

## Shifts in microbial and physicochemical parameters associated with increasing soil quality in a tropical Ultisol under high seasonal variation

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### ABSTRACT

Combination of conservation agricultural practices such as reduced tillage and complex cropping systems can improve soil quality. However, the different effects of conservation practices on soil physicochemical and microbiological parameters need to be monitored, since distinct managements, crops, regions and soil types can lead to different responses. In addition, seasonality can also affect these parameters, leading to changes associated with the environmental conditions, such as temperature and moisture. To better detect differences in soil quality between agricultural practices, the most appropriate season of sampling must be identified. Therefore, the objective of this study was to assess the changes in soil physicochemical and microbiological parameters between four different agricultural practices from a 7-year experiment along two seasons with contrasting soil water content. We analyzed the differences in physicochemical and microbiological parameters, including soil organic matter (SOM), aggregates mean weight diameter (MWD), water stability of aggregates (WSA), soil pH, total nitrogen (soil N), soil C:N ratio, microbial biomass-C (MB-C), N (MB-N), and C:N ratio (MB-C:N), basal respiration, metabolic quotient ( $q\text{CO}_2$ ) and microbial quotient (MB-C:soil C), between the agricultural practices of conventional tillage with maize monoculture (CTM), no-till with maize monoculture (NTM), no-till with annual rotation of maize and soybean monoculture (NTM/S), no-till with annual rotation of maize intercropped with *Brachiaria ruziziensis* and soybean monoculture (NTMB/S) compared to a long-term fallow (>40 years secondary forest) at the Brazilian coastal tablelands in both winter (rainy) and summer (dry) seasons. Results indicated that the physicochemical and mostly the microbiological variables were changed between seasons. Among the practices, NTMB/S and fallow showed higher, while NTM/S and CTM showed lower soil physicochemical quality. The differences between agricultural practices were most obvious in the summer. Moreover, the microbiological and physicochemical data were correlated in the summer, but not in the winter. WSA was the variable most distinctive between practices, stable between seasons and correlated with the changes in microbial biomass/activity. On the other hand,  $q\text{CO}_2$  and mainly MB-C were the microbial parameters more associated with the increase in soil quality between the practices. In sum, we show that the benefits of conservation agriculture for soil quality in this region were most obvious in the summer and depended on the agricultural practices, with NTMB/S showing the greatest conservation of soil physicochemical quality.

### 1. Introduction

There is an increasing demand for replacing conventional by conservation agricultural practices in order to improve soil and ecosystem quality, avoiding problems such as erosion and excessive  $\text{CO}_2$  emissions (Kassam et al., 2018). Soil erosion decreases crop productivity, produce sediment pollutants and increases eutrophication of rivers and lakes (Nearing et al., 2017), while excessive  $\text{CO}_2$  emissions are directly

associated with the global threat of climate change (Oertel et al., 2016). Conservation practices are management methods of soil tillage and cropping systems aiming to reduce the environmental impact and soil degradation in agriculture (Brooker et al., 2015; Daigh et al., 2018). Among the conservation practices, no-till usually increases soil quality compared to conventional tillage, since the lack of soil disturbance generally improves the physicochemical properties (Daigh et al., 2018). Regarding cropping systems, methods that increase crop complexity

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such as crop rotation, intercropping, and agroforestry are also able to improve soil quality (Brooker et al., 2015; Schwab et al., 2015).

However, it is difficult to quantify soil quality. Therefore, some soil physical and chemical parameters were identified as useful indicators of soil quality in the last decades. Among these parameters, increases in soil organic matter (SOM) content are essential for soil quality, mainly in tropical regions (Castro et al., 2015; Gomes et al., 2016), because it increases carbon (C) storage, is a source of nutrients for plant growth and is a crucial cementing agent for soil aggregation (Pulido-Moncada et al., 2018; Sarker et al., 2018). In turn, increases in the physical parameters mean weight diameter (MWD) and water stability of aggregates (WSA) also indicate increase in soil quality, since they contribute to soil physical structure, water retention and infiltration, roots penetration, soil porosity and aeration, besides SOM conservation (Fiedler et al., 2016; Sarker et al., 2018). Other chemical parameters such as soil nitrogen (N) can have positive or negative effects for soil quality, since it is an important nutrient for plant growth, but N inputs from plant residues with low C:N ratio can stimulate SOM decomposition and loss of soil C (Fustec et al., 2010; Liang et al., 2017), since microbial C:N ratio is low and decomposition of residues is limited by N availability (Sakala et al., 2000; Kamble and Baath, 2014).

On the other hand, microbiological parameters are considered unstable indicators, since they show a high temporal variability (Benintende et al., 2015). However, they are intimately associated with those physicochemical parameters, besides performing several important soil functions, such as the biogeochemical cycles (Falkowski et al., 2008; Schloter et al., 2018). The dynamics of SOM decomposition and conservation are mediated by the soil microbiome, which also actively contributes for the formation and stabilization of aggregates (Lehmann et al., 2017). In addition, investigating microbial parameters is important for monitoring ecosystem quality, since soil microbes can entrap (immobilization) or release C (mineralization) to the atmosphere depending on the agricultural management, resulting in higher microbial biomass or respiration, respectively (Schimel et al., 2007). There are many microbiological parameters used for assessing soil quality, including methods with lower (e.g. biomass, respiration, enzyme activities) or higher (e.g. community profiling by fatty acids, DNA fingerprinting, high-throughput sequencing) resolution (Bünemann et al., 2018; Schloter et al., 2018). Community profiling methods have a high potential for providing new markers useful for predicting soil quality, but are more complex and expensive. The lower resolution methods are easier and cheaper, reflecting changes in specific microbial groups (enzyme activities) or in the whole microbial community (biomass, respiration) (Schloter et al., 2018).

Seasonality is another factor influencing soil quality and ecosystem functioning. Conservation agricultural practices can show distinctive effects in soil physicochemical and microbial parameters according to seasonal variability (Agaras et al., 2014; Liu et al., 2015; Panettieri et al., 2015). Tropical regions typically have drastic changes in soil water content between winter and summer affecting both microbial and physicochemical parameters (Mendes et al., 2012; Bouskill et al., 2016; Taketani et al., 2017). Soil wet or drought are contrasting conditions that can impact both soil quality and ecosystem services (Falsone et al., 2017; O'Connell et al., 2018). Thus, it is important to analyze soil physical, chemical and microbiological parameters in both seasons to better assess the effects of the agricultural practices on soil quality.

Therefore, this study analyzed soil physicochemical and microbiological parameters in winter (wet) and summer (dry) seasons of a 7-year experiment with different tillage (conventional tillage and no-till) and cropping systems (maize monoculture, annual rotation of maize and soybean, annual rotation of maize intercropped with grass and soybean) to test if conservation agricultural practices containing increasing complexity of cropping systems and reduced tillage improve soil quality compared to conventional methods of soil tillage and cropping system in the Brazilian coastal tablelands and in which season these differences are most obvious. Additionally, we aimed to identify the

physicochemical and microbiological variables to be more distinctive between agricultural practices and stable between seasons, and infer on the ecological associations between microbial and physicochemical parameters.

## 2. Material and methods

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

In this study we analyzed soil samples (Ultisol) from an experiment at the “Jorge Sobral” Experimental Station of Embrapa Coastal Tablelands, located at Nossa Senhora das Dores municipality, Sergipe State, Brazil, 10°29'30"S and 37°11'36"W, 204 m altitude, which was set up to evaluate the effects of different soil tillage and cropping systems on soil quality and crop yields (maize – *Zea mays* and soybean - *Glycine max*) at the Brazilian coastal tablelands ecosystem. The local climate is classified as tropical with dry summers and wet winters, with average daily temperature of 26 °C and average annual rainfall of 1150 mm. Summers and winters have a low variation in average temperatures (28–24 °C, respectively), but a high variation in precipitation, with average monthly accumulation of 19 mm in December (summer climax) and average monthly accumulation of 103 mm in May (winter climax) (Weather Spark, 2020). The dry period lasts 7.5 months, from August 15th to March 31th, while the rainy period lasts 4.5 months, from March 31th to August 15th. The samplings were performed at the end of summer (March 19th) and winter (August 18th) of 2019, to detect soil physicochemical and microbiological changes after the full duration of each season. In the year of sampling, the cumulative precipitation of the dry period (08/15/2018–03/31/2019) was 252 mm, while that of the wet period (03/31/2019–08/18/2019) was 1137 mm.

The experiment was set up in 2012 in a randomized block design, with four replicates. Each plot containing an agricultural practice had an area of 20 m × 40 m. The four agricultural practices used in this study consisted in different combinations of soil tillage management (conventional tillage or no-till) and cropping systems (permanent maize monoculture; annual rotation of soybean and maize monoculture; and annual rotation of soybean monoculture and maize intercropping). In the intercropping, the grass *Brachiaria ruziziensis* was planted together with maize, persisting in the field after maize harvesting as a vegetative cover till the planting of the following year. Samplings were performed in 2019, a year in which maize was cropped in all agricultural practices. Additionally, we compared the agricultural practices with a long-term fallow area beside the experiment containing a native vegetation (secondary forest) without any human intervention for at least 40 years, characterized as a deciduous sub-deciduous tropical forest. Thus, we analyzed a total of five practices: conventional tillage with maize (CTM), no-till with maize (NTM), no-till with annual rotation of maize and soybean (NTM/S), no-till with annual rotation of maize intercropped with *B. ruziziensis* and soybean (NTMB/S), and fallow.

Crops were planted annually around May/June, in the beginning of the winter (rainy period). Seven days before sowing, the herbicide glyphosate was sprayed in the agricultural practices under no-till (NTM, NTM/S and NTMB/S) for weed control. For CTM, one disking (20 cm deep) and two harrowing (10–12 cm and 15–18 cm deep) operations were performed immediately before planting. Crops were planted with a space of 0.5 m between rows, using hybrid varieties of maize (7088 V T PRO MAX Agrocere) and soybean (MSOY 1944 RR). The seeds of *B. ruziziensis* were mixed with fertilizers on the NTMB/S practice. Fertilization was performed annually according to soil analyses, using urea (N), single superphosphate (P) and potassium chloride (K) in the proportion of 200–100–80 kg ha<sup>-1</sup> of NPK. P and K were incorporated to soil before planting, while N was added as side-dressing at the V2 growth stage of maize. N fertilization with urea was fully replaced by *Bradyrhizobium japonicum* inoculation in the years of soybean planting on the practices with legume rotation (0–100–80 kg ha<sup>-1</sup> of NPK).

Soil samples were collected at 0–20 cm depth from four random

places in each plot and mixed to obtain a composite sample. In the winter, when the crops were in the field, soil samples were collected in the inter-rows. A total of four (reflecting the agricultural practices analyzed) composite samples were obtained in each of the four blocks of the field experiment ( $4 \times 4 = 16$ ). We collected four additional samples from random points in fallow. Thus, 20 composite samples (~2 kg each) were transported to the laboratory in each season (total of 40 samples in the study). Part of the soil samples (~400 g) was sieved (2 mm) and stored at the field moisture at 4 °C prior to biological analyses, which were performed at a maximum of two weeks after sampling. Soil samples were air-dried and not pre-sieved for physical analyses. Chemical analyses were performed on the air-dried soil samples passing the 2 mm sieve after the physical analyses.

## 2.2. Soil physical and chemical analyses

The first physical analysis was mean weight diameter of aggregates (MWD), where air dried soil samples (600 g) were passed through a series of sieves with mesh diameters of 8.0, 4.0, 2.0, 0.85, and 0.5 mm, and shaken in a sieve shaker (Produtest, Brazil) for 5 min according to Arshad et al. (1996). MWD was calculated according to Green et al. (2007). For water stability of soil aggregates (WSA), we used 20 g of the aggregates retained in the 2 mm sieve from the previous analysis as described in Nimmo and Perkins (2002). The aggregates were rewetted by capillarity prior to the wet sieving procedure, which was performed in a wet sieving apparatus (Marconi, Brazil) using a 2 mm mesh sieve (Kemper and Rosenau, 1986). The sieving operation was performed for 5 min, with a period of oscillation of 3.7 cm at a frequency of 30 cycles  $\text{min}^{-1}$ . The final results were obtained after discounting the mass fraction of sand retained on the sieve after dispersion in NaOH (1 mol  $\text{L}^{-1}$ ).

Among the chemical variables, we analyzed soil organic matter (SOM), total soil nitrogen (soil N) and soil pH. For SOM, soil samples were boiled with  $\text{K}_2\text{Cr}_2\text{O}_7$  for 5 min, cooled to room temperature, reacted with orthophosphoric acid and titrated with 0.102 mol  $\text{L}^{-1}$  ferrous ammonium sulphate (Walkley and Black, 1934). Total soil N was quantified according to the Kjeldahl method for soil samples (Kjeldahl, 1883; Bremner, 1960). Conversion of SOM to total soil carbon (soil C) was obtained by dividing the values of SOM by 1.72 (Pribyl, 2010), in order to estimate soil C:N ratio. Soil pH was determined on a suspension of soil in water with a 1:2.5 ratio (Embrapa, 1997). All the physicochemical analyses were performed in laboratory duplicates.

## 2.3. Soil microbiological analyses

For soil microbial basal respiration, soil samples were adjusted to 70 % water-holding capacity and incubated at room temperature for 10 days in the dark within gas-tight glass pots containing a vial with 0.5 mol  $\text{L}^{-1}$  NaOH to capture the  $\text{CO}_2$  released from microbial respiration. After this period, the pots were opened and the NaOH solution was precipitated with 20 %  $\text{BaCl}_2$  and titrated with 0.1 mol  $\text{L}^{-1}$  HCl for quantification of emitted C– $\text{CO}_2$ , calculated according to Jenkinson and Powlson (1976). A second 10-day incubation was performed, but no differences were detected compared to the first (data not shown). The same incubation time was used in a previous study of our group in the same region (Lopes and Fernandes, 2020), and some higher respiration values were found compared to the present results, indicating that no saturation of the NaOH solution occurred during the 10 days of incubation in this study.

Microbial biomass-C (MB-C) and N (MB-N) analyses were performed using the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). The C and N contents of fumigated and non-fumigated soil samples were extracted with 0.5 mol  $\text{L}^{-1}$   $\text{K}_2\text{SO}_4$  and stored at –20 °C. For determination of MB-C, soil extracts were submitted to chemical reactions with  $\text{H}_2\text{SO}_4$  *p.a.* and a solution containing  $\text{Na}_4\text{P}_2\text{O}_7$  0.1 mol  $\text{L}^{-1}$ ,  $\text{H}_2\text{SO}_4$  0.5 mol  $\text{L}^{-1}$ ,  $\text{KMnO}_4$  0.1 mol  $\text{L}^{-1}$ , and  $\text{MnSO}_4\text{H}_2\text{O}$  0.1 mol  $\text{L}^{-1}$ , followed by spectrophotometric readings at 495 nm absorbance

(Bartlett and Ross, 1988). MB-C concentrations were calculated by subtracting the values of fumigated and non-fumigated samples and correcting by a  $K_C$  of 0.41 (Vance et al., 1987). For determination of MB-N, the same  $\text{K}_2\text{SO}_4$  soil extracts were submitted to the ninhydrin reaction protocol and read at 570 nm (Joergensen and Brookes, 1990). MB-N concentrations were calculated by the same subtraction mentioned above and corrected by a  $K_N$  of 0.54 (Brookes et al., 1985). All microbiological analyses were performed in duplicates.

We assessed these general and cheaper biological parameters (respiration, MB-C and MB-N) as potential indicators of soil quality because they can be easily used by researchers from different backgrounds of the soil sciences. Two ratios were used to further explore microbial parameters in our study. The first was microbial metabolic quotient, *i.e.*  $q\text{CO}_2$  (basal respiration/MB-C), which quantifies the emitted  $\text{CO}_2$  per unit of microbial biomass (Anderson and Domsch, 1993). The second was the ratio between MB-C and MB-N, *i.e.* MB-C:N ratio, which is used to estimate fungal and bacterial contributions for total microbial biomass, where higher values indicate increasing fungal abundance (Paul, 2007; Li et al., 2016). In addition, a mixed microbiological and chemical ratio known as microbial quotient (MB-C:soil C) was also calculated to infer on proportional increases in microbial biomass compared to soil C (Kaschuk et al., 2010).

## 2.4. Statistical analyses

All 12 microbiological and physicochemical variables were individually assessed by univariate analysis in each season regarding the differences between the five evaluated practices. For that, data were log-transformed and submitted to one-way ANOVA and Tukey pairwise comparison tests. In addition, two-way ANOVA was performed for each variable to verify the existence of significant differences between the seasons, agricultural practices and the interaction of these two factors. Linear correlations (Pearson) were identified between the physicochemical and microbiological variables. All univariate statistical analyses were performed using Past software (Hammer et al., 2001). The three variables directly associated with soil quality (SOM, MWD and WSA) were standardized by total and their averages in each practice and season were combined without weighting to illustrate with box plots the increasing soil quality of the assessed agricultural practices. Linear regressions were performed between averages of the microbiological variables and those physicochemical variables to infer on correlations between microbiological variables and soil physicochemical quality.

Multivariate analyses were used to investigate the effects of agricultural practices and seasonality on the whole set of physicochemical and microbiological parameters. Data were exported to Primer-6 software, where all multivariate analyses were performed (Clarke and Gorley, 2006). Each variable was standardized (value of each sample divided by the sum of values of all samples) to avoid bias associated with different scales. Then, data were log-transformed and submitted to biplot principal component analyses (PCA) in order to assess the ordination of samples according to the physicochemical or microbiological parameters, as well as the correlation of practices and seasons with these variables. Distance matrices were obtained using the Bray-Curtis similarity index and analyses of similarity (ANOSIM) were performed to test for significant differences between the summer and winter seasons regarding the microbiological and physicochemical variables.

The original physicochemical and microbiological matrices were split and analyzed within each season. The same approach described above was used for the four matrices to isolate the effects of the agricultural practices on the physicochemical and microbiological variables in each season. Thus, biplot PCA and ANOSIM were performed in each of the four matrices. ANOSIM was also performed considering only the three physicochemical variables directly associated with soil quality (MWD, WSA and SOM). SIMPER analysis was used to identify the variables most contributing to the differences between practices. Mantel tests were performed to detect the existence of correlations (Spearman

coefficient) between the physicochemical and microbiological matrices in each season. The correlations were further explored by identifying the set of physicochemical variables most correlated (i.e. showing the highest Spearman's correlation coefficient [ $\rho$ ] value) with the changes in the microbiological data and *vice-versa* with BEST analysis (Clarke et al., 2008). Linkage trees, a non-parametric version of multivariate regression trees (De'ath, 2002), were performed to split the samples according to physicochemical or microbiological data (Clarke et al., 2008). The choice for each node division is based on the higher ANOSIM R found in the set of compared samples and only kept if significant ( $P < 0.05$ ) on SIMPROF tests (999 permutations). The explanation of each node division was provided by the variables previously selected in BEST analyses (Clarke et al., 2008).

### 3. Results

#### 3.1. Changes in individual variables between agricultural practices and seasons

Among all 12 variables analyzed, four showed significant differences between agricultural practices ( $P < 0.05$ ) in only one season according to one-way ANOVA: MWD and  $qCO_2$  in the summer, and soil N and soil C:N ratio in the winter (Table 1). In the summer, NTMB/S showed significantly higher MWD than fallow. On the other hand, NTMB/S showed significantly lower  $qCO_2$  than NTM/S. In the winter, fallow had significantly higher contents of soil N than NTMB/S, while NTM/S had a significantly lower soil C:N ratio than all other agricultural practices, except fallow (Table 1).

Six variables were significantly different ( $P < 0.05$ ) between the practices in both seasons, one physical (WSA), two chemical (SOM and pH), two microbiological (MB-C and basal respiration) and one mixed (MB-C:soil C). WSA was the variable showing the highest variability between the different practices assessed in our study (Table 1). Fallow showed significantly higher WSA than CTM, NTM and NTM/S in the summer, and higher than CTM and NTM/S in the winter. NTMB/S was also significantly higher than NTM/S in the summer. CTM and NTM/S showed the lowest WSA among all practices in both seasons. Regarding SOM, NTMB/S was significantly higher than NTM/S in the summer, which was significantly lower than all other practices in the winter (Table 1).

With respect to the microbial parameters, soil respiration in fallow was significantly higher than in CTM and NTMB/S in the summer, and higher than in NTM and NTMB/S in the winter (Table 1). A slight increase in basal respiration was observed in most practices in the winter, except for CTM, which was drastically increased. On the other hand, a clear decrease in MB-C was observed in the winter for all practices. Fallow showed significantly higher MB-C than CTM, NTM and NTM/S in

the summer and higher than CTM and NTM in the winter (Table 1). Soil pH showed slight but significant differences, with higher values in fallow than in all agricultural practices at both seasons, except for NTM in the winter. At this season, pH was also significantly higher in CTM than in NTMB/S. The ratio MB-C:soil C was higher in fallow than in NTM/S, NTM and CTM in the summer, and higher than in CTM in the winter. Only two variables – MB-N and MB-C:N ratio – showed no differences between the practices during any season (Table 1), which is possibly associated with the high variability within practices in MB-N results (Supplementary Figure S1).

Two-way ANOVA confirmed the significant differences between agricultural practices pointed by one-way ANOVA for all variables, except for total N and  $qCO_2$ , indicating that changes in these variables within a specific season (Table 1) were not enough for detecting general changes regardless of the seasons (Fig. 1 and Supplementary Figure S1). Regarding the seasonal variability, two-way ANOVA showed that six variables were significantly different between summer and winter regardless of the practices: MWD, basal respiration and  $qCO_2$  in general increased, while soil C:N ratio, MB-C and MB-C:soil C ratio in general decreased their values in the winter (Fig. 1). The variables WSA, SOM, total N, pH, MB-N and MB-C:N ratio were not changed between seasons (Supplementary Figure S1). The only variables showing significant interaction between practices and seasons were soil C:N ratio and total N (Fig. 1 and Supplementary Figure S1). It is noteworthy that despite fallow had the highest average basal respiration among all practices, its  $qCO_2$  was comparable to the other practices in the summer and kept stable in the winter, not showing the sharp increase observed in other practices such as NTM and mainly in CTM.

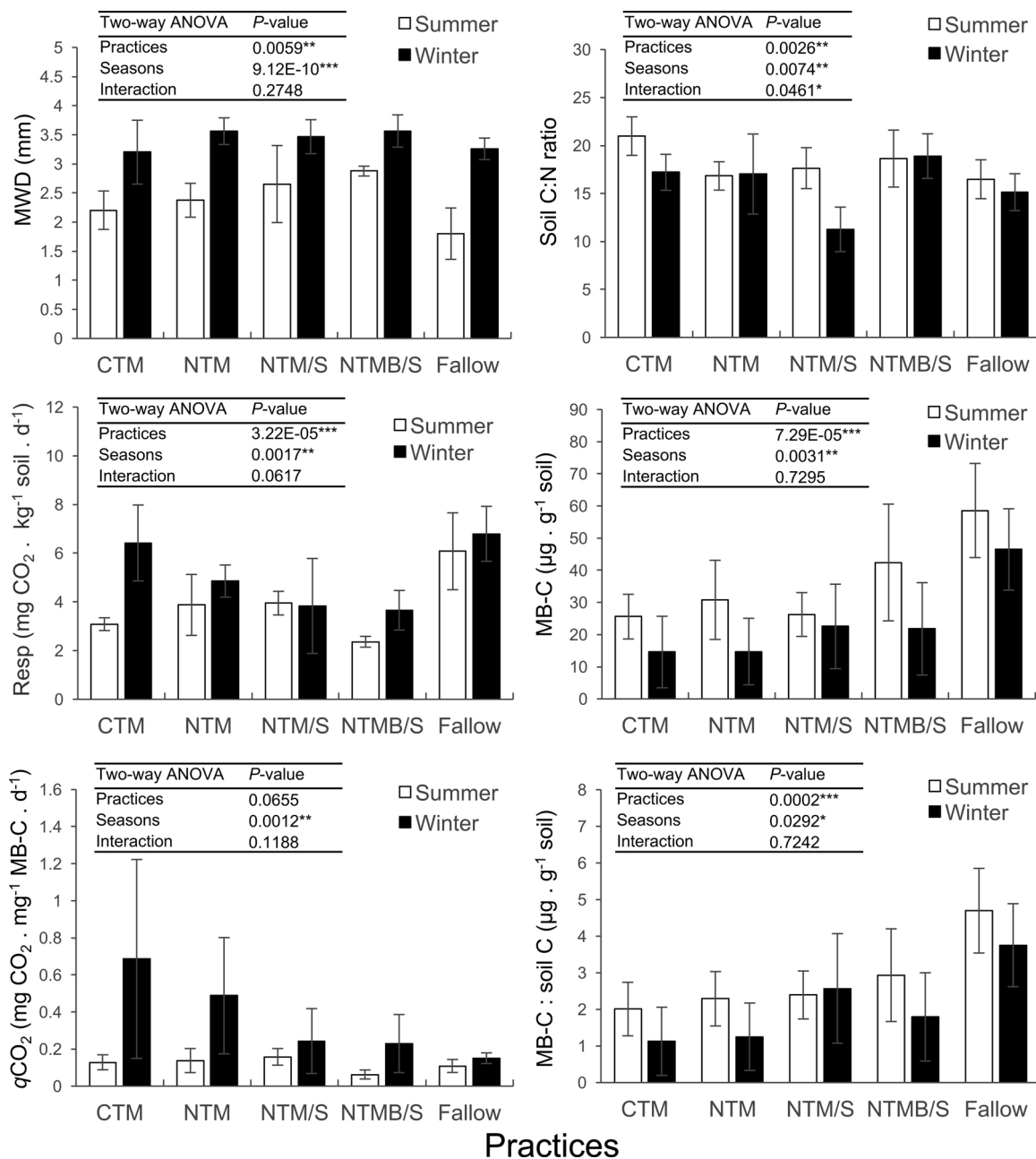
#### 3.2. Multivariate differences between practices and seasons regarding physicochemical and microbiological data

Biplot PCAs indicated that both physicochemical and microbiological data were affected by seasonality, confirmed by ANOSIM ( $P < 0.05$ ). The ordination of samples was mostly explained by PC1, which showed 76.2 and 71.2 % of the data variability for the physicochemical and microbiological variables, respectively (Fig. 2). However, the influence of the winter (rainy) and summer (dry) seasons was more effective on the microbiological than on the physicochemical variables, since the separation of summer and winter samples were higher and along PC1 for the first, while lower and along PC2 for the latter (Fig. 2). For the physicochemical data, the separation of samples along PC1 was determined by practices, with the ordination of NTMB/S and fallow samples at the right and NTM/S and CTM samples at the left of the graph (Fig. 2A). On the other hand, samples from the winter accumulated at the left, while samples of the summer accumulated at the right of the graph for microbiological data (Fig. 2B). It is noteworthy that summer

**Table 1**

Univariate statistical analysis comparing the five practices according to the variables: WSA (%), MWD (mm), SOM (g kg soil<sup>-1</sup>), total soil N (g kg soil<sup>-1</sup>), soil C:N ratio, pH (soil:water 1:2.5), basal respiration (mg C-CO<sub>2</sub> kg soil<sup>-1</sup> d<sup>-1</sup>), MB-C (μg g soil<sup>-1</sup>), MB-N (μg g soil<sup>-1</sup>), MB-C:N ratio,  $qCO_2$  (mg CO<sub>2</sub> mg<sup>-1</sup> MB-C d<sup>-1</sup>) and MB-C:soil C (μg g soil<sup>-1</sup>). Averages of the four blocks are shown for the 12 variables in each practice at both seasons. Different letters indicate significantly different according to Tukey test ( $P < 0.05$ ).

	Physicochemical						Microbiological				Mixed	
	WSA	MWD	SOM	Soil N	Soil C:N	pH	Respiration	MB-C	MB-N	MB-C:N	$qCO_2$	MB-C:soil C
	Summer											
CTM	4.1 bc	2.2 ab	22.5 ab	0.6 a	21.0 a	4.9 b	3.1 bc	25.6 b	7.3 a	3.7 a	0.13 ab	2.0 b
NTM	5.1 bc	2.4 ab	22.9 ab	0.8 a	16.8 a	4.9 b	3.8 abc	30.8 b	8.0 a	3.9 a	0.14 ab	2.3 b
NTM/S	3.4 c	2.6 ab	19.0 b	0.6 a	17.6 a	5.0 b	3.9 ab	26.2 b	7.1 a	4.0 a	0.16 a	2.4 b
NTMB/S	7.7 ab	2.9 a	24.9 a	0.8 a	18.6 a	4.6 b	2.4 c	42.4 ab	9.6 a	5.4 a	0.06 b	2.9 ab
Fallow	10.7 a	1.8 b	21.5 ab	0.7 a	16.5 a	5.4 a	6.1 a	58.6 a	9.8 a	6.7 a	0.11 ab	4.7 a
	Winter											
CTM	3.0 b	3.2 a	23.1 a	0.8 ab	17.2 a	5.0 b	6.4 ab	14.6 b	8.5 a	2.0 a	0.69 a	1.1 b
NTM	5.5 ab	3.6 a	21.1 a	0.7 ab	17.0 a	5.1 ab	4.8 b	14.7 b	15.5 a	1.4 a	0.49 a	1.2 ab
NTM/S	2.0 b	3.5 a	15.0 b	0.8 ab	11.2 b	5.0 bc	3.8 ab	22.5 ab	6.5 a	4.7 a	0.24 a	2.6 ab
NTMB/S	5.1 ab	3.6 a	21.0 a	0.6 b	19.0 a	4.8 c	3.6 b	21.8 ab	10.5 a	2.1 a	0.23 a	1.8 ab
Fallow	10.4 a	3.3 a	21.5 a	0.8 a	15.1 ab	5.3 a	6.8 a	46.5 a	13.2 a	4.9 a	0.15 a	3.7 a

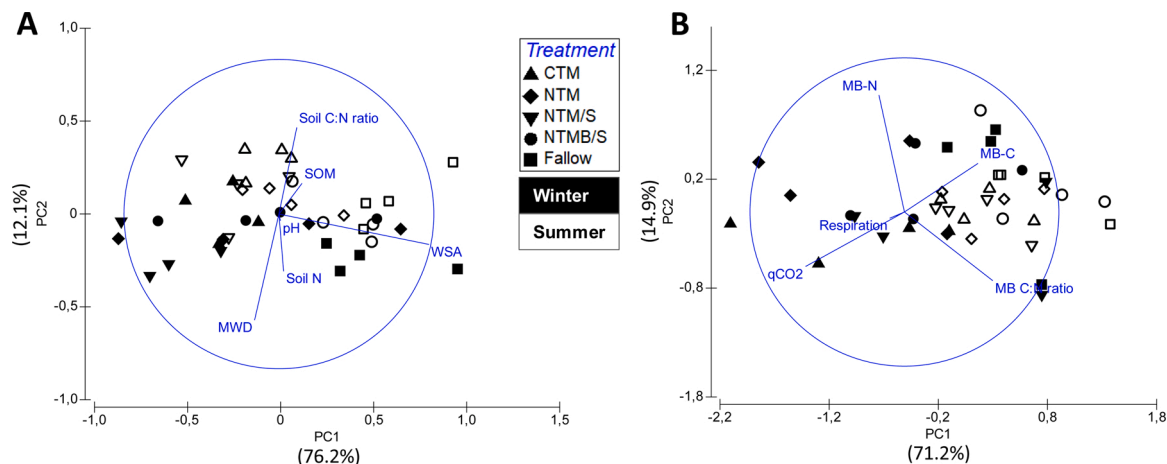


**Fig. 1. Microbiological and physicochemical variables significantly different between seasons.** Bar graphs showing the averages of variables in each agricultural practice and season. Vertical bars represent the standard deviation in each treatment. Variables shown in this figure were significantly different between summer and winter seasons according to two-way ANOVA ( $p < 0.05$ ). Results of two-way ANOVA are displayed above each bar graph, containing the  $p$ -value of each factor (seasons and agricultural practices) and their interaction.

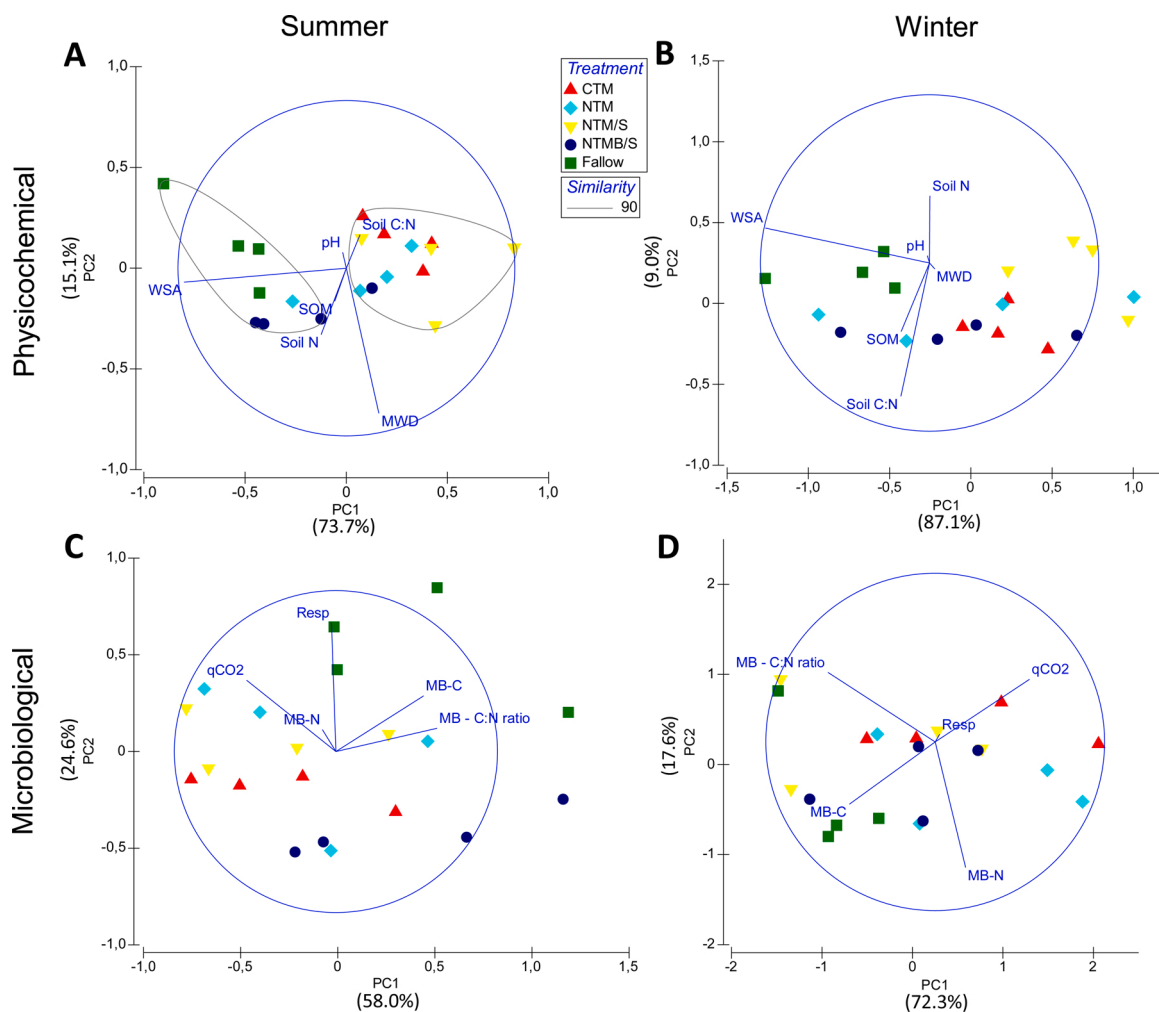
samples were more closely related between each other, whereas winter samples were more dispersed in the graph, indicating higher microbial variability among samples in the winter (Fig. 2B). For the physicochemical data, MWD and soil C:N ratio were the variables most affected by seasons, increasing their values in the winter and summer, respectively. On the other hand, WSA showed a high stability between seasons and was the main variable associated with the differences between practices, increasing its values in NTMB/S and fallow (Fig. 2A). For the microbiological data, MB-N showed a low variation between seasons. Conversely  $q\text{CO}_2$  increased in the winter, while MB-C and MB-C:N ratio increased in the summer (Fig. 2B).

Since the physicochemical and microbiological data were affected by seasonality, we analyzed the seasons separately to better identify the differences associated with agricultural practices. For the

physicochemical data in the summer, biplot PCA showed a separation of all fallow and most NTMB/S samples from the other agricultural practices (Fig. 3A). ANOSIM indicated that fallow was significantly different to NTMB/S, and both were different to CTM and NTM/S, while NTM was not different to NTMB/S (Table 2). All fallow and most NTMB/S samples clustered in a group, whereas all CTM, NTM/S and most NTM samples clustered in another group of >90 % similarity. SIMPER analysis identified WSA as the main variable differentiating most practices, with the highest percentage of contribution for the separation of fallow and NTM/S samples (54.4 %). In the winter, the separation of agricultural practices was less clear, with a higher clustering of NTMB/S with NTM and CTM samples than in the summer (Fig. 3B). Fallow was significantly different to all practices except NTM, but NTMB/S was only different to NTM/S (Table 2). Both physical variables (WSA and MWD) were highly



**Fig. 2. Principal component analyses (PCA) of microbiological and physicochemical data.** PCA showing the ordination of samples from the five agricultural practices in both seasons. A) Ordination of samples according to physicochemical data and correlations with each physicochemical variable. B) Ordination of samples according to microbiological data and correlations with each microbial variable.



**Fig. 3. Principal component analyses (PCA) of microbiological and physicochemical data separated for each season.** PCA showing the ordination of samples from the five agricultural practices for physicochemical (A–B) and microbiological variables (C–D) in the summer (A, C) and winter (B, D) seasons. A) Ordination of samples according to physicochemical data in the summer and correlations with each physicochemical variable. Groups of samples within the circles share >90 % similarity according to cluster analysis using the Bray Curtis similarity index; B) Ordination of samples according to physicochemical data in the winter and correlations with each physicochemical variable; C) Ordination of samples according to microbiological data in the summer and correlations with each microbiological variable; D) Ordination of samples according to microbiological data in the winter and correlations with each microbiological variable.

**Table 2**

Analysis of similarity (ANOSIM) between the five practices analyzed in our study according to physicochemical and microbiological data in both seasons. Different letters represent significant differences ( $P < 0.05$  and  $R > 0.4$ ).

	Physicochemical		Microbiological	
	Summer	Winter	Summer	Winter
Fallow	a	a	a	a
NTMB/S	b	b	ab	ab
NTM	bc	abc	abc	ab
NTM/S	c	c	c	ab
CTM	c	b	bc	b

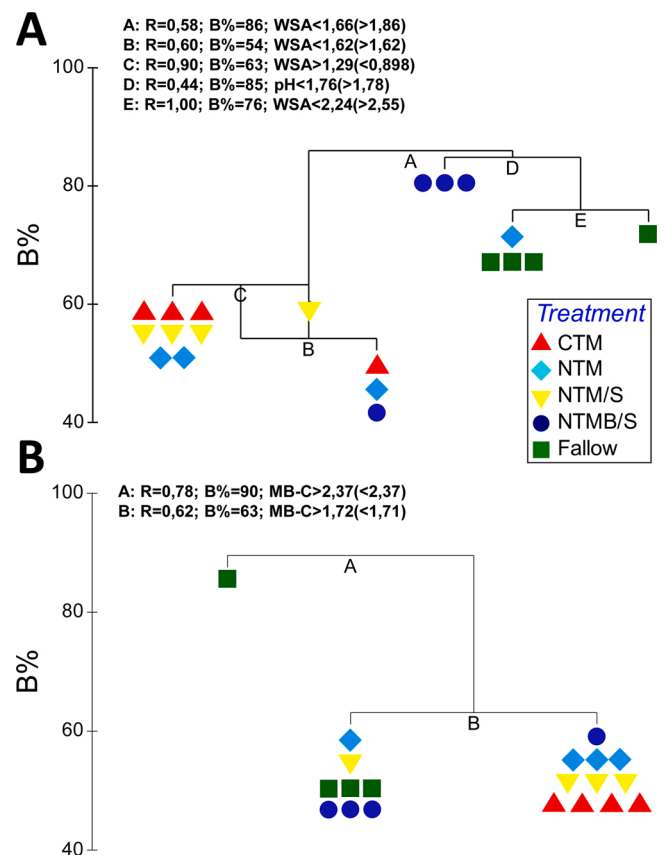
associated with the ordination of samples (PC1 and 2) in the summer, but only WSA was distinctive between practices in the winter (Fig. 3A and B), since MWD increased to similar levels among all practices at this season (Fig. 1). SIMPER analysis confirmed that WSA increased its percentage of contribution for the differentiation of practices in the winter, with the highest difference observed between fallow and CTM (69.4 %). When analyzing only the three variables directly associated with soil quality (WSA, MWD and SOM), ANOSIM revealed a similar result, with the same significant differences between practices (Table 2).

The microbiological parameters showed distinct behaviors at the two seasons. In the summer, NTMB/S and fallow samples were more distant, and separated from the other practices (Fig. 3C). However, significant differences were only observed for fallow compared to CTM and NTM/S, and between NTMB/S and NTM/S (Table 2). The highest values of respiration in fallow promoted the highest divergence compared to NTMB/S, while both showed decreased  $qCO_2$  and increased MB-C and MB-C:N ratio compared to the other practices (Fig. 3C). In the winter, the separation of practices according to microbiological data was even lower, with significant differences just detected between fallow and CTM (Fig. 3D; Table 2). SIMPER analysis indicated that distinct variables were more associated with the differences between practices along the two seasons, including basal respiration, MB-C and MB-C:N ratio. However, the variable showing the highest contribution percentages in both seasons was  $qCO_2$ , differentiating NTMB/S and NTM/S in the summer (30.8 %), and NTMB/S and CTM in the winter (32.2 %).

### 3.3. Correlations between physicochemical and microbiological data

Mantel tests identified correlations between the physicochemical and microbiological data in the summer ( $P = 0.01$ ;  $\rho = 0.42$ ), but not in the winter ( $P = 5.51$ ;  $\rho = -0.02$ ). Thus, we further explored these correlations in the summer season. BEST analysis identified WSA and pH as the set of physicochemical variables most correlated with the microbiological data ( $\rho = 0.45$ ), while MB-C and MB-N was the set of microbiological variables most correlated with the physicochemical data ( $\rho = 0.50$ ). Thus, these variables were selected for linkage tree analyses. Linkage tree in the microbiological data showed that samples were initially split in a node containing all fallow and most NTMB/S samples, and another node containing all CTM, all NTM/S and most NTM/S samples (Fig. 4A). Higher WSA values in NTMB/S and fallow explained this initial separation of samples on microbiological data. Higher WSA also explained the separation of the remaining NTMB/S sample, plus a NTM and a CTM sample, from the other nodes dominated by NTM/S and CTM samples. However, pH rather than WSA explained the microbiological separation of fallow from most NTMB/S samples (Fig. 4A).

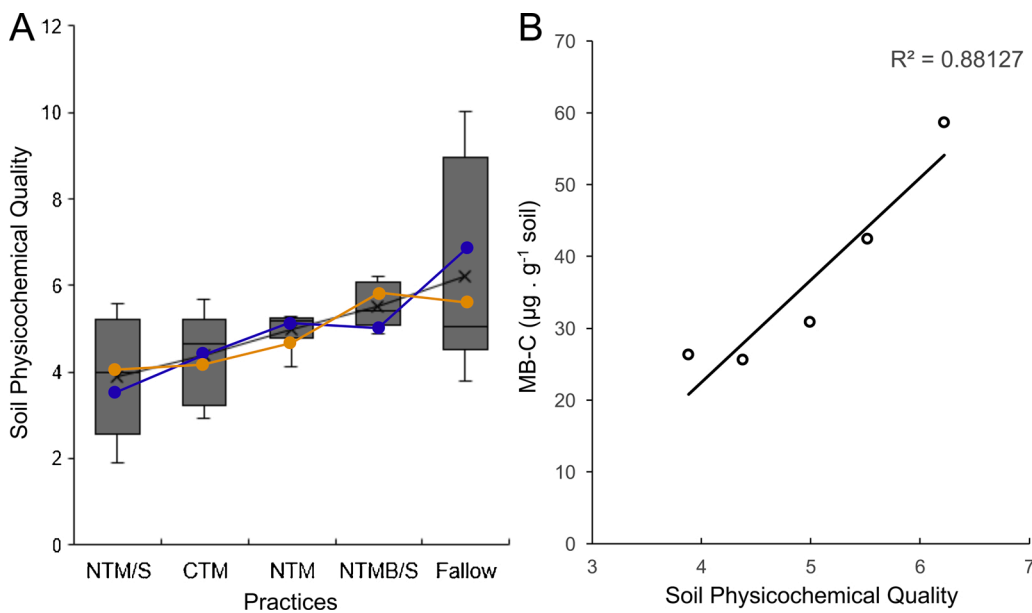
Regarding the linkage tree on the physicochemical data, an outlier sample of fallow was distinct to all others, with higher MB-C values explaining this initial node division (Fig. 4B). Accordingly, higher MB-C values also explained the separation of the other fallow samples and most NTMB/S samples from the other node, which contained all CTM samples and most NTM and NTM/S samples (Fig. 4B). Linear correlations were observed between individual physicochemical and microbiological variables in the summer as well. WSA was positively correlated



**Fig. 4. Linkage tree analyses showing division of samples according to physicochemical and microbiological data in the summer.** Optimal ANOSIM R value (relative subgroup separation) and B% (absolute subgroup separation, scaled to maximum for first division) are provided for each split, which are significant ( $P < 0.05$ ) according to SIMPROF tests (999 permutations). A) Linkage tree splitting groups of samples according to microbiological data and explained by the set of physicochemical variables selected in BEST analysis with higher  $\rho$  values (WSA = water stability of aggregates); B) Linkage tree splitting groups of samples according to physicochemical data and explained by the set of microbiological variables selected in BEST analysis with higher  $\rho$  values (MB-C = microbial biomass-C). The values of variables explaining each node split shown above the trees are post-transformed (standardization by total and log transformation).

with MB-C ( $P < 0.001$ ;  $r = 0.68$ ) – corroborating linkage trees results – and negatively correlated with  $qCO_2$  ( $P < 0.01$ ;  $r = -0.56$ ); MB-N was positively correlated with soil N ( $P < 0.05$ ;  $r = 0.54$ ) and negatively correlated with soil C:N ratio ( $P < 0.01$ ;  $r = -0.65$ ); and basal respiration was positively correlated with soil pH ( $P < 0.001$ ;  $r = 0.87$ ).

Analysis of the three physicochemical variables most associated with soil quality (SOM, WSA and MWD) confirmed the results from multivariate analyses for all physicochemical data, showing that soil quality increased in the same order: high in fallow and NTMB/S, intermediate in NTM, and low in NTM/S and CTM (Fig. 5A, Table 2). NTMB/S and NTM showed a lower variation between the three variables and two seasons compared to the other practices. Similarly to the results of multivariate regression (linkage) trees in the summer, linear regressions also showed that MB-C was the microbiological variable most associated with soil physicochemical quality in the summer ( $R^2 = 0.88$ ), indicating that this variable could be used as a predictor of soil quality in this season (Fig. 5B).



**Fig. 5. Estimation of soil physicochemical quality and correlations with microbiological parameters.** Soil physicochemical quality (SPCQ) was obtained as the combined averages of the standardized variables soil organic matter (SOM), mean weight diameter (MWD) and water stability of aggregates (WSA) in both seasons without pre-weighting. A) Box plots showing the variation in SPCQ between agricultural practices. The X symbol and black line represent the averages, while the whisker and quartile sizes indicate the variability between the three variables in both seasons for each practice. Blue line and dots represent the averages of the three variables in the winter, while orange line and dots represent the averages of the three variables in the summer. B) The microbiological variable (MB-C) showing the highest  $R^2$  on the linear regressions with SPCQ in the summer.

## 4. Discussion

### 4.1. Seasonal shifts on soil microbiological and physicochemical parameters

Our results showed that soil physicochemical parameters were in general more stable along the seasons than the microbiological, confirming previous studies that reported high seasonal variation in biological variables (Paz-Ferreiro et al., 2013; Benintende et al., 2015). However, a physical (MWD) and a chemical (soil C:N ratio) parameter were changed between the seasons according to two-way ANOVA (Fig. 1). The change in MWD was more dramatic than in soil C:N ratio and observed in all practices, increasing in the winter. This sharp increase in MWD was possibly associated with the higher water content in the winter, since it can promote soil aggregation (Wang et al., 2014). Moreover, the rhizodeposition in the winter also might have contributed to the higher MWD (Liu et al., 2019), because crops are in the field at this season and root secretion probably reaches the area of soil sampling (inter-rows), since the spacing between plants is reduced (0.5 m between rows). The decrease of soil C:N ratio in the winter was probably associated with the N fertilization before planting, as well as the possible induction of free-living diazotrophic bacteria by the plant rhizosphere, which can increase soil N (Gupta et al., 2014; Smircina et al., 2019).

Among the microbiological variables, basal respiration and  $qCO_2$  increased in the winter, possibly responding to the higher soil moisture and availability of labile C-sources present in root exudates, since both usually increase microbial activity (Yan et al., 2015; de Vries et al., 2019d). In contrast, MB-C decreased in the winter. The simultaneous decrease in MB-C and increase in basal respiration caused the increase in  $qCO_2$ , indicating that microbial metabolism destined more energy for carbon degradation than for growth at this season, resulting in higher C-mineralization than immobilization. Some studies also found higher microbial biomass and lower microbial activity in drier conditions, which might be associated with an incapacity of the soil microbiota to produce the enzymes needed for carbon degradation under low water potential, coupled with a shift in the microbial physiology in order to accumulate osmolytes, exopolysaccharides (EPS) and thicker cell walls to resist soil drought (Robertson and Firestone, 1992; Schimel et al., 2007; Allison and Goulden, 2017; Schaeffer et al., 2017; Kakumanu et al., 2019).

The decrease in MB-C might be also due to an ecological change in the soil microbiome, since multivariate analysis showed that MB-C:N

ratio – but not MB-N – decreased in the winter (Fig. 2B), suggesting that bacteria were favored in this season compared to fungi. It was previously shown that bacteria from the Proteobacteria phylum dominates the rhizosphere of many plants and have a copiotrophic or *r*-strategist lifestyle, adapted to the higher availability of labile C-sources secreted by plant roots (Fierer et al., 2007; Peiffer et al., 2013; Fernández-Gómez et al., 2019; Pérez-Jaramillo et al., 2019). On the other hand, soil fungi are in general more tolerant to desiccation and promotes higher C conservation than bacteria under drought stress (Schimel et al., 2007; Barnard et al., 2013; Malik et al., 2016). Since in the winter the soil had a higher water content and rhizosphere effect than in the summer, we infer that both these abiotic and biotic factors were responsible for the physiological and ecological changes in the soil microbiome, decreasing and increasing microbial biomass and activity, respectively.

It is worth mentioning that the most dramatic increase in both soil basal respiration and  $qCO_2$  in the winter was observed in CTM, which was the only practice submitted to plowing and disking operations. The disrupting of soil structure before planting in conventional tillage enables the soil microbiota to access particulate organic matter entrapped in soil aggregates, increasing microbial activity and hence  $CO_2$  emissions (Fiedler et al., 2016). Moreover, conventional tillage promotes higher soil aeration, which favors aerobic respiration compared to no-till (Yonemura et al., 2013; Neogi et al., 2014). In contrast, fallow showed the most similar levels of  $qCO_2$  between seasons, indicating the greater stability of the microbial communities in non-agricultural soils in the face of seasonal weather changes.

Our results also indicated that both the physicochemical and microbiological parameters showed higher differences between the five assessed practices in the summer, when the microbial community was under water and nutritional stress, since the precipitation was drastically reduced and the labile C-sources from rhizodeposition were lacking (CTM, NTM and NTM/S) or lower (NTMB/S, fallow) than in the winter (Holz et al., 2018). Therefore, the benefits or disadvantages of the compared practices were best observed in the summer, when both soil physicochemical quality and microbial activity/biomass were more associated with the agricultural practices.

### 4.2. Physicochemical and microbial changes associated with increasing soil quality

Our study showed that in the summer soil physicochemical quality



was higher in fallow and NTMB/S, intermediate in NTM and lower in CTM and NTM/S (Fig. 5A). On the other hand, in the winter soil quality was higher in fallow, intermediate in NTMB/S, NTM and CTM, and lower in NTM/S (Fig. 5A). This result was mainly influenced by WSA, which was higher in fallow followed by NTMB/S along the seasons. A previous study showed that fallow promoted higher soil physicochemical quality than agriculture in the Brazilian coastal tablelands even in the short-term (Fernandes et al., 2011). The higher similarity of NTMB/S and fallow indicated that no-till combined with the intercropping of maize and *B. rhiziziensis* was highly beneficial for soil quality in this region. The NTMB/S practice conserved soil quality compared to fallow and increased compared to the other agricultural practices because the permanent presence of *B. rhiziziensis* in the field probably promoted higher soil coverage than only plant litter of the other no-till practices (NTM and NTM/S), as well as provided a continuous C supply from roots secretion. This higher C supply may be the reason why higher SOM levels and hence higher MWD were observed in NTMB/S compared to all other practices (including fallow) in the summer, which are important parameters for soil quality (Karami et al., 2012). Intercropping was shown to also improve soil quality in other soil types and geographic regions (Cong et al., 2014; TerAvest et al., 2015; Naab et al., 2017).

Curiously, NTM/S showed soil physicochemical quality parameters as low (WSA) or even lower (SOM) than CTM (Fig. 5A, Table 2). Soil disturbance of conventional tillage was largely shown to decrease soil physicochemical quality, since it impacts soil structure and aggregation, which in turn exposes SOM decreasing its contents (Fiedler et al., 2016), while no-till generally increases these parameters (Aziz et al., 2013; Crittenden et al., 2015; TerAvest et al., 2015; Naab et al., 2017). However, we also analyzed the effect of crop rotation with legume in our study besides soil tillage. The NTM/S practice decreased soil physicochemical quality compared to NTM in the summer, and compared to both NTM and CTM in the winter (Fig. 5A). The reason for this result is unclear, but is probably associated with the lower SOM content of NTM/S compared to all other practices (Table 1). Previous studies showed that maize increased soil organic carbon compared to soybean (Huggins et al., 2007). With respect to the shifts in microbiological parameters, NTMB/S also showed some advantages compared to the other practices. This agricultural practice promoted higher SOM and lower respiration rates as well as  $qCO_2$  than all other practices (including fallow), releasing less C to the atmosphere. In addition, MB-C was also higher in fallow and NTMB/S, indicating that these practices favor microbial growth and C immobilization. The microbial quotient (MB-C:soil C) – a widely used indicator of soil quality (Kaschuk et al., 2010) – was also increased in fallow and NTMB/S compared to the other practices, mainly in the summer. Finally, MB-C:N ratio increased in these practices in the summer according to multivariate analysis (Fig. 2B), suggesting that fungi are stimulated under agricultural practices promoting higher soil quality. Recent studies have changed the previous belief that soil fungi are favored in environments with restricted and recalcitrant C-sources, showing that fungal abundance is higher in conditions with high-quality C, such as in rhizosphere-dominated soil, as observed in the NTMB/S and fallow practices (Rousk and Frey, 2015; Hu et al., 2017; Bluhm et al., 2019).

#### 4.3. Ecological relations between soil microbial and physicochemical attributes

Summer season promoted not only a higher difference in microbiological and physicochemical parameters between practices, but also a higher association between soil environment and microbial activity/biomass. At this season, the different soil attributes of the compared practices influenced the dynamics of soil microbes, which in turn possibly contributed to the differences in soil physicochemical quality. On the other hand, in the winter there was no correlation between physicochemical and microbiological variables, indicating that other factors mostly contributed to the differences in soil quality and

microbial responses, such as the higher water content and rhizodeposition.

In the summer, our results indicated that WSA was highly associated with the activity and biomass of soil microbial communities, which makes sense because the aggregates were the main hot spots for microbial life at this season, since rhizosphere and plant litter were lower or lacking (Gupta and Germida, 2015; Kuzyakov and Blagodatskaya, 2015). In turn, microbial biomass was the microbiological parameter more associated with the physicochemical differences between practices, mainly the stabilization of aggregates. Many studies showed that fungal hyphae, as well as bacterial EPS are important for the formation and stabilization of aggregates, indicating the importance of increased microbial biomass for soil aggregation (Gupta and Germida, 2015; Lehmann et al., 2017; Krause et al., 2019). Besides decreasing microbial biomass, we also found that soil disturbance increased  $qCO_2$  (CTM), which is indicative of microbial stress, and therefore can possibly contribute for decreasing aggregates stability (Anderson and Domsch, 1993). Soil disturbance was previously shown to increase microbial stress and decrease microbial biomass in the Brazilian coastal tablelands (Chaer et al., 2009; Fernandes et al., 2011).

Since in the summer soil physicochemical quality of fallow and NTMB/S were more similar between each other compared to the other practices (Fig. 5A), another soil component not directly associated with soil quality was most correlated with the differences in microbial parameters for these two practices, i.e. soil pH (Fig. 4A). Soil pH was shown to be important for determining soil bacterial diversity, community structure and biogeography (Fierer and Jackson, 2006). This factor was secondary in our study (only affecting NTMB/S vs. fallow) because we analyzed soil microbial activity and biomass, but not microbial diversity, which is usually highly correlated with pH. Future studies are needed to analyze the shifts in the structure and composition of soil microbial communities of these practices, in order to identify specific microbial groups associated with increasing soil quality and not affected by the contrasting seasons, potential useful bioindicators of sustainable agricultural practices in the Brazilian coastal tablelands ecosystem.

## 5. Conclusions

Our results showed that both physicochemical and microbiological parameters were in general changed between seasons, but the physicochemical were more stable. The differences in soil quality between practices and the correlation between microbial and physicochemical variables were more observed in the summer (dry period), possibly because this season has less influence of other factors unrelated to soil quality (e.g. soil moisture, presence of crops). Therefore, the best time for assessing differences in soil quality between agricultural practices in this region is the summer. The most conservationist practice regarding both soil tillage and crop system complexity (NTMB/S) maintained the soil quality observed in fallow, showing the highest soil quality among all agricultural practices analyzed. On the other hand, NTM/S and CTM showed the lowest soil quality among the five practices. Additionally, we identified WSA as the physicochemical variable most distinctive between agricultural practices, most stable between seasons, and most associated with soil microbial parameters, indicating its high usefulness for assessing soil quality. Regarding the microbial parameters, MB-C and  $qCO_2$  were the variables most associated with changes in soil quality between agricultural practices. MB-C was highly correlated with increases in soil physicochemical quality in the summer, suggesting it is a suitable predictor of soil quality in this region.

## Authors contributions

LDL collected and processed soil samples, performed laboratory and statistical analyses, interpreted the results and wrote the manuscript. RCFJ performed laboratory analyses. EPP designed and conducted the field experiment. MFF conceptualized the study, 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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### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.still.2020.104819>.

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