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Influence of a tropical grass (*Brachiaria brizantha* cv. Mulato) as cover crop on soil biochemical properties in a degraded agricultural soil

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ABSTRACT

The inclusion of tropical grass forage as a cover crop (CC) could be a useful tool to improve microbiological activity and, consequently, soil quality. The aim of this study was to evaluate the effect of *Brachiaria brizantha* cv. Mulato and maize (*Zea* mays) as CC on soil microbial communities and their contributions to a degraded common bean (*Phaseolus vulgaris* L.). monoculture system. Soil sampling was carried out in 2016 after six years of cumulative effect across different treatments: B. *brizantha*-B. *brizantha*-common bean (B2), *B. brizantha*-common bean (B1), maize-common bean (M) and common bean monoculture (control). B2 and B1 showed higher fluorescein diacetate hydrolysis (108.1% and 78.6%, respectively) and higher acid phosphatase activity (304.5% and 181.6%, respectively) compared with the control treatment. The metabolic efficiency was higher in treatments containing *B. brizantha* as CC, with a significantly lower metabolic quotient (respiration rate per unit microbial biomass carbon) in B2 (1.65) compared with the control (5.46). The B2 treatment also showed higher values of soil organic carbon, which was correlated with soil microbial activities. In contrast, qPCR analysis of microbial structure did not show significant differences in response to the evaluated treatments. Thus, fungal and bacterial abundance probably had less influence on the differentiation of treatments compared to microbial activity and soil chemical properties. In context of this research, the use of *B. brizantha* as CC increased soil fertility and generated a greater microbial metabolic efficiency. Our research demonstrates that *B. brizantha* cv. Mulato as CC is a suitable agricultural tool to restore soil biochemical properties.

1. Introduction

Microorganisms play an essential role in biogeochemical cycling promoting plant growth. The presence of a diverse and functional microbial community contributes to stress resistance and resilience in soils [1]. Therefore, the study of soil microbial communities is a useful measurement to assess the impact of land use change [2]. However, there is little information available about the impact of tropical forage grasses employed as cover crops (CC) on soil microbial diversity and activity in monoculture systems in valleys of northwest Argentina. This information could be used to address the urgent need to restore soil fertility and agroecosystem biodiversity in major agricultural areas. The subtropical valleys of northwest Argentina constitute a diverse, dynamic and productive territory, with congenial environment for production of a variety of crops due to presence of fertile soils and warm temperatures [3]. Subtropical agroecosystems, such as those in this region, are particularly susceptible to increased soil degradation and associated nutrient losses compared to temperate/cold regions because of the higher mineralization of organic matter [1]. Moreover, the natural vegetation was rapidly and extensively cleared for industrial agriculture in these valleys, with more than 60% of production based on tobacco or common bean monoculture [4]. These processes caused negative effects such as environmental resource degradation, fertility losses, a reduction of soil microbial diversity and low productivity [5]. Given the negative effects of predominance of monoculture in major agricultural regions of Argentina and in other parts of the world, it is important to study alternative agriculture strategies oriented towards producing high-yield crops without compromising natural resources

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and ecosystem services.

Employing certain plant species as CC in the fallow period represents a promising way to diversify agricultural systems [6]. In this regard, the use of *Brachiaria brizantha* cv. Mulato, a highly nutritious and palatable forage grass, could favor the activity and diversity of soil microorganisms due to the abundance, expansion and exploration of its roots [7]. *Brachiaria brizantha* cv. Mulato is a perennial and tiller grass, with vigorous stems reaching heights of 1.5–2 m, also characterized by its good growth rate and its deep root system. The inflorescence is a racemic panicle and crop establishment can be by sexual seed or in vegetative form, establishing quickly and the stolons rooted well. Moreover, this grass produces a high amount of stubble on the surface generating a high production of fodder in dry matter [8]. Grasses with deep root systems help pumping nutrients from the deeper layers to the surface soil horizons and their biomass extract the nutrients from the deeper layers, which are gradually released [9]. Therefore, the use of *B. brizantha* could prevent soil fertility loss under monoculture of tobacco or common bean in subtropical agricultural regions. In addition, recent studies have concluded that the use of *B. brizantha* as a CC contributed to improve the quality of chemical and physical attributes of soils [10]. We hypothesize that the use of *B. brizantha* cv. Mulato contributes to restore soil biochemical properties through an increase in fungal and bacterial abundance and microbial activity, increasing the availability of macronutrients. This effect would be higher when using *B. brizantha* as CC in comparison with the use of maize. Therefore, the objective of this research was to assess the effect of one and two cycles of *B. brizantha* cv. Mulato and maize (*Zea mays*) as CC on soil microbial activity and community composition (fungal and bacterial genes abundances) and chemical properties in a degraded common bean (*Phaseolus vulgaris* L.) monoculture system.

2. Materials and methods

2.1. Study site

A field trial was established in 2010 at the Salta Agricultural Experimental Station of the Instituto Nacional de Tecnología Agropecuaria (EEA-INTA), Cerrillos, Lerma Valley, Salta, Argentina (S 24º53′52.84´´ W 65º27′59.11´´, 1420 m. a.s.l.). The climate of the region is subtropical serrano with little or no water deficit in January and February. Mean annual precipitation is 900 mm, concentrated in spring-summer with a prolonged dry season in winter. The average temperature is 23 °C (74 °F) in summer and 15 °C (60 °F) in winter [4]. The soil texture was loam (32% sand, 44% silt, 24% clay) with 2.91% organic matter. Soil belong to Ustochrepts udic as per USDA Soil Taxonomy, Cerrillos series with A, AC and C horizons [11]. The soil in which the experiments were carried out is a degraded soil resulting from 50 years of monoculture of tobacco and common bean (intensive tillage includes several, approximately 20–30 plows to the soi). The experimental design followed a randomized complete block design with three replications. Each plot independently had a type of cover crop (*Brachiaria brizantha* cv Mulato, maize (*Zea mays*), and no CC) seeded with common bean as cash crop in summer with the experimental plots measuring 15 m wide and 50 m long with 12 rows of *B. brizantha* cv. Mulato or maize in each plot. *B. brizantha* was sown with seed drill (sowing machine) at a dose of 4–5 kg/ha and then it was cut with a mower. The stubble was left on the surface. On the ground with stubble, no agricultural work was done, no machinery entered. At the beginning of the rains, the common bean was planted with seed drill, so no tilling was done during the experiment. The four treatments were: a) *B. brizantha/B. brizantha*/common bean (B2); b) common bean/*B. brizantha*/common bean (B1); c) common bean/maize/common bean (M); d) common bean/fallow/common bean: common bean monoculture (control). Sown density was 25 seeds m^{-1} and row width was 52 cm, or 2-3 kg/ha, being seeds inoculated with *Rhizobium* spp (Rizofos Liq Soybean) at a dose of 140 ml/20 g seed. Common bean was managed using recommended production practices, including only one tillage before sowing and pesticide applications (Dimetoato 40% p/v. EC Basf at a dose of 300 ml ha^{-1} and Carbendazim 50 (2-metaxicarbamoilbencimidazol) Nufarm Limited). Weeds were controlled using pre-emergent herbicide Pivot® H Basf (imazetapir 10,59%) and Dual Gold® (S-metolacloro: 96%p/v Syngenta at a dose of 400 ml ha^{-1} and 500 ml ha^{-1}, respectively. Thirty days after sowing, a new herbicide was applied Flex® (fomesafen: 25% p/v) Syngenta at a dose of 500 ml ha^{-1}. No chemical fertilizers were used during the growth of the common bean crop.

2.2. Soil sampling

Soil samples were collected at common bean R1 stage (beginning of flowering: plants present an open flower in any internode of the main stem) in summer (February), during the 2016 crop cycle. For microbial activity analysis, sampling was done by taking soil from the roots of 10 plants in a linear meter, which constitutes 1 composite sample. In total, 6 composite samples of rhizospheric soil were collected per experimental unit from 0 to 10 cm layer [12]. Roots were gently shaken to remove loosely adhering soil, placed in plastic bags and processed immediately. For each of the biochemical parameters measured, triplicate measurements were performed. For chemical analysis, the same soil employed for microbial activity analysis was used and a subsample of 10 g from each sample was stored at -20 °C until molecular analysis. Soil samples were sieved at field moisture (2 mm), homogenized, air-dried and stored at 4 °C for further analysis.

2.3. Soil chemical properties

The soil pH and electrical conductivity (EC) were measured at soil-to-water ratio of 1:2.5. Total C was determined by wet oxidation following the Walkley and Black procedure [13]. Because these soils are free of carbonates [14], the total C content is equivalent to the soil organic C (SOC) content. Total N and extractable phosphorus (eP) were determined by micro-Kjeldhal method [15] and Bray-Kurtz method [16], respectively.

2.4. Soil microbial activities

Microbial activity was estimated by hydrolysis of fluorescein diacetate activity (FDA), according to Adam and Duncan [17]. Briefly, 2 g of soil and 15 ml of 60 mM potassium phosphate buffer pH 7.6 were placed in a 50-ml conical flask. Substrate (FDA, 1000 mg ml^{-1}) was added to start the reaction. The flasks were placed in an orbital incubator at 30 °C and 100 rpm for 20 min. Once removed from the incubator, 15 ml of chloroform/methanol (2:1 v/v) was immediately added to stop the reaction. The contents of the conical flasks were then centrifuged at 447 × *g* for 5 min. Finally, the supernatant was filtered and measured at 490 nm on a spectrophotometer.

Acid phosphatase (AP) was assayed using 1 g soil, 4 ml 0.1 M universal buffer (pH 6.5), and 1 ml 25 mM *p*-nitrophenyl phosphate [18]. After incubation at 37 ± 1 °C for 1 h, the enzyme reaction was stopped by adding 4 ml 0.5 M NaOH and 1 ml 0.5 M $CaCl_2$ to prevent the dispersion of humic substances. Absorbance was measured in the supernatant at 400 nm.

Dehydrogenase activity (DHA) was determined according to García et al. [19]. Briefly, 1 g of soil at 60% field capacity was exposed to 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water at 22 °C for 20 h in the dark. The INTF (iodonitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtering through a Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

2.5. Microbial biomass and respiration

Microbial biomass C was determined employing the chloroform fumigation-incubation technique [20]. Soil microbial respiration was determined as potentially mineralizable C (CO_2-C respiration) [21]. The amount of CO_2 released was measured from chloroform-treated and untreated soil samples (ca. 20 g). Treated samples were previously fumigated with chloroform, inoculated with fresh soil, and incubated with NaOH 0.2 M at room temperature in the dark for no longer than two weeks. Released CO_2 was estimated using HCl 0.2 N. For the quantification of microbial respiration, flasks without soil served as the control treatment.

2.6. Fungal and bacterial gene abundances

DNA was extracted from 0.5 g of soil. Extraction was performed with the soil NucleoSpin® Soil Kit for soil (Macherey-Nagel) using the manufacturer protocol. DNA yield and purity were measured by using a microvolume fluorospectrometer (NanoDrop Technologies, Delaware).

The bacterial 16S rRNA gene copy numbers (primer set 338F/518R [22]) and fungal 18S rRNA gene copy numbers (primer set NS1-F/Fung R [23]) of all samples were determined following the protocol by Liu et al. [24]. PCR amplification was quantified in a Line-Gene 9600 Plus by flourometric monitoring with Power SYBR Green PCR master mix (Applied Biosystems). The reaction was performed in a 25 µl volume containing 10 ng DNA, 0.2 mg ml^{-1} BSA, 0.2 µM each primer and 12.5 µl of SYBR premix EX TaqTM (Takara Shuzo, Shiga, Japan). The standard curves were constructed using plasmids from cloned rRNA genes (Takara) separately for bacteria and fungi. Negative (ultrapure water) and positive DNA controls (*Pseudomonas aeruginosa*, 10-fold serially diluted) were also included. The number of copies of the standards was calculated from the concentration of extracted plasmid DNA. Standard curves were generated using triplicate 10-fold dilutions of plasmid DNA ranging from 2.07×10^2 to 2.07×10^8 copies for the bacterial 16S rRNA gene, and 4.60×10^2 to 4.60×10^8 copies of template for fungal 18S rRNA gene per assay. An amplification efficiency of quantification was obtained of 105% for the bacterial 16S rRNA gene and of 98.2% for the fungal 18S rRNA gene, with a R^2 value and a slope of 0.998 and −3.197, and 0.993 and −3.365, respectively. Melting curve analysis was conducted following each assay to confirm specific amplification. A 2% agarose gel electrophoresis was further performed on the PCR amplification products and blanks to check whether an appropriate size was achieved. We used a negative control master mix to test possible inhibitory effects on quantitative PCR amplification caused by coextracted humic substances. The amplification efficiencies were calculated by using the formula Eff = [10 (-1/slope)-1] [25]. A relative fungal-to-bacterial ratio was directly calculated from the qPCR assays [22].

2.7. Statistical analyses

Statistical analyses were performed using INFOSTAT Professional v. 2012 (UNC, Argentina). Analyses of variance (ANOVA) were used with LSD (least significant difference) to test differences among treatments ($p \leq 0.05$). In all cases, residuals were tested for normality with the Shapiro-Wilks' test. Correlation analyses between microbial activities and chemical variables were performed using INFOSTAT software. In addition, a principal component analysis (PCA) was performed to determine separation among treatments, and to identify variables that best contributed to the separation of treatments. Finally, a generalized procrustes analysis was performed to study the relationship between variable groups.

Table 1
Mean values of soil organic carbon (SOC), total nitrogen (TN), C/N ratio, extractable phosphorus (eP), pH and electric conductivity (EC) across different treatments: B. *brizantha*- B. *brizantha*-common bean (B2), common bean B. *brizantha*/Common bean (B1), common bean/Maize/common bean (M) and common bean monoculture (control). Different letters within a column reflect significant differences ($p \leq 0.05$).

Treatments	SOC (mg C g^{-1})	TN (mg N g^{-1})	C/N	eP (mg P g^{-1})	pH	EC (dS/m)
B2	1.09 a	0.11 a	9.67 ab	16.33 a	6.90 b	0.25 b
B1	0.95 ab	0.11 a	9.33 bc	14.00 a	7.37 a	0.35 a
M	0.99 ab	0.10 a	8.67 bc	14.67 a	7.40 a	0.36 a
C	0.82 b	0.08 b	10.33 a	15.00 a	7.30 a	0.32 ab
p value	0.0058	< 0.0001	0.0113	0.3803	0.0001	0.0234

3. Results

3.1. Soil chemical properties

Most soil chemical properties were affected by the inclusion of *B. brizantha* (Table 1). An important increase of SOC was recorded under B2 treatment, which was 32.9% higher than in the control. Also, total N showed significant differences, being 25% lower in the control compared to the rest of the treatments. Contrary, no significant differences were observed for eP among treatments. Values of pH and EC were significantly lower in B2 compared to the other treatments.

3.2. Soil microbial activities

Soil enzyme activities also were affected by CC treatments (Fig. 1). For FDA, B2 and B1 were 108.1% and 78.6% higher than the control, respectively. Also, B2 and B1 were 83.2% and 56.8% higher than M, respectively. Similar results were observed for AP activity, with B2 being 43.6%, 127.9% and 304.5% higher than B1, M and the control, respectively. The opposite trend was observed for DHA, with B2 being 56.3% and 42.2% lower than M and the control, respectively.

3.3. Components of metabolic quotient

Microbial respiration did not show significant differences among treatments in this study (Table 2). Regarding MBC, B2 treatment showed the highest mean value (0.4 mg g^{-1}) as compared to B1 (0.1 mg g^{-1}), M (0.2 mg g^{-1}) and the control (0.1 mg g^{-1}). Metabolic quotient showed the lowest value in B2 which was on average 244.2% lower than the rest of the treatments.

3.4. Abundance of fungal and bacterial communities

The inclusion of CC did not have significant effect on either fungal or bacterial abundances (Fig. 2). The mean value of fungal abundance varied between 3.2×10^{14} (B1) and 0.5×10^{14} (M) 18S rDNA copy numbers g^{-1}. No significant differences were observed between treatments B2, B1 and the control. However, fungal abundance tended to be highest in B1 and lowest in M. The mean value of bacterial abundance varied between 2.0×10^{15} (M) and 0.5×10^{15} (control) 16S rDNA copy numbers g^{-1}. Except in M, where a significant increase in bacterial abundances was observed, the fungal:bacterial ratio did not show significant differences among treatments. Significantly higher fungal:-bacterial ratio were observed at control (0.42), B1 (0.39) and B2 (0.31) as compared to M (0.03) (data not shown).

3.5. Integrated multivariate relationships

PC1 and PC2 of the principal component analysis (PCA) (Fig. 3) accounted for 64.3% and 23.2% of the variance, respectively. Along PC1, B2 was separated from B1 and even more from the control. The M

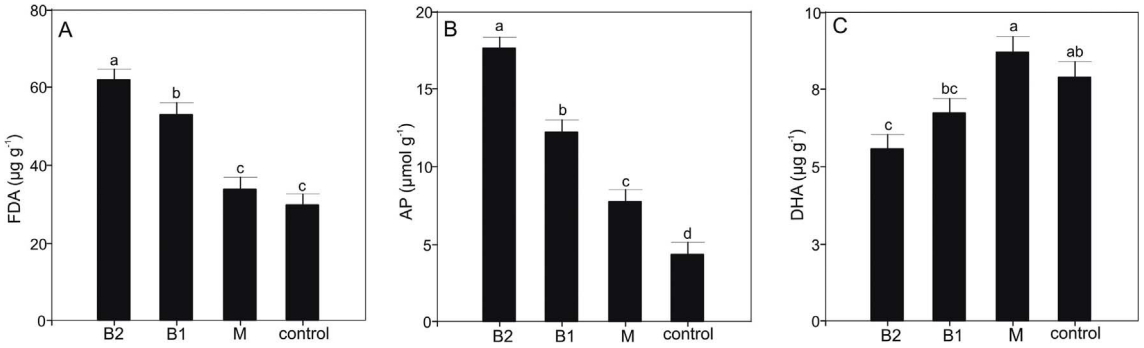

Fig. 1. Mean values of fluorescein diacetate (FDA) hydrolysis (A), acid phosphatase (AP) activity (B) and dehydrogenase activity (DHA) (C) across different treatments: *B. brizantha- B. brizantha*-common bean (B2), common bean *B. brizantha*/common bean (B1), common bean/maize/common bean (M) and common bean monoculture (control). Different letters indicate values that are significantly different ($p \leq 0.05$). Error bars indicate standard error.

Table 2
Mean values of microbial respiration, microbial biomass carbon (MBC) and metabolic quotient (qCO_2) across different treatments: B. *brizantha*- B. *brizantha*-common bean (B2), common bean B. *brizantha*/Common bean (B1), common bean/Maize/common bean (M) and common bean monoculture (control). Different letters within a column reflect significant differences ($p \leq 0.05$).

Treatments	Microbial Respiration (mg g^{-1})	MBC (mg g^{-1})	qCO_2
B2	0.65 ± 0.12 a	0.40 ± 0.03 a	1.65 ± 0.14 b
B1	0.61 ± 0.12 a	0.10 ± 0.03 b	6.16 ± 0.14 a
M	0.54 ± 0.12 a	0.20 ± 0.03 b	5.42 ± 0.18 a
C	0.39 ± 0.12 a	0.10 ± 0.03 b	5.46 ± 0.18 a
p value	0.3003	< 0.0001	0.0017

treatment was close to the control along PC1, and was separated from the rest of the treatments along PC2. Most variables of microbial activity and chemical parameters were responsible for the separation of treatments, particularly enzymes activities, microbial respiration and SOC. Values of 18S and 16S rDNA copy did not influence the separation of treatments along PC1. Correlation analysis (Table 3) showed a significant, positive relationship between soil enzymes activities and pH, EC, SOC, and eP. In addition, microbial respiration and MBC positively correlated with SOC and EC.

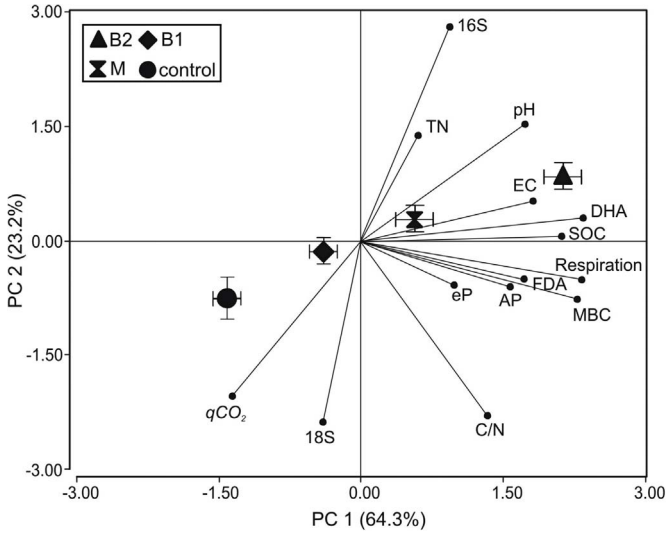

Fig. 3. Principal component (PC) analysis including microbial activities and diversity variables and chemical parameters across different treatments: *B. brizantha- B. brizantha*-common bean (B2), common bean *B. brizantha*/common bean (B1), common bean/maize/common bean (M) and common bean monoculture (control). Error bars indicate standard error.

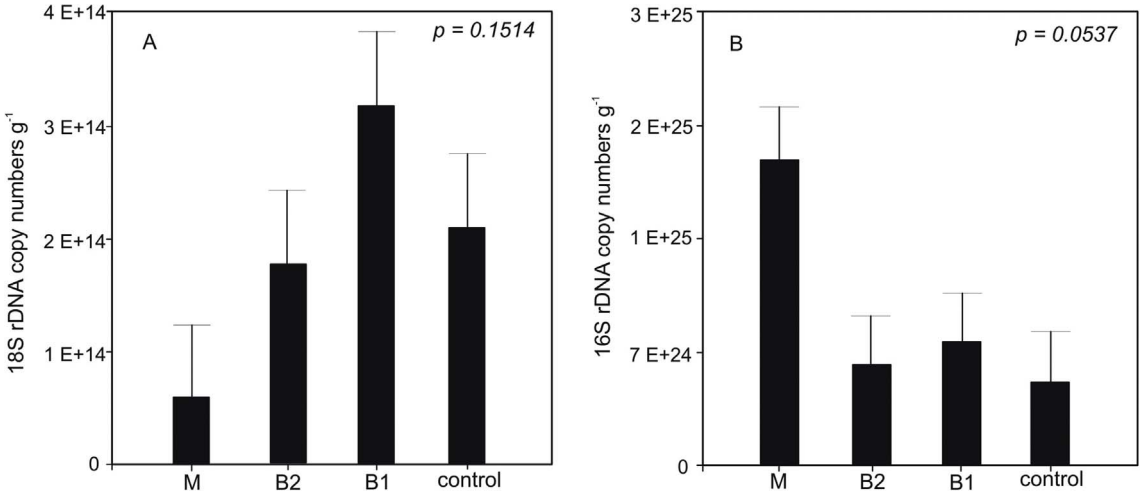

Fig. 2. Mean values of fungal (18S) and bacterial (16S) rDNA copy numbers g^{-1} for different treatments: *B. brizantha- B. brizantha*-common bean (B2), common bean *B. brizantha*/common bean (B1), common bean/maize/common bean (M) and common bean monoculture (control). Different letters indicate values that are significantly different ($p \leq 0.05$). Error bars indicate standard error.

Table 3
Correlation analysis between microbial functionality parameters and chemical parameters.

	pH	EC (dS/m)	SOC (mg C g^{-1})	TN (mg N g^{-1})	eP (mg P g^{-1})
FDA (μg/g)	0.21*	0.34*	0.44*	0.22*	0.2
DHA (μg/g)	0.47*	0.37*	0.43*	0.06	0.06
AP (μmol/g/h)	0.38*	0.02	0.29*	0.17	0.21*
Microbial Respiration (mg g^{-1})	0.24*	0.40*	0.48*	0.01	0.21
MBC (mg g^{-1})	0.08	0.52*	0.42*	0.13	0.03

*Significant at $P < 0.05$.

4. Discussion

4.1. Soil chemical properties in relation to the inclusion of Brachiaria brizantha

After six years of diversification of a common bean monoculture system through the inclusion of the tropical forage *Brachiaria brizantha* cv. Mulato and maize, there was some evidence for a shift in soil chemical properties and macronutrients content. Specifically, B2, B1 and M showed an increase in SOC compared to control treatment, probably related to nutrient content in crop residues, taking into account that this soil is the result of 50 years of intensive monoculture of tobacco and common bean. According to this, it was demonstrated that forage radish as CC results in higher SOC content and distribution in soil surface compared with fallow treatments, contributing to a substantial increase of carbon during growth and later biomass decomposition [26]. SOC increase was more evident in B2 treatment compared to B1 in our work, suggesting that two cycles of *B. brizantha* cv. Mulato in the crop sequence had a positive effect. Concerning TN, it is also important to consider that two consecutive cycles of *B. brizantha* did not negatively impact soil nitrogen content, even considering that it is a fodder plant with high nitrogen demand. *B. brizantha* cv. Mulato increases nitrifying bacterial populations compared to *B. humidicola* cv. Tully [27]. Thus, the inclusion of *B. brizantha* cv. Mulato would be a suitable tool to maintain the nitrogen content in cover cropping schemes in subtropical regions. In this regard, B2 and control treatments were the closest to the optimal C:N ratio of 10:1 [28], suggesting that compared to other treatments, these treatments could have a higher potential to transform organic matter into mineral nitrogen. However, further studies are needed to proved this. Regarding eP, our study also revealed that the inclusion of B2 did not negatively impact on eP content, despite the substantial phosphorus demand of *B. brizantha* [29]. Moreover, the high AP activity registered under B2 may generate an increase of eP in the long term. In this regard, several authors have reported a positive correlation between AP activity and phosphorus content in soil [30,31]. Thus, implementation of this type of forage species may increase soil phosphorus availability compared to the conventional common bean, by promoting the turnover of organic phosphorus. Inclusion of CC apparently supports the preservation of organic matter and associated nutrients in the soil.

4.2. Effects of Brachiaria brizantha on soil microbial activity

The inclusion of *B. brizantha* cv. Mulato in the rotation produced a marked increase in total microbial activity estimated as FDA hydrolysis, and enhanced the efficiency in the decomposition of soil organic matter. FDA hydrolysis has been widely used as indicator to measure overall microbial activity, because the ubiquitous lipase, protease, and esterase are involved in this reaction [32]. Our results suggest that two consecutive cycles of *B. brizantha* cv. Mulato enhance microbial activity even more than observed for maize as CC. In relation to this, *B. brizantha* has been previously employed in the phytoremediation of contaminated soils through an increase of microbial diversity [33]. The increase of microbial activity recorded in our work may be related to the amount of root exudates and energy sources after two cycles of *B. brizantha.* Moreover, the abundant biomass of the aerial parts produces coverage of the superficial soil, minimizing the growth of weeds and enhancing soil microbial activity and biochemical process rates. Higher values of FDA hydrolysis were found to be related to high deposition of plant residues [34]. The recorded increase in FDA hydrolysis could probably indicate a higher proportion of macronutrients, such as carbon and nitrogen, since a positive correlation has been reported between FDA and these nutrients [35]. A similar trend recorded for FDA was observed in our results for AP activity, revealing a higher activity of this enzyme in the two consecutive cycles of *B. brizantha.* Also, B2, B1 and M increase AP activity as compared to control treatment. Similarly, a tropical perennial gramineae (*Paspalumnatatu*) as CC was found to stimulate the AP activity compared to fallow [36]. Although it has been reported that DHA is a sensitive indicator to soil quality, in our work this parameter did not show a clear response. In fact, previous studies conducted by our research group [37,38] in different trials showed the same trend, suggesting an unclear response of DHA to agricultural management. DHA reflects the total range of oxidative activity of soil microflora and can be inhibited by various soil chemical fertilizers and pesticides [39]. Thus, it is possible that certain chemical products may affect the DHA response in treatments under *B. brizantha*. One parameter that sheds light on the effect of agricultural diversification on the soil ecosystem function is the metabolic quotient (qCO_2). A lower qCO_2 reflects improved soil biophysical conditions, while a higher qCO_2 indicates soil degradation under intensive land use [40]. The decrease in qCO_2 observed under two cycles of *B. brizantha* treatments indicates a greater metabolic efficiency compared to conventional system (fallow), maize as CC, even to a single *Brachiaria* cycle. Accordingly, higher metabolic efficiency was observed in plots under cover crops and direct seeding compared to plots under conventional tillage, where qCO_2 was 32% lower under direct seeding [41]. Since B2 showed the lowest value of qCO_2 compared with B1, M and the control, our results suggest that the inclusion of two cycles of *B. brizantha* in the fallow period may be a suitable tool to improve efficiency in the microbial use of carbon sources. In support of these findings, a decrease in qCO_2 was found in plots under species commonly used as CC (oat, vetch,lupin, radish or wheat) compared to fallow [42]. The results of our study showed a similar effect using drought-tolerant tropical forage, which can be cultivated in zones with saline soils or hydric limitations.

4.3. Effects of Brachiaria brizantha on soil fungal and bacterial communities

Six years of *B. brizantha* cv Mulato used as CC did not show significant differences in both fungal and bacterial communities abundance values. Our data showed that M had a tendency to decrease fungal biomass and to increase bacterial biomass, resulting in a low F/B ratio. In contrast, we found a higher abundance of fungal communities in B1 treatment, resulting in a high F/B ratio. This result may be due to the fact that bacteria are more resistant than fungus to changes in soil environment. The abundance of bacteria in soil is associated with high soil moisture, pH, N and faster rates of carbon mineralization [43]. A higher F/B ratio was observed to be associated with an improvement in soil quality through the inclusion of vetch as CC [44]. It is widely accepted that high F/B ratio indicates more sustainable agroecosystems and low impacts on the environment, in which organic matter composition and C mineralization dominate the nutrient supply to plants [45]. Microbial soil communities dominated by fungi can improve carbon storage and contributeto the slow mineralization of organic matter [46]; agricultural management that contributes to the growth of fungal biomass may thus increase carbon sequestration. In this sense, high F/B ratio usually occurs in unaltered grassland ecosystems, grasslands lacking long-term fertilization histories, and agroecosystems

under reduced tillage [47]. It is probable that these relationships may occur in diversified systems, such as B2 and B1. There is little literature that relates the soil physiological efficiency and microbial community structure. Thus, quantifying the abundance of both fungal and bacterial taxonomic groups can provide information about mechanisms involved in the sustainability of local productive systems. Nevertheless, microbial community structure includes different specific groups of bacteria and fungi which can be explored by advanced molecular techniques that exceed this work. Further in depth studies are needed to examine possible variations in soil microbial structure when *B. brizantha* is used as CC in degraded agricultural soils.

4.4. Relationships between soil microbiological and chemical properties

Particularly, FDA hydrolysis, AP activity, microbial respiration and SOC were dominant variables responsible for treatment differentiation. This agrees with previous studies conducted by our work group that showed that soil management primarily impacts microbial functions [48,49]. It has been suggested that enzyme activities are sensitive indicators to evaluate changes in farming systems [50]. In this regard, microbial activities were positively correlated to SOC, indicating that the carbon input through crop residues and root exudates may stimulate microbial enzymes synthesis. Related to this, a decrease in carbon mineralization was observed due to lower soil enzymes activities associated to grasses defoliation [51]. Considering the positive correlation between FDA hydrolysis and TN and SOC observed in our research, the higher soil microbial activity in *B. brizantha* treatments may be a key factor to increase soil macronutrient cycling mechanism efficiency. In this regard, the high qCO_2 observed in B1, M and control treatments reflects a high energy demand to maintain microbial metabolic activity in relation to the energy necessary to synthesize microbial biomass. This effect could probably be reversed with the inclusion of two cycles of *B. brizantha* due to an increase in MBC. The MBC content in soil is a fast reacting and sensitive carbon pool which correlates positively with fertility and productivity [52].

Molecular analysis of both soil fungal and bacterial community did not significantly correlate with the other variables after six years of assay implementation. This may be linked to the limited amount of changes observed in the structure of the microbial communities in relation to those observed in microbial functions. In fact, SOC, TN, EC and pH have been reported as important parameters influencing microbial biomass [53]. Even though bacterial community is important to separate M treatment along PC2, this effect did not show any correlation with microbial activity. Indeed, maize as CC showed a generally low FDA and AP activity and a higher qCO_2, which may be due to a highly inefficient consumption of carbon sources compared to *B. brizantha* (B2) as CC.

In conclusion, this research demonstrates that the inclusion of *B. brizantha* cv. Mulato as CC in a degraded agricultural system may affect soil microbial functionality through the contribution of abundant stubble generated by *B. brizantha* and to increasing microbial efficiency in the use of these carbon sources. Two consecutive cycles of *B. brizantha* stimulated the activity of microbial enzymes and a high metabolic efficiency that favor organic matter decomposition, without a negative impact on soil nitrogen and phosphorus content. In the middle term, compared to microbial community structure, microbial activities seemed to better reflect the changes generated by crop-plant species rotation cycle. Our results show that the use of *B. brizantha* as CC improves soil biochemical properties. Therefore, the use of this forage species as CC can contribute to the sustainability of mixed agricultural systems in the study region and constitute a promising alternative to diversify degraded agroecosystems.

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Abbreviations

qCO_2: metabolic quotient
TN: total nitrogen
eP: extractable phosphorus
EC: electric conductivity
SOC: soil organic carbon
AP: acid phosphatase
FDA: fluorescein di acetate
DHA: dehydrogenase activity
MBC: microbial biomass carbon