β*-glucosidase enzyme activity was based on the methodology of Tabatabai (1994). Soil samples (1 g) were weighed and placed in a 50 mL Erlenmeyer flask, then 0.25 mL of toluene, 4 mL of MUB pH 6, and, except for the blank, 1 mL of 0.025 M PNG were added. They were incubated for one hour at 37°C, then 1 mL of CaCl2 0.5 M and 4 mL of THAM pH 12, and only in the blank, 1 mL of PNG 0.025 M were added. They were shaken and filtered through Whatman nº 2 filter paper, and the yellow color was read in a molecular absorption spectrophotometer at 410 nm. The activity of the *β*-glucosidase enzyme will be expressed in mg p-nitrophenol kg^{-1} soil h^{-1}.

Arylsulfatase

The activity of the arylsulfatase enzyme was based on the methodology of Tabatabai (1994). 1 g was weighed and placed in a 50 mL Erlenmeyer flask, then 0.25 mL of toluene, 4 mL of acetate buffer pH 5.8, and, except for the blank, 1 mL of 0.05 M PNS were added. It was incubated for one hour at 37°C, then 1 mL of 0.5 M $CaCl_2$, 4 mL of 0.5 M NaOH, and only in the blank, 1 mL of 0.05 M PNS were added and filtered through Whatman nº 2 filter paper, and the yellow color was read in a molecular absorption spectrophotometer at 410 nm. The activity of the arylsulfatase enzyme will be expressed in mg *p*-nitrophenol kg^{-1} soil h^{-1}.

Acid phosphatase

The acid phosphatase enzyme activity was based on the methodology of Tabatabai (1994). 1 g was weighed and placed in a 50 mL Erlenmeyer flask, then 0.25 mL of toluene, 4 mL of MUB pH 6.5, and except for the blank, 1 mL of 0.05 M PNF were added. It was incubated for one hour at 37°C, then 1 mL of $CaCl_2$ 0.5 M, 4 mL of NaOH 0.5 M, and only in blank, 1 mL of PNF 0.05 M was added. This was shaken and filtered through Whatman nº 2 filter paper, and the yellow color was read in a molecular absorption spectrophotometer at 410 nm. The acid phosphatase enzyme activity will be expressed in mg *p*-nitrophenol kg^{-1} soil h^{-1}.

Urease

The urease enzyme activity was based on the methodology of Tabatabai and Bremner (1972). 5 g of soil was weighed, adding 0.2 mL of toluene, 9 mL of buffer (pH 9), and 1 mL of solution with urea (0.2 mol L^{-1}), and incubated for 2 hours in an oven with a temperature of 37°C. After this period, 35 mL of KCI-Ag_2SO_4 was added to stop the reaction, stirred for a few minutes, and left for about 5 minutes at room temperature. After this period, the solution was completed with KCI-Ag_2SO_4 to 50 mL and stirred for a few minutes.

From the solution, 20 mL was pipetted and taken to nitrogen still, adding 0.2 g of MgO. In the nitrogen distiller, the distillate is collected in a beaker with a boric acid solution (H_3BO_3) containing methyl red ($C_{15}H_{15}N_3O_2$) and bromocresol green ($C_{21}H_{14}Br_4O_5S$) as indicators, titrated with a standardized solution of H_2SO_4 (0.005 mol L^{-1}). A control sample was performed for each sample, with urea being added only after the KCl-Ag_2SO_4. The urease activity is expressed in ug N-NH_4^+ g dry soil $^{-1}$ h^{-1}.

Fluorescein diacetate (FDA)

The urease enzyme activity was based on the methodology of Diack (1997). 3 g of soil was weighed, and 30 mL of a buffer solution with fluorescein was added. The tube was capped and incubated in rotation at 35ºC. After this period, 2 mL of acetone was added to stop the reaction. The suspended soil was stirred for 5 min; the supernatant was filtered with Whatman nº 42 filter paper and determined with a molecular absorption spectrophotometer at 490 nm. The fluorescein concentration is expressed in mg F g dry soil^{-1} day^{-1}.

Statistical analysis

Data were analyzed using analysis of variance, and means were compared using the Tukey test (5%). The data analysis was performed with the Sisvar 5.8 software (Ferreira 2019). For the principal component analysis, the Paleontological Statistics Software Package – PAST4 software (Hammer *et al.* 2013) was used.

Results

Farm of associated group of southwest goiano producers (Rio Verde, GO)

The C-BM showed a statistical difference for corn + *U. brizantha* cv. Paiaguás in the rainy period, differing from the other treatments. In the dry period, treatments with Corn + *U. ruziziensis*, Corn + *U. brizantha* cv. Marandu, and Corn + *U. brizantha* cv. Paiaguás exhibited a difference when compared to conventional corn treatment (Table 1).

In the N-BM component, both in the rainy and dry periods, with Corn + *U. ruziziensis* and Corn + *U. brizantha* cv. Marandu showed differences between the other treatments with higher Nitrogen efficiency in the soil (Table 1).

No differences were observed for the *q*CO$_2$ attribute in either collection period. In the rainy period, the intercropping of corn with *U. brizantha* cv. Paiaguás showed the highest contents for C-BM and *q*Mic, the intercropping of corn with *U. ruziziensis* showed the highest content of N-BM, and the intercropping did not show differences for BSR compared to monoculture. In the dry period, the intercrops were superior to monoculture. In the attributes C-BM and *q*Mic, the intercropping of corn with *U. ruziziensis* showed superiority to monoculture and similarity with sorghum with *U. ruziziensis* with N-BM, and the intercropping did not show a difference compared to BSR with monocropping (Table 1).

No differences were observed in the rainy period for the attributes *β*-glucosidase, acid phosphatase, arylsulfatase, urease, and FDA, and in the dry period for the attributes *β*-glucosidase, acid phosphatase, urease, and FDA. For the arylsulfatase enzyme, the intercropping of corn with *U. brizantha* cv. Paiaguás showed superiority to corn and sorghum intercropping with *U. ruziziensis*, with no difference from monocropping (Table 2).

In the analysis of principal components for the biological attributes of the soil, they represent 74.45 and 77.06% of the total variance of the rainy and dry periods, respectively. In the rainy period, the intercropping of sorghum with *U. ruziziensis* was correlated with BSR, *q*CO$_2$, *β*-glucosidase, and urease. The intercropping of corn with *U. brizantha* cv. Paiaguás correlated with C-BM, *q*Mic, and FDA, and corn with *U. brizantha* cv. Marandu, with the N-BM. Corn monocropping and intercropping with *U. ruziziensis* correlated with acid phosphatase and arylsulfatase (Fig. 3). In the dry period, the intercropping of sorghum *with U. ruziziensis* correlated with C-BM, *q*Mic, and acid phosphatase. The intercropping of corn with *U. ruziziensis* correlated with N-BM and FDA, and the intercropping of corn with *U. brizantha* cv. Marandu and Paiaguás with BSR and arylsulfatase. Monocropping correlated with the *β*-glucosidase enzyme (Fig. 4).

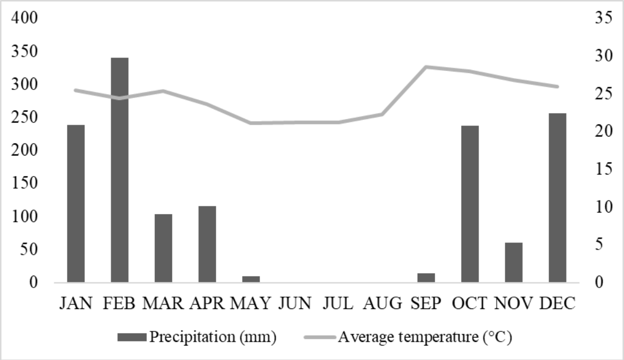

Fig. 1: Monthly temperature and rainfall data (during the experiment, 2020) in the Experimental farm of the Associated Group of Producers of Southwest of Goiás (GAPES) in Rio Verde, GO, Brazil. Source: Authors, 2022

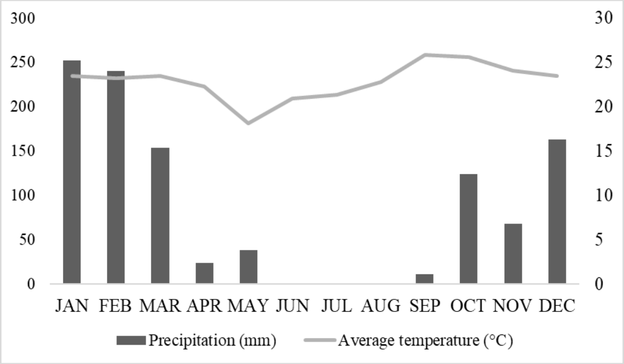

Fig. 2: Monthly temperature and rainfall data (during the experiment, 2020) in the Boa Esperança farm in Montividiu, GO, Brazil. Source: Authors, 2022

Boa esperança farm (Montividiu, GO)

In the rainy period, the intercropping of sorghum with *U. ruziziensis*, corn with *U. ruziziensis*, and corn with *U. brizantha* cv. Paiaguás showed higher levels of C-BM than monocropping. The intercropping showed no difference in

Table 1: Soil biological attributes (0-10 cm deep) in two periods (rainy and dry) after three years of cover crop cultivation in a no-tillage system on the experimental farm of the Associated Group of Producers of Southwest of Goiás (GAPES), Rio Verde, GO, Brazil

Crops	C-BM	N-BM	BSR	qCO$_2$	qMic
	mg C kg^{-1} soil	mg N kg^{-1} soil	mg C-CO$_2$ kg^{-1} soil h^{-1}	mg C-CO$_2$ g^{-1} BMS-C h^{-1}	%
			Rainy Period		
Corn	165.68 c	127.31 b	1.69 ab	0.11 a	1.09 b
Corn + *U. ruziziensis*	171.25 c	173.95 a	1.59 ab	0.12 a	1.30 b
Corn + *U. brizantha* cv. Marandu	233.30 bc	206.28 a	1.35 b	0.09 a	1.70 b
Corn + *U. brizantha* cv. Paiaguas	359.15 a	119.56 b	1.56 ab	0.13 a	3.04 a
Sorghum + *U. ruziziensis*	269.26 b	131.66 b	1.89 a	0.12 a	1.84 b
CV (%)	12.98	10.47	11.39	17.59	23.02
	Dry Period				
Corn	271.43 b	37.23 bc	5.47 ab	0.46 a	2.31 b
Corn + *U. ruziziensis*	416.70 a	60.92 a	4.66 b	0.41 a	3.70 a
Corn + *U. brizantha* cv. Marandu	498.12 a	26.75 bc	5.77 ab	0.47 a	4.10 a
Corn + *U. brizantha* cv. Paiaguas	457.07 a	22.80 c	6.74 a	0.53 a	3.63 a
Sorghum + *U. ruziziensis*	521.55 a	45.41 ab	5.18 ab	0.39 a	4.00 a
CV (%)	12.51	22.23	14.52	16.62	13.06

Means followed by the same letter in the column do not differ by Tukey's test ($p < 0.05$)

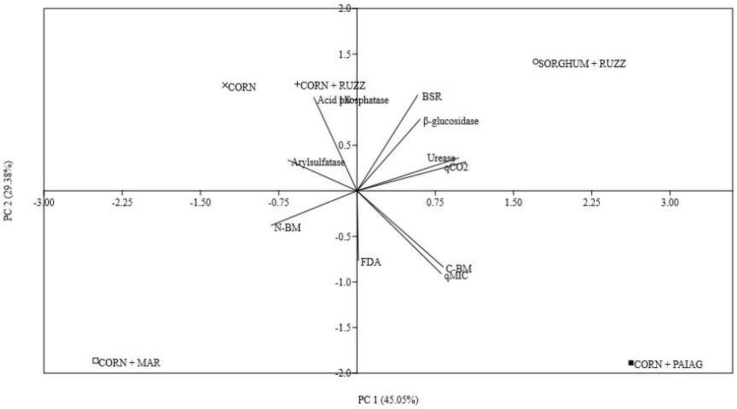

Fig. 3: Principal component analysis of the biological attributes of the soil (0-10 cm deep) during the rainy period after three years of cover crop cultivation in the no-tillage system on the experimental farm of the Associated Group of Producers of Southwest of Goiás (GAPES), Rio Verde, GO, Brazil

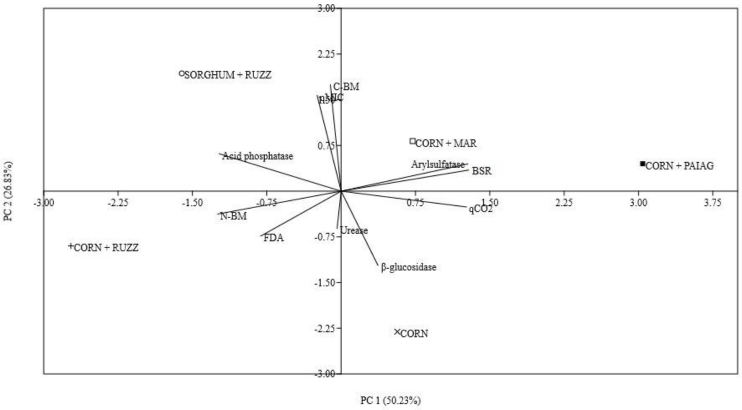

Fig. 4: Principal component analysis of the biological attributes of the soil (0-10 cm deep) during the dry period after three years of cover crop cultivation in the no-tillage system on the experimental farm of the Associated Group of Producers of Southwest of Goiás (GAPES), Rio Verde, GO, Brazil

monocropping with N-BM. The intercropping of corn with *U. brizantha* cv. Marandu demonstrated superiority to the other intercropping systems and monocropping systems with BSR and qCO$_2$ and corn and sorghum with *U. ruziziensis* with qMic. In the dry period, the intercropping, except for sorghum with *U. ruziziensis*, did not differ from the monocropping in C-BM. About N-BM, corn in monocropping and intercropped with *U. brizantha* cv. Paiaguás and *q*Mic with corn intercropping with *U. ruziziensis* had the highest contents. The BSR showed no difference between the intercropping and monocropping and only the intercropping of *U. brizantha* cv. Marandu showed inferiority to other intercropping and monocropping with *q*CO_2 (Table 3).

No differences were observed for the acid phosphatase, arylsulfatase, urease, and FDA attributes in the rainy period. The intercropping of corn and sorghum with *U. ruziziensis* was superior to monocropping and similar to the intercropping of corn with *U. brizantha* cv. Paiaguás concerning to *β*-glucosidase. In the dry period, no differences were observed for *β*-glucosidase, acid phosphatase, urease, or FDA. The arylsulfatase enzyme showed the superiority of corn intercropping with *U. ruziziensis* with other intercrops, with no difference from monocropping (Table 4).

In the analysis of principal components for the biological attributes of the soil, they represent 77.44 and 73.94% of the total variance in the rainy and dry periods, respectively. In the rainy period, the intercropping of corn and sorghum with *U. ruziziensis* correlated with C-BM, *q*Mic, *β*-glucosidase, urease, and FDA. The intercropping of corn with *U. brizantha* cv. Marandu correlated with BSR and *q*CO_2. The monocropping correlated with acid phosphatase and arylsulfatase (Fig. 5). The intercropping of sorghum with *U. ruziziensis* correlated with urease and FDA, corn with *U. ruziziensis* with C-BM, *q*Mic, and arylsulfatase, corn with *U. brizantha* cv. Paiaguás and Marandu with acid phosphatase and *β*-glucosidase. The monocropping correlated with N-BM, BSR, and *q*CO_2 (Fig. 6).

Discussion

About C-BM, in the rainy period, the intercropping of corn with U. brizantha cv. Paiaguás showed superiority concerning the other intercropping and monocropping systems, and in the dry period, the intercropping system was superior to the monocropping (Table 1). In the absence of a cover crop, only spontaneous vegetation reduces the C-BM content (Carneiro *et al.* 2008). Duarte *et al.* (2014) noted the superiority of the intercropping *Mucuna pruriens* and millet regarding C-BM contents, validating this attribute's management difference. There is a quick influence on biological attributes due to the plant cycle and the addition of plant residues (Hoffmann *et al.* 2018, Miranda *et al.* 2020).

Regarding N-BM, the intercropping of corn with *U. ruziziensis* showed superiority to monocropping in both periods. In the dry period, there was similarity between sorghum and *U. ruziziensis* (Table 1). According to Souza *et al.* (2010), low forage height or absence can cause a reduction in N-BM under water stress conditions, which may be correlated with the chemical composition of the residues (Tian *et al.* 1992).

The intercropping systems did not differ from monocrop in both periods for BSR (Table 1). In a study by Duarte *et al.* (2014), basal soil respiration was not different in one of the analyzed experiments evaluating the management of millet, *Canavalia ensiformis, M. pruriens, Cajanus cajan*, and *Crotalaria juncea*.

No differences were observed for the *q*CO_2 in both periods (Table 1). Despite the superiority of the two intercropping systems in the rainy period and the intercropping system in the dry period, there was no impact on *q*CO_2. According to Cunha *et al.* (2011), the more effective the C-BM, due to the assimilation of C from the soil, the lower the value of *q*CO_2.

Concerning *q*Mic, in the rainy period, the intercropping of corn with *U. brizantha* cv. Paiaguás was superior to the other intercropping systems and monocrop, and in the dry period, the intercropping system was superior to the monocropping (Table 1). The lowest *q*Mic content observed was 1.09% in corn monocropping. In a study by Jakelaitis *et al.* (2008), the *q*Mic values ranged between 0.9 and 1.8% when assessing corn monocropping, intercropped corn, and native vegetation. They stated that values less than 1% indicate that there is some limiting factor to the microbiological activity in the soil, which did not occur in this work.

In both periods, no differences were observed for the *β*-glucosidase, acid phosphatase, urease, and FDA, in addition to the absence of a difference for the arylsulfatase enzyme in the rainy period (Table 2). According to Green *et al.* (2007) and Ferreira *et al.* (2017), the sowing system can increase the enzymatic activity values in the superficial layer. As we observed in this work, the differences between the evaluated managements may be in deeper layers.

For the arylsulfatase enzyme in the dry period, the intercropping of corn with *U. brizantha* cv. Paiaguás showed superiority to corn and sorghum intercropping with *U. ruziziensis*, with no difference from the monocropping (Table 2). According to Rodrigues *et al.* (2022), arylsulfatase was the most sensitive indicator to detect changes in the soil with evaluated crops, responding to the water regime and the presence of brachiaria. Mendes *et al.* (2005), in Rio Verde, Goiás, Brazil, observed significant increases in the activity of this enzyme just one year after the adoption of the no-tillage system, showing the enzyme's ability to show minimal changes, even before the carbon of microbial biomass and organic matter from the soil.

In the rainy period, the intercropping of sorghum with *U. ruziziensis*, corn with *U. ruziziensis,* and corn with *U. brizantha* cv. Paiaguás had higher levels than monocropping for C-BM (Table 3). Notably, the C-BM levels found in this work only with intercropping with corn indicate positive responses of the management adopted with microbial diversity (Duarte *et al.* 2014). Gallo *et al.* (2019), evaluating the C-BM contents in monocropping and intercropped corn, observed higher contents in corn intercropped with *C. juncea* and *C. cajan* than in corn monocropping.

In the dry period, the intercropping, except for

Table 2: Soil enzymatic activity (0-10 cm deep) in two periods (rainy and dry) after three years of cover crops in a no-tillage system on the experimental farm of the Associated Group of Producers of Southwest of Goiás (GAPES), Rio Verde, GO, Brazil

Crops	β- glucosidase	Acid Phosphatase	Arylsulfatase	Urease	FDA
	mg p-nitrophenol kg^{-1} soil h^{-1}			ug N-NH_4^+ g dry $soil^{-1}$ h^{-1}	mg F g dry $soil^{-1}$ day^{-1}
			Rainy Period		
Corn	197.84 a	711.21 a	174.21 a	19.78 a	227.29 a
Corn + *U. ruziziensis*	213.88 a	706.36 a	171.01 a	18.63 a	200.00 a
Corn + *U. brizantha* cv. Marandu	195.61 a	706.21 a	171.76 a	17.20 a	240.83 a
Corn + *U. brizantha* cv. Paiaguas	204.05 a	693.18 a	170.55 a	20.92 a	232.08 a
Sorghum + *U. ruziziensis*	215.09 a	714.69 a	170.61 a	20.92 a	231.45 a
CV (%)	5.13	2.78	2.23	17.38	24.21
	Dry Period				
Corn	222.41 a	726.96 a	180.97 ab	22.78 a	95.62 a
Corn + *U. ruziziensis*	222.96 a	734.24 a	178.59 b	21.05 a	129.16 a
Corn + *U. brizantha* cv. Marandu	221.46 a	728.33 a	182.40 ab	23.36 a	102.29 a
Corn + *U. brizantha* cv. Paiaguas	222.99 a	725.45 a	184.66 a	19.90 a	87.50 a
Sorghum + *U. ruziziensis*	220.44 a	735.15 a	180.30 b	20.76 a	86.04 a
CV (%)	1.27	1.49	0.97	14.28	39.05

Means followed by the same letter in the column do not differ by Tukey's test ($p < 0.05$).

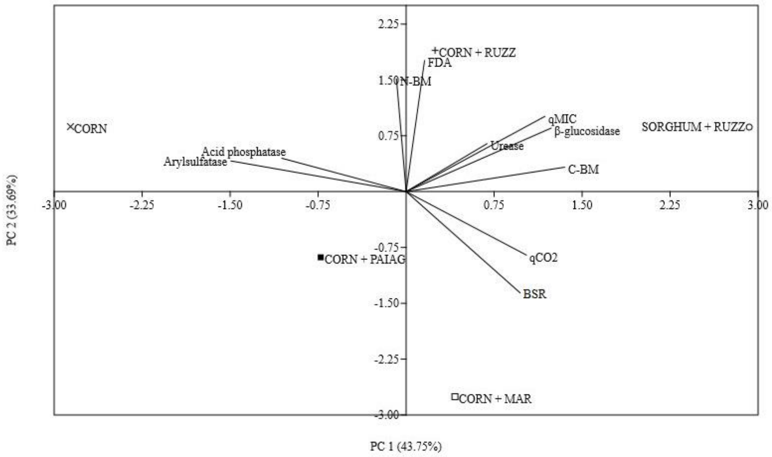

Fig. 5: Principal component analysis of the biological attributes of the soil (0-10 cm deep) during the rainy period after three years of cover crop cultivation in the no-tillage system on the Boa Esperança Farm, Montividiu, GO, Brazil

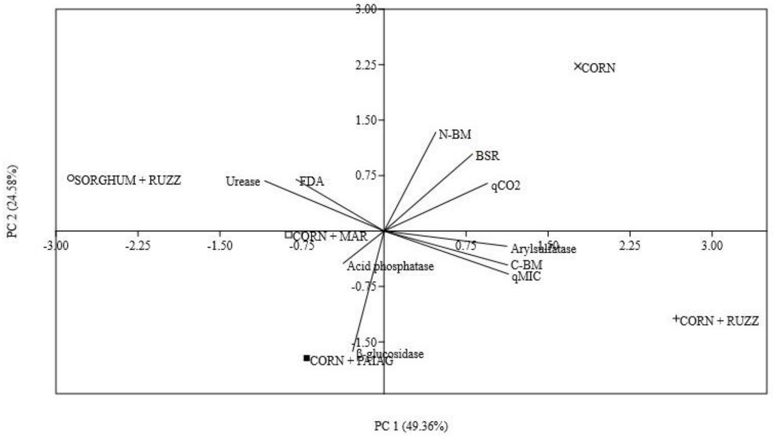

Fig. 6: Principal component analysis of the biological attributes of the soil (0-10 cm deep) during the dry period after three years of cover crop cultivation in the no-tillage system on the Boa Esperança Farm, Montividiu, GO, Brazil

sorghum with *U. ruziziensis,* did not differ from the monocropping for C-BM (Table 3). Hoffmann *et al.* (2018) observed differences in the transition of collection periods. According to Mendes *et al.* (2009), stressful soil conditions, such as the collection period, can increase C-BM values. In the rainy period, the intercropping showed no difference in

Table 3: Soil biological attributes (0-10 cm deep) in two periods (rainy and dry) after three years of cover crop cultivation in a no-tillage system on the Boa Esperança Farm, Montividiu, GO, Brazil

Crops	C-BM	N-BM	BSR	qCO$_2$	qMic
	mg C kg^{-1} soil	mg N kg^{-1} soil	mg C-CO$_2$ kg^{-1} soil h^{-1}	mg C-CO$_2$ g^{-1} BMS-C h^{-1}	%
			Rainy Period		
Corn	260.22 c	174.68 ab	1.78 d	0.22 d	3.25 b
Corn + *U. ruziziensis*	327.70 ab	191.42 a	2.05 c	0.27 b	4.37 a
Corn + *U. brizantha* cv. Marandu	297.37 bc	142.40 ab	2.63 a	0.30 a	3.47 b
Corn + *U. brizantha* cv. Paiaguas	334.04 ab	133.80 b	2.26 b	0.24 cd	3.62 b
Sorghum + *U. ruziziensis*	370.86 a	163.97 ab	2.32 b	0.27 bc	4.33 a
CV (%)	7.96	14.81	3.65	4.70	7.27
	Dry Period				
Corn	364.17 ab	146.11 a	2.29 a	0.22 a	3.58 b
Corn + *U. ruziziensis*	395.75 a	99.90 b	1.67 a	0.18 ab	4.46 a
Corn + *U. brizantha* cv. Marandu	300.82 b	130.55 a	1.09 a	0.08 b	2.36 c
Corn + *U. brizantha* cv. Paiaguás	347.25 ab	54.85 c	1.39 a	0.14 ab	3.54 b
Sorghum + *U. ruziziensis*	182.39 c	87.89 b	1.39 a	0.12 ab	1.64 c
CV (%)	11.29	7.93	37.22	35.98	12.23

Means followed by the same letter in the column do not differ by Tukey's test ($p < 0.05$)

Table 4: Soil enzymatic activity (0-10 cm deep) in two periods (rainy and dry) after three years of cover crop cultivation in a no-tillage system on the Boa Esperança Farm, Montividiu, GO, Brazil

Crops	β- glucosidase	Acid Phosphatase	Arylsulfatase	Urease	FDA
	mg p-nitrophenol kg^{-1} soil h^{-1}			ug N-NH$_4^+$ g dry soil^{-1} h^{-1}	mg F g solo seco^{-1} day^{-1}
				Rainy Period	
Corn	198.27 c	720.45a	176.87 a	19.66 a	85.00 a
Corn + *U. ruziziensis*	214.76 ab	725.90 a	171.93 a	17.83 a	95.00 a
Corn + *U. brizantha* cv. Marandu	202.03 bc	713.63 a	168.31 a	18.09 a	55.55 a
Corn + *U. brizantha* cv. Paiaguás	208.30 abc	722.72 a	173.37 a	17.04 a	69.72 a
Sorghum + *U. ruziziensis*	217.16 ab	706.06 a	165.58 a	21.76 a	89.16 a
CV (%)	3.07	1.30	3.05	12.75	30.91
	Dry Period				
Corn	220.76 a	730.30 a	184.84 ab	20.55 a	123.61 a
Corn + *U. ruziziensis*	222.99 a	736.36 a	186.67 a	18.99 a	89.16 a
Corn + *U. brizantha* cv. Marandu	223.36 a	724.84 a	184.76 ab	21.59 a	107.77 a
Corn + *U. brizantha* cv. Paiaguás	225.14 a	737.27 a	183.08 bc	20.55 a	126.11 a
Sorghum + *U. ruziziensis*	222.11 a	742.12 a	181.72 c	21.85 a	129.16 a
CV (%)	1.44	1.67	0.66	7.89	27.30

Means followed by the same letter in the column do not differ by Tukey's test ($p < 0.05$)

monocropping compared to N-BM, and in the dry period, corn monocropping and intercropping with *U. brizantha* cv. Paiaguás did not differ (Table 3). Brandão Junior (2005) and Fernandes Junior (2021), evaluating different types of management, did not observe significant differences.

In the rainy period, the intercropping of corn with *U. brizantha* cv. Marandu showed superiority to the other intercropping systems and monocropping concerning BSR, and in the dry period, there was no difference between the intercropping and monocropping (Table 3). The behavior of the rainy period was also observed by Cunha *et al.* (2011), where intercropping provided higher levels of soil respiration, which provided a greater amount of labile C in the soil. In their second experiment, Duarte *et al.* (2014) observed the superiority of the intercropping of millet and *M. pruriens* to the cultivation of millet alone, as observed in the rainy period. In a study by Gallo *et al.* (2019), greater releases of microbial respiration were observed in corn alone and intercropped with *M. pruriens*, *C. cajan*, and *C. juncea*, with this behavior observed in the dry period. For the BSR, the variable behavior of the tests is evident, mainly according to the collection period, not presenting conclusive indications. According to Gonçalves *et al.* (2019), the BSR does not allow conclusions to be drawn, as the high values may be related to an efficient production system or some disturbance.

In the rainy period, the intercropping of corn with *U. brizantha* cv. Marandu showed the superiority of qCO$_2$ to the other intercropping systems and the monocropping, and in the dry period, the intercropping system, except for corn of *U. brizantha* cv. Marandu showed no difference (Table 3). The qCO$_2$ contents tend to be higher when the C-BM is lower. Duarte *et al.* (2014) observed no difference between the soil coverages evaluated. In Gallo *et al.* (2019), single corn had the highest values compared to intercropping.

In the rainy period, intercropping corn and sorghum with *U. ruziziensis* was superior to the other intercropping systems and monocropping. In the dry period, corn with *U. ruziziensis* had the highest values of qMic (Table 3). Cunha *et al.* (2011) found the influence of cover crops on this attribute, using *C. juncea*, *C. cajan*, *M. pruriens*, and sorghum, and in work by Gallo *et al.* (2019), with corn intercropped with *C. cajan* and *C. juncea*.

No differences were observed for *β*-glucosidase, acid phosphatase, urease, and FDA in the rainy and dry periods. There was no difference in the arylsulfatase enzyme in the rainy period (Table 4). Note the lack of response to intercropping systems and monocropping for urease, acid phosphatase, and FDA, validating the lack of sensitivity of the parameter. The study by Mendes *et al.* (2005) observed variable behaviors in the properties evaluated regarding *β*-glucosidase enzymes and the absence of a difference for acid phosphatase with no-tillage with sorghum and off-season corn. Mendes *et al.* (2018) showed high levels of activity of *β*-glucosidase and arylsulfatase enzymes in treatments with the presence of brachiaria (intercropped and not), in addition to the equivalence of monocropping of corn and brachiaria and corn intercropped with brachiaria.

The intercropping of corn and sorghum with *U. ruziziensis* was superior to monocropping and similar to the intercropping of corn with *U. brizantha* cv. Paiaguás in the rainy period for *β*-glucosidase activity. In the dry period, the enzyme arylsulfatase showed the superiority of corn and *U. ruziziensis* intercropping concerning other intercropping systems, with no difference in monocropping (Table 4). According to Mendes *et al.* (2021b), in 20 years of studies with bioindicators in the Cerrado region, the enzymes arylsulfatase and *β*-glucosidase were the most efficient indicators of soil quality due to the management system. Rodrigues *et al.* (2022), with samples obtained in March during the rainy period, observed that the activity of *β*-glucosidase and arylsulfatase responded positively and significantly to the management system.

Principal component analysis

In the analysis of the main components for the biological attributes of the soil, two main components were used, which together represented 75.45, 77.06, 77.44 and 73.94% of the total variance of the rainy and dry periods in Rio Verde and Montividiu, respectively. According to Regazzi (2000), the amount of principal components that explain 70% or more of the proportion of the total variance is used so that your assessment can be validated.

In Rio Verde, intercropping correlated with all biological attributes in the rainy period, and the monocropping showed similarity with corn and *U. ruziziensis* intercropping for acid phosphatase and arylsulfatase enzymes (Fig. 3). In the dry period, it maintained the same behavior except for *β*-glucosidase, which was associated with monocropping (Fig. 4). The response of the intercropping regarding the biological attributes was evidenced, where the enzymes arylsulfatase > *β*-glucosidase > acid phosphatase, following this order, according to Rodrigues *et al.* (2022), are the most sensitive to detect changes in the soil. According to Mendes *et al.* (2018), brachiaria can keep the soil biologically more active, and the *β*-glucosidase and arylsulfatase enzymes are the most sensitive to minor differences. Carneiro *et al.* (2013) studied an integrated crop-livestock system that promoted improvements in the carbon contents of microbial biomass and soil carbon stocks.

In Montividiu, intercropping is correlated with biological attributes in the rainy period, except acid phosphatase and arylsulfatase, which are associated with monocropping (Fig. 5). In the dry period, the intercropping system correlated with biological attributes except for N-BM, basal soil respiration, and $q\mathrm{CO_2}$, which were associated with monocropping (Fig. 6). For Mendes *et al.* (2018), the superiority of soybean/brachiaria rotation in relation to soybean/fallow is evidenced, as for C-BM and the enzymes *β*-glucosidase, arylsulfatase, and acid phosphatase, but higher levels of *β*-glucosidase from the soybean/ *U. ruziziensis* rotation compared to soybean/corn and soybean/corn + *U. ruziziensis*.

Despite the variable behavior, the presence of cover crops reinforces the importance of agrobiodiversity for soil health, and the best way to transform the soil into a biologically active and productive land is to offer diversified cover crops in an adequate quantity for microbial communities that reside in it (Mendes *et al.* 2021a).

Conclusion

It is concluded that the management influenced the biological attributes and enzymatic activity. The carbon and nitrogen of the microbial biomass, and qMic, got the best response in the intercroppings in the study area in Rio Verde, GO, Brazil.

The results of carbon of the microbial biomass, basal soil respiration, $\mathrm{qCO_2}$, and qMic were better in intercropping than monocropping in the rainy period in the area evaluated in Montividiu, GO, Brazil.

The *β*-glucosidase and arylsulfatase enzymes showed high sensitivity to management. The *β*-glucosidase enzyme in the rainy period in Rio Verde, GO, showed high efficiency on *U. ruziziensis* for soil biological components.

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Author Contributions

MVAV, HRFB, ELS, MACC, and DCS planned the experiments; MVAV and HRFB collected samples in the field; MVAV and HRFB performed the analysis; MVAV, HRFB, ELS, and MACC interpreted and discussed the results; MVAV and HRFB statistically analyzed the data; MVAV and ELS wrote and revised the text.

Conflicts of Interest

All other authors declare no conflicts of interest.

Data Availability

Not applicable.

Ethics Approval

Not applicable.

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