

# Different no-till grain production systems with *Urochloa* spp. affect soil microbial community structure, biomass and activity in a tropical Ultisol

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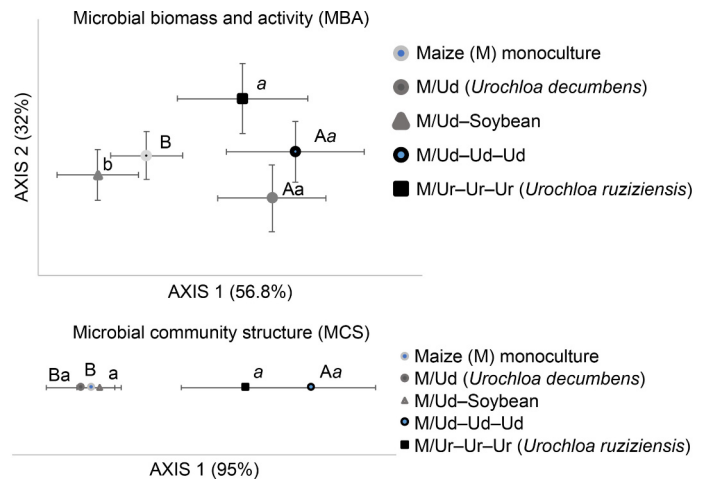
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## ABSTRACT

- Integrated grain cropping systems promote soil health (SH) and sustainability.
- Microbial biomass and activity (MBA) and community structure (MCS) are key to SH.
- Integration of maize with *Urochloa* pastures strongly impacts MBA and MCS.
- MBA is more sensitive than MCS to shifts in grain cropping systems.
- Systems under continuous *Urochloa* increased microbial activity and AMF abundance.

Tropical soils are prone to degradation. Adoption of conservation agricultural practices is essential to improve soil health, which is influenced by soil microbes. In this study we analyzed shifts in microbial biomass and activity (MBA) and microbial community structure (MCS) based on fatty acid methyl esters (FAMES) between five no-till agricultural practices: maize monoculture (MM); maize annually intercropped with *Urochloa decumbens* (M/Ud); M/Ud with soybean rotation every other year (M/Ud-S); M/Ud keeping the pasture for the next two years (M/Ud-Ud-Ud); and maize intercropped with *U. ruziziensis* keeping the pasture for the next two years (M/Ur-Ur-Ur). Results indicated that MBA was affected by the inclusion of *Urochloa* intercropping and by rotation with soybean. Systems under a longer residence time with *Urochloa* in the field had higher  $\beta$ -glucosidase activity and soil basal respiration, indicating a greater microbial activity. MCS was less affected than MBA by the investigated cropping systems. MCS changed only in the continuous pasture systems, which were enriched in arbuscular mycorrhizal fungi (AMF). Additionally, the continuous pasture systems had lower microbial stress ratios than the other agricultural practices. In sum, our study showed that utilization of *Urochloa* spp. under longer periods in no-till agricultural practices contributes to increase microbial activity, AMF abundance and decrease microbial stress ratio. These changes are primarily beneficial for soil health.

**Keywords** crop-pasture integration, crop rotation, no-tillage, soil health, microbial ecology.



## 1 Introduction

The increase in world population demanded the search for grain production systems with higher crop yields while causing lower damage to the environment (Roesch-McNally et al., 2018). Therefore, soil utilization in agricultural systems

needs to be managed to improve soil health, which is the capacity of the soil to perform its ecological functions without impacting the ecosystem (Bünemann et al., 2018; Lehmann et al., 2020). Since soil health correlates with various soil properties, the study of impacts caused by agricultural production systems on soil health is complex, so a set of chemical, physical and biological soil indicators have been evaluated (Bai et al., 2018; Fierer et al., 2021). These indicators must be sensitive to changes in land use, but

resistant to temporary changes not associated with agricultural management, such as the standing annual crop or season (Bünemann et al., 2018; Lopes et al., 2021).

Soil physico-chemical properties such as soil organic matter (SOM), available P, pH, water storage, aggregates mean weight diameter (MWD), water stability of aggregates (WSA), soil density (SD) and mechanical penetration resistance (PR) have been used to assess the feasibility of agricultural systems because they are directly associated with soil quality and health (Bünemann et al., 2018; Lopes et al., 2021). SOM is particularly important, since it directly influences soil chemical, physical and microbiological properties, in addition to being an important agent in carbon (C) sequestration (Reeves, 1997; Bongiorno et al., 2019). Soils with improved physical properties are less impacted by climatic variations, have greater water retention ability and promote better soil aeration and root growth (Nouri et al., 2019). Soil biological indicators (bioindicators) are also very important for assessing soil health, since soil microbes mediate many processes in the soil environment, including SOM decomposition, nutrients cycling, aggregates formation, etc (Schloter et al., 2018; Lehmann et al., 2020; Fierer et al., 2021). Therefore, bioindicators such as soil microbial biomass, basal respiration, enzyme activities, and microbial community structure/functions have been widely used in soil health studies (Fierer et al., 2021).

Previous studies have shown that the utilization of conservation agricultural systems including crop rotation, no-till, intercropping and crop-livestock integration resulted in improvements in soil quality and health (Sant-Anna et al., 2017; Laroca et al., 2018; Peralta et al., 2018; Alhameid et al., 2019; Menezes et al., 2019; Lopes and Fernandes, 2020; Li et al., 2021). For example, crop rotation and no-till increased soil C content, microbial biomass, urease and  $\beta$ -glucosidase activities in a study in South Dakota, USA (Alhameid et al., 2019). In addition, crop rotational diversity increased the disease suppressiveness of the soil microbiome in Michigan, USA (Peralta et al., 2018). Intercropping of pastures with legumes increased soil C stocks (Sant-Anna et al., 2017; Laroca et al., 2018), microbial biomass and  $\beta$ -glucosidase activity (Laroca et al., 2018; Menezes et al., 2019) in Brazilian soils. Intercropping of maize and pigeon pea increased microbial biomass and fungal relative abundance in the Brazilian Coastal Tablelands (Lopes and Fernandes, 2020). The abundance of arbuscular mycorrhizal fungi is also generally increased in intercropping systems (Li et al., 2021).

Intercropping with pasture species of the *Urochloa* genus (previously *Brachiaria*) in no-till grain production systems have been shown to promote substantial improvements in soil quality and health in the tropics, with decreases in SD and increases in SOM, WSA and microbial activity (Bonetti et al., 2018; Rego et al., 2020; Sousa et al., 2020). In our

previous study based on the same field experiment used in the current work, intercropping between maize and *Urochloa* under no-till was shown to improve soil quality and health compared to other conservation systems in the Brazilian Coastal Tablelands (Lopes et al., 2021). Here, we have extended that investigation by testing a longer residence time of *Urochloa* spp. in the field. In addition, we aimed to compare the effects of different *Urochloa* species, since previous studies showed that the quantity and quality of soil C, soil microbial community structure, biomass, activity and functions, are different where different plant species are cultivated (Huggins et al., 2007; Jesus et al., 2016; Finney et al., 2017; Lopes and Fernandes, 2020; Parhizkar et al., 2021).

Therefore, in this work we assessed the changes in microbial biomass and activity (MBA) and microbial community structure (MCS) between different grain production systems under no-till in the Brazilian Coastal Tablelands, including maize monoculture (MM), maize annually intercropped with *Urochloa decumbens* (M/Ud), M/Ud with soybean rotation every other year (M/Ud-S), M/Ud keeping the pasture only (no additional crops) for the next two years (M/Ud-Ud-Ud), and maize intercropped with *U. ruziziensis* keeping the sole pasture for the next two years (M/Ur-Ur-Ur). We tested the following hypotheses: (I) the residence time of *Urochloa decumbens* in the system impacts MBA and MCS; (II) the insertion of soybean rotation every other year affects MBA and MCS; and (III) the use of different *Urochloa* species affects MBA and MCS.

## 2 Materials and methods

### 2.1 Field experiment

The experiment reported in this work was conducted in an experimental farm of the Brazilian Agricultural Research Corporation (Embrapa) located in Nossa Senhora das Dores-SE, Brazil (10°27' S and 37°11' W). The soil in that location is a dystrophic Ultisol. The average annual temperature is 26°C and the average annual precipitation is 1150 mm, which is highly concentrated (around 70%) between April and August.

The experiment was arranged in a randomized block design, each block with four replicates (400 m<sup>2</sup> plots, with 3 m between them, and 10 m between the blocks) containing five agricultural practices managed under no-till: maize monoculture (MM), maize annually intercropped with *Urochloa decumbens* (M/Ud), M/Ud with soybean rotation every other year (M/Ud-S), M/Ud with extended permanence of the pasture for the next two years (M/Ud-Ud-Ud), and maize intercropped with *U. ruziziensis* with extended permanence of the pasture for the next two years (M/Ur-Ur-Ur).

The maize genotype DKB 177 PRO3 was planted at a density of 70 000 seeds ha<sup>-1</sup>, while soybean genotype FT 4280 IPRO was planted at a density of 320 000 seeds ha<sup>-1</sup> on June 3rd and 4th, 2020. For both crops, we used a distance of 0.5 m between rows. In M/Ud, maize and *U. decumbens* were sown together annually. The *U. decumbens* seeds were planted at the same height of fertilizers, below the maize seeds. Soils were fertilized with 150 kg N ha<sup>-1</sup>, 80 kg P ha<sup>-1</sup> and 116 kg K ha<sup>-1</sup> before planting, and 150 kg N ha<sup>-1</sup> at maize V4 stage (four leaves fully unfolded). For soybean there was no N fertilization, but seed inoculation with *Bradyrhizobium japonicum* instead. Weeds were controlled with the herbicide glyphosate before planting and 30 days after plant emergence. The MM, M/Ud and M/Ud–S agricultural practices had been used continuously in this experiment since 2009, while the practices with extended *Urochloa* cropping (M/Ud–Ud–Ud and M/Ur–Ur–Ur) started in 2018 (Table 1 and Supplemental Fig. 1).

## 2.2 Soil sampling and analyses of microbial biomass and activity

On December 6th 2020, after soybean and maize harvesting, soil samples were collected for microbial community structure (MCS), biomass, basal respiration and enzyme activity

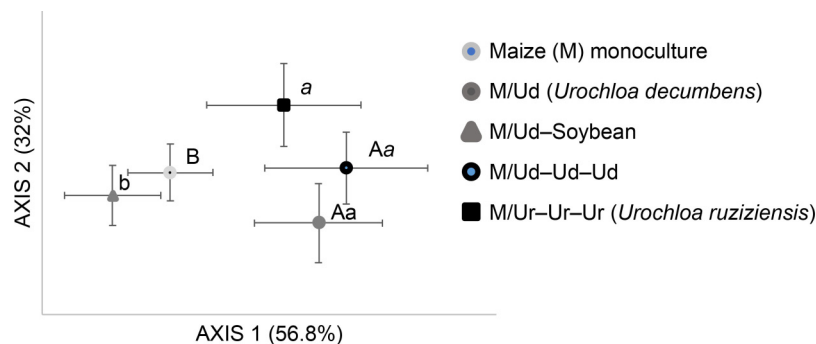
analyses. Soil samples were passed through a 2 mm sieve and stored at 4°C until the analyses, which were performed within two weeks after sampling. For soil basal respiration, 32 g of soil were weighed and moisture was adjusted to 80% soil water holding capacity. Samples were stored in hermetically sealed glass jars containing open vials with 5 mL NaOH (1 mol L<sup>-1</sup>) for 10 days in the dark at 25°C. After this period, the evolved CO<sub>2</sub>-C was quantified by titrating the NaOH with HCl (0.1 mol L<sup>-1</sup>) (Jenkinson, 1976).

We estimated microbial biomass by quantifying microbial biomass C using the difference between the values of organic C extracted in fumigated and non-fumigated samples, which were quantified in a spectrophotometer at 495 nm (Vance et al., 1987). The activity of the enzymes β-glucosidase, acid phosphatase and arylsulfatase were determined from assessment of the p-nitrophenol released by these enzymes in soil samples. Analyses were carried out in duplicates. Samples were incubated with buffered solutions of substrates specific to each enzyme for 1 h at 37°C. After incubation, readings were performed in a spectrophotometer at a 410 nm absorbance. Results were expressed in µg p-nitrophenol h<sup>-1</sup> g<sup>-1</sup> soil using a standard curve of known values of p-nitrophenol (Tabatabai, 1994).

For fluorescein diacetate hydrolysis (3',6'-diacetylfluorescein, FDA) assay 1 g of soil from each sample was incubated

**Table 1** History of the grain production systems from 2017 to 2020.

Production systems/ year	MM	M/Ud	M/Ud–S	M/Ud–Ud–Ud	M/Ur–Ur–Ur
2017	M	M/Ud	M/Ud	M/Ud	M/Ur
2018	M	M/Ud	S	M/Ud	M/Ur
2019	M	M/Ud	M/Ud	Ud	Ur
2020	M	M/Ud	S	Ud	Ur



**Fig. 1** Non-metric multidimensional scaling (NMDS) graph of microbial biomass and activity (MBA) in soil samples under different soil management systems: maize in monoculture (MM), maize intercropped with *Urochloa decumbens* (M/Ud), maize intercropped with *Urochloa decumbens* and annual rotation with soybean (M/Ud–S), M/Ud and M/Ur followed for the next two years by *Urochloa decumbens* pasture (M/Ud–Ud–Ud) and *Urochloa ruziziensis* pasture (M/Ur–Ur–Ur). Different letters in the figure indicate significant differences between the agricultural practices. Uppercase letters compare the effect of continuous residence time of *Urochloa decumbens* (MM vs. M/Ud vs. M/Ud–Ud–Ud) on microbial biomass and activity (MBA), lowercase letters indicate whether the insertion of the soybean crop in rotation with M/Ud (M/Ud vs. M/Ud–S) caused changes in MBA, and lowercase letters in italics compare whether *Urochloa* species (M/Ud–Ud–Ud vs. M/Ur–Ur–Ur) differ in MBA by Multiresponse Permutation Procedures (MRPP) ( $p < 0.01$ ). Horizontal and vertical bars indicate  $\pm 1$  S.D. from centroids of treatment means along axes 1 and 2, respectively.

in phosphate buffer (6.0 mmol L<sup>-1</sup>, pH 7.6) with sodium fluorescein diacetate under shaking for 2.5 h. Acetone was added after this time to stop reactions. Absorbance readings were performed at 490 nm and results expressed in µg fluorescein h<sup>-1</sup> g<sup>-1</sup> soil using a standard curve of known amounts of fluorescein.

### 2.3 Analysis of FAMES for microbial community structure

Fatty acid methyl ester (FAME) analysis was used to investigate changes in soil MCS with the method proposed by Schutter and Dick (2000), where the mild alkaline methanolysis reaction was performed directly on the soil samples. Three g of soil was weighed in glass centrifuge tubes, 15 mL KOH (0.2 mol L<sup>-1</sup>) prepared in methanol was added, and the tubes were vortexed for 15 s. Then the tubes were submitted to a water bath for 1 h at 37°C and at every 10 min the tubes were shaken. Then 3 mL of acetic acid (1 mol L<sup>-1</sup>) was added, tubes were stirred, 10 mL of hexane was added and the tubes were centrifuged for 10 min (480 g). The hexane phase, containing the FAMES, was collected into a new glass tube and dried with ultrapure N<sub>2</sub> at 38°C. The fatty acids were resuspended in hexane, transferred to 2 mL GC-vials and analyzed in a gas chromatograph equipped with a capillary column (5% biphenyl-95% dimethylpolysiloxane, 25 mol L<sup>-1</sup> and flame ionization detector, programmed to promote an increment of 5°C per min, from 120°C to 270°C). Injector and detector temperatures were 250°C and 280°C, respectively. A standard curve prepared from a solution of tridecanoic acid methyl ester (Supelco) was used to convert chromatographic areas to nanomoles of FAMES and the results were expressed as mol percent (mol%) of FAMES in the sample.

FAMES with masses < 4% of the FAME 16:0 mass were excluded from the data matrix. Although FAMES are not necessarily taxon-specific, higher relative abundance of some FAME chemical groups are typically found in major microbial groups, as follows: FAMES 15:0i, 15:0a, 16:0i, 17:0i and 17:0a were used as biomarkers for Gram-positive bacteria; FAMES 17:0cy and 18:1ω7c for Gram-negative bacteria; FAMES 16:0 10-Me, 17:0 10-Me and 18:0 10-Me for actinomycetes; FAME 16:1ω5c for arbuscular mycorrhizal fungi (AMF); and FAMES 18:1ω9c and 18:2ω6c for fungi (Zelles, 1999; Kaiser et al., 2010; Willers et al., 2015).

### 2.4 Statistical analyses

Two data matrices were separately used for multivariate analyses: (i) data of soil microbial biomass, respiration and enzymes activity, representing microbial biomass and activity (MBA); and (ii) data of relative abundance of FAMES, representing microbial community structure (MCS). Differences among soil samples regarding MCS and MBA were graphi-

cally represented by a non-metric multidimensional scaling (NMDS) ordination based on a Sorensen distance matrix (Sokal, 1979; Mccune and Grace, 2002). Before that, MCS and MBA data were standardized by sample totals for each variable to account for different scales among the variables. The NMDS plots were generated in the PC-ORD software v. 6.04 (McCune and Mefford, 1999) in autopilot mode.

Pearson correlations between sample scores and individual variables in each matrix were performed to characterize changes in MCS and MBA under the different grain production systems. The ratio between FAMES 17:0cy and 16:1ω7c was also calculated and used as a marker of microbial stress in Pearson correlation analyses, but it was not included as a variable in the main MCS matrix used for NMDS ordination. Multivariate tests were used to evaluate the effect of the residence time of *Urochloa decumbens*, the insertion of soybean rotation in the M/Ur system, and the choice of *Urochloa* species (Ud vs. Ur) on MCS and MBA, using the multiresponse permutation procedure (MRPP) (Mielke and Berry, 2007).

Univariate statistical analyses were performed in each individual variable belonging to MBA and MCS, to test if they differ between the five agricultural practices assessed. The values of the individual variables were submitted to analysis of variance (ANOVA) and Tukey's HSD pairwise test using the Past software (Hammer et al., 2007).

## 3 Results

### 3.1 Changes in soil microbial biomass and activity between grain production systems

A two-dimensional graphic solution was selected by the NMDS, which represented 88.8% of the total variability of the MBA data, with 56.8% of this variability associated with axis 1 and 32% with axis 2 (Fig. 1). The gradient of MBA along NMDS axis 1 was positively correlated with changes in β-glucosidase ( $p < 0.01$ ), arylsulfatase ( $p < 0.01$ ) and soil basal respiration ( $p < 0.05$ ); and negatively correlated with microbial biomass-C ( $p < 0.01$ ) (Table 2). Although NMDS axis 2 showed a lower percentage of variability than axis 1, its gradient was positively correlated with basal respiration ( $p < 0.01$ ); microbial biomass-C ( $p < 0.01$ ); acid phosphatase ( $p < 0.01$ ); arylsulfatase ( $p < 0.05$ ) and fluorescein diacetate hydrolysis ( $p < 0.05$ ) (Table 2).

According to MRPP, the longer residence time of *U. decumbens* in the grain production systems influenced MBA, although only MM (0 months with Ud) differed from the other two treatments – those being M/Ud (9 months with Ud) and M/Ud–Ud–Ud (30 months with Ud) (Fig. 1). Therefore, MBA was more affected by the inclusion of *Urochloa* in the system than by the residence time of *Urochloa* in the field. It



is noteworthy that MRPP results agreed with the graphic separation of these three treatments along NMDS axis 1, but not along axis 2. The inclusion of soybean as a rotation crop with M/Ud influenced MBA (MRPP,  $p < 0.01$ ), with separation between M/Ud and M/Ud-S treatments clearly observed along NMDS axis 1, but not along axis 2 (Fig. 1). The choice of the *Urochloa* species (*U. decumbens* vs *U. ruziziensis*) had no effect on MBA in the cropping systems where they were kept as pastures only (no other crops) for the following two years (M/Ud-Ud-Ud vs M/Ur-Ur-Ur) (Fig. 1).

**Table 2** Pearson correlation coefficients ( $r$ ) between microbial biomass and activity (MBA) individual variables and NMDS axes 1 and 2.

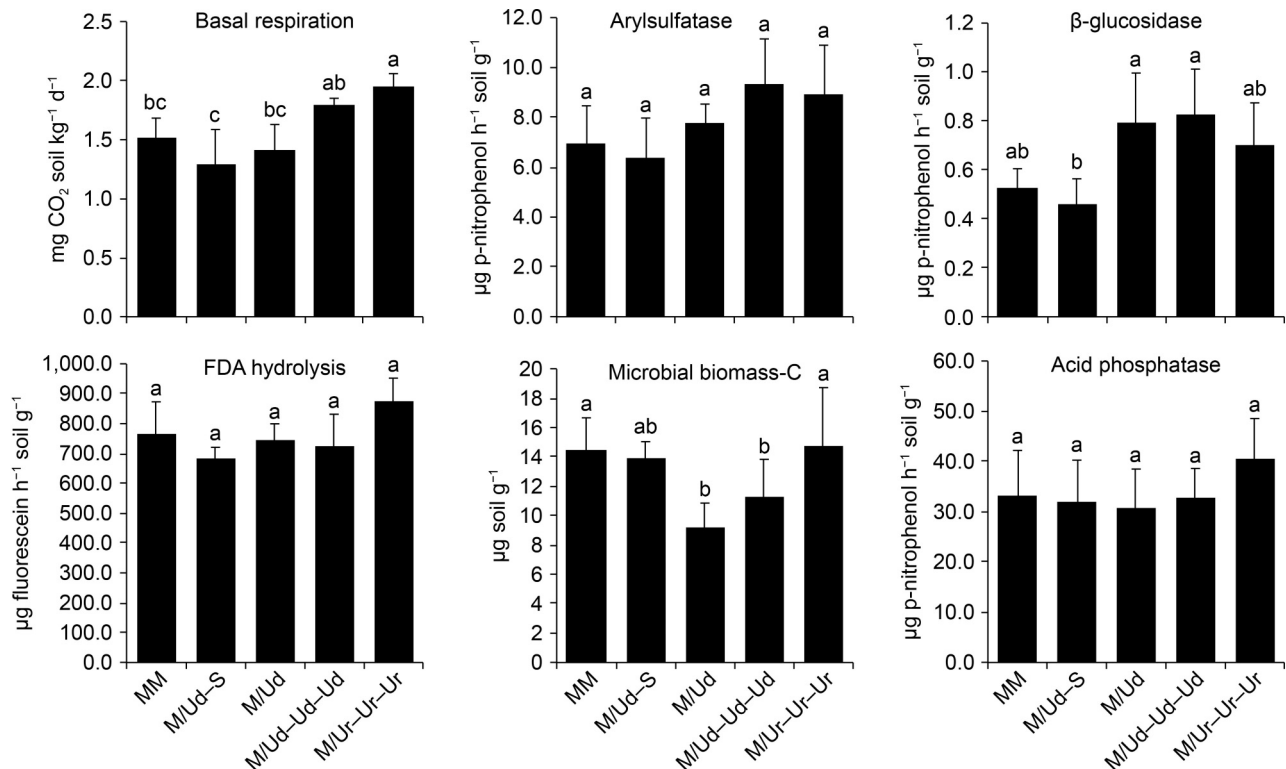
MBA variables	Axis 1	Axis 2
Basal respiration	0.44*	0.79**
Microbial biomass-C	-0.73**	0.61**
$\beta$ -glucosidase	0.96**	-0.09
Arylsulfatase	0.55**	0.45*
Fluorescein diacetate hydrolysis	0.19 <sup>ns</sup>	0.43*
Acid phosphatase	-0.05 <sup>ns</sup>	0.61**

\*  $|r| > 0.41$ , significant at 5%; \*\*  $|r| > 0.52$  significant at 1%.

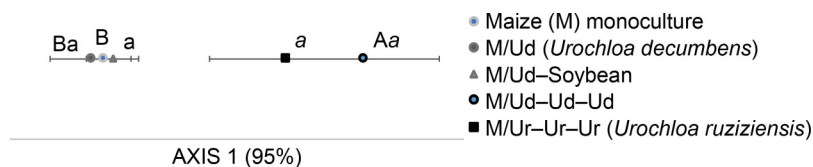
The individual MBA variables respiration and  $\beta$ -glucosidase in general had higher values in the systems with *Urochloa* spp. without the soybean rotation (Fig. 2). Soil respiration was significantly larger ( $p < 0.01$ ) in the continuous pasture systems than in the soybean rotation system, while  $\beta$ -glucosidase activity was significantly higher in the systems with *U. decumbens* intercropping than in the system with soybean rotation ( $p < 0.05$ ) (Fig. 2). On the other hand, microbial biomass-C was significantly larger ( $p < 0.05$ ) in the continuous pasture system with *U. ruziziensis* and in the MM system compared to M/Ud and M/Ud-Ud-Ud (Fig. 2).

### 3.2 Changes in soil microbial community structure between grain production systems

A one-dimensional NMDS graphical solution represented 95% of the variability in the microbial community structure (MCS) data (Fig. 3). A MCS gradient, from the left to the right of this graph, was characterized by relative increments in biomarkers for arbuscular mycorrhizal fungi ( $p < 0.01$ ), Gram-negative bacteria ( $p < 0.01$ ) and fungi ( $p < 0.05$ ), while the markers for Gram-positive bacteria ( $p < 0.01$ ) and actinomycetes ( $p < 0.01$ ) increased in the opposite direction – from the right to



**Fig. 2** Bar charts showing the mean values of the individual variables composing the microbial biomass and activity (MBA) in the different grain production systems: basal respiration, arylsulfatase,  $\beta$ -glucosidase, fluorescein diacetate (FDA) hydrolysis, microbial biomass-carbon and acid phosphatase. Error bars indicate the standard deviation among the samples for each treatment – maize in monoculture (MM), maize intercropped with *Urochloa decumbens* (M/Ud), maize intercropped with *Urochloa decumbens* and annual rotation with soybean (M/Ud-S), M/Ud and M/Ur followed for the next two years by *Urochloa decumbens* pasture (M/Ud-Ud-Ud) and *Urochloa ruziziensis* pasture (M/Ur-Ur-Ur), respectively. Different letters indicate significant differences according to Tukey's pairwise test ( $p < 0.05$ ).



**Fig. 3** Non-metric multidimensional scaling (NMDS) plot of microbial community structure (MCS) in soil samples, accessed using fatty acid methyl ester (FAME) profiles, under different soil management systems: maize in monoculture (MM), maize intercropped with *Urochloa decumbens* (M/Ud), maize intercropped with *Urochloa decumbens* and annual rotation with soybean (M/Ud-S), M/Ud and M/Ur followed for the next two years by *Urochloa decumbens* pasture (M/Ud-Ud-Ud) and *Urochloa ruziziensis* pasture (M/Ur-Ur-Ur). Different letters in the figure indicate significant differences between the agricultural practices. Uppercase letters indicate the effects of continuous residence time of *Urochloa decumbens* (MM vs. M/Ud vs. M/Ud-Ud-Ud) on microbial biomass and activity (MBA), lowercase letters show whether the insertion of soybean in crop rotation with M/Ud (M/Ud vs. M/Ud-S) caused changes in MBA, and lowercase letters in italics compare whether *Urochloa* species (M/Ud-Ud-Ud vs. M/Ur-Ur-Ur) differ in MBA by Multiresponse Permutation Procedures (MRPP) ( $p < 0.01$ ). Horizontal bars indicate  $\pm 1$  S.D. from centroids of treatment means along axis 1.

the left (Table 3, Fig. 3). The microbial stress marker, represented by the 17:0cy/16:1 $\omega$ 7c FAME ratio, was negatively correlated with the MCS along axis 1 ( $p < 0.01$ ) (Table 3).

The residence time of *U. decumbens* on the production systems influenced MCS, with a significant difference between the system with *U. decumbens* for a longer period (M/Ud-Ud-Ud; 30 months with Ud) compared to those under MM (0 months with Ud) and M/Ud (9 months with Ud) (Table 3, Fig. 3). Neither the inclusion of soybean in the rotation with M/Ud (M/Ud vs. M/Ud-S) nor the choice of the *Urochloa* species (M/Ud-Ud-Ud vs. M/Ur-Ur-Ur) affected MCS (MRPP,  $p > 0.05$ ) (Table 3, Fig. 3). On the other hand, the NMDS clearly separated samples of the continued *Urochloa* spp. systems (right) from all other agricultural practices (left) (Fig. 3). Therefore, it is possible to associate an increase in the relative abundance of AMF, Gram-negative bacteria and fungi, and a decrease in the relative abundance of Gram-positive bacteria, actinomycetes and the microbial stress biomarker in the practices that kept *Urochloa* in the field for two years (Fig. 3).

The individual MCS variables (microbial groups) confirmed the NMDS ordination. The relative abundance of AMF was significantly ( $p < 0.05$ ) higher in the systems with a longer residence time of pasture in the field compared to all other practices, regardless the *Urochloa* species (Fig. 4). On the other hand, the relative abundance of actinomycetes and the stress biomarker 17:0cy/16:1 $\omega$ 7c were significantly lower ( $p < 0.05$ ) in the continuous pasture systems compared to all other practices (Fig. 4). The relative abundance of actinomycetes was significantly lower ( $p < 0.05$ ) in the continuous pasture with *U. decumbens* compared to all other agricultural practices, except for the continuous pasture with *U. ruziziensis* (Fig. 4).

## 4 Discussion

The presence of *Urochloa* influenced soil microbial biomass and activity, stimulating soil microbial respiration and

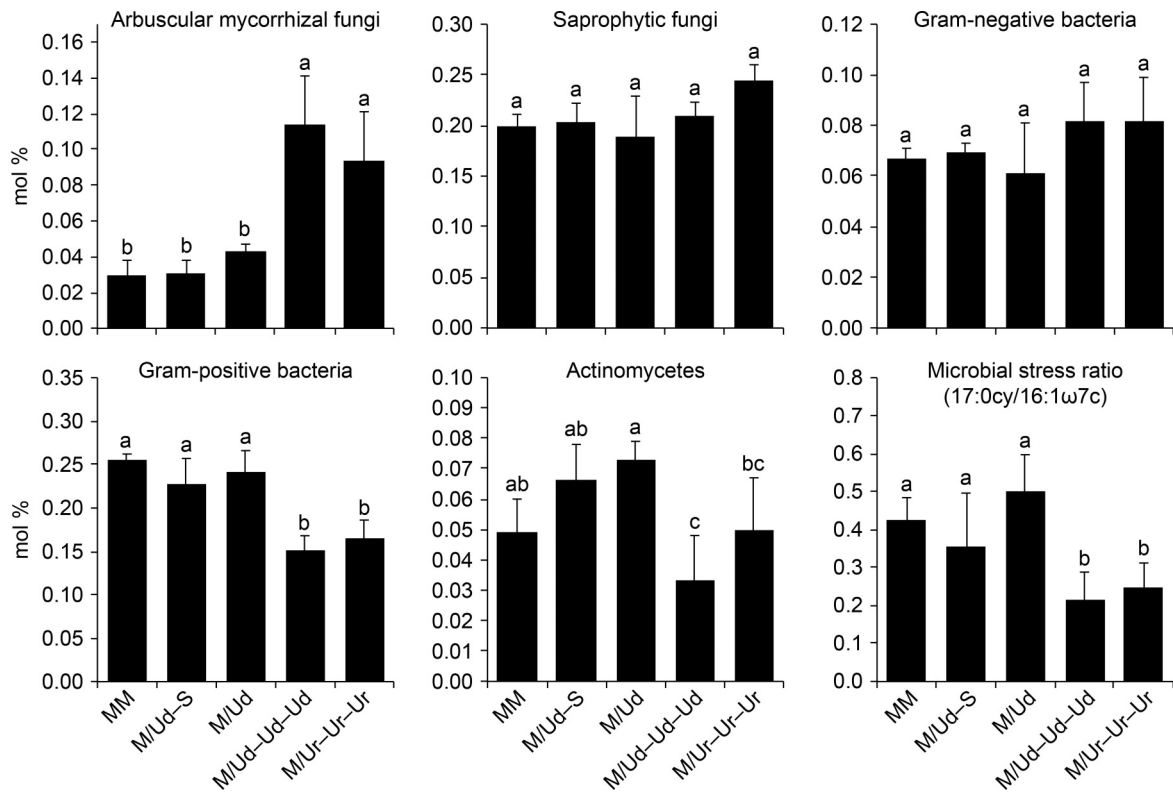
**Table 3** Pearson correlation coefficients ( $r$ ) between the sum of mol% contribution of fatty acid methyl esters (FAME) used as biomarkers for microbial groups and microbial stress ratio (17:0cy/16:1 $\omega$ 7c) along NMDS axis 1.

FAME biomarkers	NMDS axis 1
Gram-positive bacteria	-0.92**
Actinomycetes	-0.79**
Arbuscular mycorrhizal fungi	0.94**
Gram-negative bacteria	0.54**
Saprophytic fungi	0.43*
17:0cy/16:1 $\omega$ 7c	-0.76**

\*  $|r| > 0.41$ , significant at 5%; \*\*  $|r| > 0.52$ , significant at 1%.

enzyme activity. Among the assessed enzymes,  $\beta$ -glucosidase participates in the C cycle acting in the final stage of cellulose degradation ( $\beta$ -1,4-D-glucose polymer) (Eriksson and Wood, 1985). Management systems with pastures generally provide a greater deposition of plant residues and hence increase soil C (Delelegn et al., 2017; Mariscal-Sancho et al., 2018; Chen et al., 2019; Sarto et al., 2020), which was probably the reason for the higher concentrations of  $\beta$ -glucosidase in the systems with *Urochloa*. Microbial activity is intimately associated with soil basal respiration, which is decreased when the soil enzymes that participate in the decomposition of C compounds are inhibited (Ameloot et al., 2014; Zhang et al., 2014; Chen J et al., 2018; Liang et al., 2018; Chen et al., 2019). The agricultural practices with longer *Urochloa* residence time in general had higher soil respiration and  $\beta$ -glucosidase activity, indicating that the continuous pasture systems promoted increases in soil microbial activity.

In contrast, microbial biomass-C was larger in the agricultural practice where *U. decumbens* was not present (MM). The season when the soil is collected influences microbial biomass-C results, as reported in previous studies that detected increased soil microbial biomass under drought conditions (Schaeffer et al., 2017; Lopes et al., 2021). The MM practice does not have the pasture to protect soil



**Fig. 4** Bar charts showing the mean values of the sum of mol% contribution of fatty acid methyl esters (FAME) used as biomarkers for microbial groups (Gram-negative bacteria, Gram-positive bacteria, actinomycetes, saprophytic fungi and arbuscular mycorrhizal fungi) and microbial stress ratio (17:0cy/16:1ω7c) in the different grain production systems. Error bars indicate the standard deviation among the samples of each treatment—maize in monoculture (MM), maize intercropped with *Urochloa decumbens* (M/Ud), maize intercropped with *Urochloa decumbens* and annual rotation with soybean (M/Ud-S), M/Ud and M/Ur followed for the next two years by *Urochloa decumbens* pasture (M/Ud-Ud-Ud) and *Urochloa ruziziensis* pasture (M/Ur-Ur-Ur), respectively. Different letters indicate significant differences according to Tukey’s pairwise test ( $p < 0.05$ ).

surface in the summer. Moreover, previous studies showed that the MM practice has a low amount of litter in the summer, which causes higher variations in soil moisture (Coelho et al., 2013; Shen et al., 2018). Soils covered with litter have greater moisture compared to uncovered soils (Deutsch et al., 2010). Lower soil water potential inhibits microbial activity and shifts the microbial physiology toward accumulation of osmolytes, exopolysaccharides and thicker cell walls, in order to resist soil drought (Schimel et al., 2007; Schaeffer et al., 2017; Kakumanu et al., 2019). Therefore, the lower soil basal respiration and higher microbial biomass in the practice without *U. decumbens* intercropping (i.e. MM) was probably due to the lower soil moisture caused by the absence of soil coverage (pasture and litter) in the drought season. The larger microbial biomass in the system under extended pasture with *U. ruziziensis* compared to that with *U. decumbens* suggests that the two *Urochloa* species have different dynamics in this respect, despite they did not differ significantly when the multiple variables of MBA and MCS were analyzed together.

The contrasting amounts of C deposition between the compared agricultural systems also explain the differences

in MBA between the system without and with soybean rotation. Soybean litter amount and residence time on the field are much lower compared to that of maize and *Urochloa* (Pacheco and Barros, 2014). A previous study in this field experiment showed that 30 days after soybean harvest, 85% of soil surface was uncovered, 8% was covered with mulch and 7% was covered with green vegetation, while corresponding values for maize systems integrated with either *U. decumbens* or *U. ruziziensis* were 0%–2%, 60%–63% and 37%–38% (Pacheco and Barros, 2014) – which is a dramatic difference in soil coverage between the systems with or without soybean rotation. The reason for these differences is probably because legumes such as soybean produce less biomass and have a lower C:N ratio than maize and *Urochloa*, thus accelerating the decomposition of residues (Huggins et al., 2007). The main MBA variable affected by soybean rotation was soil respiration, which was significantly lower compared to that in the continuous pasture systems. These differences in respiration reflect the contrasting C depositions in the practice with soybean rotation (minimal) and in the practices under continuous pasture (maximal). In addition to the different C inputs, the differences

in N fertilization may also have contributed to the differences in MBA between the systems with and without soybean rotation, since the soybean system had no chemical N fertilization and previous studies have showed that inorganic N addition affects the soil microbiome (Zhong et al., 2015; Fernandes et al., 2021).

Soil MCS is usually affected by the history of land use, including the soil tillage systems and the crop sequence (Jangid et al., 2011; Ashworth et al., 2017). In the present study, the larger residence time of *Urochloa* in grain production systems influenced MCS. A previous study showed that conversion of forest to pasture affected MCS, increasing the relative abundance of many microbial groups when compared to a forest in the first stage of regeneration (Pedrinho et al., 2019). Another study showed that pasture conversion caused increases in the relative abundance of total bacteria, fungi and arbuscular mycorrhizal fungi compared to chili–corn and watermelon–corn rotation systems (Flores-Rentería et al., 2020). Conversion of no-till agricultural practices to fallow dominated by pasture also increased the relative abundance of Gram-negative bacteria and arbuscular mycorrhizal fungi (Lopes and Fernandes, 2020). In our study, the systems with a longer residence time of *Urochloa* pasture in the field also increased the relative abundance of arbuscular mycorrhizal fungi. The increases in the relative abundance of arbuscular mycorrhizal fungi is beneficial to plants, since it improves the water and nutrient absorption of the root system (Chen M et al., 2018). In addition, arbuscular mycorrhizal fungi produce glomalin, which contributes to the formation and stability of soil aggregates (Wilkes et al., 2021). Greater soil aggregation is essential to improve soil quality in the tropics (Lopes et al., 2021), since it increases water infiltration and retention, soil macroporosity and aeration, protection of soil organic matter, and decreases soil compaction and erosion – which are pivotal for successful crop yield (Lehmann et al., 2017; Nouri et al., 2019). Therefore, the longer residence time of *Urochloa* pasture in the field stimulated soil microbial activity and increased the relative abundance of arbuscular mycorrhizal fungi, which may ameliorate soil physico-chemical properties for crop production in this region.

In contrast to the increases in relative abundance of arbuscular mycorrhizal fungi in the systems under a longer residence time of *Urochloa*, the relative abundance of Gram-positive bacteria and actinomycetes increased in the agricultural practices without continuous *Urochloa* (MM, M/Ud and M/Ud–S). This result is concordant with the findings of Brennan and Acosta-Martinez (2017) who identified an enrichment of actinomycetes and Gram-positive bacteria in systems without vegetation coverage. The reason for the greater relative abundance of these microbial groups in the systems with uncovered soils is probably due to lower soil moisture,

since drought stress can shape the bacterial community increasing the Gram-positive/Gram-negative bacterial ratio (Nazih et al., 2001; Uhlířová et al., 2005). Indeed, we observed an enrichment of the microbial stress ratio 17:0cy/16:1w7c in these systems than in the continuous pastures. The change in the microbial community composition may be related to the inherent resistance provided by the thicker cell wall of Gram-positive bacteria and actinomycetes (Harris, 1981; Schimel et al., 2007). Interestingly, there are many actinomycetes associated with plant abiotic stress resistance, mainly *Streptomyces* spp. (Viaene et al., 2016). Therefore, the enrichment of these bacteria in the practices without continuous *Urochloa* may help plants to tolerate the higher drought stress in the cropping season. On the other hand, the lower relative abundance of the microbial stress ratio in the systems under continuous pasture suggests that the longer residence time of *Urochloa* in the field alleviates the drought stress and promotes a more conducive environment for soil biological activity and enrichment of microbes that are beneficial for plant growth and soil health.

## 5 Conclusions

MBA was affected by the presence of *Urochloa* and its residence time on the field in integrated grain production systems, as well as by the inclusion of soybean in rotation with maize integrated with *U. decumbens*. Systems with intercropping between maize and *Urochloa* without soybean rotation in general had higher  $\beta$ -glucosidase activity and soil basal respiration, and lower microbial-biomass C. On the other hand, MBA did not differ between cropping systems with subtle differences in mulch pattern, i.e., between the two *Urochloa* species. MCS was less affected by different grain cropping systems than MBA. Only the systems with longer residence time of *Urochloa* spp. in the field had a different MCS compared to the others. Arbuscular mycorrhizal fungi (AMF) were enriched and microbial stress ratio was depleted on the systems under a longer *Urochloa* residence time, regardless of the *Urochloa* species. The greater microbial activity, larger AMF relative abundance and lower microbial stress ratio suggest that the introduction and longer residence time of *Urochloa* in no-till grain production systems have a positive effect on soil health and conservation.

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## Declaration of competing interest

The authors declare no conflict of interest.

## Electronic supplementary material

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