

# Changes in microbial community structure and physiological profile in a kaolinitic tropical soil under different conservation agricultural practices



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## ABSTRACT

Conservation management practices are essential for developing a sustainable agriculture. However, changes in agricultural practices impact soil microbial communities, which in turn influence soil ecosystem ecology and functioning. Investigations about the effect of agricultural practices on microbial community structure and functional profile in tropical soils are limited to a few types of land use and soil management. In this study, we used a 9-year old field experiment with a kaolinitic Kandiuudalfs located in Northeastern Brazil to assess the impact of six agricultural practices (three tillage methods combined with two cropping systems) and fallow plots dominated by *Brachiaria* sp. on soil phospholipid fatty acid (PLFA)-based microbial community structure and stress status, community-level physiological profile (CLPP), microbial biomass-C (MB-C), basal respiration and pH. The three tillage methods assessed were conventional tillage (CT; plowing + disking), chisel plowing (CP; no disking) and no-till (NT; no plowing nor disking), while the two cropping systems were monoculture (MM; sole maize) and intercropping (MP; maize with pigeonpea). Statistical analyses were performed to assess the differences in the following contrasts: agriculture (all agricultural practices) vs fallow; conventional tillage vs conservation tillage (CP + NT); chisel plowing vs no-till; and maize monoculture vs intercropping. Microbial community structure was more affected by the different agricultural practices and fallow than CLPP, but changes did not correspond to increasing in soil physical quality and crop system complexity. On the other hand, CLPP showed lower differences between fallow vs agricultural soils and was unaffected by tillage methods, but was changed according to cropping systems - properly reflecting the increases in crop system complexity. Fallow soils decreased amino acid degradation capability, microbial stress status, gram-positive bacteria and actinomycetes biomarkers, and increased arbuscular mycorrhizal fungi (AMF) and gram-negative bacteria biomarkers, MB-C and basal respiration. Conventional tillage unexpectedly led to increases in AMF and decreases in stress biomarkers compared to conservation tillage. Intercropping decreased soil pH and increased MB-C compared to monoculture. Our results showed that microbial community structure and physiological profile are distinct regarding their responses to the agricultural practices analyzed, as well as on their correlations with the soil and ecosystem benefits promoted by conservation practices. Therefore, taxonomic and functional approaches of microbial community profiling are potentially useful, but need to be combined with soil physicochemical data in order to help understanding the complex effects of using sustainable agriculture practices on the soil environment.

## 1. Introduction

Conservation management practices consist in using alternative methods of soil preparation and cropping systems in order to increase soil quality, environmental sustainability and agricultural yields. No-till and chisel plowing are examples of alternative methods of soil preparation promoting lower soil disturbance compared to conventional tillage (Daigh et al., 2018). Intercropping is an alternative crop system

compared to monoculture, where two or more crops are simultaneously planted in the field (Brooker et al., 2015). Legumes are commonly used in intercropping for increasing soil nitrogen, which is essential for plant nutrition and growth (Fustec et al., 2010). Soil tillage and plant cover impact many physical and chemical factors important for soil quality. In turn, soil microbial ecology is affected by these physicochemical factors, including nutrient availability, aeration, moisture, pH and temperature (Fierer and Jackson, 2006). Because distinct microbial groups

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respond differently to these factors, agricultural management has a great potential to modify the structure, biomass, activity and functions of soil microbial communities (Fierer and Jackson, 2006; Fierer et al., 2007; Chaer et al., 2009; Philippot et al., 2013; Souza et al., 2015; Cesarano et al., 2017). On the other hand, microbial groups differ in their potential abilities to impact relevant processes and properties that affect soil quality.

Soil fungi are pivotal for the formation and stabilization of macroaggregates because they entangle soil particles and smaller aggregates with their hyphae (Tisdall and Oades, 1982; Bossuyt et al., 2001; Lehmann et al., 2017). Arbuscular mycorrhizal fungi (AMF) are especially important, since they produce glomalin - a glycoprotein associated with macroaggregates stabilization (Wright and Upadhyaya, 1996; Lovelock et al., 2004). On the other hand, bacteria highly contribute on soil microaggregates formation, mainly by exopolysaccharides (EPS) biosynthesis (Lehmann et al., 2017; Krause et al., 2019). The formation and maintenance of soil organic matter (SOM) are also associated with specific microbial groups showing distinct litter decomposition efficiencies (Strickland et al., 2009). Some fungal (e.g. Basidiomycetes) and bacterial (e.g. Caulobacteraceae and Comamonadaceae) taxa are directly associated with the decomposition of recalcitrant fractions of plant residues such as lignin (Hatakka, 2001; Wilhelm et al., 2019). Finally, some microbial groups such as plant-growth promoting rhizobacteria (PGPR) and AMF can improve plant nutrition and health (Philippot et al., 2013).

Microorganisms have several physiological acclimation mechanisms to cope with environmental stresses. Phenotypic plasticity responding to environmental stresses has a cost at the organismal level, which can affect carbon, energy and nutrient flows in the ecosystem (Schimel et al., 2007). Due to the potential impact of microbial community composition and stress status on the ecosystem function, many authors suggested to use these variables as soil quality indicators (Bossio et al., 2005; Aboim et al., 2008; Chaer et al., 2009; Fernandes et al., 2011). Correlations between the microbial community-level physiological profile (CLPP) and relevant variables for soil quality (e.g. aggregate stability, infiltration rate, available water and cation exchange capacity) were observed in a coastal tableland soil (Chaer et al., 2009). Similarly, microbial parameters such as fungal/bacterial ratio, microbial biomass and stress status analyzed by phospholipid fatty acid analysis (PLFA) have been shown to respond to short-term shifts in soil management in this region (Fernandes et al., 2011).

Despite their potential for monitoring ecosystem functioning changes, taxonomy- and function-based approaches for profiling microbial communities may differ in their ability to discriminate land use systems, since many taxonomic groups may be functionally redundant and/or physiological changes may be faster than shifts in community structure (Allison and Martiny, 2008). Moreover, investigations regarding the relationship between microbial community structure and functional profile in tropical soils are limited to a few types of land use and management factors (Waldrop et al., 2000; Bossio et al., 2005; Mendes et al., 2015; Souza et al., 2015).

Therefore, in this study we assessed the impact of different conservation agricultural practices on the functional and taxonomic profiles of soil microbial communities in order to investigate the usefulness of these approaches to discriminate the compared land use systems. Our goal was to test if microbial community structure and physiological profile are changed between conservation agricultural practices in a kaolinitic soil of the Brazilian coastal tablelands. Additionally, we aimed to understand if these taxonomic and functional changes are consistent between each other and corresponding to the increases in soil quality promoted by the different tillage methods and cropping systems. For that, we analyzed the CLPP (Biolog Ecoplates) and PLFA-based community structure of soil samples from a field experiment comprising a factorial combination of different soil managements and maize cropping systems, besides fallow plots dominated by *Brachiaria* sp. Our results indicated that both microbial community structure and

physiological profile are changed between different agricultural practices in the Brazilian coastal tablelands, but showing distinct responses and not consistent with changes in soil quality promoted by conservation tillage, despite CLPP reflected the increases in cropping system complexity.

## 2. Material and methods

### 2.1. Study site, field experiment and soil sampling

Soil samples (fine-loamy kaolinitic isohyperthermic Typic Kandiuudalfs) were collected in 2010 from an experiment at the Umbauba Experimental Station (Umbauba, Sergipe State, Brazil, 11°16'S and 37°26'W, 105 m altitude), installed to assess the effects of three tillage methods (conventional tillage, CT; chisel plowing, CP; no-till, NT) combined with two maize (*Zea mays*) cropping systems (maize monoculture, MM; maize intercropping, MP) on crop productivity and soil quality. Therefore, six agricultural practices were assessed in our study: CT MM, CT MP, CP MM, CP MP, NT MM, and NT MP. The intercropping (MP) consisted of simultaneous cultivation of maize and pigeonpea (*Cajanus cajan*).

The experiment was set up in a split-plot randomized blocks design, with four replicates, where the tillage treatments are the main plots (20 m × 8 m) and the cropping systems are the sub-plots (10 m × 8 m). The tillage and cropping system treatments were started in April 2001 and April 2007, respectively. Before 2007, the intercropping between maize and pigeonpea was used in all tillage treatments. Additionally, fallow plots (occupying both sub-plots) are present in each of the four blocks. Thus, each of the four blocks has four main plots, from which three are occupied by agricultural practices and one is occupied by fallow. In the fallow plots, the agriculture previously performed in this field was fully interrupted, with no human intervention since April 2001. The spontaneous vegetation of these plots is dominated by the grass *Brachiaria decumbens*. More information regarding previous agricultural practices performed in this field before the experiment set up is available in Fernandes et al. (2011).

The local climate is classified as tropical with dry summers (according to the Koppen Climate System), with average daily temperature of 24.3 °C and average annual rainfall ranging from 1200 to 1500 mm. At 0–20 cm depth, soil had 74% sand, 17% silt and 9% clay (sandy loam). Before the experiment set up, soil analyses (0–20 cm depth) showed the following values: 10.4 g kg<sup>-1</sup>C, 6.5 mg dm<sup>-3</sup> P (Mehlich-1), 1.33 cmol<sub>c</sub> dm<sup>-3</sup> Ca, 0.67 cmol<sub>c</sub> dm<sup>-3</sup> Mg, 0.12 cmol<sub>c</sub> dm<sup>-3</sup> K, 0.17 cmol<sub>c</sub> dm<sup>-3</sup> Al, 4.9 cmol<sub>c</sub> dm<sup>-3</sup> CEC at pH 7.0, 44% of base saturation and pH (water 1:2.5) of 5.1. Lime was incorporated up to 20 cm depth throughout the field to raise pH to ~6.0. Furrow opening, fertilization (120 kg ha<sup>-1</sup> of N as urea, 80 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> as superphosphate triple and 75 kg ha<sup>-1</sup> of K<sub>2</sub>O as potassium chloride) and sowing are performed manually every year. In the plots under CT, soil is prepared with one plowing and two disking operations, promoting high soil disturbance and topsoil inversion. On the other hand, CP promote minimal while NT promote no topsoil inversion, and therefore these two tillage systems were included in the category “conservation tillage”, as opposed to CT.

Weeds are efficiently controlled by topsoil inversion in CT. On the other hand, weeds are controlled by glyphosate spraying in NT and CP 10–12 days before sowing, since these tillage methods do not promote topsoil inversion and thus weeds need to be chemically controlled. The operations of CP are the same used in NT, except that for the first method, soil is chisel-plowed immediately before planting, every year. This operation is generally used to reduce the high subsurface soil density (1.5 to 1.6 kg dm<sup>-3</sup>), which is commonly found in most coastal tableland soils (hardsetting soils). Chiseling was performed using a plow with shanks spaced 40 cm apart and at a depth of about 30 cm. No secondary tillage was performed in CP.

From 2001 to 2006, maize was sown with a spacing of 80 cm

between rows and 20 cm between plants in the same row (62,500 plants ha<sup>-1</sup>). During this period, pigeonpea was sown between maize rows, with plants separated by 10 cm (125,000 plants ha<sup>-1</sup>). From 2006 to 2010, pigeonpea was sown in the same row of maize. Maize rows had the same spacing as used from 2001 to 2006 (80 cm). However, maize plants were separated by 50 cm (50,000 plants ha<sup>-1</sup>) to fit pigeonpea plants between them. The samples analyzed in the present study were collected in July 2010, about three months after soil preparation and crop sowing. For agricultural plots, soil samples (0–20 cm depth) were taken randomly from six points per sub-plot, at a distance of 20 cm from crop rows, and mixed to obtain composite samples (3 tillage methods × 2 cropping systems × 4 blocks = 24 samples). For fallowed plots, two composite samples were obtained from 12 random points comprising both sub-plots in each of the four blocks (2 × 4 = 8 samples). Then, a total of 32 composite samples were used in our study. The soil samples were stored at field moisture at 4 °C, for up to six days prior to laboratory analyses. Immediately before the analyses, samples were passed through 2 mm mesh sieves.

## 2.2. Community-level physiological profile analysis

The community-level physiological profile (CLPP) of soil samples was assessed by measuring the oxidation of 31 individual carbon (C)-sources in Biolog Ecoplates, according to Girvan et al. (2003) with minor modifications. Each well of the plate was inoculated with 150 µL of soil suspension (10<sup>-2</sup> dilution in sterile NaCl 0.85%) and incubated at 28 °C. The plates were read at 596 nm absorbance after 0, 24, 48 and 72 h of inoculation. Absorbance readings at 0 h were subtracted from following readings. At each reading time, absorbance values in the control wells were further subtracted from the absorbance values in each of the 31 sole C-sources. Negative values were set to zero. To minimize the effects of different inoculum densities, data were normalized by dividing the net absorbance values by their respective average well color development (AWCD) values (Garland and Mills, 1991).

## 2.3. Phospholipid fatty acid analysis

Microbial community structure was determined by analyzing the phospholipid fatty acid (PLFA) composition in the soil extracts according to White and Ringelberg (1998) and modified by Butler et al. (2003). The data analyzed corresponded to the molar percentages (mol %) of individual fatty acids relative to the total amount of these compounds in each sample. Only peaks with areas 2% higher than that of PLFA 16:0 were considered within each sample. Calculations of mol% were performed after excluding peaks that did not match this criterion. After this exclusion, about 18 peaks were present in all samples, which represented around 96–98% of the total peak areas in the original chromatograms. Variations in the microbial community structure were characterized by using the following fatty acid biomarkers: 15:0i, 15:0a, 16:0i, 17:0i and 17:0a for gram-positive bacteria (GPB); 17:0cy, 19:0cy and 18:1ω7c for gram-negative bacteria (GNB); 16:0 10-Me, 17:0 10-Me and 18:0 10-Me for actinomycetes, 16:1ω5c for arbuscular mycorrhizal fungi (AMF), and 18:1ω9c and 18:2ω6c for saprophytic fungi (Van Aarle et al., 2002; Zelles, 1999; Kaiser et al., 2010). The ratio between fungal and bacterial fatty acids (F/B ratio) was expressed by dividing the molar masses of fungal biomarkers (18:1ω9c + 18:2ω6c) by the bacterial ones (actinomycetes + GPB + BGN). The ratios between PLFAs with cyclic rings and their monoenic precursors (19:0cy/18:1ω7c and 17:0cy/16:1ω7c) were used to evaluate the degree of environmental (nutritional and desiccation) stresses experienced by soil microbial communities (Guckert et al., 1986; Chaer et al., 2009; Fernandes et al., 2011).

## 2.4. Soil microbial biomass-C, basal respiration and soil pH measurement

Microbial biomass-C (MB-C) was estimated by the fumigation-extraction method (Vance et al., 1987), with the quantification of C in soil extracts made according to Bartlett and Ross (1988). MB-C was calculated using a Kc of 0.38 (Vance et al., 1987).

Soil basal respiration was determined in soil samples (60% of water-holding capacity) incubated in gas-tight glass vials in the presence of NaOH solution (1.0 M) for 10 days at 28 °C. The amount of C-CO<sub>2</sub> evolved and trapped in the NaOH solution in this period was quantified by titration of residual alkali with 0.1 mol L<sup>-1</sup> HCl (Jenkinson and Powlson, 1976). Soil pH was determined on a 1:2.5 suspension of soil in water (Embrapa, 1997).

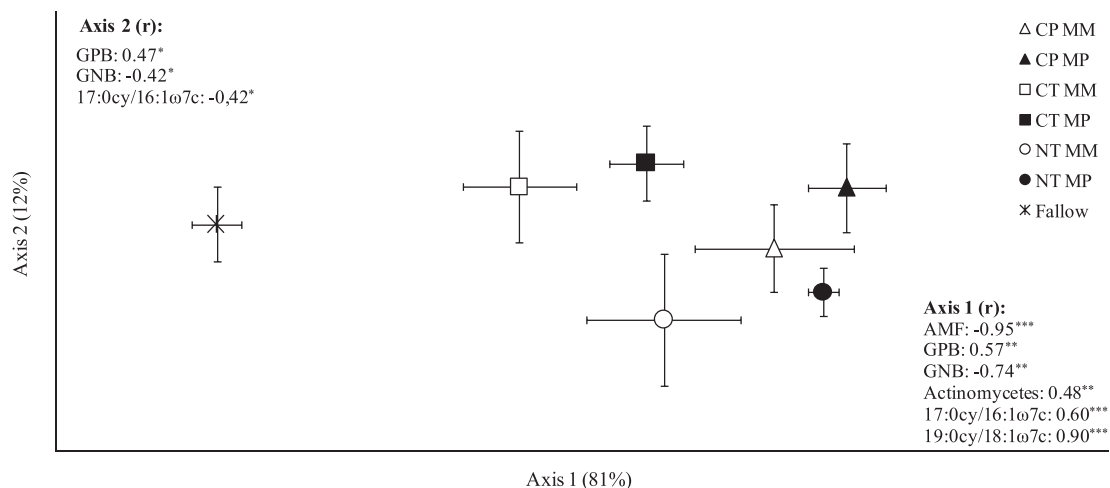
## 2.5. Statistical analyses

Linear combinations of agricultural practices were previously established to evaluate the effects of the following orthogonal contrasts: (I) agricultural lands vs fallow [(μ<sub>fallow</sub>) - (μ<sub>NT-MM</sub> + μ<sub>NT-MP</sub> + μ<sub>CP-MM</sub> + μ<sub>CP-MP</sub> + μ<sub>CT-MM</sub> + μ<sub>CT-MP</sub>) / 6]; (II) conventional tillage vs conservation tillage [(μ<sub>CT-MM</sub> + μ<sub>CT-MP</sub>) / 2 - (μ<sub>NT-MM</sub> + μ<sub>NT-MP</sub> + μ<sub>CP-MM</sub> + μ<sub>CP-MP</sub>) / 4]; (III) chisel plowing vs no-till [(μ<sub>NT-MM</sub> + μ<sub>NT-MP</sub>) / 2 - (μ<sub>CP-MM</sub> + μ<sub>CP-MP</sub>) / 2] and (IV) maize monoculture vs intercropping of maize and pigeonpea [(μ<sub>NT-MM</sub> + μ<sub>CP-MM</sub> + μ<sub>CT-MM</sub>) / 3 - (μ<sub>NT-MP</sub> + μ<sub>CP-MP</sub> + μ<sub>CT-MP</sub>) / 3].

Microbial community structure and physiological profile were graphically described by non-metric multidimensional scaling (NMDS) with Sorensen distances (Sokal, 1979). The analyses were performed in the “autopilot” mode, using the “slow and thorough” option in PC-ORD v.6.0 (McCune and Mefford, 2011). The number of dimensions interpreted was selected considering the criteria for stress and stability of graphics solutions. Individual PLFAs or C-sources were clustered in microbial groups (Zelles, 1999) and substrate biochemical classes (Chazarenc et al., 2010). Pearson correlations between NMDS sample scores and microbial groups or substrate biochemical classes were performed to characterize the changes in the structure and functional profiles of microbial communities, respectively. Multiresponse permutation procedures (MRPP) were used to test the hypothesis of no effect of contrasts of agricultural practices on the microbial community structure and CLPP (Mielke and Berry, 2000). Mantel tests were used for assessing correlations between the community structure, CLPP and stress status ratios (Mantel, 1967). For these tests, sample dissimilarities were expressed as Sorensen distances. All tests were performed using the multivariate statistical program PC-ORD 6.0 (McCune and Mefford, 2011).

The contrasts abovementioned were also used to assess the effects of the agricultural practices on soil pH, MB-C, basal respiration, and individual components of PLFA and CLPP, clustered according to the microbial groups and substrate biochemical classes as previously described.

The proportion of the variability in microbial community structure and CLPP data associated with a specific contrast of agricultural practices was estimated by the ratio between the sum of squares explained by each contrast and the total sum of squares (TSS), obtained by sum of squares multivariate regression trees (SS-MRT) (De'ath, 2002), using Euclidean distances and a minimum group size of three samples. TSS was calculated from the whole set of samples, whereas the sum of squares explained by each contrast was estimated after modeling data from sample subsets including only those relevant to each comparison. For all the contrasts of practices, trees with two terminal nodes were analyzed. SS-MRT analysis was performed in S-Plus software v.4.0 (Statistical Sciences, Seattle, WA) containing a library of functions for multivariate regression tree analyses, formerly available at Ecological Society of America's Electronic Data Archive (Ecological Archives E083-017-S1) (De'ath, 2002).



**Fig. 1.** NMDS plot representing differences in PLFA-based microbial community structure of soil samples under different tillage methods, maize cropping systems and fallow. CP, CT and NT stand for chisel plowing, conventional tillage, and no-till, respectively, whereas MM and MP stand for maize monoculture and intercropping between maize and pigeonpea, respectively. The six agricultural practices correspond to the combination of tillage methods and cropping systems (CT MM, CT MP, CP MM, CP MP, NT MM, NT MP), while fallow plots had no human intervention since the experiment setup. Horizontal and vertical bars indicate  $\pm 1$  S.D. from sample score centroids along axes 1 and 2, respectively. Values between parentheses in front of axis headings denote the percentage of total variability in PLFA data represented along each axis. Pearson correlation coefficients ( $r$ ) between PLFA biomarkers or stress ratios (17:0cy/16:1ω7c and 19:0cy/18:1ω7c) and sample scores along axes 1 and 2 were listed whenever these correlations were significant at  $P < 0.05$  (\*, \*\*, \*\*\*:  $P < 0.05$ ,  $P < 0.01$  e  $P < 0.001$ , respectively).

### 3. Results

#### 3.1. Shifts in microbial community structure and stress status between agricultural practices

Ninety-three percent of the variability in microbial community structure was represented by a 2-D NMDS ordination, with 81% of this variability displayed along axis 1, and only 12% along axis 2 (Fig. 1). The highest distinction was observed between fallow and the agricultural samples, which were closely related between each other (Fig. 1). Agricultural samples clustered more according to soil tillage (CT, CP and NT) than to maize cropping system (MM or MP). Conservation tillage samples (CP and NT) were closer to each other compared to CT samples. However, among the tillage methods CT showed the community composition more closely related to that of fallow - as observed along axis 1, while the most distinct tillage system compared to fallow was CP (Fig. 1). On the other hand, the most distinct maize cropping system compared to fallow was MP (Fig. 1).

According to the Pearson correlation coefficient ( $r$ ), the community structure gradient observed from the left to the right of axis 1 was characterized by increases in GPB ( $P < 0.01$ ) and actinomycetes ( $P < 0.01$ ), and decreases in AMF ( $P < 0.001$ ) and GNB ( $P < 0.01$ ) biomarkers (Fig. 1). The two fatty acid ratios used as microbial stress markers (17:0cy/16:1ω7c and 19:0cy/18:1ω7c) also increased in the same direction ( $P < 0.001$ ). A less remarkable structural gradient was observed in the upward direction along the axis 2, which was associated with increases in GPB ( $P < 0.05$ ), and decreases in GNB ( $P < 0.05$ ) and 17:0 cy/16:1ω7c stress ratio ( $P < 0.05$ ).

MRPP results indicated that community structure was significantly different between all the four contrasts: agriculture vs fallow, conventional vs conservation tillage, chisel plowing vs no-till, and maize monoculture vs intercropping of maize and pigeonpea (Table 1). The contrast agriculture vs fallow impacted almost all microbial groups, resulting in significantly higher levels of AMF and GNB, and lower levels of GPB and actinomycetes biomarkers in fallow compared to agricultural soils (Table 2). The stress ratios 17:0 cy/16:1ω7c and 19:0 cy/18:1ω7c (nutritional and desiccation stress on bacterial communities) were also higher in cropped than in fallowed areas (Table 2).

Curiously, topsoil layer inversion by plowing and disking of CT significantly increased AMF and decreased stress ratios compared to the

**Table 1**

Multiresponse permutation procedures (MRPP) results for the effects of the compared contrasts of agricultural practices on microbial community structure and community-level physiological profile, assessed by phospholipid fatty acid analysis (PLFA) and oxidation of single C-sources in Biolog Ecoplates, respectively.

Contrasts of agricultural practices	P-value	
	PLFA	CLPP
Agriculture (MM, MP, CT, CP, NT) × Fallow	< 0.001	< 0.001
Conventional tillage (CT) × conservation tillage (CP + NT)	< 0.001	0.119
Chisel plowing (CP) × No-till (NT)	0.004	0.258
Maize monoculture (MM) × Intercropping (MP)	0.033	0.015

soils under conservation tillage methods (NT and CP) (Table 2). In areas without topsoil inversion, chiseling promoted a significant decrease in GNB biomarkers and in the ratio of 17:0 cy/16:1ω7c, but not in 19:0 cy/18:1ω7c (Table 2). Differences in microbial community structure due to chiseling were observed along both axes in NMDS plot. Regarding the agricultural practices, despite the most significant differences (lower P-values) in community structure were observed between the tillage methods (Table 1), significant differences in specific microbial groups were more observed between the cropping systems (Table 2). The intercropping of pigeonpea and maize significantly increased the 19:0cy/18:1ω7c ratio and biomarkers of fungi, actinomycetes and GPB, and reduced those of GNB and FMA, compared with maize monoculture (Table 2).

#### 3.2. Community-level physiological profile changes between agricultural practices

NMDS represented 87% of the variability in the CLPP data (Fig. 2), with a more balanced distribution of this variability between the two axes (54% at axis 1 and 33% at axis 2) than observed for community structure. Differently to the observed in the structure of microbial communities, the NMDS ordination in CLPP was mostly determined by maize cropping systems, separating samples in clusters of MM or MP (Fig. 2). Interestingly, the physiological profile of MP was more closely related to fallow, also differing from the effects of treatments on

**Table 2**

Effects of different contrasts of agricultural practices on specific microbial groups, stress ratios (Stress17 and Stress19) and fungal/bacterial ratio, assessed by phospholipid fatty acid biomarkers.<sup>d</sup>

Contrasts	Fungi <sup>a</sup>	AMF <sup>b</sup>	GPB <sup>c</sup>	GNB <sup>d</sup>	ACT <sup>e</sup>	Stress17 <sup>f</sup>	Stress19 <sup>g</sup>	F/B <sup>h</sup>
mol %								
Agriculture (CT, CP, NT, MM, MP) vs fallow								
Fallow	10.4	5.5	38.5	33.7	2.0	0.38	0.88	0.14
Agriculture	11.1	3.8	41.5	28.4	2.3	0.48	1.73	0.16
P-value	ns	< 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	ns
Conventional (CT) vs conservation tillage (CP + NT)								
Conventional tillage	11.6	4.3	41.5	28.3	2.3	0.40	1.49	0.17
Conservation tillage	10.9	3.5	41.4	28.4	2.3	0.52	1.84	0.16
P-value	ns	< 0.001	ns	ns	Ns	< 0.001	< 0.001	ns
Chisel plowing (CP) vs no-till (NT)								
Chisel plowing	10.8	3.4	42.8	27.0	2.4	0.46	1.93	0.16
No-till	10.9	3.7	40.1	29.8	2.3	0.57	1.76	0.16
P-value	ns	ns	ns	< 0.001	Ns	< 0.001	ns	ns
Maize monoculture (MM) vs intercropping with pigeonpea (MP)								
Intercropping	11.7	3.5	42.1	27.5	2.5	0.52	1.78	0.17
Maize monoculture	10.6	4.1	40.8	29.2	2.2	0.44	1.67	0.15
P-value	< 0.05	< 0.01	ns	< 0.01	< 0.01	< 0.01	ns	< 0.05

<sup>a</sup> Saprophytic fungus biomarkers (PLFAs 18:1 $\omega$ 9c + 18:2 $\omega$ 6c).

<sup>b</sup> Arbuscular mycorrhizal fungus biomarkers (PLFA 16:1 $\omega$ 5c).

<sup>c</sup> Gram positive bacterium biomarkers (PLFAs 15:0i + 15:0a + 16:0i + 17:0i + 17:0a).

<sup>d</sup> Gram negative bacterium biomarkers (PLFAs 17:0cy + 19:0cy + 18:1 $\omega$ 7c).

<sup>e</sup> Actinomycetes biomarkers (PLFAs 16:0 10-Me + 17:0 10-Me + 18:0 10-Me).

<sup>f</sup> Stress ratio between PLFAs 17:0cy and 16:1 $\omega$ 7c.

<sup>g</sup> Stress ratio between PLFAs 19:0cy and 18:1 $\omega$ 7c.

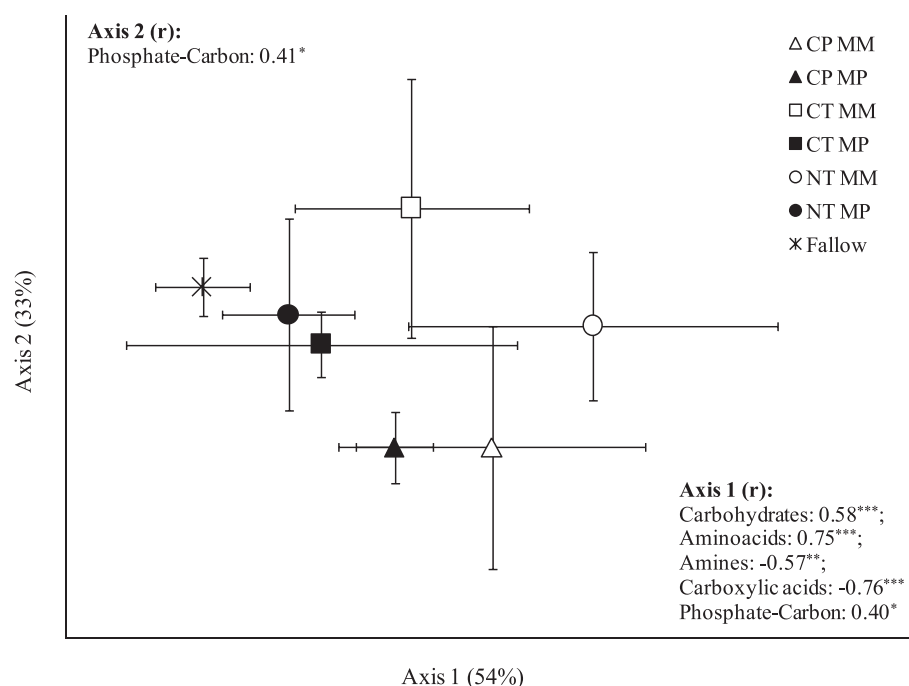
<sup>h</sup> Ratio between saprophytic fungus biomarkers and summed GPB, GNB and ACT biomarkers.

microbial community structure (Figs. 1 and 2).

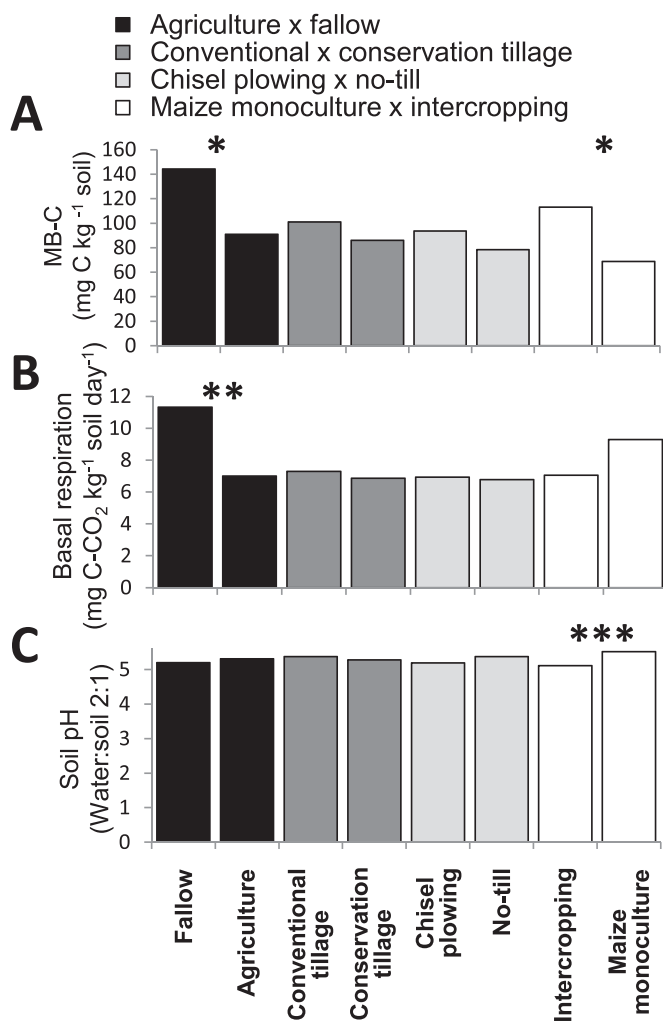
A CLPP gradient displayed from the left to the right along axis 1 (Fig. 2) was characterized by increases in the ability of using carbohydrates ( $P < 0.001$ ), amino acids ( $P < 0.001$ ) and phosphate-carbon substrates ( $P < 0.05$ ), and decreases in carboxylic acids ( $P < 0.001$ ) and amines ( $P < 0.01$ ). The distribution of soil samples upwards axis 2 was associated with an increase in the utilization of phosphate-carbon substrates ( $P < 0.05$ ).

CLPP significantly changed with respect to the contrasts agricultural

lands vs fallow and maize monoculture vs intercropping, but not to conventional vs conservation tillage and chisel plowing vs no-till (Table 1). The microbial communities in fallowed plots showed a decreased capacity for amino acid utilization compared with the cropped areas ( $P < 0.05$ ), while no significant differences were observed for the other C-sources. Soil microbial communities in the intercropping system had a higher capacity to oxidize carboxylic acids than those assembled under maize monoculture ( $P < 0.05$ ).



**Fig. 2.** NMDS plot representing differences in community-level physiological profile (analyzed using Biolog Ecoplates) of soil samples under different tillage methods, maize cropping systems and fallow. CP, CT and NT stand for chisel plowing, conventional tillage, and no-till, respectively, whereas MM and MP stand for maize monoculture and intercropping between maize and pigeonpea, respectively. The six agricultural practices correspond to the combination of tillage methods and cropping systems (CT MM, CT MP, CP MM, CP MP, NT MM, NT MP), while fallow plots had no human intervention since the experiment setup. Horizontal and vertical bars indicate  $\pm 1$  S.D. from sample score centroids along axes 1 and 2, respectively. Values between parentheses in front of axis headings denote the percentage of total variability in CLPP data represented along each axis. Pearson correlation coefficients (r) between relative degradation of substrate biochemical classes and sample scores along axes 1 and 2 were listed whenever these correlations were significant at  $P < 0.05$  (\*, \*\*, \*\*\*:  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ , respectively).



**Fig. 3.** Effects of different contrasts of agricultural practices on microbial biomass-C (MB-C) (A), basal respiration (B) and soil pH (C). The values disposed in the bar charts represent the averages of the samples comprising the combination of agricultural treatments in each contrast. “Agriculture” comprises all agricultural treatments: CT, CP, NT, MM and MP. “Conservation tillage” comprises the treatments CP and NT. The symbols \*, \*\* or \*\*\* means significantly different at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ , respectively.

### 3.3. Distinctions in soil microbial biomass, basal respiration and pH between agricultural practices

Fallow resulted in average increases of about 60% in both MB-C ( $P < 0.05$ ) and basal respiration ( $P < 0.01$ ) compared to agricultural lands (Fig. 3A and B). A similar significant increase in MB-C (64%) was observed when comparing intercropping with maize monoculture ( $P < 0.05$ ). Soil pH was also significantly different between the two cropping systems ( $P < 0.001$ ), with intercropping resulting in lower soil pH (Fig. 3C). However, respiration was not significantly different between the two cropping systems, despite the difference in averages were higher than that in the contrasts between tillage systems (conventional vs conservation tillage and chisel plowing vs no-till). No significant changes in MB-C, basal respiration and pH were observed in the contrasts conventional vs conservation tillage and chisel plowing vs no-till in the 0–20 cm depth layer analyzed.

Microbial metabolic quotient ( $qCO_2$ ) - an indicator of microbial stress - was calculated as the ratio between soil basal respiration and soil MB-C (Anderson and Domsch, 1993). A high variability was observed for this variable and none of the contrasts of agricultural practices led to significant differences in  $qCO_2$  (data not shown).

### 3.4. Correlations and differences between microbial community structure, physiological profile and stress status

According to Mantel test, responses of microbial community structure and physiological profile to agricultural practices were not correlated ( $r = 0.078$ ;  $P = 0.44$ ). On the other hand, microbial community structure was highly correlated with the stress ratios 17:0cy/16:1ω7c ( $r = 0.25$ ;  $P < 0.001$ ) and 19:0cy/18:1ω7c ( $r = 0.81$ ;  $P < 0.001$ ), whereas CLPP was not significantly correlated with those stress ratios ( $-0.047$  and  $P = 0.58$ ;  $0.063$  and  $P = 0.52$ , respectively).

Microbial community composition was more affected by the four contrasts of agricultural practices under comparison in our study than CLPP. The contrasts explained about 70% of the variability in PLFA data and only 22% in CLPP. Actually, although 33% of CLPP variability was represented by NMDS axis 2 (Fig. 2), no clear separation of samples according with treatments was observed along this axis, except among the different maize cropping systems under conventional tillage. The individual contribution of contrasts to explain PLFA data variability was higher for the contrast agricultural plots vs fallow (41%), followed by conventional vs conservation tillage (17%), intercropping vs maize monoculture (7%), and chisel plowing vs no-till (6%). For CLPP, a higher overlap was observed between samples from different treatments, and individual contribution of contrasts was higher for the contrast maize monoculture vs intercropping (8%) - confirming the clustering of samples in NMDS (Fig. 2) - and lower for chisel plowing vs no-till (3%).

It is noteworthy that the changes between agriculture and fallow, as well as between the different soil tillage methods were more effective in shifting the structure of microbial communities than their physiological profiles (Figs. 1 and 2). Although both microbial community structure and CLPP were affected by maize cropping systems, they showed distinct responses to this factor. The composition of microbial communities in the two cropping systems were closer to each other than to fallow. However, fallow samples were closer to maize monoculture than to intercropping samples (Fig. 1), while the opposite was true for CLPP (Fig. 2). In addition, the maize cropping system was the main factor influencing the clustering of samples in CLPP, while soil tillage was the main factor contributing to the clustering of samples in PLFA-based community structure, as previously mentioned (Figs. 1 and 2).

Analyzing the shifts in specific microbial groups (PLFA) and their association with the changes in biochemical classes of substrates (CLPP), we observed a convergent significant increase in GPB and actinomycetes biomarkers ( $P < 0.01$ ) and amino acids utilization ( $P < 0.05$ ) in the agricultural lands compared to fallow. Other associations between PLFA-microbial groups and CLPP-biochemical classes of substrates were not clear, since MM and MP samples inverted their similarity with fallow samples in the NMDS plots of the two compared approaches (Figs. 1 and 2).

## 4. Discussion

### 4.1. Shifts in the soil microbiome between agricultural lands and fallow

This study investigated the ecological changes in soil microbial communities (community structure and physiological profile) associated with the introduction of conservation agricultural practices, *i.e.* decrease in soil physical disturbance (conservation vs. conventional tillage) and increase in crop system complexity (intercropping vs. monoculture). In addition, all the agricultural soils were compared to fallow - plots of the experiment without any human intervention for nine years. Our previous results revealed that soil physicochemical quality increased in fallow compared to all agricultural soils after just three years of the experiment setup, with significant higher values of soil organic C, water stability of aggregates and mean weight diameter of aggregates (Fernandes et al., 2011). The present study indicates that soil microbial biomass, basal respiration, community structure and

physiological profile were also changed between agricultural lands and fallow. In addition, fallow changed the abundance of many individual microbial groups and decreased stress biomarkers compared to agricultural soils. These results show that introducing fallow on this field promoted not only the highest levels of soil physicochemical quality, but also the highest ecological changes in the soil microbiome.

The permanent presence of *Brachiaria decumbens* as the dominant plant (but not unique) in fallow may be one of the most important factors contributing to the high changes in the soil microbiome, since its abundant root system evenly distributed in the field provides a continuous supply of labile C-sources from rhizodeposition for soil microbiota inhabiting plant rhizosphere, which virtually dominates fallow topsoil. On the other hand, agricultural fields are occupied by annual crops and arranged in spaced rows, decreasing rhizosphere and increasing bulk soil areas in both time and space, respectively. This is probably the reason why the highest changes in microbial community structure, biomass and activity were observed in fallow, since rhizosphere drastically modifies these attributes compared to bulk soil (DeAngelis et al., 2009; Philippot et al., 2013; Mendes et al., 2015; Lopes et al., 2016).

We observed an increase in gram-negative bacteria and arbuscular mycorrhizal fungi in fallow compared to agricultural soils. Root symbiotic microbes, such as rhizobia (members of Proteobacteria, a phylum of gram-negative bacteria) and arbuscular mycorrhizal fungi are expected to be found in higher amounts in the rhizosphere-dominated soil of fallow. In addition, several studies have shown that the rhizosphere microbiome is dominated by Proteobacteria (DeAngelis et al., 2009; Uroz et al., 2010; Peiffer et al., 2013; Lopes et al., 2016; Fernández-Gómez et al., 2019), which possibly explains the enrichment of those microbial groups in soils under fallow. On the other hand, the gram-positive Actinobacteria phylum was shown to be higher in the bulk soil than in the rhizosphere microbiome in some studies (Fierer et al., 2007; Lopes et al., 2016; Fernández-Gómez et al., 2019), which is consistent with the increase in actinomycetes and gram-positive bacteria biomarkers in agricultural compared to fallow soils in our study. Besides the higher soil organic C content, the increases in total microbial biomass and abundance of specific bacterial/fungal groups could also have contributed to the higher soil aggregation in fallow soils (Fernandes et al., 2011), since bacterial EPS and fungal hyphae were shown to contribute on aggregates formation and stabilization (Bossuyt et al., 2001; Lehmann et al., 2017; Krause et al., 2019).

The higher degradation capacity of amino acids in agricultural than in fallow soils also might be associated with the niche and ecological behavior of the mentioned microbial groups enriched in each condition, since both arbuscular mycorrhizal fungi and the gram-negative bacteria from Proteobacteria in general use simple sugars (copiotrophs), while the microbial community in agricultural bulk soils have to use other C-sources for their nutrition (Fierer et al., 2007). Metagenomic prediction suggested that amino acid metabolism is higher in bulk soil than in sugarcane rhizosphere (Lopes et al., 2016). In addition, Actinobacteria was shown to better degrade complex C compounds and resist to abiotic stresses more severe in bulk soil, such as soil drought (Roes-Hill et al., 2011; Kavamura et al., 2013).

Despite the rhizosphere effect resulting from the permanent presence of *B. decumbens* in fallow may be the main factor contributing to the higher differences in the microbial parameters assessed between fallow and agricultural soils, other factors could also contribute to this distinction. For example, fallow plots received no chemicals (fertilizers nor pesticides) during the nine years of experiment, which can be a selecting factor for microbial communities in agricultural soils (Rossi et al., 2018). Additionally, the physical disturbance faced by agricultural soils is higher than under fallow, which could also affect microbial community structure and activity (Chaer et al., 2009).

#### 4.2. Soil tillage methods affect more microbial community structure than physiological profile

After seven years of the experiment setup, fallow kept showing the highest soil quality, but the conservation tillage methods (no-till and chisel plowing) also significantly increased soil physical quality compared to conventional tillage, showing higher values of aggregates mean weight diameter and macroporosity, and lower values of microporosity and soil bulk density (Fernandes et al., 2010). Our results show that tillage systems also changed microbial community structure, as an effect of topsoil inversion (conservation vs conventional tillage) or just chiseling (chisel plowing vs no-till). However, this change was not consistent with increases in soil physical quality, since the composition of microbial communities under conventional tillage (lower soil quality) was the nearest to fallow (higher soil quality) among all tillage systems - despite all agricultural practices were closer to each other than to fallow. Nevertheless, among the conservation tillage practices, no-till was closer to fallow than chisel plowing. The higher closeness of fallow samples to conventional tillage (among the three tillage systems) could be associated with the absence of herbicides in this treatment instead of an effect of soil disturbance, since pesticides can affect the soil microbiome and both conventional tillage and fallow lack herbicides application (Rossi et al., 2018).

The higher abundance of arbuscular mycorrhizal fungi in conventional than in conservation tillage was also intriguing, since the opposite trend was observed between fallow and agricultural soils, and soil disturbance is generally negative to arbuscular mycorrhizal fungi density and diversity (Säle et al., 2015; Pontes et al., 2017). However, it was shown that in some cases long-term (ten years) management without plowing can reduce arbuscular mycorrhizal propagules density (Curaqueo et al., 2011). Moreover, the effect of soil physical disturbance on arbuscular mycorrhizal fungi abundance is species-specific, with negative (*Scutelospora* sp.), neutral (*Gigaspora* sp.) or positive (*Glomus intraradices* and *Glomus claroideum*) effects (Jansa et al., 2003). Long-term soils under no-till usually increases residual P concentration in the most superficial soil layers (Jansa et al., 2003). High concentrations of this nutrient are deleterious for the development of mycorrhizal associations (Grant et al., 2005). Thus, conventional tillage probably increased the abundance of these symbiotic fungi compared to conservation tillage because plowing and disking dilute P concentration in the superficial layers. On the other hand, the lack of P fertilization possibly explains the enrichment of arbuscular mycorrhizal fungi in fallow compared to agricultural soils. Other variables unexpectedly higher in conservation than in conventional tillage systems were the microbial stress ratios. Soil disturbance may also be associated with these results, since it can increase substrate availability for soil microbiota on topsoil layer, both by improving plant residue distribution and by disrupting soil aggregates and exposing its entrapped particulate organic matter, avoiding nutritional limitations (Chaer et al., 2009).

Despite changes in soil microbial community structure were observed between the different tillage practices under investigation in our study, no differences were detected for microbial biomass, basal respiration and CLPP, suggesting that increases in soil physical quality do not affect these variables in our conditions. The lack of changes in CLPP suggests that the different communities assembled in the compared tillage systems were functionally redundant, i.e. performed similar functions. Perhaps these changes would only be observed after more years of experiment, when the soil quality between the conventional and conservation tillage would potentially be more contrasting, as observed when comparing to fallow. However, it is not possible to directly associate the changes in those microbial variables in soils under fallow with its increased soil physicochemical quality, since other factors (e.g. permanent and widespread rhizosphere effect) also possibly contributed to this result. Functional redundancy was also observed between the soil microbiome of conventional tillage and no-till in southern Brazil using shotgun metagenomics, although specific

differences were identified (Souza et al., 2015).

#### 4.3. Microbial biomass and physiological profile of intercropping are closer to fallow than maize monoculture

In contrast to the observed between the different tillage methods in agricultural soils, the compared cropping systems (maize monoculture or intercropping with pigeonpea) were different not only regarding microbial community structure. Intercropping increased microbial biomass and changed microbial community physiological profile, which was more closely related to fallow than maize monoculture. Therefore, the communities in monoculture and intercropping soils were not functionally redundant. Furthermore, the changes in microbial community structure and physiological profile were not consistent, since community composition of monoculture samples were closer to fallow than those of intercropping, and the opposite was observed in CLPP.

It is noteworthy that differences in soil pH were also detected between the two cropping systems, with higher acidity observed in intercropping soils. As a legume, pigeonpea roots establish symbiotic interactions with rhizobia able to perform biological nitrogen fixation (BNF), such as *Bradyrhizobium* spp. (Tewari et al., 2020). Thus, decreases in soil pH under the intercropping system may be associated with the higher BNF-driven  $H^+$  extrusion from pigeonpea roots. In addition, the increased availability of organic N can cause a higher N net mineralization, which also decreases soil pH (Robertson and Groffman, 2014). Differences in soil pH are probably associated with shifts in microbial community structure of the compared cropping systems, since it is a major factor determining soil bacterial community composition (Fierer and Jackson, 2006; Rousk et al., 2010). It was previously shown that bacteria in general prefer higher soil pH than fungi (Rousk et al., 2010; Grosso et al., 2016), which agrees with our results of increasing relative abundances of fungi and gram-negative bacteria in the soil samples with lower (intercropping) and higher (monoculture) pH, respectively.

The effect of soil pH could be also important for the functional differences in microbial communities. However, since the separation of samples between the two cropping systems was even higher for CLPP, and intercropping samples were closer to fallow than monoculture samples, other factors certainly were more important than pH in this process. Plant diversity was shown to affect beta-diversity of soil microbial communities (Prober et al., 2015). Intercropping has a higher complexity for containing more than one plant species, which: I) provides a higher diversity of C-sources deposited in soil, creating more microbial niches (Fierer et al., 2012); and II) exerts a selection of different microbial communities in the rhizosphere soil, able to perform distinct functions (Berg and Smalla, 2009). Since fallow also has other plant species besides the dominant *B. decumbens*, it is possible to infer that the higher plant diversity in intercropping and fallow promoted a higher similarity of their microbial community physiological profiles, besides stimulating microbial growth and biomass. In addition to the greater diversity of C-sources, intercropping also increased the amount of soil organic C, which possibly contributed to the higher microbial biomass in this treatment (Fernandes et al., 2011).

#### 4.4. Distinct responses of PLFA and CLPP to agricultural practices and correlations with soil quality

Our results showed that both PLFA-based microbial community structure as well as CLPP-based microbial community function are affected by most of the agricultural practices analyzed. However, the responses of the two approaches are different. Community structure is in general more changed than CLPP, responding to the three tillage systems, as well as to the two cropping systems assessed, besides showing higher differences between agricultural lands and fallow. On the other hand, microbial community functions were not changed as an

effect of the tillage managements – indicating a high functional redundancy (similar functions performed by the microbial communities) between them – but was a better predictor of changes in the complexity of cropping systems, since intercropping was closer to fallow than maize monoculture.

Microbial community structure (and stress ratios) was not a suitable predictor of the benefits promoted by conservation agricultural practices because it does not follow the same trend of increasing soil physicochemical quality resulting from conservation tillage methods nor the increasing of cropping systems complexity, since the agricultural practices more closely related to fallow (higher plant diversity and soil quality) were conventional tillage and maize monoculture (lower soil quality and plant diversity, respectively). These results suggest that other specific edaphic and anthropic factors such as pH, phosphorus availability and concentration, or application of herbicides could be more important for the structuring of microbial communities (Jansa et al., 2003; Fierer and Jackson, 2006; Curaqueo et al., 2011; Rossi et al., 2018). CLPP was also not an adequate predictor of changes in the intensity of soil management, since it was unaffected by soil tillage systems. However, it was a good predictor of increasing complexity of cropping systems, since intercropping samples were closer to fallow samples than maize monoculture.

In sum, it is possible to state that the assessed microbial parameters are more sensitive and better reflect the benefits of conservation cropping systems than conservation tillage. Based on our data, microbial community functional profile has a high potential to predict increases in conservation agricultural practices. Nevertheless, the lack of distinction between tillage systems questions its usefulness for this aim. Previous studies showed a strong correlation between tillage intensity/frequency and CLPP in this same soil (Chaer et al., 2009). However, in that study all the plots were continuously mowed throughout the course of the experiment to minimize the interference of vegetation on the microbial responses to tillage frequency. The lack of differences in CLPP can also be due to the culture-dependent nature of this approach, reflecting only part of the actual soil microbial community (Preston-Mafham et al., 2002). However, it was detected a high functional redundancy between soil microbial communities under conventional tillage and no-till assessed by shotgun metagenomics, a culture independent method (Souza et al., 2015). Maybe the microbial functional differences with respect to tillage systems would be detected using more specific approaches, such as metatranscriptomics or metaproteomics, which reflect the active functions of the soil microbiome, not only its potential functions. Future studies using these approaches can elucidate this question, which could improve our understanding on the effect of conservation agricultural practices in the soil microbiome and potentially find useful methods for predicting increases in soil quality.

## 5. Conclusions

In this study, we analyzed the changes in soil microbial community structure and physiological profile, besides microbial biomass, basal respiration and PLFA stress ratios, associated with different contrasts of agricultural practices, including the comparison of conservation tillage (no-till and chisel plowing) vs conventional tillage, maize monoculture vs intercropping with pigeonpea, and all these agricultural soils vs fallow plots dominated by *Brachiaria decumbens*. Among the contrasts assessed in our study, agricultural lands vs fallow was responsible for the highest changes in the microbial parameters analyzed, followed by maize monoculture vs intercropping, and lastly the contrasts regarding soil tillage methods. Considering the approaches for assessing soil microbiome changes to conservation agricultural practices, microbial community structure was mostly affected by soil tillage systems, while physiological profile was only affected by maize cropping systems. However, the changes in microbial community composition were not consistent with the increasing in soil physical quality resulting from the distinct tillage managements, indicating that specific edaphic and



anthropic factors also affect community structuring. On the other hand, the changes in microbiome functional profile were consistent with the increases in cropping system complexity, despite it was not affected by soil tillage methods. In sum, our results demonstrate that both microbial community structure and physiological profile are changed between distinct agricultural practices in the Brazilian coastal tablelands. However, they respond differently to the compared contrasts and the shifts are not consistent with increases in soil quality promoted by conservation tillage. Therefore, it is important to combine microbiome functional and taxonomic approaches with physicochemical data in order to better understand the effect of conservation agricultural practices in soil and ecosystem quality.

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## Authors contributions

LDL collected and processed soil samples, performed laboratory analyses, interpreted the results and wrote the manuscript. MFF conceptualized the study, performed laboratory and statistical analyses, interpreted the results and wrote the manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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